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Overview

A Systematic Review and Meta-Analysis of Prognostic Biomarkers in Anal Squamous Cell Carcinoma Treated With Primary Chemoradiotherapy

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Abstract

Recent studies suggest that the treatment response and survival from head and neck tumours can be stratified according to biomarker status, particularly human papillomavirus (HPV) status and p16 expression, but the evidence for predictive biomarkers in anal squamous cell carcinoma (ASCC) remains limited. The aim of this study was to determine which biomarkers were associated with locoregional recurrence (LRR), overall survival and disease-free survival (DFS) in ASCC. A systematic search was undertaken of the MEDLINE, Embase, Cochrane Library, CINAHL and Web of Science databases using validated terms for ASCC, biomarkers and prognosis. Biomarkers were included in the meta-analysis if they were reported by at least four studies and provided sufficient data to permit the calculation of survival effect estimates. HPV status, p16, p53 and epidermal growth factor receptor (EGFR) met the inclusion criteria for meta-analysis and were reported by 17 retrospective cohort studies describing 1635 patients. When compared with HPV-negative tumours, HPV-positive tumours were associated with reduced LRR (pooled hazard ratio = 0.27 [95% confidence interval 0.16–0.48]; $P < 0.001$), improved overall survival (hazard ratio =0.26 [0.12–0.59]; $P = 0.001$) and DFS (hazard ratio = 0.33 [0.16–0.70]; $P = 0.003$). Likewise, p16-positive tumours were associated with reduced LRR (hazard ratio = 0.26 [0.13–0.52]; $P < 0.001$), improved overall survival (hazard ratio = 0.44 [0.24–0.81]; $P = 0.009$) and DFS (hazard ratio = 0.44 [0.23–0.83]; $P = 0.012$) when compared with p16-negative tumours. HPV-positive/p16-positive tumours had improved overall survival when compared with HPV-negative/p16-negative tumours (hazard ratio = 0.27 [0.15–0.48], $P < 0.001$), but not HPV-negative/p16-positive tumours (hazard ratio = 0.64 [0.21–1.90]; $P = 0.421$). p53 mutation was associated with worse DFS (hazard ratio = 1.63 [1.33–2.01]; $P = 0.003$). There was no association between EGFR status and any survival outcome. HPV status, p16 and p53 expression are of prognostic utility in ASCC. Future studies should prospectively validate these findings with a view to conducting subsequent randomised controlled trials where patients are stratified according to biomarker status and randomised to different treatment regimens.

Key words: Anus neoplasms; biomarkers; papillomaviridae; prognosis

Statement of Search Strategies Used and Sources of Information

A comprehensive systematic search was undertaken in accordance with the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines. The PubMed/MEDLINE Ovid, Embase, Cochrane Library and CINAHL databases were searched from 1990 to the present day. Key words and medical sub-heading (MeSH) terms used in the search strategy covered three concepts: ASCC, biomarkers and prognosis. Key words and MesH terms within each concept were then separated by the Boolean operator 'AND'. The most recent search was carried out on 17 December 2018. The review was registered with PROSPERO at https://www.crd.york.ac.uk/prospero (CRD42018102841).

Introduction

The incidence of anal squamous cell carcinoma (ASCC) is increasing across the developed world, with incidence rates in the UK nearly doubling between 1990 and 2010 [1,2]. Following the seminal work of Nigro *et al.* [3] in the 1970s, chemoradiotherapy (CRT) has become the standard primary

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treatment modality for ASCC. Survival outcomes have remained unchanged in the last 20 years, despite several trials having been conducted over this time period that have compared the effectiveness of alternative chemotherapy regimens against the original mitomycin and 5-fluorouracil regimen described by Nigro [4]. According to data from the ACTII trial, 3-year locoregional control and overall survival from ASCC are currently 70–80% and 80–85%, respectively [5]. Given that CRT is the standard of care for ASCC, the identification of biomarkers that can accurately predict treatment response and thus long-term survival could be an important translational question if significant improvements in survival outcomes are to be achieved.

In a previous systematic review of biomarkers in ASCC, 13 potential prognostic molecular biomarkers were identified [6]. However, only p53 and p21 were shown to be associated with outcome in more than one study. More recent studies in the field of head and neck squamous cell carcinoma (HNSCC) have established that infection with human papillomavirus (HPV) is associated with improved survival outcomes [7,8], although the biological basis for this survival advantage is not clearly understood and may in part be explained by the increased radiosensitivity of HPV-positive tumours [9]. Improvements in survival outcomes for HPV-positive patients treated with CRT have also been noted in ASCC, but have only been reported by a limited number of studies [10,11]. HPV is a well-characterised aetiological agent in the development of orogenital squamous cell carcinomas, with 85–90% of ASCCs demonstrating HPV-positive status [12], of which HPV16 is the most common subtype [12,13]. The presence of HPV DNA alone provides insufficient evidence for HPV infectivity in the pathway to malignancy, as the virus may be biologically inactive [14]. Therefore, the tumour suppressor gene p16 is frequently used as a surrogate marker of the activity of HPV-associated viral oncoproteins and is relatively straightforward to detect with immunohistochemistry [14,15].

A key difference between HPV-positive and HPV-negative HNSCC tumours is the frequency of mutations in the TP53 gene that encodes p53. Although TP53 mutations rarely occur in HPV-positive tumours (0–10%), they occur much more frequently in HPV-negative tumours (80–100%) [16,17]. Analogous findings in squamous cell carcinomas of the anal canal have recently been reported; Meulendijks *et al.* [18] reported disruptive TP53 mutations in 80% of HPV-negative/p16-negative tumours in their series. Aberrant p53 expression is known to reduce sensitivity to radiotherapy, but evidence for its prognostic value in HNSCC remains inconclusive [19].

In summary, there is increasing evidence to suggest that the response to CRT and survival in HNSCC may be stratified according to biomarker expression, particularly HPV and p16. The data for the effect of these biomarkers on outcome in ASCC are more limited and provide the rationale for this study. The aim was to identify and assess the effect of biomarkers on locoregional recurrence (LRR), overall survival and disease-free survival (DFS) in ASCC after treatment with primary CRT.

