Title: Pretreatment elevated mean corpuscular volume as an indicator for high risk esophageal second primary cancer in patients with head and neck cancer

Abstract

Objective

Esophageal cancer is the most common second primary cancer (SPC) in patients with head and neck cancer (HNC). Esophageal SPC has a negative impact on survival. Elevated mean corpuscular volume (MCV) is an accepted predictor of esophageal cancer risk. The aim of this study was to elucidate the usefulness of elevated MCV as an indicator of a high risk for esophageal SPC.

Methods

We retrospectively reviewed the medical records of patients with oropharyngeal, hypopharyngeal, and laryngeal squamous cell carcinoma who underwent chemoradiotherapy between 2003 and 2012. We excluded patients younger than 20 years or who had received treatment for esophageal cancer and who had a histologically unproven lesion. Patients were divided into two groups according to their MCV. The cut-off for MCV was defined by receiver operating characteristics curve analysis. The

primary endpoint was the cumulative incidence of esophageal SPC.

Results

A total of 295 patients were included. The median follow-up period for surviving patients was 7.4 years and the optimal cut-off point was 99.0 fL. One hundred ninety-five patients (66%) had an MCV < 99.0 fL and 100 (34%) had an MCV ≥ 99.0 fL. The 5-year cumulative incidence in patients with an MCV < 99.0 fL and ≥ 99.0 fL was 8.7% and 27%, respectively. In the multivariate analysis, an MCV ≥ 99.0 fL (HR=2.2; 95%CI, 1.1-4.2) was an independent risk factor.

Conclusion

MCV ≥ 99.0 fL was found to be a risk factor for esophageal SPC. We, therefore, recommend that patients with an MCV ≥ 99.0 fL should undergo intensive monitoring.

Keywords

Head and neck cancer; second primary cancer; esophageal cancer; mean corpuscular volume; aldehyde dehydrogenase; field cancerization.

Introduction

The risk of second primary cancer (SPC) in patients with head and neck cancer (HNC) is higher than that in the general population [1, 2]. The sites of SPC formation in the upper aerodigestive tract are exposed to common carcinogens such as alcohol and tobacco, and this process is referred to as “field cancerization” [2, 3]. Esophageal cancer is the most common SPC in patients with HNC [3]. The reported prevalence of synchronous or metachronous esophageal cancer ranges from 0% to 22% [1]. Esophageal SPC has a negative impact on the survival of patients with HNC, with the 3-year overall survival rate estimated to range from 0% to 15% [4], while early diagnosis and treatment of an esophageal SPC may improve the prognosis of patients with HNC [3, 5, 6]. Superficial esophageal cancers are resected by minimally invasive techniques such as endoscopic submucosal dissection [3].

Head and neck cancer and esophageal cancer have common risk factors such as alcohol and smoking [7, 8]. Elevated mean corpuscular volume (MCV) is also an accepted predictor of esophageal cancer risk [7, 9]. MCV is a measure of the average volume of a red blood cell. An elevated MCV correlates with deficiencies in folic acid and vitamin B12. In addition, MCV is particularly elevated in heavy drinkers with inactive aldehyde dehydrogenase-2 (ALDH2) [10, 11]. However, few studies have been conducted to

evaluate the influence of elevated MCV in patients with HNC [12], and there have been no reports on risk factors for esophageal SPC in patients with HNC.

The aim of this study was to elucidate the usefulness of elevated MCV as an indicator of a high risk for esophageal SPC in patients with HNC.

Material and methods

Study design

We retrospectively reviewed the medical records of patients with oropharyngeal, hypopharyngeal, or laryngeal squamous cell carcinoma who underwent chemoradiotherapy in Hokkaido University Hospital between January 2003 and December 2012. We excluded patients younger than 20 years as well as those who had received treatment for esophageal cancer, had a histologically unproven lesion, or for whom pretreatment MCV data were unavailable. An esophageal SPC was defined as a malignancy proven by histological study. An esophageal SPC was classified as a synchronous SPC if identified within 6 months of primary HNC diagnosis. The data for esophageal and other-site SPCs were collected from the medical records up to December 2020. This study was approved by the Institutional Review Board of Hokkaido University Hospital (No. 021-0088) and the study was performed in accordance with the tenets of

the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Treatment

Pretreatment MCV was determined from initial pretreatment blood counts. A receiver operating characteristics (ROC) curve for incidence of esophageal SPC during the observation period was plotted to verify the optimal cut-off point for pretreatment MCV.

Every patient was evaluated by clinical examination, contrast-enhanced computed tomography (CT) imaging, esophagogastroduodenoscopy, and positron emission tomography, where possible. Clinical stage was determined before treatment by the Cancer Board consisting of head and neck surgeons, radiation oncologists, diagnostic radiologists, dentists, and medical oncologists. Tumor-node-metastasis status was classified according to the Union for International Cancer Control (UICC), 7th edition.

HPV status of oropharyngeal cancer was determined by p16 immunostaining. We have performed p16 immunostaining at our institution since 2009. In patients with oropharyngeal cancer who were treated between 2003 and 2008, we checked for HPV status by p16 immunostaining if specimens were available.

