# Prognostic Significance of HPV DNA and p16$^{INK4a}$ in Anal Cancer: A Systematic Review and Meta-Analysis

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## ABSTRACT

We conducted a systematic review and meta-analysis of observational studies evaluating survival in patients with anal cancer, according to human papillomavirus (HPV) DNA, p16$^{INK4a}$, and combined HPV DNA/p16$^{INK4a}$ status. We systematically searched PubMed, EMBASE, and Cochrane Library databases to identify studies published in English until July 25, 2018, directly providing or allowing estimation of survival of patients with anal cancer according to the presence of HPV DNA and/or overexpression of p16$^{INK4a}$. We estimated pooled HRs and 95% confidence intervals (CI) for overall survival (OS) using a random-effects model. We included 16 studies, comprising 1,724 patients with anal cancer tested for HPV DNA (65% positive), and 567 patients tested for p16$^{INK4a}$ (87% positive). The pooled HR for OS was 0.54 (95% CI, 0.33–0.89) for HPV DNA positive versus negative, 0.37 (95% CI, 0.24–0.57) for p16$^{INK4a}$ positive versus negative, and 0.36 (95% CI, 0.22–0.58) for HPV DNA positive/p16$^{INK4a}$ positive versus HPV DNA positive/p16$^{INK4a}$ negative patients with anal cancer. Patients with HPV DNA or p16$^{INK4a}$ positive anal cancer have significantly better OS compared with HPV DNA or p16$^{INK4a}$ negative. This points to the possible value of HPV DNA and/or p16$^{INK4a}$ testing when planning the management and follow-up strategy for patients diagnosed with anal cancer.

## Introduction

Anal cancer is a rare malignancy (1). The incidence varies globally but is increasing in the majority of countries (2). Among multiple etiologic factors for anal cancer, the most important is human papillomavirus (HPV) infection (3), and HPV is detected in more than 80% of anal cancers (4).

The HPV oncoproteins E6 and E7 become overexpressed in transforming HPV infections, initiating and maintaining the transformed phenotype throughout HPV-related carcinogenesis. E7 increases the activity of the demethylase KDM6B resulting in overexpression of the cellular protein p16$^{INK4a}$ (5). Consequently, p16$^{INK4a}$ is considered an indicator of transforming high-risk HPV (hrHPV) infections (6, 7). Both HPV DNA and p16$^{INK4a}$ have been identified as positive prognostic factors in other HPV-related cancers, with better survival of patients with HPV DNA and/or p16$^{INK4a}$ positive cancers compared with negative (8–12). A better understanding of the prognostic value of HPV DNA and p16$^{INK4a}$ in anal cancer could improve the treatment strategies of this malignancy, as clinically introduced treatment deescalation approaches for oropharyngeal cancers (13).

Two reviews and meta-analyses of the role of HPV DNA in anal cancer survival have previously been published (14, 15). In this meta-analysis of overall survival (OS) according to HPV DNA status we included three times more cases of anal cancer (>1,700). To our knowledge there is only one previous meta-analysis estimating the role of p16$^{INK4a}$ in OS from anal cancer (15), and we included additional 100 cases in our meta-analysis. In addition to two meta-analyses estimating the combined effect of HPV DNA and p16$^{INK4a}$ (15, 16), we estimate the effect of adding p16$^{INK4a}$ testing in HPV DNA positive anal cancers.

## Materials and Methods

### Search strategy and selection criteria

In this systematic review and meta-analysis, we performed a literature search in PubMed, EMBASE, and Cochrane Library databases for studies published until July 25, 2018. We combined search terms for anal cancer, HPV, p16$^{INK4a}$, and survival, using text words and Medical Subject Headings (PubMed and Cochrane Library) or Emtree headings (EMBASE; Supplementary Fig. S1). We reviewed the reference lists of included studies to identify other relevant publications. Peer-reviewed studies in English, reporting one or more survival outcomes for anal cancer in relation to HPV DNA and/or p16$^{INK4a}$ status on five or more cases were eligible for inclusion. If study populations overlapped, we used data from the publication with the most detailed information on a particular survival outcome. If two studies included the same population but reported different survival outcomes [i.e., OS and disease-free survival (DFS)], we included them both. However, for a given outcome, a study population was included only once. We used "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) guidelines to outline our study (17).