Materials and Methods

Search Strategy

A comprehensive systematic search was undertaken in accordance with the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines [20]. The PubMed/MEDLINE Ovid, Embase, Cochrane Library and CINAHL databases were searched from 1990 to the present day. Key words and medical sub-heading (MeSH) terms used in the search strategy covered three concepts: ASCC, biomarkers and prognosis. Key words and MesH terms within each concept were then separated by the Boolean operator 'AND' (see Supplementary Appendix S1). The most recent search was carried out on 17 December 2018. The review was registered with PROSPERO at https://www.crd.york.ac.uk/prospero (CRD42018102841).

Study Selection

Identified studies were subsequently screened and duplicate records removed. Studies were deemed eligible for inclusion if they were full-text English-language articles and reported outcomes from patients with ASCC stratified according to biomarker status who received CRT as their initial treatment modality. Where multiple studies described the same cohort of patients, the largest and most complete data were included. Abstracts and conference proceedings were also excluded during the preliminary screening because of the high risk of incomplete data. Studies were also excluded if they were review articles, contained a cohort of fewer than 10 patients, animal studies, involved patients with non-ASCCs or did not report the prognostic value of the biomarkers being studied or used abdominoperineal excision as the primary treatment modality. A supplemental hand search of selected reviews and the reference lists of all the included studies in the review was undertaken to identify potentially eligible studies missed in the original search. Articles that reported on the prognostic value of a biomarker that had been reported by at least four studies, and provided sufficient information to calculate survival effect estimates, were included in the meta-analysis in order to maximise statistical power and account for heterogeneity between studies.

Data Extraction

Data from selected studies were independently extracted by two reviewers (IP and TM) using a dedicated data extraction form (see Supplementary Appendix S2). The first author's name, country, year of publication, number of participants, study design, tumour location, TNM staging, staging modalities, CRT regimens, biomarker detection techniques, cut-off values used to define a positive result, follow-up protocols, duration of follow-up, survival outcome measures (and their definition) and statistical analyses of the survival end points were all recorded. Disagreement was resolved by discussion with the senior author (DEM).

Assessment of Quality

The Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) [21] was used as the criteria to assess the methodological quality of studies included in the review. The REMARK criteria evaluate the following six key areas: sample definition, adequacy of baseline clinical data, description of immunohistochemical techniques, definition of survival outcomes, adequacy of statistical analysis with reference to cut-off values used to stratify groups and reporting of the relationship between the biomarkers and classical prognostic factors.

Outcome Measures

For the purposes of this review, LRR was defined as the time from the beginning of CRT to when locoregional failure was detected at either the primary site of the tumour or mesorectal, pelvic or inguinal lymph nodes either by radiological imaging or biopsy. Overall survival was defined as the time from the beginning of CRT to when death from any cause occurred. DFS was defined as the time from the beginning of CRT to when disease recurrence was detected, either LRR or distant metastases, or to when death occurred from any other cause. A complete clinical response (cCR) was considered to be a secondary outcome measure and was defined as the absence of any residual tumour at 6 months after the completion of CRT on both clinical examination and radiological assessment.

Statistical Analysis

The summary measure for the dichotomous outcomes of LRR, overall survival and DFS was the hazard ratio with 95% confidence intervals. Only those studies providing an estimate of hazard ratios and associated 95% confidence intervals for biomarker expression were included in the meta-analysis of LRR, overall survival and DFS. Where studies did not report hazard ratios and their associated 95% confidence intervals, the summary time to event data were manipulated in accordance with the methods described by Tierney *et al.* [22] to calculate these values. Only meta-analyses of the unadjusted effect estimates were carried out for the following reasons: (i) there were insufficient adjusted estimates reported by individual studies for each of the survival outcome measures to allow any meaningful meta-analysis, (ii) confounders included in the multivariate models of survival differed between individual studies, (iii) the number of covariables and the sample size differed between studies, thus increasing the potential for unstable regression modelling and (iv) multicollinearity due to the highly correlated nature of HPV status and p16 expression was rarely accounted for by regression modelling in most studies. The chi-squared test of heterogeneity was used to measure the heterogeneity of hazard ratios between studies. Summary estimates for hazard ratios were computed by either the fixed or random-effects models, depending on the evidence for heterogeneity between studies.

Results

Description of Studies

The search strategy yielded 4005 citations from the electronic databases and 64 full-text articles were reviewed (Figure 1). These articles assessed 41 unique biomarkers, of which 10 biomarkers were reported by at least two studies (Figure 2). Sufficient prognostic data were provided on HPV status, p16, p53 and epidermal growth factor receptor (EGFR) expression by 17 studies, reporting on 1635 patients, to allow the calculation of survival effect estimates and subsequent meta-analysis (Table 1).

Biomarker Assessment

All studies included in the meta-analysis were retrospective cohort studies where biomarker status was determined from tissue biopsies after treatment for ASCC had been started. Ten studies assessed the prognostic value of HPV status [10,11,18,23,24,26–28,34,36], of which four studies reported outcomes according to the HPV16 subtype [11,23,27,34]. The prognostic value of p16 expression was assessed by 10 studies [11,18,23–28,32,34], with combined HPV status/p16 expression assessed by three studies [18,24,26]. Two studies described the same cohort of patients [25,36], but were still included in the meta-analysis as one study assessed the prognostic value of HPV status and the other study assessed p16 expression. Of the 10 studies that assessed HPV status, four studies used chromogenic *in situ* hybridisation to confirm HPV subtypes after the detection of HPV DNA with polymerase chain reaction (PCR) [18,24,26,27]. Cut-off points for p16 expression varied between studies, although a positive result was typically taken to be moderate to intense staining in most tumour-containing cells. Six studies reported on the prognostic value of p53 [18,25,29,30,34,35], of which five studies used immunohistochemistry and one study used fluorescent *in situ* hybridisation. Five studies assessed the prognostic effect of EGFR using immunohistochemistry [25,30–33].

Study Quality

Most of the REMARK criteria were fulfilled by each of the included studies (see Supplementary Appendix S3). Statistical analysis and interpretation were the domains where criteria were least frequently fulfilled, with only one study undertaking checking assumptions, sensitivity analyses or internal validation [18]. No study gave the rationale for sample size, except Ajani *et al.* [30]. Time to event data had to be manipulated in six studies to allow the calculation of hazard ratios and associated confidence intervals, as these values were not originally presented in the univariate analysis.