The irradiation dose was from 65 to 70 Gy (median 70 Gy). Although the concomitant chemotherapy consisted of various regimens, approximately 74% of all patients received cisplatin, 20% received docetaxel, and 4.1% received carboplatin.

Statistics

All statistical analyses were performed using the JMP Pro 16.0.0 statistical software (SAS Institute, Cary, NC). MCV was reported as the median value and inter quartile ranges (Q1–Q3). Correlations between MCV and clinicopathological characteristics were evaluated using the Kruskal-Wallis test or Wilcoxon test. The primary outcome was cumulative incidence of esophageal SPC. The time of interest was defined as from the first day of treatment to the date of esophageal SPC diagnosis or last follow-up for which data were available. The cumulative incidence of esophageal SPC was estimated using the Kaplan-Meier method, and differences in probabilities between curves were evaluated by log-rank test. The level of statistical significance was set at $p < 0.05$. Univariate HRs and corresponding 95% confidence intervals (CI) were calculated according to the Cox proportional hazards model, including age, gender, alcohol consumption, smoking, anatomical site, clinical stage, and MCV. Alcohol consumption was converted to ethanol per week and used to divide subjects into four categories of never or rare (≤ 70 g/week),

light (71–140 g/week), moderate (141–280 g/week), and heavy (> 280 g/week). An ever smoker was defined as a patient who had smoked at least 1 cigarette a day for at least 1 year. Multivariate Cox regression analysis was performed for all parameters with a P-value < 0.2 based on the univariate analysis. Variance inflation factor (vif) was estimated to exclude multicollinearity; a vif value < 5 was considered as satisfactory.

The cumulative incidence of other-site SPC was calculated in the same manner as that for esophageal SPC.

Results

Patient characteristics and pretreatment MCV

The clinical characteristics of the 295 patients enrolled for this study are shown in Table 1. The median follow-up period for surviving patients was 7.4 years. Fifty-four of the 116 patients with oropharyngeal cancer underwent p16 immunostaining. The number of p16-positive and -negative patients was 23 (43%) and 31 (57%), respectively. Table 2 shows the relationships between clinicopathological characteristics and MCV. Overall, the median pretreatment MCV was 95.7 fL in all patients. The median pretreatment MCV was higher in male than in female patients (P < 0.001), in ever drinkers than in never drinkers (P < 0.001), in ever smokers than in never smokers (P=0.0015), and in p16-

negative than in p16-positive patients ($P < 0.001$).

ROC curve analysis showed the optimal cut-off point (0.64) was 99.0 fL, with a sensitivity of 0.57 and specificity of 0.70. Based on this cut-off value, patients were divided into two groups. One hundred ninety-five patients (66%) had an MCV < 99.0 fL and 100 (34%) had an MCV ≥ 99.0 fL. Table 3 shows the number of patients with MCV ≥ 99.0 fL in each clinicopathological category.

Esophageal SPC

Forty-four of the 295 patients had esophageal SPC. The number of synchronous and metachronous esophageal SPCs was 21 and 23, respectively. The median time to metachronous esophageal SPC was confirmed from the first day of treatment to be 3.7 (Q1–Q3, 2.3–6.2) years. The 5-year cumulative incidence of esophageal SPC was 14% (Fig. 1a). The cumulative incidence of esophageal SPC was higher in patients with an MCV ≥ 99.0 than those with an MCV < 99.0 fL (Fig. 1b, $P < 0.001$). The 5-year cumulative incidence in patients with an MCV < 99.0 fL and ≥ 99.0 fL was 8.7% and 27%, respectively. Table 4 shows the univariate and multivariate analyses of variables predictive of esophageal SPC. In the univariate analysis, alcohol consumption (moderate drinkers (HR=8.5; 95%CI, 1.8–41), heavy drinkers (HR=8.7; 95%CI, 2.1–36)),

hypopharyngeal cancer (HR=6.5; 95%CI, 2.2–19), and MCV ≥ 99.0 (HR=2.9; 95%CI, 1.6–5.2) were significant predictor of esophageal SPC. In the multivariate analysis, hypopharyngeal cancer (HR=4.7; 95%CI, 1.3–17), and MCV ≥ 99.0 fL (HR=2.20; 95%CI, 1.0–3.8) were independent risk factors for esophageal SPC. Multicollinearity was assessed and collinearity between variables was found not to be a concern on multivariable analysis.

Other-site SPCs

Overall, 114 SPCs were diagnosed during the follow-up period (Table 5). The second most frequent SPC was HNC (n=24), followed by lung cancer (n=17) and stomach cancer (n=10). The cumulative incidence of head and neck SPCs was higher in the patients with an MCV ≥ 99.0 fL than in those with an MCV < 99.0 fL (Fig. 2a). An elevated MCV was not associated with the cumulative incidence of lung, stomach, or other-site SPCs (Fig. 2b, c, d).

Discussion

In the current study, the cumulative incidence of esophageal SPC was higher in patients with an MCV ≥ 99.0 fL than in those with an MCV < 99.0 fL. Studies have shown the

3-year overall survival rate of patients with esophageal SPC to be 0–15% [4], while, the 3-year overall survival rates for patients with early and advanced esophageal SPCs were reported to be 77.7% and 21.7%, respectively [6]. Early detection of esophageal SPC would improve treatment outcomes for patients with HNC; therefore, clarifying the indicators of a high risk for esophageal SPC is important.

