The Presence of HPV DNA in Neck Lymph Node Metastasis Correlates with Improved Overall Survival of Patients with Oropharyngeal Cancer Undergoing Surgical Treatment

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Key Words

Head and neck cancer · Human papilloma virus · Oropharyngeal squamous cell carcinoma · Neck Lymph Node Metastasis

ABSTRACT

Background:

Few studies have addressed how human papilloma virus (HPV) infection in oropharyngeal squamous cell carcinoma (OPSCC) affects the outcome of surgical therapy, and the relationship between the presence of HPV DNA and neck lymph node (LN) metastasis has not been well established.

Methods: A total of 65 patients who underwent surgery as a first-line therapy for OPSCC were enrolled in this study. In HPV-positive patients, the presence of HPV DNA in metastatic neck LN lesions was evaluated.

Results: The HPV-positive patients had significantly better overall survival (OS) than the HPV-negative patients (log-rank P=0.04), whereas HPV infection status did not significantly affect disease-free survival (DFS) (log-rank P=0.65). In all of the HPV-positive OPSCC patients who developed cervical LN metastasis, the same HPV DNA type was found in both the primary tumour and the metastases.

Conclusions: The present results suggest that HPV infection is a determining factor for good prognosis in patients undergoing first-line surgical therapy for OPSCC.

INTRODUCTION

Head and neck cancer (HNC) is the fifth most common form of cancer worldwide[1] and the eighth most common cause of cancer-related death.[2] Human papilloma virus (HPV) infection not only causes cervical cancer but also penile cancer, anal cancer, and HNC. The incidence of HPV-associated HNC as a whole and that of HPV-associated oropharyngeal squamous cell carcinoma (OPSCC) are both significantly increasing, with approximately 60–80% of OPSCC cases in the United States[3,4] and 93% of those in Sweden currently reported as HPV-positive.[5] A recent multi-centre joint study in Japan also found that approximately 50.3% of OPSCC cases were HPV-positive.[6] Numerous studies have already reported that HPV infection is a good prognostic factor for OPSCC,[7-18] but most of these studies involved patients undergoing radiotherapy or chemoradiotherapy. In contrast, few studies have addressed how HPV infection affects the outcomes of surgical therapy. The presence of neck lymph node (LN) metastases is one of the most important factors determining the prognoses of head and neck squamous cell carcinoma (HNSCC) patients. Neck LN metastasis typically occurs at an earlier stage in HPV-associated OPSCC relative to non-HPV-associated OPSCC;[19] however, very few studies have investigated the association between neck LN metastasis and the presence of HPV DNA in cervical LN metastatic lesions in HNC. Therefore, in the current study, the relationship between HPV infection status and prognosis in patients who underwent first-line surgical therapy for OPSCC and the presence of HPV DNA in neck LN metastatic lesions in these patients were evaluated.

METHODS

This study was approved by the Ethics Committee for Biomedical Research of the Jikei Institutional Review Board at Jikei University School of Medicine, Tokyo, Japan. All patients provided written informed consent. Between September 2006 and February 2012, tumours were obtained from OPSCC patients who underwent surgery at the Department of Head and Neck Surgery, Jikei University

Hospital. A total of 65 OPSCC patients were included in this study; among these, we evaluated 18 pairs of primary HPV-positive OPSCC and the associated lymph node metastases. Clinical information was abstracted from clinical and surgical charts. Based on postoperative staging, tumour node metastasis (TNM) classification and cancer stage were determined according to the 7th UICC TNM classification and stage groupings. [20]

Samples

Primary and metastatic lymph node tumour samples were excised from each participant and stored at -80° C. Each cancer specimen was divided in two, with one half being used for pathological confirmation that the sample was composed of more than 70% cancer cells and the other half being used for DNA extraction.

HPV Detection and Genotyping

Polymerase Chain Reaction: HPV type (within the alpha-papillomavirus genera) was identified by multiplex PCR using a TaKaRa Human Papillomavirus Typing Set (Takara Bio, Inc., No. 6603) and a Human Papillomavirus PCR Detection Set (Takara Bio, Inc., No. 6602). The Takara Human Papillomavirus PCR Typing Set is designed to identify a broad range of HPV types via HPV type-specific PCR using two pairs of consensus primers that are designed to amplify the E6 and E7 homologous regions of HPV (228–268 bp). The malignant HPV16, 18, 33, 52b and 58 types are amplified using the HPVpU-1M/ HPVpU-2R primer pair, while the benign HPV6 and 11 types are amplified using the HPVpU-31B/HPVpU-2R primer pair. The PCR products were digested using the restriction enzymes *AccI, AfaI, AvaI, AvaII* and *BglII,* available as Enzyme Set A, and then submitted to agarose gel electrophoresis to identify the resident HPV types. The Human Papillomavirus PCR Detection Set offers a set of primers that have been designed to detect HPV16, 18 and 33. These HPV types have all been detected in cervical carcinoma. This primer set facilitates simple and sensitive detection of HPV16, 18 and 33 through the amplification of viral DNA. Included in this set are primers specific to the E6 regions of HPV16, 18 and 33. The amplification product sizes are 140 bp for HPV16 and 18 and 141 bp for HPV33. A sample was reported as HPV-positive if any of the high-risk HPV DNA types (types 16, 18, 26, 31, 33, 35, 52, and 58) were detected.