Clinical and Treatment Characteristics

The range of median ages of patients included in the meta-analysis was 55.0–65.5 years and 995 of 1533

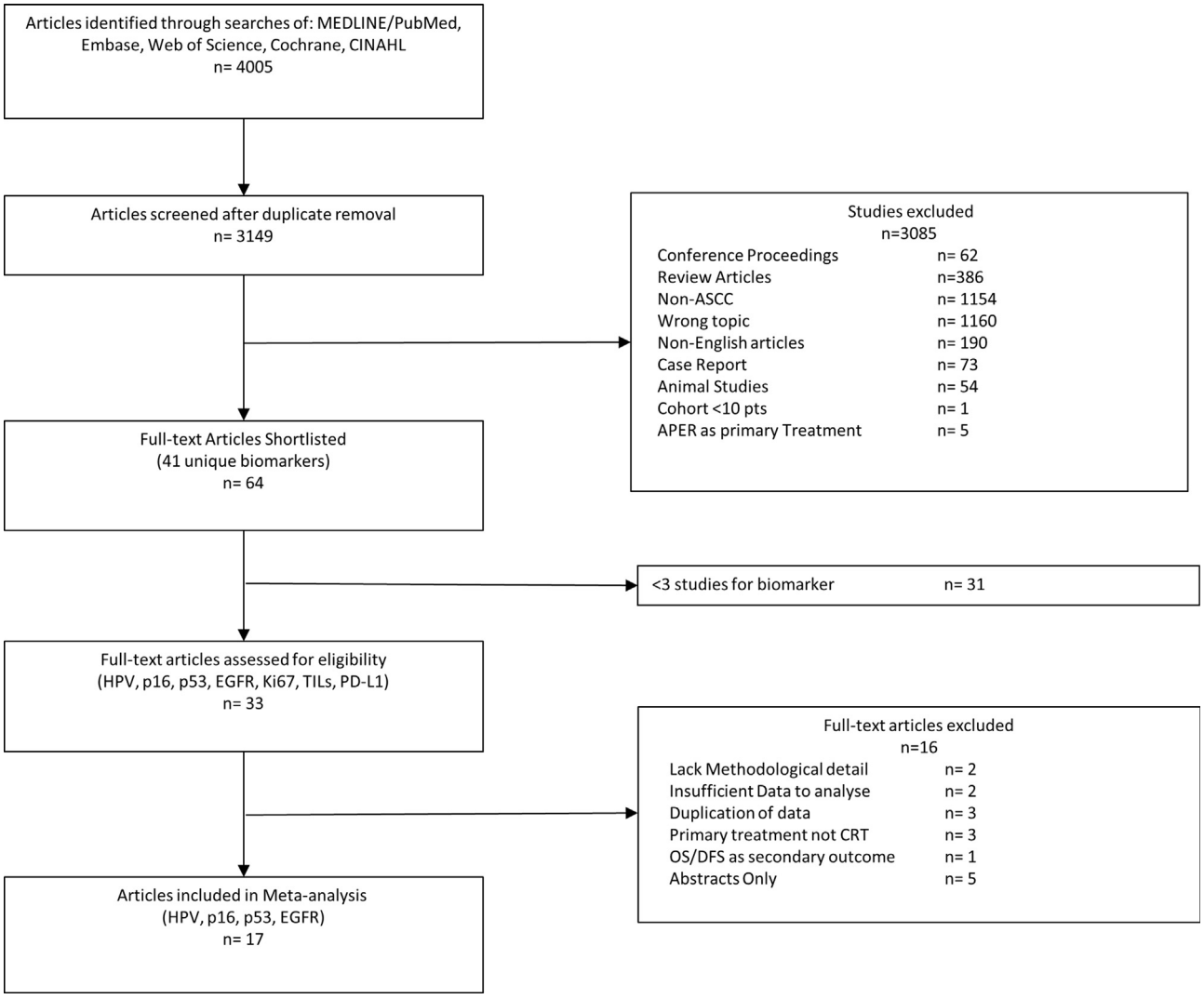

Fig 1. PRISMA flow diagram showing the selection and screening process for eligible studies.

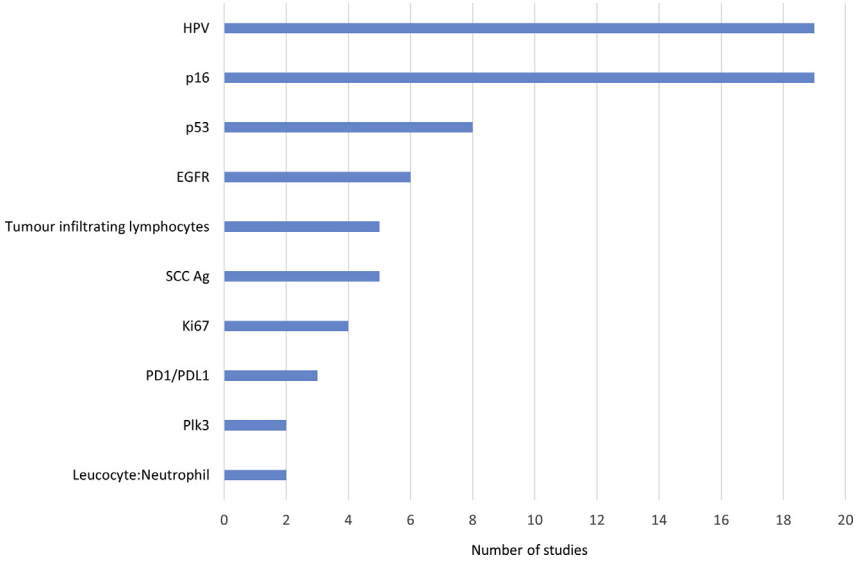

Fig 2. Biomarkers assessed for their prognostic utility in two or more studies.