MCV was reported to a predictor for esophageal squamous cell carcinoma and found to be a biomarker for alcohol abuse accompanied by inactive heterozygous aldehyde dehydrogenase 2 (ALDH2) [7, 13]. A combination of inactive ALDH2 and heavy alcohol consumption is a well-known risk factor for esophageal cancer [14]. Ethanol is mainly metabolized to acetaldehyde by alcohol dehydrogenase and further oxidized to acetate by aldehyde dehydrogenase (ALDH) [15]. At least 4‑5 classes of ALDH isoenzymes exist and ALDH2 is the most important, possessing a high affinity to acetaldehyde [16]. A single point mutation in the ALDH2 gene results in the ALDH2*2 allele, which is characterized by a reduced ability to metabolize acetaldehyde [17]. After alcohol consumption, the blood concentration of acetaldehyde is 19 and 6 times higher in individuals homozygous or heterozygous for the *2 allele of ALDH2, respectively, as compared with those with wild-type homozygotes [10]. Growing evidence exists in support of the notion that acetaldehyde rather than alcohol itself is carcinogenic [16, 18].

The International Agency for Research on Cancer certified acetaldehyde associated with alcohol consumption as a group I carcinogen for the esophagus and head and neck region [8]. The heterozygous ALDH2*2 allele itself is not a carcinogen, and past studies reported that the ALDH2*2 allele was not associated with a risk for esophageal cancer among never drinkers [19, 20].

The combination of heavy alcohol consumption and the heterozygous ALDH2*2 allele is known to result in high exposure to acetaldehyde. A previous study reported that the relationship between alcohol consumption and elevated MCV is mediated mainly by exposure to acetaldehyde [10]. Therefore, elevated MCV may be used as a surrogate marker for high exposure to acetaldehyde. Alcohol and smoking are well known carcinogens for the esophagus and head and neck region [8]. However, neither alcohol consumption or smoking was significantly associated with esophageal SPC in the multivariate analysis (Table 4). In the current study, the rate of moderate or heavy drinkers and smokers was 64% (156/245) and 89% (216/244), respectively (Table 2). We do not think that either alcohol consumption or tobacco smoking is an appropriate indicator as a majority of patients were both drinkers and smokers. Rather, we consider an MCV $\geq$ 99.0 fL to be a stronger and more appropriate indicator for esophageal SPC. If ALDH2 gene status were examined, alcohol consumption would become a reliable

indicator.

In the current study, an elevated MCV was also significantly associated with the cumulative incidence of head and neck SPC. Although the relationship between MCV and the incidence of HNC has not been reported, acetaldehyde or the combination of alcohol consumption and the heterozygous *2 allele is a known risk factor for HNC [17]. The relationship between elevated MCV and head and neck SPC is consider to be similar to that of esophageal SPC. Intensive follow-up is also needed for the head and neck region in patients with elevated MCV. Early detection of head and neck SPC may improve treatment outcomes [21], while an elevated MCV is not associated with incidence of other-site SPC. This may be due to the fact that acetaldehyde is not a significant carcinogenic factor except for the esophagus and head and neck region.

Smoking was also found to be associated with elevated MCV (Table 2). It is speculated that one of the mechanisms by which smoking affects MCV is through the compensatory response to reduced oxygen capacity [11, 13]. Inhalation of acetaldehyde in tobacco smoke may also affect MCV as acetaldehyde is one of the major chemical constituents of tobacco smoke [22]. Therefore, elevated MCV may be one indicator for lung cancer as tobacco smoking is a well-known risk factor. In the current study, although no significant difference was observed, the cumulative incidence of lung-SPC

was higher in patients with an MCV ≥ 99.0 fL (Fig 2b). Patients with HPV-negative oropharyngeal cancer had a higher MCV than did those with HPV-positive oropharyngeal cancer (Table 2), which is in agreement with the results of a previous study [12]. We think the lower rate of ever drinkers among patients with HPV-positive oropharyngeal cancer can explain these results. The number of heavy drinkers was 14 of 20 (70%) and 4 of 28 patients (14%) in HPV-negative and -positive groups, respectively.

In the current study, an MCV ≥ 99.0 fL and anatomical site (hypopharynx) were both associated with the cumulative incidence of esophageal SPC. A past study reported that hypopharyngeal cancer is a risk factor for esophageal SPC [23]. On the other hand, there have been no reports to date that an elevated MCV is a risk factor for esophageal SPC. Several studies have recommended intensive endoscopic monitoring for patients with hypopharyngeal cancer due to the high incidence rates of esophageal SPC [1, 24]. Intensive endoscopic monitoring would improve the detection rate and treatment outcomes of esophageal SPCs [1, 3]. In the current study, MCV ≥ 99.0 fL was found to have as great an impact as anatomical site. We, therefore, suggest patients with an elevated MCV should also undergo intensive endoscopic monitoring to improve treatment outcomes.

We acknowledge several limitations to this study. First, the cut-off point for

pretreatment MCV was determined by ROC curve analysis. In the current study, the number of esophageal SPCs was 44. A larger sample size would provide a more valid cut-off point. Second, follow-up strategies for esophageal SPC were not defined. Early-stage esophageal SPC may not be detected during the observation period; therefore, a prospective study is required to validate our results.