Statistical Analysis

Student’s t test, the χ 2 test and Fisher’s test were used to evaluate differences in patient characteristics after stratification by HPV status. The disease-free survival (DFS) curve end point was defined by recurrence (local relapse, new lymph node metastasis or distant metastasis) during follow-up. All patients were initially evaluated within 2 months after surgery, and they were then examined periodically (every 0.5–2 months) on an outpatient basis to ensure they had not relapsed. The examinations consisted of standard tests, including endoscopy and computed tomography of the chest and neck. Recurrence was established when a new carcinoma was detected as a primary tumour or in a cervical lymph node or when distant metastases were observed by computed tomography. DFS curves were generated using the Kaplan-Meier method and compared using log-rank tests. All statistical analyses were performed using Stata 9.1 (Stata Corp., College Station, Tex., USA). A p value <0.05 was considered statistically significant.

RESULTS

HPV Detection and Genotyping by PCR

Overall, 25 of the 65 OPSCC patients (38%) were HPV-positive. Of the 25 HPV-positive patients, 23 were positive for HPV16, 2 were positive for HPV18, and 18 were N1 or more; in the latter group, each patient had the same type of HPV DNA in their primary tumour and their metastases.

The patients' characteristics are listed in Table 1. In all patients, surgical margins of the primary were negative and there were not extracapsular extension of lymph node metastasis.

Kaplan-Meier Overall Survival and Disease-Free Survival Curves stratified by HPV Status

A total of 65 patients for whom specimens and clinical information could be obtained were included. Tumour recurrence and death occurred in 29 patients (44.6%) and 15 patients (23.0%), respectively, during the median follow-up period of 41.0 months. The 5-year estimated overall survival (OS) rate was 67% (95% CI: 0.48–0.81), and the DFS rate was 76% (95% CI: 0.68–0.81) at 5 years. When analysed according to HPV status, the 5-year estimated OS rates were 80% (95% CI: 0.44–0.94) for the HPV-positive patients and 60% (95% CI: 0.41–0.76) for the HPV-negative patients. The DFS rates at 5 years were 60% (95% CI: 0.38–0.76) for the HPV-positive patients and 36% (95% CI: 0.14–0.60) for the HPV-negative patients. HPV status was significantly associated with OS (log-rank test, $p = 0.03$) but not with DFS (log-rank test, $p = 0.42$) (Fig. 1, 2).

Patterns and Treatment of Disease Recurrence

After undergoing surgery as a first-line therapy, 10 of the 25 HPV-positive patients experienced disease recurrence (40%); of these, 6 patients could be salvaged by chemoradiotherapy and one could be salvaged by surgical treatment. In contrast, 19 of the

40 HPV-negative patients experienced disease recurrence (47.5%); of these, only 7 patients could be salvaged. Disease recurrence patterns were then compared according to tumour HPV status. Twelve patients had multiple sites of disease at the time of first recurrence, with a similar distribution between HPV-positive and HPV-negative patients (30% vs. 47%, P=0.55). Among the patients with any history of distant metastases (6 patients), the lung was the most common site of metastasis regardless of HPV status. The distribution of distant metastatic sites also did not differ with respect to HPV status. (Fig. 3, 4).

Time to Disease Recurrence

The median follow-up time after disease recurrence was 21.4 months (range, 0.2-105.8 months). The HPV-positive patients had significantly improved OS after recurrence compared to the HPV-negative patients (3-year OS 18.8% vs. 80.0%, P=0.04) (Fig. 3). The median survival time was longer for the HPV-positive patients than the HPV-negative patients (29.6 months vs. 17.1 months). (Fig. 5).