Table 1
Table of characteristics

Reference	Year	Study type	Country	Number of patients	Gender	Age (median, range)	Tumour location	T-category	N-category	Biomarkers studied	Biomarker-positive (*n*, %)	Analysis method	Cut-off value
[23]	2017	RC	Frankfurt, Germany	150	Male: 66 Female: 84	NS	NS	T1: 0 T2: 105 T3: 0 T4: 45	N0: 97 N+: 53	HPV16 p16	68/135 (50.4) 77 (51.3)	PCR IHC	N/A Weighted score > 6 (moderate intensity and >50% stained positive)
[24]	2014	RC	Heidelberg, Germany	90	Male: 13 Female: 77	55 (22–94)	NS	T1: 16 T2: 48 T3: 17 T4: 9	N0: 68 N1: 7 N2: 9 N3: 6 Nx: 0	HPV (all) p16 HPV (all)/p16	75 (83.3) 75 (83.3) 70 (77.8)	CISH & PCR IHC	Diffuse tumoral staining
[25]	2013	RC	Sussex & Kent, UK	153	Male: 60 Female: 93	65.5 (32.4–92.7)	NS	T1: 11 T2: 46 T3: 40 T4: 26 Tx: 10	N0: 79 N1: 22 N2: 27 N3: 4 Nx: 0	p16 p53 EGFR	137 (89.5) 28 (19.0) 90 (60.1)	IHC IHC IHC	>5% moderate/strong >5% strongly positive >5% moderate/strong (grading system 0–3 used: absent, weak, moderate, strong)
[26]	2015	RC	Mannheim, Germany	106	Male: 43 Female: 63	59.5 (31–86)	AC: 81 PA: 25	T1: 20 T2: 59 T3: 19 T4: 8 Tx: 0	N0: 70 N+: 36	HPV (all) p16 HPV (all)/p16	72 (67.9) 74 (69.8) 63 (59.4)	CISH & PCR IHC	N/A Diffuse, continuous staining
[18]	2015	RC	Amsterdam, The Netherlands	107	Male: 50 Female: 57	60 (34–86)	AC: 107 (all)	T1: 4 T2: 53 T3: 32 T4: 18	N0: 49 N1: 30 N2: 18 N3: 9 Nx: 1	HPV (all) p16 p53	93 (86.9) 97 (90.7) 14 (13.1)	CISH & PCR IHC IHC	N/A >70% strong nuclear staining 0% or >70% strong nuclear staining
[27]	2014	RC	Calgary, Canada & Tampa, USA	53	Male: 13 Female: 40	59 (34–83)	AC: 53 (all)	NS	N0: 31 N1: 0 N2: 3 N3: 3 Nx: 16	HPV16 p16	42 (79.2) 28/35 (80.0)	CISH & PCR IHC	N/A Moderate to intense staining >50% cells

(*continued on next page*)

Table 1 (*continued*)

Reference	Year	Study type	Country	Number of patients	Gender	Age (median, range)	Tumour location	T-category	N-category	Biomarkers studied	Biomarker-positive (*n*, %)	Analysis method	Cut-off value
[10]	2014	RC	Milan, Italy	50	Male: 13 Female: 37	62 (55–69)	NS	T0: 0 T1: 3 T2: 24 T3: 15 T4: 8	N0: 19 N1: 22 N2: 4 N3: 5 Nx: 0	HPV (all)	42 (84.0)	PCR	N/A
[11]	2011	RC	Jeonju, South Korea	47	Male: 22 Female: 25	65 (44–90)	NS	T1 + T2: 31 T3 + T4: 16	N0: 29 N+: 19	HPV16 p16	35 (74.5) 39 (83.0)	PCR IHC	N/A Strong and diffuse nuclear and cytoplasmic staining >80% of cells
[28]	2014	RC	Herlev, Denmark	143	Male: 37 Female: 106	63 (36–97)	AC: 143 (all)	T1 + T2: 85 T3 + T4: 58	N0: 87 N+: 56	HPV (all) p16	120/137 (87.6) 131/141 (92.9)	PCR IHC	N/A >70% staining
[32]	2016	RC	Multicentre, Canada & USA	183	Male: 60 Female:123	NS	AC: 183 (all)	T1: not included T2: 135 T3 + T4: 38	N0: 137 N+: 46	EGFR-Ki67 coexpression	91 (49.7)	IHC	> Median value of Ki67 within EGFR high and low areas
[33]	2015	RC	Liberec, Czech Republic	17	Male: 3 Female: 14	57 (40–81)	NS	TNM staging not used	TNM staging not used	EGFR	8/16 (50.0)	IHC	Weak, moderate, strong (grading system 0–3 used: absent, weak, moderate, strong)
[34]	2018	RC	Belem, Brazil	78	Male: 14 Female: 64	63.4 (34–86)	AC: 78 (all)	T1: 6 T2: 30 T3: 30 T4: 12	N0: 60 N+: 18	HPV p16 p53	59 (75.6) 57 (73.1) 21 (26.9)	PCR IHC PCR & FISH	N/A NS NS
[35]	1999	RC	Multicentre, USA	64	Male: 21 Female: 43	NS	AC: 64 (all)	T0: 0 T1: 7 T2: 23 T3: 26 T4: 8	N0: 53 N1: 11	p53	31 (48.4)	IHC	>5% staining
[36]	2015	RC	Sussex & Kent, UK	110	Male: 47 Female: 63	62.4 (34–93)	NS	T1: 9 T2: 34 T3: 29 T4: 18 Tx: 8	N0: 59 N1: 17 N2: 19 N3: 3 Unknown: 12	HPV16	100 (90.9)	PCR	NS
[29]	1999	RC	Toronto, Canada & Michigan, USA	49	Male: 22 Female: 27	NS	AC: 49 (all)	NS	N0: 43 N1: 1 N2: 3 N3: 2	p53	27 (55.1)	IHC	>5% staining

Table 1 (*continued*)

Reference	Year	Study type	Country	Number of patients	Gender	Age (median, range)	Tumour location	T-category	N-category	Biomarkers studied	Biomarker-positive (*n*, %)	Analysis method	Cut-off value
[30]	2009	RC	Houston, USA	30	Male: 8 Female: 22	NS	AC: 30 (all)	NS	NS	p16 p53 EGFR	NS NS 15 (50.0)	IHC IHC IHC	100% staining (median) >5% staining (median) Intense staining
[31]	2013	RC	Frankfurt, Germany	103	Male: 46 Female: 57	NS	NS	T1 +T2: 70 T3 + T4: 31 Tx: 2	N0: 57 N+: 37 Nx: 9	EGFR	76 (73.8)	IHC	Intermediate/ intense staining (grading system 0 –3 used: absent, weak, intermediate, intense)