Conclusion

An MCV ≥ 99.0 fL was found to be a risk factor for esophageal SPC in patients with HNC. We, therefore, recommend patients with an elevated MCV should undergo intensive endoscopic monitoring.

FIGURE LEGENDS

Table 1. title: Patient characteristics.

Table 2. title: Median values and interquartile range (Q1-Q3) for mean corpuscular volume (MCV) according to clinicopathological characteristics.

never or rare (≤ 70 g ethanol/week), light (71–140 g ethanol/week), moderate (141–280 g ethanol/week), heavy (> 280 g ethanol/week).

Table 3. title: The number of patients with MCV ≥ 90 fL in each clinicopathological category.

never or rare (≤ 70 g ethanol/week), light (71–140 g ethanol/week), moderate (141–280 g ethanol/week), heavy (> 280 g ethanol/week).

Table 4. title: Univariate and multivariate Cox regression analyses for esophageal second primary cancer.

never or rare (≤ 70 g ethanol/week), light (71–140 g ethanol/week), moderate (141–280 g ethanol/week), heavy (> 280 g ethanol/week).

Table 5. title: All second primary cancers diagnosed during the follow-up period.

Figure 1. Kaplan-Meier estimates of cumulative incidence of esophageal second primary cancer. MCV = mean corpuscular volume.

Figure 2. Kaplan-Meier estimates of cumulative incidence of (a) head and neck, (b) lung, (c) stomach, and (d) other-site second primary cancers. MCV = mean corpuscular volume. SPC = second primary cancer

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Characteristic	
Overall	295
Gender	
Male	262
Female	33
Age, years	
Median (range)	61 (37–79)
Follow-up period, years	
Median (range)	7.4 (0.37–17)
Site	
Oropharynx	116
Hypopharynx	108
Larynx	71
T classification	
1–2	143
3–4	147
N classification	
0	94
1–3	196
Stage	
I–II	63
III–IV	227
HPV	
Negative	23
Positive	31
Unknown	62

	n	Median MCV (Q1–Q3)	P-value
Overall	295	95.7 (91.6-100.5)	
Gender			<0.001
Male	262	96.2 (92.1–101)	
Female	33	92.2 (87.2–96.0)	
Age			0.074
<61	148	95.1 (91.0–99.8)	
61≤	147	96.2 (92.1–101.3)	
Alcohol consumption			<0.001
Never or rare	67	91.6 (89.0–95.0)	
Light	22	95.0 (90.5–99.9)	
Moderate	30	94.7 (92.0–102)	
Heavy	126	98.2 (93.3–102)	
Smoking status			0.0015
Never	28	92.9 (88.7–95.0)	
Ever	216	95.9 (91.9–100.5)	
Site			0.11
Oropharynx	116	94.8 (90.7–99.5)	
Hypopharynx	108	96.3 (92.4–101.6)	
Larynx	71	95.8 (91.6–100.2)	
T classification			0.94
1-2	143	95.7 (91.9–100.2)	
3-4	147	95.4 (91.4–100.3)	
N classification			0.50
0	94	95.9 (92.3–100.2)	
1–3	196	95.4 (91.3–100.4)	
Stage			0.42
I-II	63	95.8 (92.8–100.5)	
III-IV	227	95.4 (91.3–100.2)	
Hb (g/dL)			0.65
< 12	37	95.8 (91.8–105.5)	