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### Data extraction

Two authors (A. Urbute and C.L. Rasmussen) reviewed titles, abstracts, and full-texts, and extracted data independently. Any inconsistency in the evaluation of study eligibility or data extraction was discussed with a third author (F. Verdoodt or S.K. Kjaer), until consensus was reached. Where available, we extracted the following variables from individual studies, including first author, publication year, country, year of sample collection, age at diagnosis (mean, median, and range), sex, type of specimen, tumor histology, tumor grade, clinical stage, treatment modalities, follow-up time, HPV DNA testing method and primers, HPV genotypes tested, $p16^{INK4a}$ testing method, definition of $p16^{INK4a}$ overexpression, number of evaluators in $p16^{INK4a}$ staining, sample size, number of HPV DNA positive and negative cancers, number of $p16^{INK4a}$ positive and negative cancers, and survival outcome and estimates. We extracted data on different survival outcomes, including OS, cancer-specific survival (CSS), DFS, and progression-free survival (PFS). Recurrence-free survival (RFS) was analyzed together with DFS and disease-specific survival (DSS) together with CSS due to similar definitions. OS is the length of time from the date of anal cancer diagnosis until death from any cause. DSS (or CSS) is the length of time after the date of diagnosis or primary treatment for anal cancer ends that the patients have not died from anal cancer. Meanwhile, RFS (or DFS) is the length of time after primary treatment for anal cancer without any signs or symptoms of that cancer. We contacted authors of individual studies if relevant information for the statistical analyses was missing.

### Quality assessment of individual studies

In the absence of a standardized tool for assessing the quality of observational studies, we did not assign summary scores to individual studies (18). Instead, we discussed the quality of each included study at plenary meetings, using a set of predefined criteria based on the Newcastle–Ottawa Quality Assessment Scale for observational studies (19). The result of these evaluations was included in the design of sensitivity analyses and a narrative discussion in the article.

### Statistical analysis

To evaluate the association between HPV DNA or $p16^{INK4a}$ and survival from anal cancer, we used the HRs with 95% confidence intervals (CI) from individual studies. When HRs were not reported in the study (20–23), we estimated the HR from the survival probabilities using the method proposed by Moodie and colleagues (24), which allows taking into account studies with different lengths of follow-up time. For two studies, reporting only survival curves (21, 23), we manually extracted data and subsequently estimated the HR. If an individual study reported the HR estimate with a different reference group (i.e., marker-positive as the reference group), we calculated the inverse HR and CI to obtain results concordant with other studies (i.e., marker-negative as the reference group).

We used a random-effects model to pool HRs for OS according to HPV DNA status, $p16^{INK4a}$ status, and combined HPV DNA/$p16^{INK4a}$ status. Each study was weighted using the inverse of its variance, which includes the estimated within-study variance plus the between-study variation assessed with the DerSimonian and Laird estimator ($\tau^2$; ref. 25). $I^2$ statistic was used to assess the statistical heterogeneity; it describes the percentage of total variation that is caused by heterogeneity rather than chance (26). The significance of heterogeneity was described by Cochran Q test and $P < 0.05$ (26). Pooled analyses are presented graphically using forest plots.

In the main analysis, we pooled studies reporting adjusted HRs with corresponding 95% CIs when available, otherwise, unadjusted. In addition, we conducted two separate meta-analyses: (i) including studies that reported adjusted HR (if more than two studies available); (ii) including studies that reported unadjusted estimates. We performed several sensitivity analyses: restricted to studies where HPV DNA was tested by PCR, and including only those studies, which reported HRs. In another sensitivity analysis, we excluded one study that included a combination of *in situ* (7/75) and invasive anal cancer, including 19 anal adenocarcinomas (22). Finally, in one more sensitivity analysis, we excluded a study which tested nine cases for HPV DNA by *in situ* hybridization (ISH), while the remaining 63 were tested for $p16^{INK4a}$ by IHC (27).

All statistical analyses were performed using the package "meta" (28) in R statistical software (version 3.5.1; ref. 29).

## Results

We identified 1,097 relevant records in the databases. After excluding duplicates, we reviewed titles of 819 unique studies, 233 abstracts, and 52 full texts. One study was included through manual review of reference lists (22). Altogether, we included 16 articles reporting different survival outcomes on 14 study populations. Number and reason for exclusion of studies are presented in a PRISMA chart (Supplementary Fig. S2).