AC, anal canal; CISH, chromogenic *in situ* hybridisation; EGFR, epidermal growth factor receptor; FISH, fluorescent *in situ* hybridisation; HPV, human papillomavirus; IHC, immunohistochemistry; N/A, not applicable; NS, not stated; PA, perianal; PCR, polymerase chain reaction; RC, retrospective cohort study.

patients (64.9%) were women. The median follow-up ranged from 27.9 to 72.0 months. Tumour location was specified in nine studies, of which eight only included patients whose tumours were located in the anal canal. The combined T-category distribution was T1/T2 in 851 patients (55.5%), T3/T4 in 520 patients (33.9%) and unknown or not specified in 163 patients (10.6%). The N-category distribution was N0 in 938 patients (61.2%), N+ in 490 patients (31.9%) and unknown or not specified in 105 patients (6.9%). Among studies where the proportion of biomarker-positive patients was recorded, 698 of 908 patients (76.9%) were HPV-positive, 715 of 907 patients (78.8%) were p16-positive and 226 of 303 patients (74.6%) were HPV-positive/p16-positive. EGFR-positive tumours were noted in 273 of 456 patients (59.9%), with overexpression of a mutated p53 variant reported in 113 of 373 patients (30%). Chemotherapy regimens and follow-up protocols are shown in Table 2.

Survival Outcomes

In total, 10 different survival outcome measures were reported (Table 2). The most frequently reported outcome measures were LRR in five studies, overall survival in 15 studies and DFS in 11 studies. Of the 11 studies reporting DFS, this was originally reported as recurrence-free survival (RFS) in three studies [25,27,36] and as progression-free survival (PFS) in three studies [11,24,33]. However, the studies reporting RFS and PFS were considered to be reporting DFS as the same definition was used. Two studies reported on distant metastasis-free survival (DMFS) [11,31], with one study reporting a trend towards improved DMFS in tumours with intermediate/intense EGFR expression compared with absent/weak expression [31].

Locoregional Recurrence

Only HPV status and p16 expression were assessed in the meta-analyses of LRR, as this outcome was reported by an insufficient number of studies assessing p53 and EGFR expression. HPV-positive tumours were associated with reduced LRR compared with HPV-negative tumours (pooled hazard ratio = 0.27 [95% confidence interval 0.16–0.48]; $P < 0.001$) (Figure 3A). Likewise, p16-positive tumours had lower LRR when compared with p16-negative tumours (hazard ratio = 0.26 [95% confidence interval 0.13–0.52]; $P < 0.001$) (Figure 3B). HPV-positive/p16-positive tumours were associated with reduced LRR compared with both HPV-negative/p16-negative tumours (hazard ratio = 0.21 [95% confidence interval 0.12–0.37]; $P < 0.001$) and HPV-negative/p16-positive tumours (hazard ratio = 0.33 [95% confidence interval 0.11–0.95]; $P = 0.04$) (Figure 3C,D). There was insufficient data to permit a comparison with HPV-positive/p16-negative tumours.

Overall Survival

HPV-positive tumours had improved overall survival when compared with HPV-negative tumours (hazard ratio = 0.26 [0.12–0.59]; $P = 0.001$) (Figure 4A). Meta-regression by HPV subtype did not reveal any difference in the effect estimates between studies that analysed

Table 2
Summary of chemoradiotherapy regimes, follow-up and survival outcomes

Reference	Radiotherapy dosage (Gy)	Chemotherapy regimen (n – where specified)	Median follow-up (months, range)	Modalities used for follow-up	Survival outcome measures							
					cCR	LRR	OS	DFS	RFS	PFS	DSS	DMFS
[23]	53.4 (46.8–64.8)	5-FU + MMC	40 (1–205)	Clinical Proctoscopy CT MRI			X	X				
[24]	54 (45–63.2)	5-FU + MMC (69) 5-FU + CDDP (1) 5-FU + carboplatin (1) CDDP + etoposide (1) None (18)	48.6 (2.8–169.1)	Clinical Proctoscopy CT MRI		X	X			X		
[25]	50.4	5-FU + MMC	27.9	NS			X		X			
[26]	50.4–54	5-FU + MMC	48 (0–205)	NS		X	X					
[18]	64.8 (59.4–70.2)	5-FU + MMC (44) Capecitabine + MMC (47) None (16)	NS	NS		X	X					
[27]	55 (median)	5-FU + MMC + folinic acid (41) None (12)	59	Clinical CT MRI			X		X			
[10]	Node positive: 59.4 (50.4–59.4) Node negative: 39.6 (30.6–41.4)	5-FU + CDDP (49) 5-FU + MMC (1)	48 (4.8–165.6)	Clinical EUS MRI CT			X	X				
[11]	54 (40–70.2)	5-FU + MMC (40) 5-FU + cisplatin (7)	51.7 (5.1–136)	NS		X	X			X		X
[28]	60 (combined chemotherapy) 64 (radiotherapy alone)	5-FU + CDDP 5-FU + MMC None (75)	51.2 (0.4–144.4)	Clinical Proctoscopy			X				X	
[32]	NS	5-FU + MMC or FU + CDDP	72 (2.4–133.2)	NS			X	X				
[33]	60 (48–68)	5-FU + MMC (7) 5-FU + CDDP (5) CDDP (1)	57	NS			X			X		
[34]	50.4	5-FU + MMC	NS	Clinical Proctoscopy CT MRI	X							
[35]	45–50.4	5-FU or 5-FU + MMC	57 (0.84–100.2)	NS		X	X					
[36]	50.4	5-FU + MMC	28	NS			X		X			
[29]	24–28	MMC + 5-FU	54 (7.2–80.4)	Clinical examination			X	X				
[30]	55	CDDP + 5-FU	NS	Proctoscopy CT	X			X				
[31]	50.4 (41.4–60)	5-FU + MMC	44 (2–264)	DRE Proctoscopy CT MRI			X					X

5-FU, 5-fluorouracil; cCR, complete clinical response; CDDP, cisplatin; CT, computerised tomography; DFS, disease-free survival; DMFS, distant metastasis-free survival; DRE, digital rectal examination; DSS, disease-specific survival; EUS, endoscopic ultrasound; LRR, locoregional recurrence; MMC, mitomycin C; MRI, magnetic resonance imaging; NS, not specified; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival.