Discussion

HPV-positive HNC differs from HPV-negative HNC over a range of characteristics. For example, almost all cases of HPV-positive HNC are found to originate in the oropharynx, particularly in the palatine and lingual tonsils, are not associated with smoking or drinking; and are more common in comparatively young age groups[3-6]. Additionally, the primary tumour is discovered comparatively early or while it is still small, although in

many cases it has already progressed to cervical LN metastasis, and these LN lesions frequently cause cystic changes.[19] In this study, it was age, smoking, the use of neck dissection and micro surgery to have been significantly different (table 1). Although no difference in stage the use of microsurgery would mean difference in anatomical area and extent of the tumor are different since the reconstructive needs of the patients are determined by these two factors later on skills. One of the most important differences associated with HPV status may be that HPV-positive HNC has a better prognosis than HPV-negative HNC. However, the majority of previous studies investigating these differences have done so in the context of radiotherapy[8,12,14] or chemoradiotherapy,[13,15,18] while few have addressed surgical therapy. Those studies that have investigated HPV status in relation to surgical therapy have primarily examined surgical therapy in combination with another form of therapy;[7,10,17] the only study to have investigated differences in prognosis after surgical therapy alone was that reported Licitra et al.[11] In Licitra's study, 90 OPSCC patients who received surgical therapy were divided into groups according to who underwent surgery alone and who underwent surgery in addition to postoperative radiation, and HPV-positive and HPV-negative patients were further compared within these groups. It was found that HPV-positive patients who underwent surgical therapy had a better prognosis than did HPV-negative patients. According to the literature, evidence is already being established that HPV-positive HNC has a good prognosis when treated with radiation or chemoradiotherapy, although the underlying mechanism is unclear. Weinberger et al.[10] reported that HPV-positive tumours have a low recurrence rate, which dramatically

improves OS and DFS, but recent prospective data from the Radiation Therapy Oncology Group 0129 and 0522 trials demonstrated that time to disease recurrence does not differ according to tumour HPV status.[21] The current study also reports that recurrence rate and time to disease recurrence does not differ according to tumour HPV status in 64 patients who underwent surgery as a first-line therapy for OPSCC. However, the salvage rate for patients with HPV-positive tumours was higher than that for the patients with HPV-negative tumours. Theresa et al. [22] reported that surgical salvage was associated with improved OS for 65 cases of recurrent locoregional OPSCC and 43 cases of distant metastatic OPSCC; this relationship existed independent of tumour HPV status. This result is consistent with a study reported by Patel et al.[23] in which HPV status was not associated with either OS or RFS (relapse-free survival) in 34 patients who underwent salvage surgery for locally recurrent or persistent OPSCC after chemoradiotherapy. In the current study, because the majority of salvage therapy was not surgery but rather chemoradiotherapy, the HPV-positive patients who underwent surgery as a first-line therapy for OPSCC had a survival advantage.

However, it should be noted that cervical LN metastasis is the most important factor determining the prognosis of HNSCC patients, a relationship that is highly relevant to treatment design. For cervical cancer, it has already been reported that the presence of HPV DNA in regional LNs is a biomarker of latent metastasis[29,31,32,34,39]—in that report, Slama et al.[24] found HPV DNA in 100% of pelvic LN metastatic lesions and stated that the low rate of HPV DNA detection in past reports was a result of the techniques and analytical

methods used to respectively collect and assess cancer specimens (Table 2). For OPSCC, neck LN metastasis has been reported to occur at an earlier stage in HPV-positive patients than in HPV-negative patients;[19,25] however, very few studies have investigated the association between neck LN metastasis and the presence of HPV DNA in cervical LN metastatic lesions in HPV-associated OPSCC. Yasui et al.[26] investigated the clinical significance of HPV status in neck LN metastasis from cancers of unknown primary tumours and concluded that a tumour's HPV status remains unchanged after metastasis. Mirghani et al.[27] tested 45 regional LNs for HPV DNA in 11 HPV-positive and 3 HPV-negative OPSCC patients. In the referenced study, HPV DNA was not detected in the LNs of HPV-negative tumour patients, whereas HPV DNA was detected in all metastatic LNs of HPV-positive tumour patients. These results are similar to those reported in the current study, in which it was found that primary tumours and neck LN metastatic lesions contained the same type of HPV DNA. These results indicate that tumour HPV status remains unchanged after metastasis in HPV-positive OPSCC.

Furthermore, in women with cervical cancer, although numerous publications have suggested that the presence of HPV DNA in LNs could be an early indicator of relapse and poor prognosis,[28-32] the significance of this association remains controversial. In patients with OPSCC, this relationship has not been well investigated to date. In a few articles, Mrghani et al.[33] advocated that an HPV-positive status in a LN free of pathological tumours may not be related to occult metastasis but rather to immune system cells. As a consequence, HPV DNA is not a reliable marker of occult metastasis. Understanding the

prognostic significance of the presence of HPV DNA in LNs requires additional studies with long-term follow-up and standardised molecular and pathological protocols.