Characteristics of the included studies ($N = 16$) are presented in Supplementary Table S1. The studies were published through 2007–2018 and conducted mainly in Europe ($n = 10$; refs. 21, 23, 30–37) and North America ($n = 4$; refs. 27, 38–40). Mean or median age of the participants ranged from 52 to 72 years. All of the studies included only squamous cell carcinoma of the anus, except for Koerber and colleagues (34) who did not specify the histology, and Scapulatempo-Neto and colleagues (22) who also included adenocarcinomas. The majority of studies included patients without distant metastases (i.e., stage I–III; refs. 20, 23, 30, 31, 33–40). One study also comprised *in situ* and stage IV tumors (22), one study comprised of stages I–IV (32), and one included only patients with stage IV at presentation or after the initial treatment (27). Patients were initially treated with a combination of chemotherapy and radiation (CRT; refs. 20, 21, 23, 30–40), or CRT and a combination of CRT with other treatment modalities (22). In most studies, HPV DNA was tested by PCR-based methods (20–22, 31, 32, 34–37), and in one study by chromogenic ISH (40). The study by Jhaveri and colleagues did not present details on HPV DNA testing methods (39). $p16^{INK4a}$ protein overexpression was detected by IHC (20, 22, 23, 30, 31, 33, 34, 36–38, 40). In one study 63 of 72 samples tested for $p16^{INK4a}$ by IHC and 9 of 72 samples tested for HPV DNA by ISH as a surrogate for $p16^{INK4a}$ (27). The majority of studies ($n = 14$) reported OS by HPV DNA, $p16^{INK4a}$, or HPV DNA/$p16^{INK4a}$ (20–23, 27, 31, 32, 34–40), and some of the studies reported other survival outcomes (i.e., DFS, DSS, and PFS; refs. 20, 23, 30, 31, 33–36, 40).

For the studies included in the meta-analysis on HPV DNA and OS from anal cancer, the prevalence of HPV DNA infection varied from 66% to 91%, not including the study by Jhaveri and colleagues (53%; ref. 39). Similarly, for the studies included in the meta-analysis on $p16^{INK4a}$ and OS from anal cancer, the prevalence of $p16^{INK4a}$ positive cases varied from 76% to 94%. OS of HPV DNA