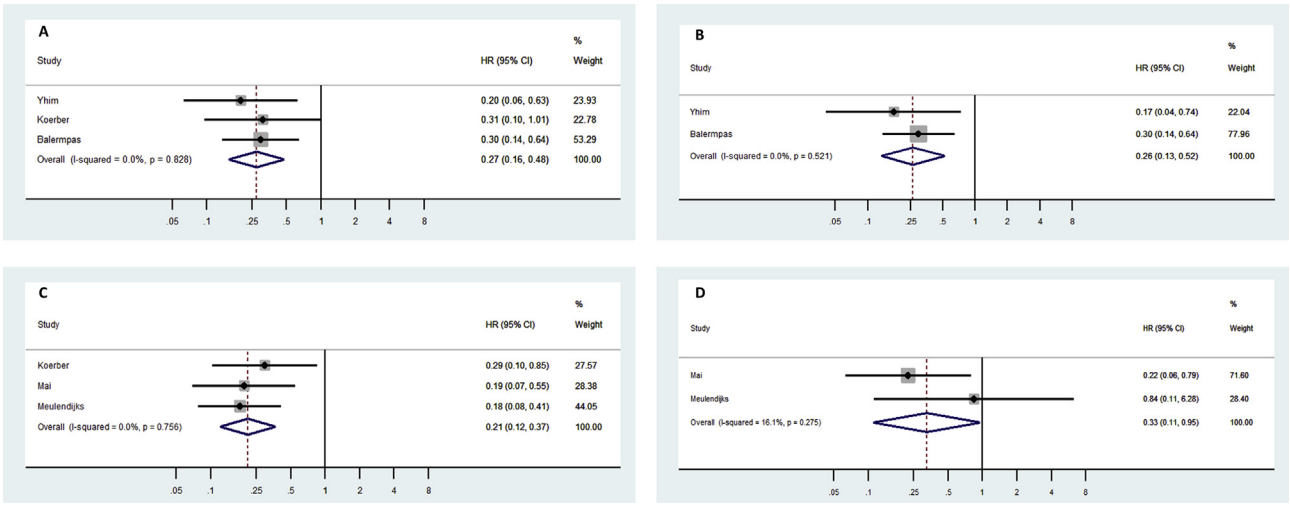

Fig 3. Forest plots for the association between biomarker status and locoregional recurrence (LRR). (A) Human papillomavirus (HPV) status and LRR, (B) p16 status and LRR, (C) comparison between HPV-positive/p16-positive and HPV-negative/p16-negative and LRR, (D) comparison between HPV-positive/p16-positive and HPV-negative/p16-positive and LRR.

overall survival according to HPV16 status and those studies that included all HPV subtypes (hazard ratio = 0.25 [95% confidence interval 0.07–0.86] versus hazard ratio = 0.32 [95% confidence interval 0.12–0.59]; $P = 0.978$)]. Overall survival was better among p16-positive tumours compared with p16-negative tumours (hazard ratio = 0.26 [95% confidence interval 0.13–0.52]; $P < 0.001$) (Figure 4B). HPV-positive/p16-positive tumours were associated with improved overall survival compared with HPV-negative/p16-negative tumours (hazard ratio = 0.27 [95% confidence interval 0.15–0.48], $P < 0.001$) (Figure 4C), but there was no difference in overall survival when compared with HPV-negative/p16-positive tumours (hazard ratio = 0.64 [95% confidence interval 0.21–1.90], $P = 0.421$) (Figure 4D). There were insufficient data to allow comparisons between any other combinations of HPV status and p16 expression.