CONCLUSIONS

The present results suggest that HPV infection is a determining factor of good prognosis in patients undergoing first-line surgical therapy for OPSCC. HPV status remained unchanged after cervical LN metastasis in HPV-positive OPSCC. However, whether the presence of HPV DNA in neck LNs is a biomarker for latent neck LN metastatic lesions remains controversial.

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Figure legends

Figure 1. Kaplan-Meier curves of Overall survival by HPV

Figure 2. Kaplan-Meier curves of Disease-free survival by HPV

Figure 3. HPV positive for 25 patients treated with up-front surgery.

Figure 4. HPV negative for 40 patients treated with up-front surgery.

Figure 5. Time to death from the time of disease recurrence of oropharyngeal cancer compared according to HPV.

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TABLES

Table 1. Patients' characteristics stratified by HPV status

Variable	HPV-positive (n=25)	HPV-negative (n=40)	P value
Age: Mean±SD	57.4±9.7	65.7±10.2	0.002*
Sex: Male/Female	19/6	34/6	0.36 †
Tumour Location Lateral (Tonsil) Upper (Soft palate) Anterior (Base of tongue) Posterior (Rear wall)	 21 (82%) 0 (0%) 4 (18%) 0 (0%)	 31 (78%) 3 (8%) 6 (14%) 0 (0%)	 0.37 †
Tumour Differentiation: Well/Moderate/Poor	 1/18/6	 8/26/6	 0.16 †
T stage: T1/T2/T3/T4	2/13/5/5	2/24/9/5	0.80 †
N stage: N0/N1/N2/N3	4/6/15/0	18/6/16/0	0.06 †
Stage: Ⅰ/Ⅱ/Ⅲ/Ⅳ	0/3/5/17	0/13/10/17	0.10 †
Tobacco (pack-year)	16.3±16.8	32.0±22.8	0.004*
Drinking (-/+)	10/15	11/29	0.29 †
Neck Dissection (-/+)	1/24	9/31	0.04 †
Microsurgery (-/+)	2/23	12/28	0.036 †

*Student's t test †Chi-square test

Table 2. Relationship between HPV status and LN metastasis

Authors	No.patients	HPV detection method	HPV detection in pN+	Primary
Park et al.[35] 1996	79	PCR E6	13/31 (42%)	Cervix
Baay et al.[36] 1997	50	PCR E6	7/15 (46.5%)	Cervix
Czegledy et al.[37] 1998	31	PCR E6	10/11 (91%)	Cervix
Hernadi et al.[30] 2003	39	PCR E6	11/11 (100%)	Cervix
Chan et al.[38] 2005	15	PCR L1/E6	3/3 (100%)	Cervix
Fule et al.[39] 2006	150	PCR E6	78/120 (65%)	Cervix
Lee et al.[32] 2007	57	PCR	10/11 (91%)	Cervix
Lucaszuk et al.[33] 2007	116	PCR E6/L1	61/61 (100%)	Cervix
Slama et al.[40] 2011	46	PCR L1	8/8 (100%)	Cervix
Mirghani et al.[34] 2013	14	PCR E6	9/9 (100%)	Oropharynx
Yasui et al.[27] 2014	37	PCR E2/E6	19/19 (100%)	Oropharynx