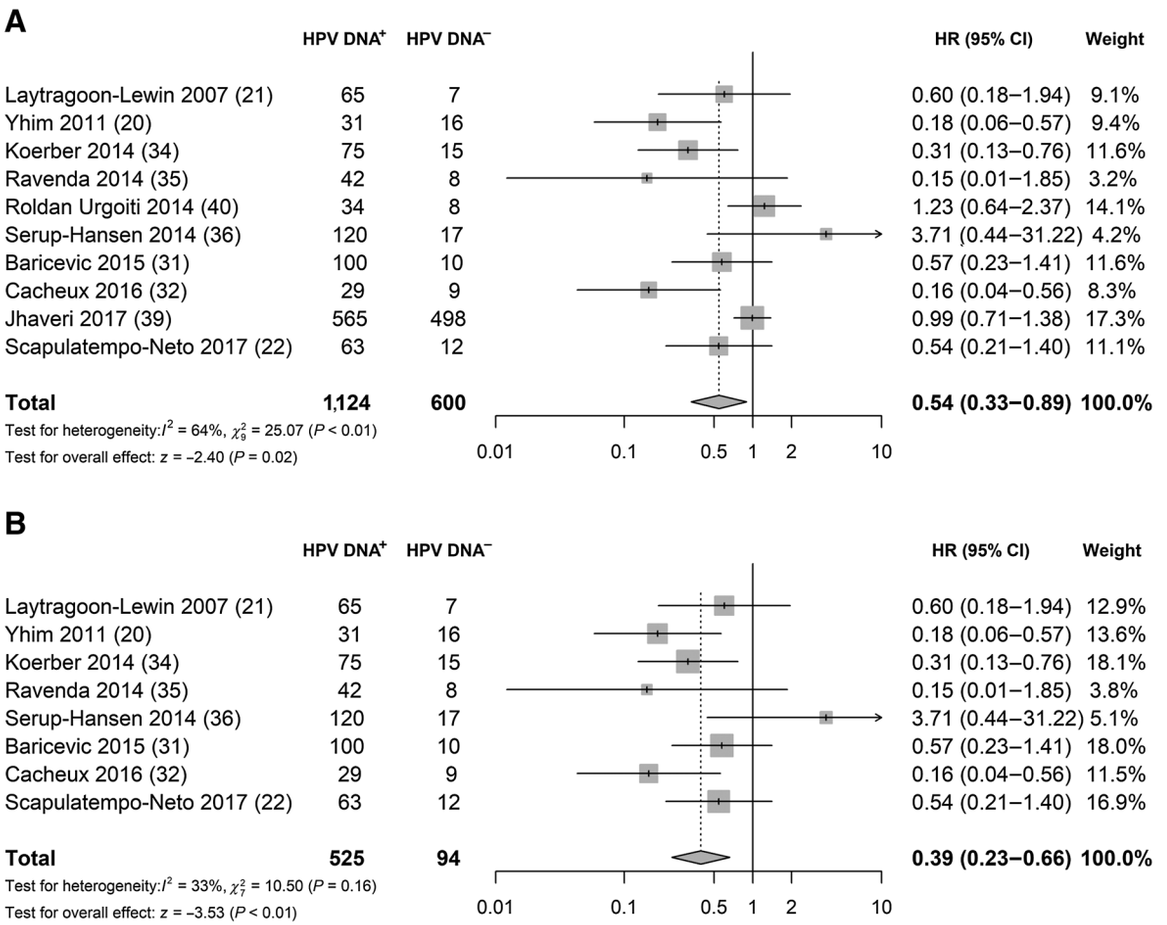


**Figure 1.**

Forest plot for OS between patients with HPV DNA positive versus HPV DNA negative (reference group) anal cancer. **A,** Main pooled analysis. **B,** Sensitivity pooled analysis with the studies that tested for HPV DNA by PCR.

positive versus HPV DNA negative patients is shown in **Fig. 1**, with individual and pooled HRs and corresponding CIs. We included 10 studies in the meta-analysis comprising 1,724 patients; 1,124 (65%) were HPV DNA positive and 600 HPV DNA negative (20–22, 31, 32, 34–36, 39, 40). Most studies reported HRs below 1, except for two studies (36, 40). The pooled HR was 0.54 (95% CI, 0.33–0.89), showing a better survival for HPV DNA positive patients. Heterogeneity among the studies was substantial ($I^2$ = 64%; **Fig. 1A**). When we pooled the studies with unadjusted estimates ($n$ = 10; refs. 20–22, 31, 32, 34–36, 39, 40), the results remained virtually the same [pooled HR 0.53 (95% CI, 0.35–0.81), heterogeneity was lower ($I^2$ = 57%)]. We also made a pooled analysis including only studies reporting HRs adjusted for prognostic factors of anal cancer ($n$ = 3; refs. 31, 32, 36), the pooled HR estimate was only slightly higher (pooled HR 0.56; 95% CI, 0.13–2.35). The HRs in the studies included in this analysis were adjusted for sex (31, 32, 36), tumor (31) or node (31, 32) stage, age (31), $p16^{INK4a}$ (36), and other factors (32).

In a sensitivity analysis including only those studies which reported HRs in the article ($n$ = 7; refs. 31, 32, 34–36, 39, 40), the pooled HR remained virtually unchanged, but the association did not reach statistical significance (pooled HR 0.62; 95% CI, 0.34–1.13). In addition, including only studies which tested for HPV DNA by PCR ($n$ = 8; refs. 20–22, 31, 32, 34–36), the pooled HR decreased (pooled HR 0.39; 95% CI, 0.23–0.66) and the heterogeneity was low ($I^2$ = 33%; **Fig. 1B**). When we excluded the study that also comprised *in situ* anal cancer cases and adenocarcinomas (22), the pooled HR did not change from the main meta-analysis (pooled HR 0.54; 95% CI, 0.31–0.94).

The results of six studies reporting other survival outcomes (DFS, PFS, and DSS) in relation to HPV status are described in **Table 1** (20, 31, 34–36, 40). HPV DNA was a positive prognostic factor for survival from anal cancer in five studies (**Table 1**), and in four of them, results reached statistical significance (20, 34–36).