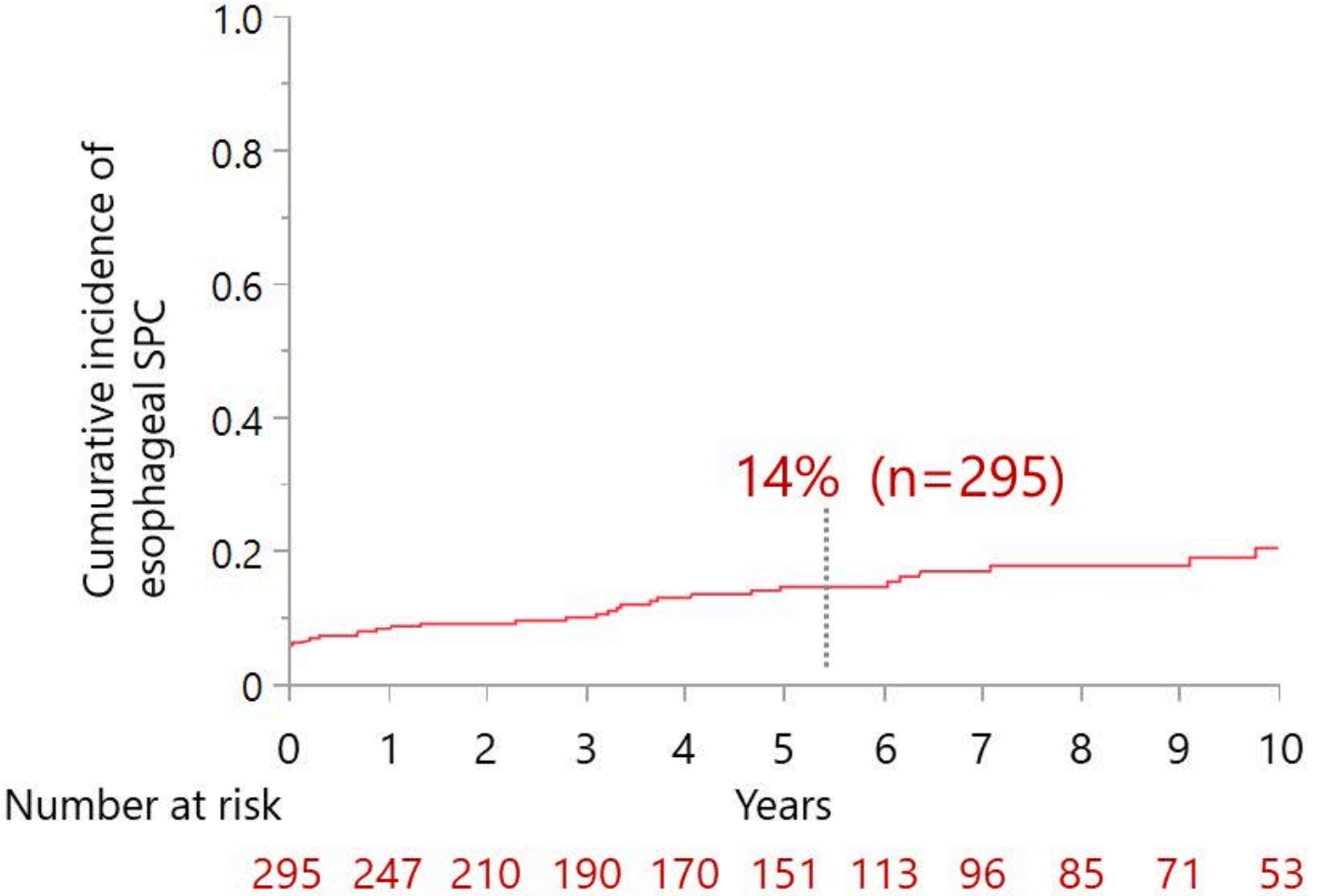
12 ≤	263	95.8 (91.6–100.2)	
p16			<0.001
Negative	23	98.7 (94.8–101.5)	
Positive	31	90.9 (88.5–95.0)	

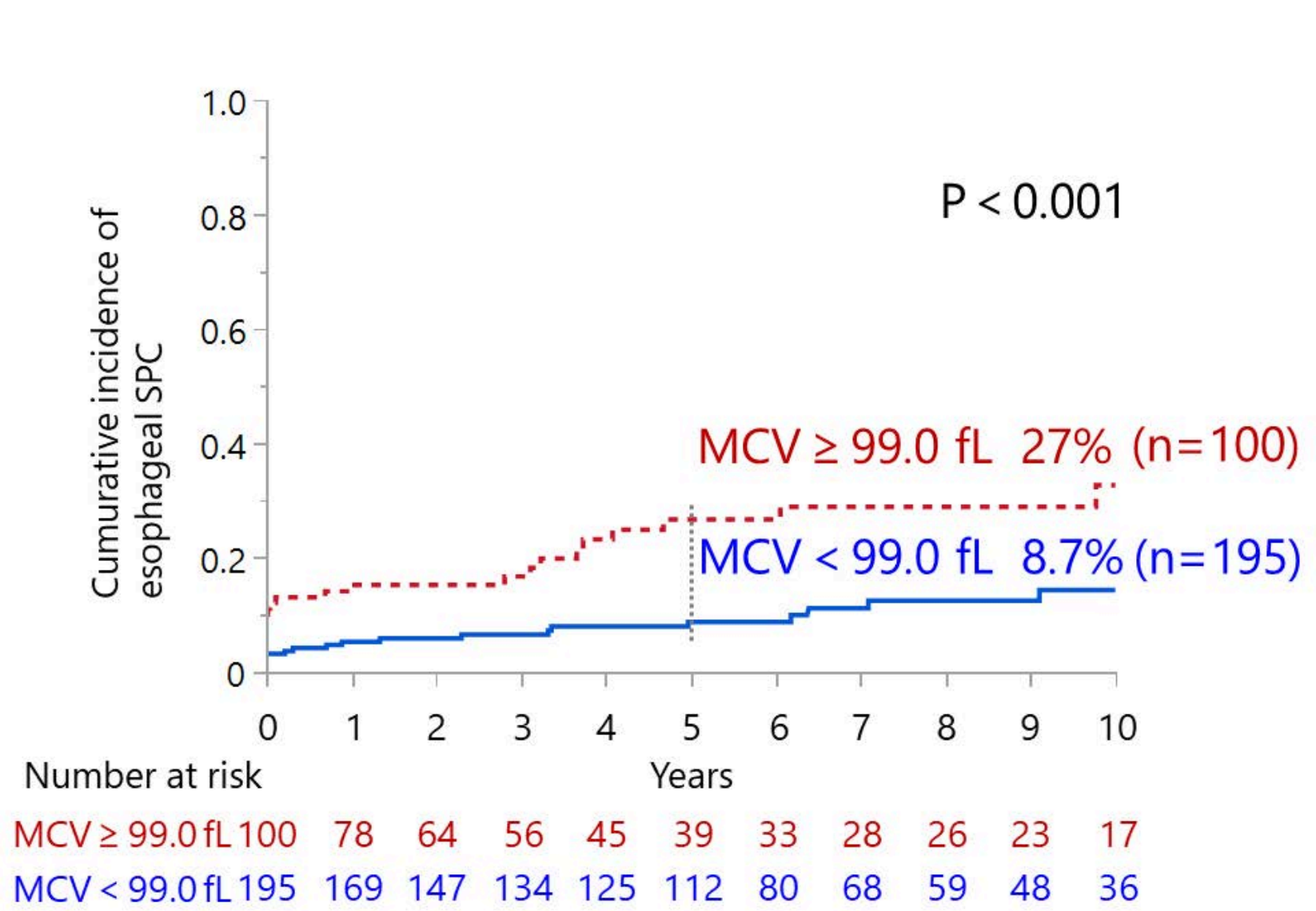
	n	MCV ≥ 90 fL, n (%)
Overall	295	100 (34)
Gender		
Male	262	96 (37)
Female	33	4 (12)
Age		
< 61	148	42 (28)
61 ≤	147	58 (39)
Alcohol consumption		
Never or rare	67	9 (13)
Light	22	6 (27)
Moderate	30	10 (33)
Heavy	126	55 (44)
Smoking status		
Never	28	4 (14)
Ever	216	76 (35)
Site		
Oropharynx	116	32 (28)
Hypopharynx	108	43 (40)
Larynx	71	25 (35)
T classification		
1-2	143	47 (33)
3-4	147	50 (34)
N classification		
0	94	32 (34)
1-3	196	55 (28)
Stage		
I-II	63	21 (33)
III-IV	227	76 (33)
Hb (g/dL)		
< 12	37	12 (32)