Current study	64	PCR E6/E7	24/24 (100%)	Oropharynx

Table 3. List of abbreviation

OPSCC: oropharyngeal squamous cell carcinoma

LN: lymph node

HNC: head and neck cancer

HPV: human papilloma virus

HNSCC: head and neck squamous cell carcinoma

TNM: tumour node metastasis

DFS: disease-free survival

OS: overall survival

PCR: polymerase chain reaction

RFS: relapse-free survival

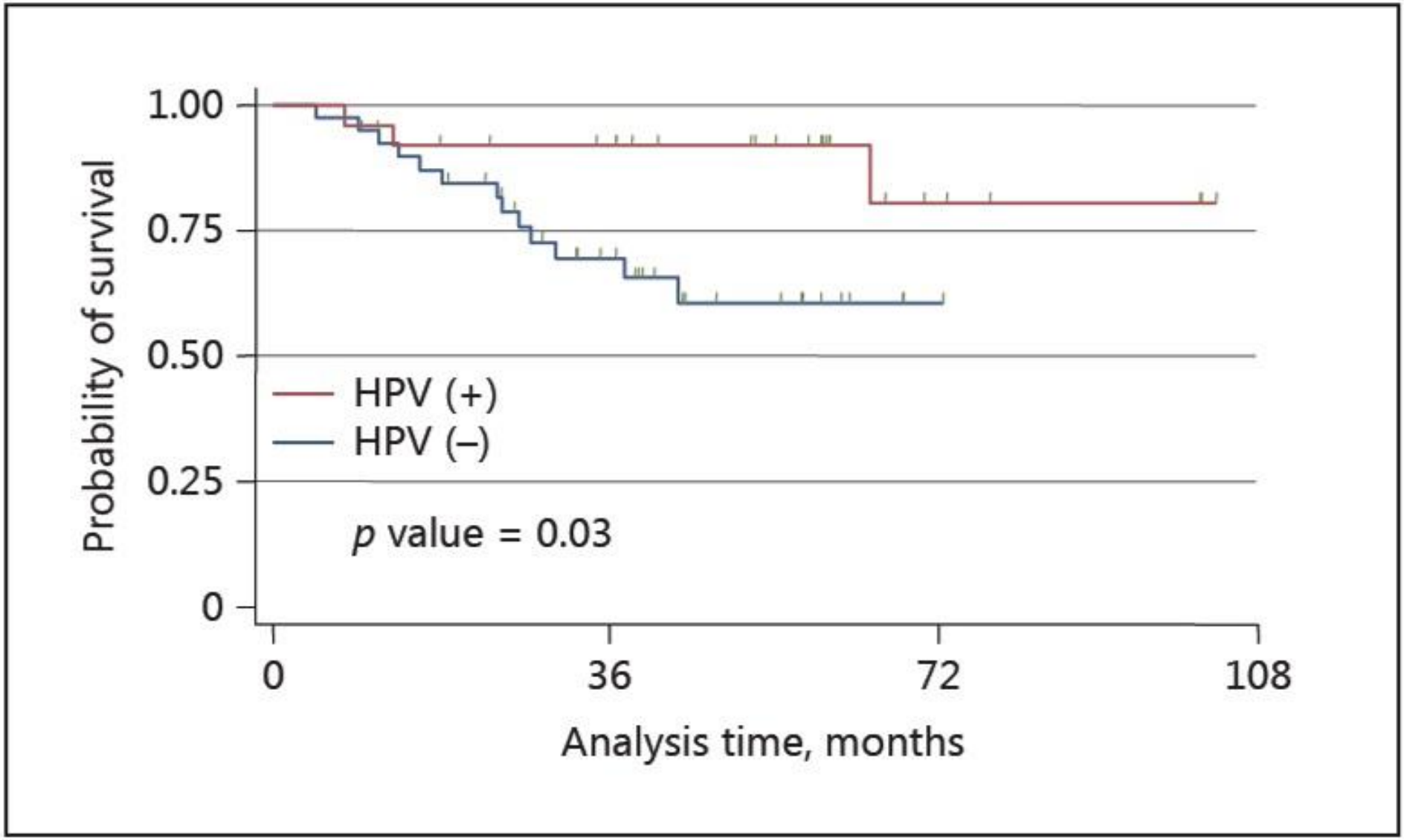

Fig. 1. Kaplan-Meier curves of overall survival by HPV.