The comparison of OS between $p16^{INK4a}$ positive and $p16^{INK4a}$ negative patients is shown in **Fig. 2**. We included seven studies evaluating $p16^{INK4a}$ as a prognostic factor for anal cancer survival in the meta-analysis (20, 22, 27, 31, 34, 36, 40). Altogether, they comprised 567 patients; 494 (87%) were $p16^{INK4a}$ positive and 73 (13%) $p16^{INK4a}$ negative. Individual HRs ranged from 0.07 (95% CI, 0.01–0.55) to 0.58 (95% CI, 0.24–1.38). The pooled HR was 0.37

**Table 1.** Other survival outcomes reported according to HPV DNA status.

| First author (ref), year of publication | Survival outcome | Measure | Covariates included in multivariate model | HR (95% CI)[a] | *P* |
|---|---|---|---|---|---|
| Baricevic and colleagues (31), 2015 | DFS | Multivariate HR | Age, sex, N stage, T stage | 0.60 (0.27–1.32)[b] | 0.201 |
| Ravenda and colleagues (35), 2014 | DFS | Multivariate HR | Clinical stage | 0.10 (0.02–0.50) | NA |
| Koerber and colleagues (34), 2014 | PFS | Univariate HR | NA | 0.33 (0.16–0.68) | NA |
| Roldan Urgoiti and colleagues (40), 2014 | PFS | Univariate HR | NA | 1.22 (0.69–2.18) | 0.49 |
| Yhim and colleagues (20), 2011 | PFS | Multivariate HR | N stage | 0.30 (0.12–0.74)[b] | 0.009 |
| Serup-Hansen and colleagues (36), 2014 | DSS | Univariate HR | NA | 0.27 (0.12–0.62) | 0.002 |

Abbreviations: N, node; NA, not applicable; T, tumor.
[a]Reference group was HPV-negative patients.
[b]Estimates calculated for the reciprocal reference group by the authors.

(95% CI, 0.24–0.57), indicating better survival for $p16^{INK4a}$ positive patients. We found no evidence of heterogeneity among the studies ($I^2 = 0\%$). Pooling only the unadjusted HRs of the above mentioned studies, the pooled HR was 0.34 (95% CI, 0.23–0.50; $I^2 = 0\%$).

In a sensitivity analysis including only those studies which directly reported HRs (27, 31, 34, 36), the pooled HR remained virtually unchanged (pooled HR 0.30; 95% CI, 0.18–0.51). In addition, including only studies who tested for $p16^{INK4a}$ by IHC (20, 22, 31, 34, 36, 40), the pooled HR was very similar to the main analysis (pooled HR 0.37; 95% CI, 0.24–0.57). When we excluded a study that also included *in situ* anal cancer cases and adenocarcinomas (22), the pooled HR did not change markedly either (pooled HR 0.32; 95% CI, 0.20–0.52).

Six studies reported other survival outcomes (DFS, PFS, and DSS) in relation to $p16^{INK4a}$ status; their results are presented in **Table 2** (20, 30, 33, 34, 36, 40). In all of the studies, $p16^{INK4a}$ positive patients had better survival than $p16^{INK4a}$ negative; the results were statistically significant in five studies (20, 33, 34, 36).

**Figure 3** shows the comparison of OS between HPV DNA positive/$p16^{INK4a}$ positive and HPV DNA positive/$p16^{INK4a}$ negative patients, with individual and pooled HRs with corresponding CIs. We included four studies in the meta-analysis comprising 263 patients; 210 (79.8%) were HPV DNA positive/$p16^{INK4a}$ positive and 53 (20.2%) HPV DNA positive/$p16^{INK4a}$ negative (23, 34, 37, 38). Individual HRs ranged from 0.29 (95% CI, 0.14–0.64) to 0.74 (95% CI, 0.16–3.36). The pooled HR was 0.36 (95% CI, 0.22–0.58), showing a better survival for HPV DNA positive/$p16^{INK4a}$ positive patients compared with HPV DNA positive/$p16^{INK4a}$ negative patients. There was no evidence of heterogeneity among the studies ($I^2 = 0\%$).