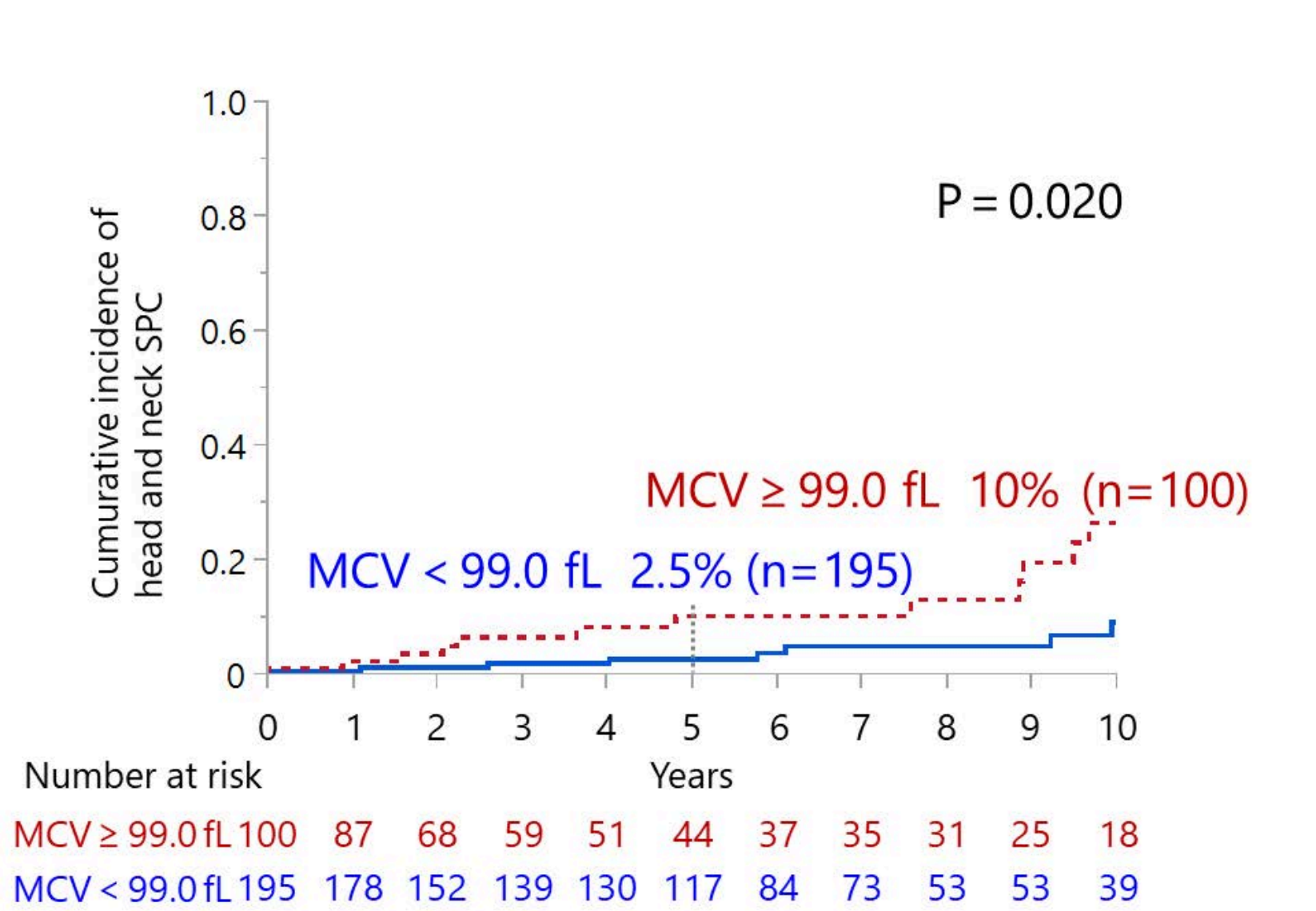
12 ≤	263	88 (33)
p16		
Negative	23	11 (48)
Positive	31	4 (13)