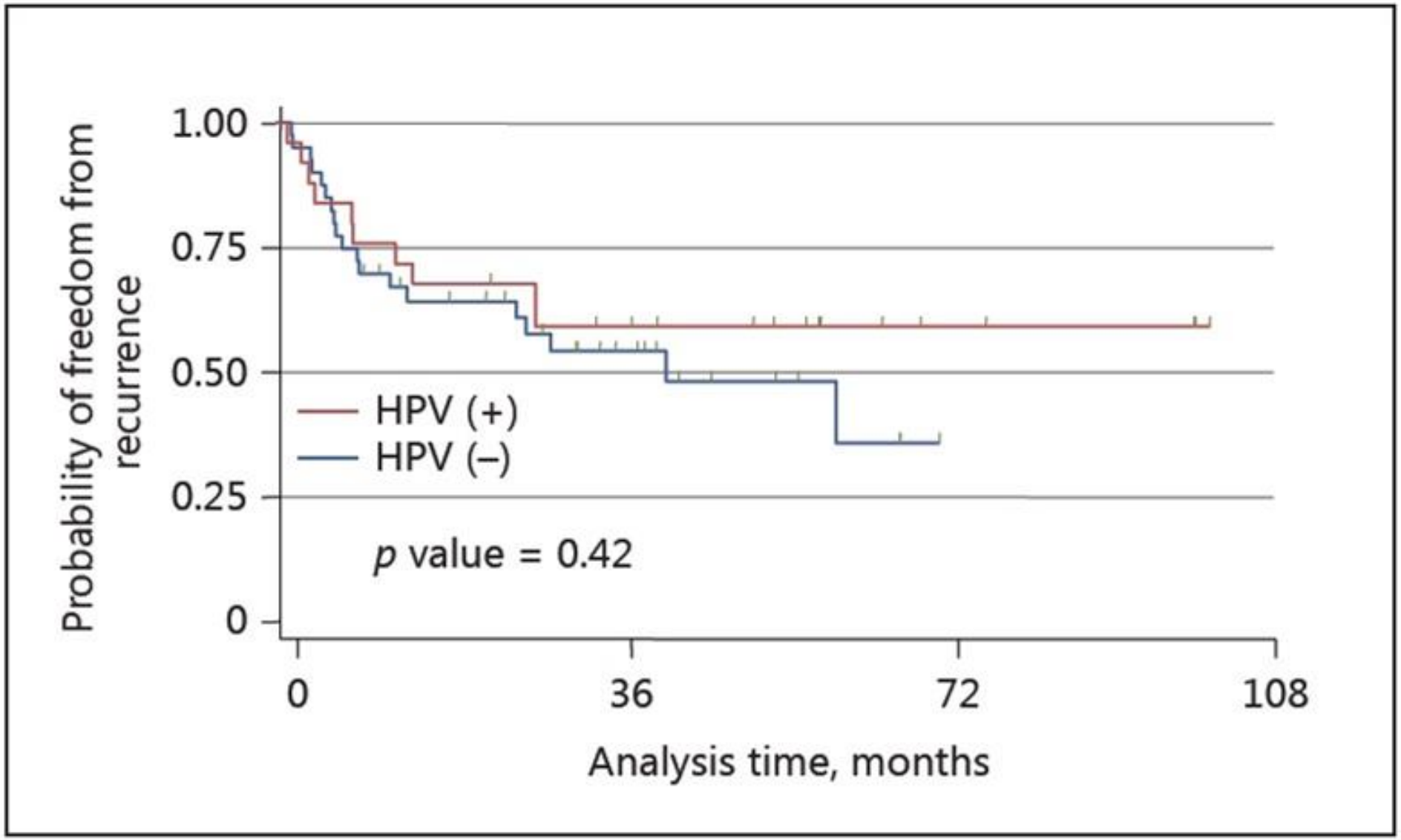

Fig. 2. Kaplan-Meier curves of disease-free survival by HPV.