## Discussion

According to this meta-analysis, patients with HPV DNA positive anal cancers had almost two times better OS compared with HPV DNA negative. However, when based only on studies using PCR to detect HPV DNA, OS was almost three times better for patients with HPV DNA positive tumors, compared with HPV DNA negative. We also found that patients with $p16^{INK4a}$ positive anal cancer had approximately three times superior OS compared with $p16^{INK4a}$ negative. Among patients with HPV DNA positive anal cancer, $p16^{INK4a}$ positivity provided an additional survival benefit.

Our results are consistent with the results of previous meta-analyses about the prognostic significance of HPV DNA (14, 15) or $p16^{INK4}$ (15) alone. Regarding the combined effect of the HPV DNA and $p16^{INK4a}$

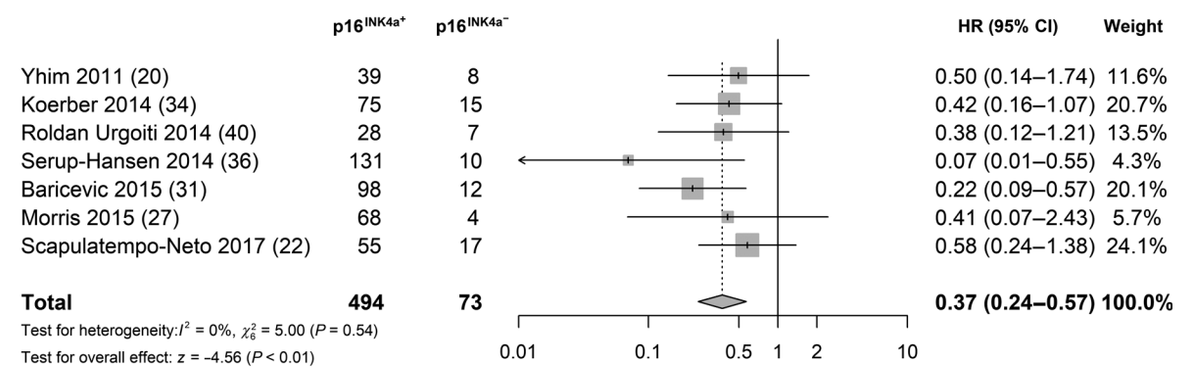


**Figure 2.**
Forest plot for OS between $p16^{INK4a}$ positive and $p16^{INK4a}$ negative (reference group) patients.

**Table 2.** Other survival outcomes reported according to $p16^{INK4a}$ status.

| First author (ref), year of publication | Survival outcome | Measure | Relative survival rates: $p16^{INK4a}$ positive | Relative survival rates: $p16^{INK4a}$ negative | HR (95% CI)[a] | *P* |
|---|---|---|---|---|---|---|
| Balermpas and colleagues (30), 2017 | DFS | 5-year relative survival[b] | 79% | 55% | NA | 0.088 |
| Gilbert and colleagues (33), 2013 | DFS | Multivariate HR[c] | NA | NA | 0.22 (0.10–0.51)[d] | <0.001 |
| Koerber and colleagues (34), 2014 | PFS | Univariate HR | NA | NA | 0.33 (0.16–0.68) | NA |
| Roldan Urgoiti and colleagues (40), 2014 | PFS | Univariate HR | NA | NA | 0.37 (0.75–1.86) | 0.27 |
| Yhim and colleagues (20), 2011 | PFS | 4-year relative survival | 53% | 25% | NA | 0.014 |
| Gilbert and colleagues (33), 2013 | DSS | 5-year relative survival[b] | 62% | 14% | NA | <0.001 |
| Serup-Hansen and colleagues (36), 2014 | DSS | Univariate HR | NA | NA | 0.13 (0.06–0.31) | <0.001 |

Abbreviation: NA, not applicable.
[a]Reference group was $p16^{INK4a}$ negative patients.
[b]Estimates read manually from Kaplan–Meier curves.
[c]Adjusted for T stage and N stage in the model.
[d]Estimates calculated for the reciprocal reference group by the authors.