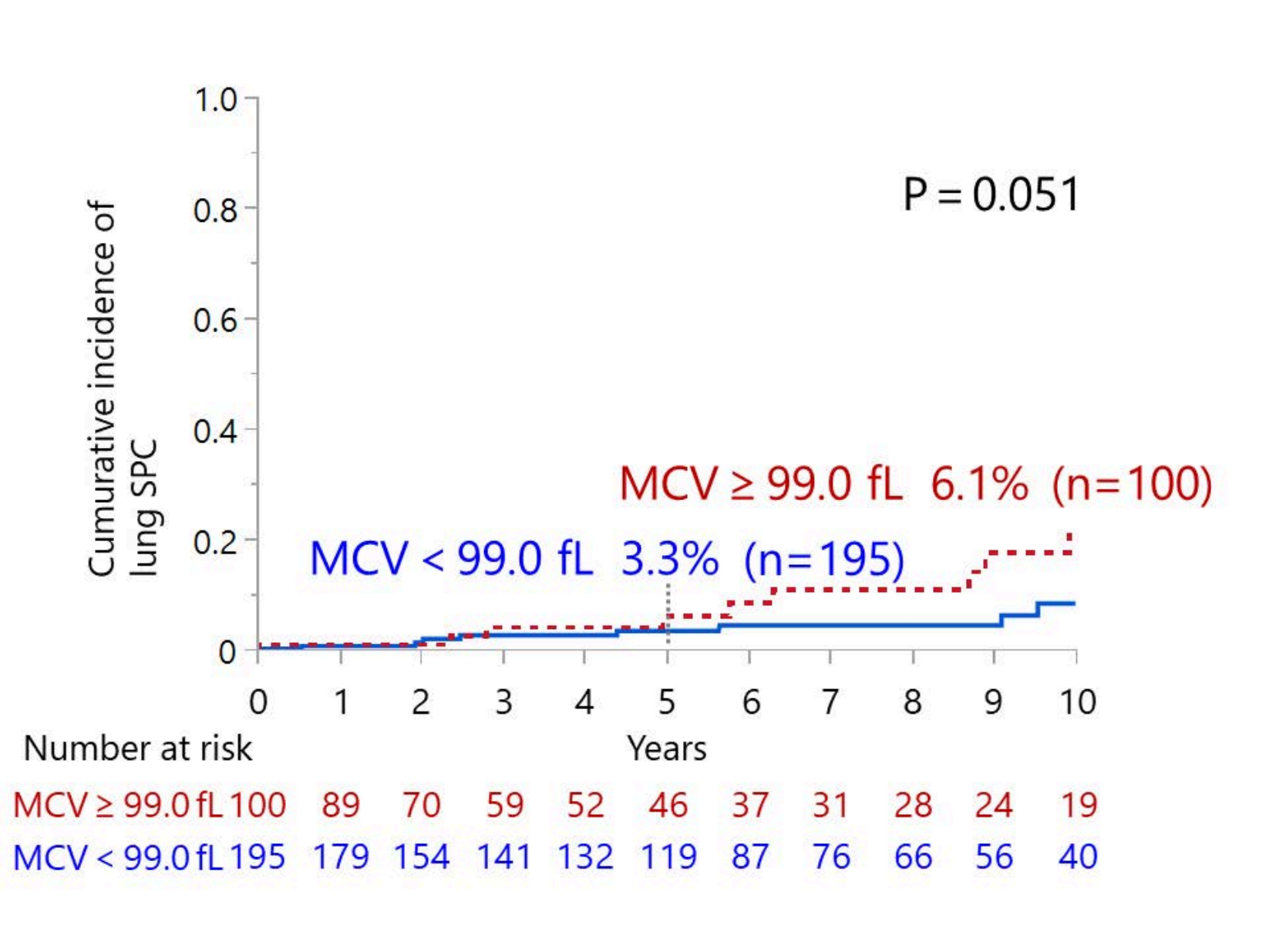
		Univariate analysis		Multivariate analysis	
	n	HR (95% CI)	P-value	HR (95% CI)	P-value
Gender			0.080		0.28
Female	33	1		1	
Male	262	5.9 (0.81–42.7)		3.2 (0.40–25)	
Age			0.73		
<61	148	1			
61≤	147	1.1 (0.62–2.0)			
Alcohol consumption			0.014		0.13
Never or rare	67	1		1	
Light	22	2.9 (0.41–20)	0.29	2.9 (0.41–20)	0.29
Moderate	30	8.5 (1.8–41)	0.0075	5.8 (1.2–28)	0.031
heavy	126	8.7 (2.1–36)	0.0032	5.3 (1.2–24)	0.028
Smoking status			0.51		
Never	28	1			
Ever	216	1.5 (0.46–4.9)			
Site			<0.001		0.046
Larynx	74	1		1	
Oropharynx	118	2.6 (0.83–8.1)	0.10	3.1 (0.8–12)	0.10
Hypopharynx	109	6.5 (2.2–19)	<0.001	4.7 (1.3–17)	0.015
T classification			0.89		
1–2	143	1			
3–4	147	1.0 (0.57–1.9)			
N classification			0.14		0.96
0	94	1		1	
1–3	196	1.7 (0.84–3.4)		0.98 (0.43–2.2)	
Stage			0.43		
I–II	63	1			
III–IV	227	1.4 (0.64–2.9)			
Hb (g/dL)			0.13		0.10
12≤	259	1		1	
<12	35	1.9 (0.84–4.3)		2.0 (0.86–4.9)	
MCV (fL)			<0.001		0.044
<99	195	1		1	
99≤	100	2.9 (1.6–5.2)		2.0 (1.0–3.8)	