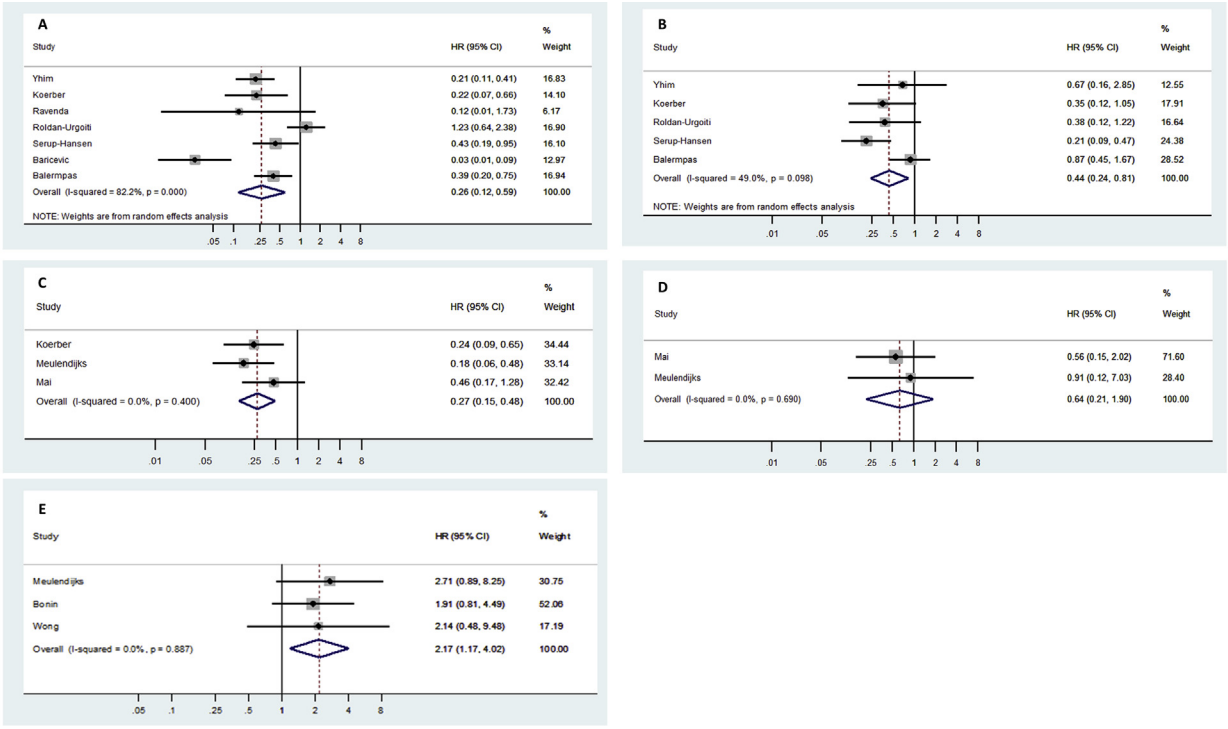

Fig 4. Forest plots for the association between biomarker status and overall survival. (A) Human papillomavirus (HPV) status and overall survival, (B) p16 status and overall survival, (C) comparison between HPV-positive/p16-positive and HPV-negative/p16-negative and overall survival, (D) comparison between HPV-positive/p16-positive and HPV-negative/p16-positive and overall survival, (E) p53 status and overall survival.

Mutated p53 expression was associated with worse overall survival (hazard ratio 2.17 [95% confidence interval 1.17–4.02]; $P = 0.014$) compared with wild-type p53 (Figure 4E).

Disease-free Survival

HPV-positive tumours had improved DFS when compared with HPV-negative tumours (hazard ratio = 0.33 [95% confidence interval 0.16–0.70]; $P = 0.003$) (Figure 5A). Meta-regression by HPV subtype did not reveal any difference in the effect estimates between studies that analysed DFS according to HPV16 status and those studies that included all HPV subtypes (hazard ratio = 0.44 [95% confidence interval 0.19–1.09] versus hazard ratio = 0.19 [95% confidence interval 0.09–0.42]; $P = 0.266$). p16-positive tumours had better DFS when compared with p16-negative tumours (hazard ratio = 0.44 [95% confidence interval 0.23–0.83]; $P = 0.012$) (Figure 5B). Mutated p53 expression was associated with a worse DFS (hazard ratio 1.60 [95% confidence interval 1.13–2.28]; $P = 0.009$) compared with wild-type p53 (Figure 5C). There was no association between EGFR expression and DFS (hazard ratio = 1.31 [95% confidence interval 0.34–5.07]; $P = 0.694$) (Figure 5D).

Complete Clinical Response

Two studies reported cCR as an outcome measure [30,34], but only Soares *et al.* [34] stated that treatment response was assessed at 6 months after the completion of CRT. Ajani *et al.* [30] stated that treatment response was assessed every 2 months until there was certainty whether a cCR had been obtained or persistent tumour remained. This disparity in outcome definition precluded any meaningful meta-analysis from being undertaken.

Discussion

An array of potentially prognostic biomarkers has been studied in ASCC. This review sought to determine the prognostic impact of the most frequently studied biomarkers on which there were available survival data, specifically HPV status, p16, p53 and EGFR expression. The identification of HPV and its surrogate marker of infectivity, p16 overexpression, were both strongly associated with a reduction in LRR and improved overall survival and DFS when analysed as individual biomarkers. When assessed in combination, HPV-positive/p16-positive tumours had markedly improved oncological outcomes compared with HPV-negative/p16-negative tumours, and reduced LRR when compared with HPV-negative/p16-positive tumours. Wild-type p53 was also shown to be associated with improved DFS, although EGFR expression seemed to have no prognostic impact. However, these findings should be interpreted with caution due to the limited number of studies included in some of the meta-analyses.

The survival benefit conferred by HPV infection mirrors the findings from several large meta-analyses in the head and neck literature that have consistently shown improvements in overall survival and disease-specific survival estimates in HPV-positive HNSCCs [7,37]. Subgroup analysis by HPV subtype did not show significant differences in overall survival or DFS between studies that determined HPV status according to the presence of the HPV16 subtype and those studies where HPV status was determined by the presence of any HPV subtype. Similar findings have been reported in a recent meta-analysis of HNSCC [37] and can probably be explained by the fact that the HPV16 subtype is present in over 80% of HPV-positive ASCC cases [13,18,24]. Previous systematic reviews have explored prognostic

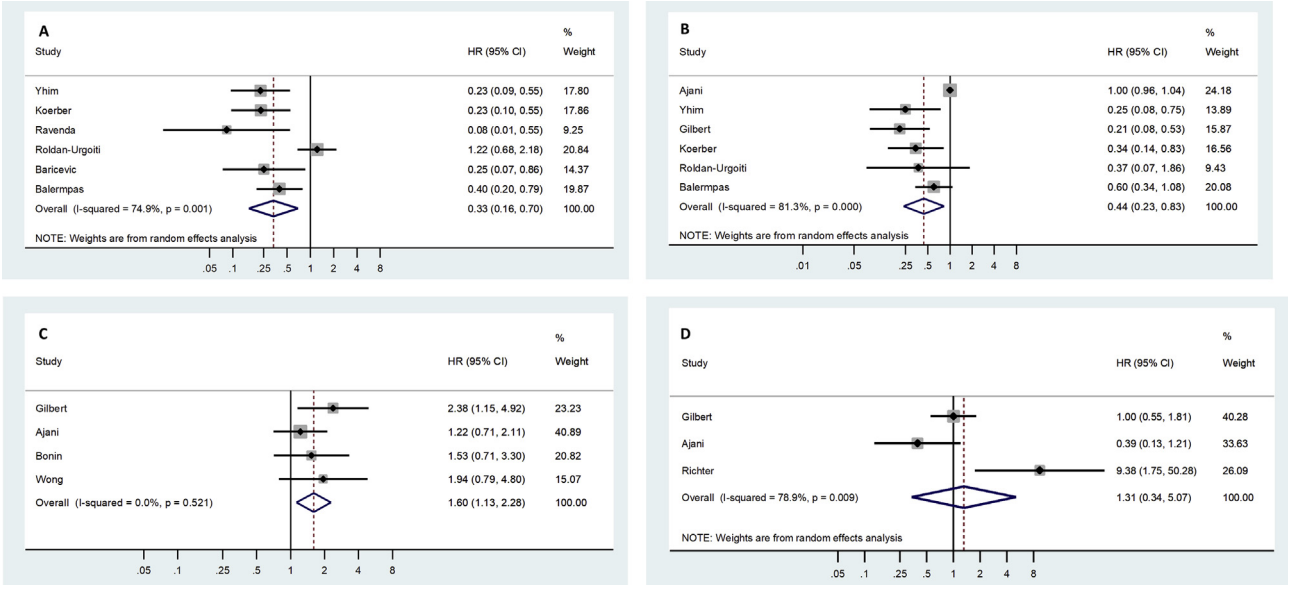

Fig 5. Forest plot for the association of biomarker and disease-free survival (DFS). (A) Human papillomavirus (HPV) status and DFS, (B) p16 status and DFS, (C) p53 status and DFS, (D) epidermal growth factor receptor (EGFR) and DFS.