status, we updated and included more cases than previous meta-analyses (15, 16), and found a better OS of HPV DNA positive/$p16^{INK4a}$ positive anal cancers compared with HPV DNA positive/$p16^{INK4a}$ negative. Thus, our results suggest an improved prediction of survival by combined HPV DNA and $p16^{INK4a}$ testing compared with HPV DNA testing alone, which may be clinically valuable. This result is in-line with Sun and colleagues (16), who also found better survival of HPV DNA positive/$p16^{INK4a}$ positive anal cancers compared with HPV DNA positive/$p16^{INK4a}$ negative. However, their result was based on fewer studies and did not reach statistical significance. The previous meta-analysis of Sun and colleagues has also shown a significantly better survival for patients with HPV DNA positive/$p16^{INK4a}$ positive compared with HPV DNA negative/$p16^{INK4a}$ positive and HPV DNA negative/$p16^{INK4a}$ negative anal cancers (16). Parwaiz and colleagues confirmed the better survival for HPV DNA positive/$p16^{INK4a}$ positive compared with HPV DNA negative/$p16^{INK4a}$ negative anal cancer (15). In contrast, Parwaiz and colleagues did not find that survival was statistically significantly better for HPV DNA positive/$p16^{INK4a}$ positive compared with HPV DNA negative/$p16^{INK4a}$ positive, however, the results were only based on two studies (15). These results are in-line with a meta-analysis of oropharyngeal cancer, which has also indicated that combined positivity for hrHPV DNA and $p16^{INK4a}$ was more accurate than hrHPV DNA positivity alone in predicting HPV-induced cancer (6). Similarly, in anal cancer, it may be possible that presence of HPV is not sufficient to accept it as an agent that has driven the carcinogenic process, and thus treatment decisions based on HPV DNA alone may not be optimal and could induce a risk for undertreatment.

More than 80% of anal cancers are HPV DNA positive (4). It is therefore important to highlight that testing for HPV DNA alone might not give a valid risk assessment. Some studies consider the expression of $p16^{INK4a}$ among HPV DNA positive as an indicator of a transforming hrHPV infection (6, 7). The results of our meta-analysis confirm that patients who had tumors positive for both HPV DNA and $p16^{INK4a}$, had improved OS compared with those who were not expressing $p16^{INK4a}$ among HPV DNA positive. It has also been shown that higher than the median viral load of HPV DNA is associated with better OS compared with lower than the median viral load of HPV DNA among HPV DNA positive anal cancer cases (23, 30). Thus, testing for HPV DNA positivity alone may not be enough.

Improved survival for patients with HPV DNA and especially $p16^{INK4a}$ positive anal cancer suggests that some biological mechanism may play a role. It is suggested that altered DNA repair, reduced hypoxic regions, and increased cellular immune response in HPV-associated cancers contribute to increased sensitivity to

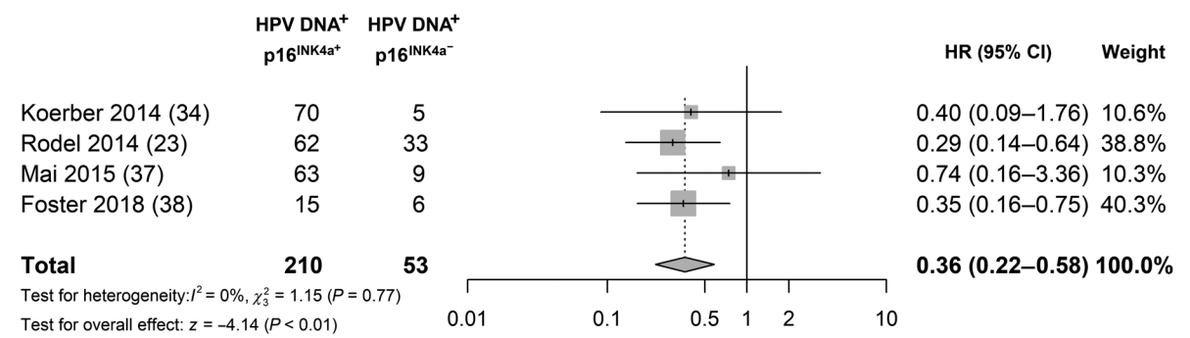


**Figure 3.**
Forest plot for OS between HPV DNA positive/$p16^{INK4a}$ positive and HPV DNA positive/$p16^{INK4a}$ negative (reference group) patients.

CRT (41). Research in head and neck cancer have shown that the highest sensitivity to radiation is during the M-phase of the cell cycle, and a higher percent of HPV-transformed epithelial cells accumulate in this phase (42). In addition, $G_2$-phase is shorter, resulting in less time for repairing the double-strand breaks induced by the radiation (43). This research may be extrapolated to HPV-associated anal cancers, as they are caused mainly by the same HPV genotypes (4, 44). In addition, $p16^{INK4a}$ overexpression in transforming HPV infections supplements the theory, because $p16^{INK4a}$ impairs the DNA repair system (42).