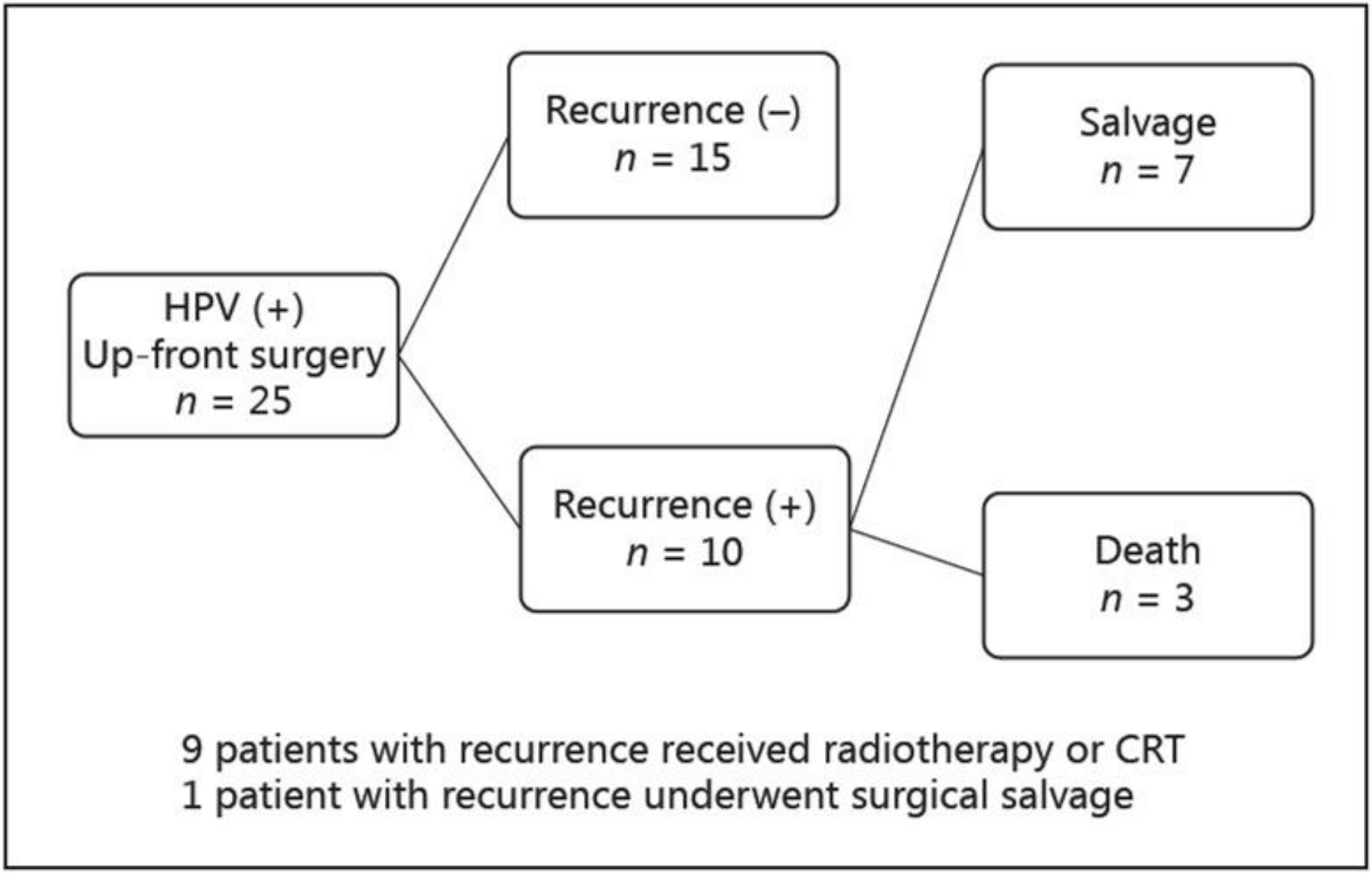

Fig. 3. HPV-positive patients (n = 25) treated with up-front surgery. CRT, chemoradiotherapy.

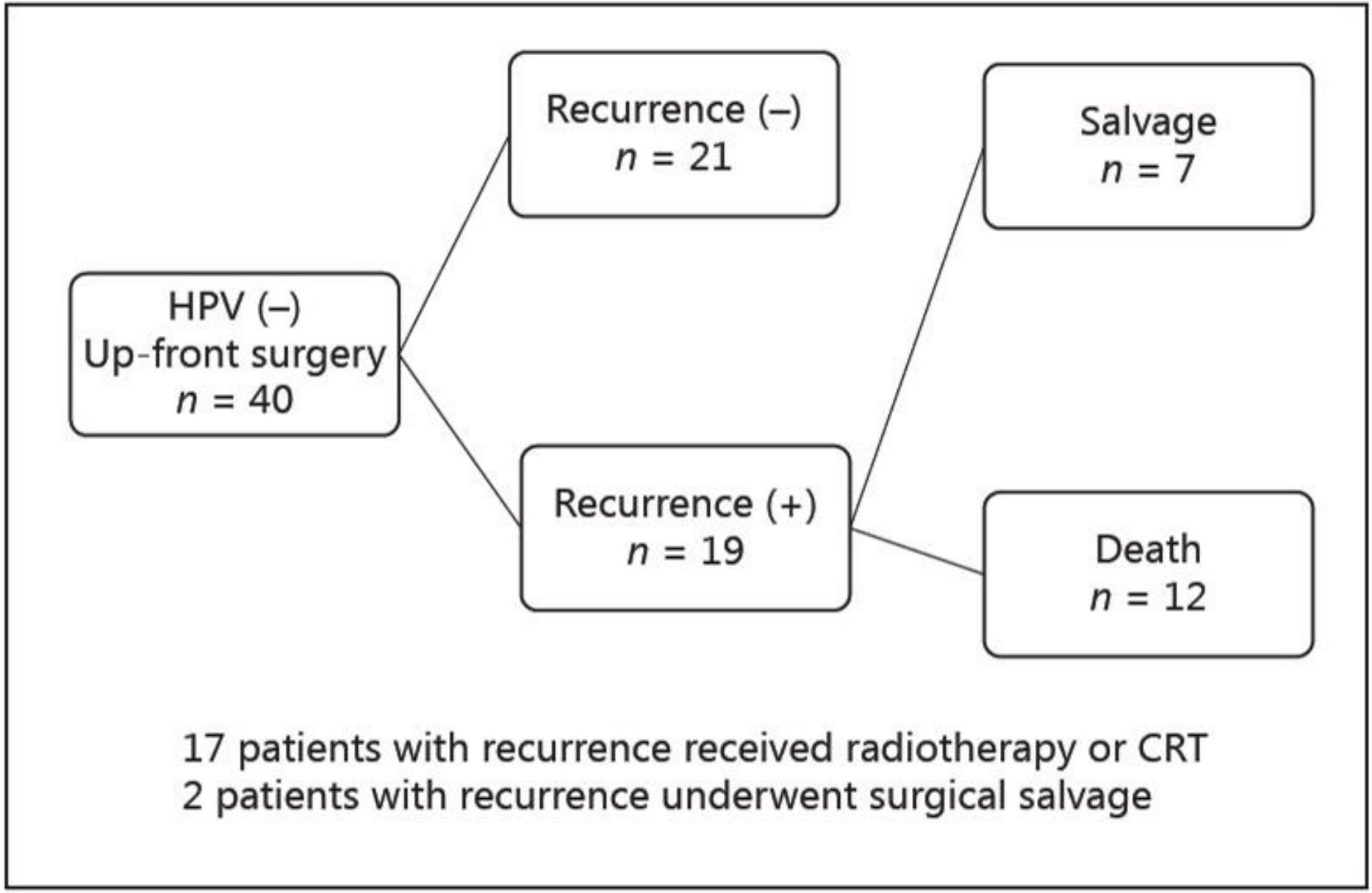

Fig. 4. HPV-negative patients ($n = 40$) treated with up-front surgery. CRT, chemoradiotherapy.