biomarkers in ASCC, but this is the first review to show that HPV status and p16 expression, both individually and in combination, predict oncological outcomes. Lampejo *et al.* [6] identified 13 potential prognostic biomarkers, but none of these biomarkers included HPV or p16. A recently published review by Sun *et al.* [38] assessing the combined prognostic impact of HPV and p16 concluded that patients with HPV-negative/p16-negative or HPV-negative/p16-positive tumours had considerably worse survival than patients with HPV-positive/p16-positive tumours. However, there was a number of methodological flaws in their review related to the definition and reporting of survival outcome measures. Specifically, a composite disease recurrence end point was used that did not differentiate between LRR and DFS. When compared with HPV-positive/p16-positive tumours in the current review, both HPV-negative/p16-negative and HPV-negative/p16-positive tumours had worse LRR, but only HPV-negative/p16-negative tumours had worse overall survival. This would suggest that patients with HPV-negative/p16-positive tumours have an intermediate prognosis between that of HPV-positive/p16-positive and HPV-negative/p16-negative tumours. Only nine patients from one study were HPV-positive/p16-negative, so it is difficult to draw conclusions about prognosis with this biomarker combination. Although p16 overexpression is considered to be a surrogate marker of HPV infectivity, and the two biomarkers are highly correlated, HPV-negative/p16-positive tumours are not HPV induced and p16 overexpression may be driven by mutations in upstream regulators of p16, such as pRb. Future biomarker profiling of ASCCs should therefore include HPV PCR, as p16 expression may not be a completely reliable surrogate marker of HPV status when used in isolation.

The adverse prognostic effect of aberrant p53 expression reflects the findings from a recent biomarker meta-analysis in vulvar cancer [39]. It is now recognised that TP53 mutation is a key underlying step in the tumorigenesis of HPV-orogenital squamous cell carcinomas and is associated with resistance to CRT [40–42]. However, the evidence for p53 as a prognostic biomarker for other tumours, specifically HNSCC, remains inconclusive [19]. This has been attributed to significant variability in immunohistochemical methods related to antibody selection and cut-off values [43]. The lack of between-study heterogeneity in the meta-analysis of p53 expression in this review may be reflective of the same cut-off value being used by nearly all studies and lends weight to the prognostic utility of p53 in ASCC. Nevertheless, there is no consensus on the optimal method to determine p53 expression, which has a low concordance with TP53 mutational analysis [18].

EGFR is a cell signalling molecule that when overactivated causes dysregulated cell growth and inhibition of cell apoptosis [44], but was not shown to be of prognostic value in this review. Only Richter *et al.* [33] have shown a reduction in survival with EGFR overexpression. This may suggest that EGFR overexpression alone does not necessarily result in the upregulation of the downstream KRAS/BRAF/ERK/MAPK pathway. Concomitant EGFR overexpression with tumour proliferation markers, such as Ki67, may identify patients in whom there is a downstream signalling effect, although this has only been shown by one study to date [32].

The studies included in this review fulfilled most of the REMARK criteria and could thus be considered of reasonable methodological quality. All studies used PCR to detect the presence of HPV, which is a more sensitive means of detection than the use of *in situ* hybridisation techniques alone [36]. The use of consistent definitions for survival end points helped to maximise the reliability of the meta-analyses. A particular strength of the review was the manipulation of time to event data to allow the calculation of hazard ratios and associated confidence intervals in studies where effect estimates were not presented, as opposed to using odds or risk ratios that would have resulted in unreliable summary effect estimates. A major limitation of this review was the retrospective nature of the included studies, which could have led to reporting bias. Furthermore, the studies may have been subject to selection bias as cohorts were drawn from institutional databases where inclusion criteria may have been dependent on the availability of pretreatment biopsy specimens or a desire to include cases to maximise the prognostic effect of a biomarker by ensuring a certain proportion of cases with the exposure or outcome of interest. A range of cut-off values was used to determine biomarker expression, potentially resulting in misclassification of the effect estimate. Unadjusted effect estimates were used to derive summary hazard ratios and although the rationale for doing so has been outlined in the methodology, there was no adjustment for potential confounding variables and effect modifiers. All of these factors may have contributed to the significant between-study heterogeneity observed in several of the meta-analyses. Finally, it must be appreciated that the field of biomarker research is rapidly evolving and that there were insufficient data to undertake a meta-analysis of additional biomarkers that may be of prognostic importance in ASCC. There is now increasing focus on the adaptive immune response beyond HPV status/p16 expression, in particular the role of tumour-infiltrating lymphocytes [45,46] and squamous cell carcinoma antigen [47]. Unfortunately, a lack of ASCC cell lines makes it difficult to assess the effect of the tumour microenvironment on radiosensitivity at the current time [48].

Conclusion

This review provides evidence that HPV infection, p16 overexpression and wild-type p53 expression are associated with improved survival outcomes from ASCC treated with primary CRT. There is insufficient evidence at the current time to suggest whether any other biomarkers have a prognostic impact. These findings should first be validated by means of a large prospective cohort study where standardised criteria are used for patient inclusion, reporting of biomarker status, staging, treatment, surveillance and the definition of oncological outcomes. Such a study could then inform the design of future trials where

stratification according to biomarker status would occur before randomisation to treatment regimens.

Conflicts of Interest

The authors declare no conflict of interest. No funding was provided for the conduct of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clon.2019.06.013.

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