The studies included in this HPV DNA meta-analysis showed some heterogeneity. For instance, in the adjusted model in the study by Serup-Hansen and colleagues, survival for patients with HPV DNA positive anal cancers were worse than for patients with HPV DNA negative cancers (36). It may be a consequence of $p16^{INK4a}$ being included in the adjusted model, as all the other studies pointed to the opposite direction. However, several sensitivity analyses performed in our meta-analysis confirmed the overall results, and for example when we performed an analysis including only studies where HPV DNA was tested by PCR, we found a better survival for HPV DNA positive anal cancers than in the main meta-analysis. Jhaveri and colleagues did not provide information on the HPV-testing method and the prevalence of HPV in this study was among the lowest (i.e., 53.2%; ref. 39), and in the study by Roldan Urgoiti and colleagues HPV DNA was tested by ISH (40). Because other tests, such as ISH, are generally less sensitive in detecting HPV DNA than PCR, a tumor that is in fact HPV-driven may be missed. Potential misclassification of HPV DNA positivity may have caused neutral HR. To better understand the source of heterogeneity between the studies, a meta-regression analysis would have been relevant. However, this was not possible due to the insufficient number of studies.

In contrast to studies assessing the effect of HPV DNA, the individual studies estimating $p16^{INK4a}$ effect on survival from anal cancer showed less heterogeneity, with no obvious outliers.

A limitation of this meta-analysis is the diverse outcome measures (i.e., adjusted and unadjusted HRs or relative-survival proportions). We therefore conducted several sensitivity analyses, which did not show substantially different results from the main analyses. We were not able to stratify our analyses according to factors commonly associated with survival (e.g., clinical stage and sex), although some studies reported multivariate HRs, where age, sex, or clinical stage were included as covariates in the models. We conducted a meta-analysis including only these studies providing adjusted estimates, and the results supported the hypothesis that HPV is an independent prognostic factor in anal cancer survival. It would, however, be relevant to conduct an individual patient meta-analysis to answer the remaining questions about the effect of sex, age, stage, and other potentially important confounding factors in the survival of HPV DNA or $p16^{INK4a}$ positive and negative anal cancers.

As pointed out in the literature, the validity of tests for publication bias when only a few studies are available is uncertain (45, 46). Therefore, we did not conduct such analyses. Instead, we did several sensitivity analyses to assess the potential impact of publication bias, as suggested by Debray and colleagues (45).

Despite the mentioned limitations, our study also has important strengths. We updated the previous meta-analyses of survival from anal cancer in relation to HPV DNA status (14, 15) with almost 1,100 additional cases, with additional 100 cases in relation to $p16^{INK4a}$ status, and supplemented the meta-analyses on the combined HPV DNA/$p16^{INK4a}$ status by adding a pooled estimate of the value of adding p16 testing to HPV DNA testing. We also performed several pooled analyses, including only studies reporting HRs, adjusted HRs, unadjusted HRs, or excluding the studies with outlying characteristics, which strengthened our findings. The comprehensive review of the literature and data extraction were conducted by two authors independently, and in case of any disagreements discussed with the other authors.

In conclusion, this meta-analysis suggests that HPV DNA positive or $p16^{INK4a}$ positive anal cancers have a better prognosis than their negative counterparts. Our results also show an additional survival benefit of combined testing for HPV DNA and $p16^{INK4a}$. These findings may open a window for more personalized medicine, based on the tumor molecular features. Combined HPV DNA and $p16^{INK4a}$ testing is the most suitable approach when planning the individual management and follow-up strategy for the patient diagnosed with anal cancer. However, further studies are needed to examine the potential clinical and economic value of particularly combined testing for HPV DNA and $p16^{INK4a}$, but also of HPV E6/E7 mRNA testing (to detect transforming HPV infections).

## Disclosure of Potential Conflicts of Interest

E.-S. Prigge reports receiving a commercial research grant and lecture fees from MSD Sharp & Dohme GmbH. S.K. Kjaer reports receiving a commercial research grant and speakers bureau honoraria from Merck. No potential conflicts of interest were disclosed by the other authors.

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