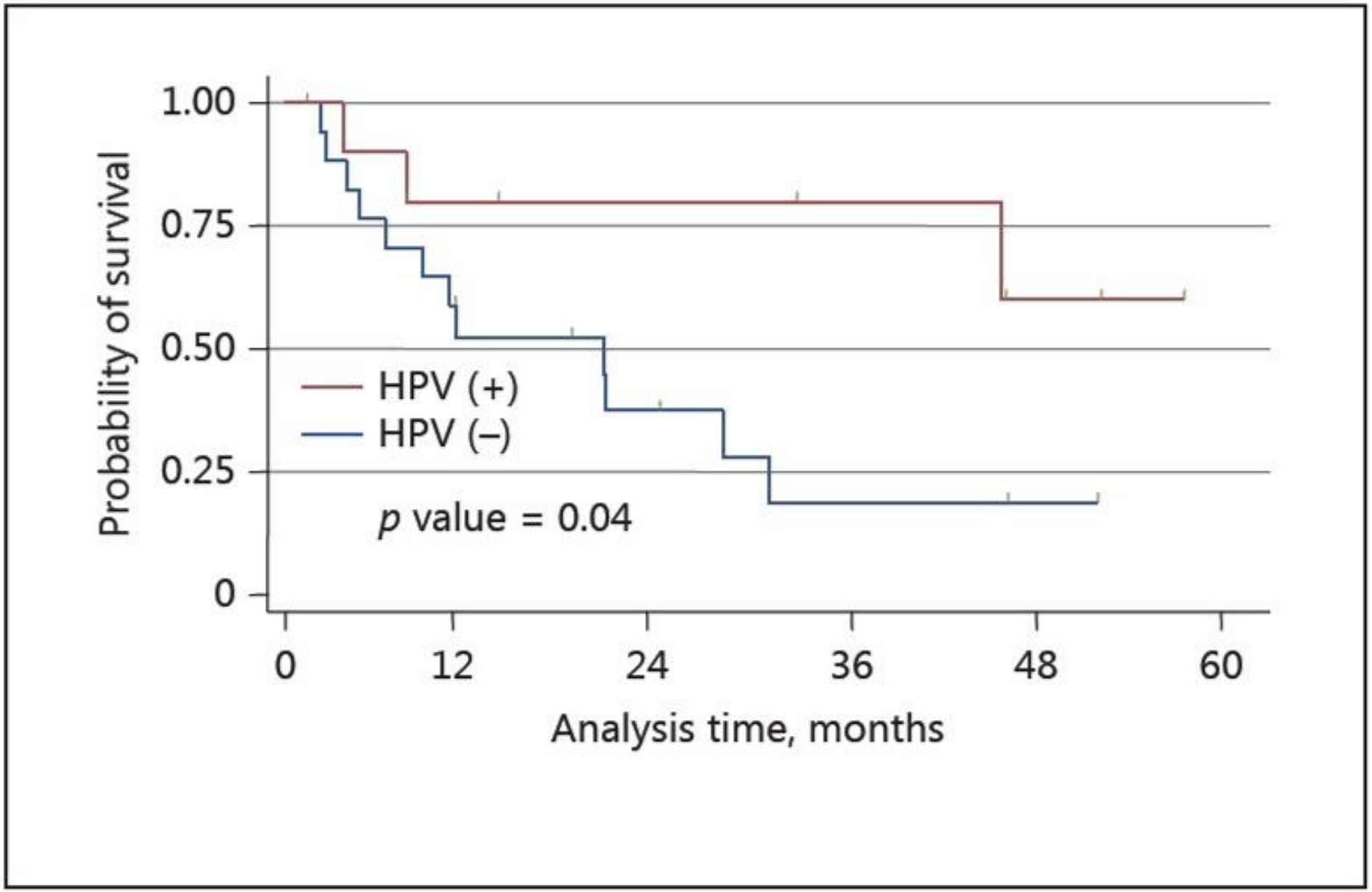

Fig. 5. Time to death from the time of disease recurrence of oropharyngeal cancer compared according to HPV.