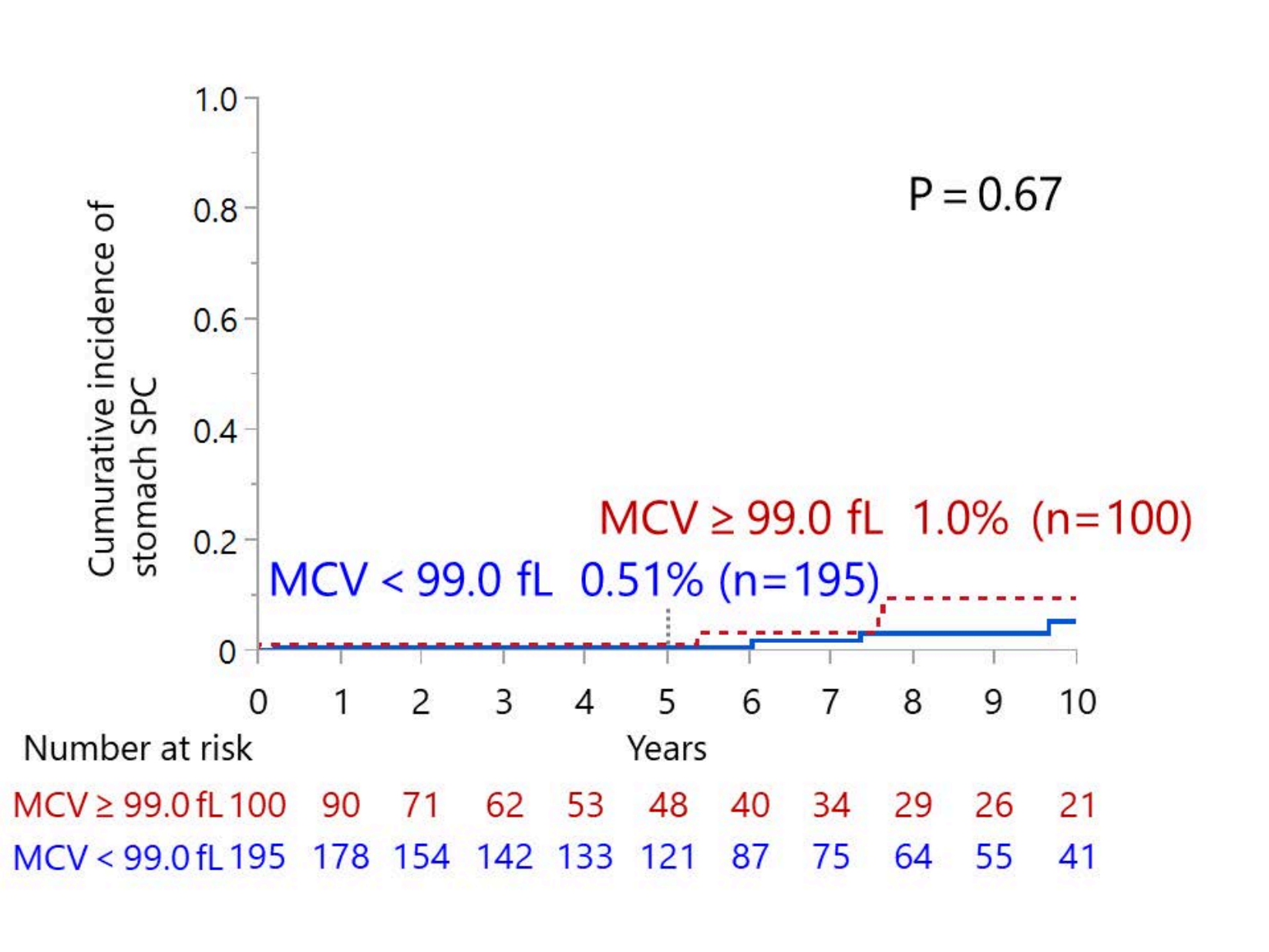
Site	
Overall	114
Esophagus	44
Head and neck	24
Lung	17
Stomach	10
Colon and rectum	4
Bladder	3
Prostate	3
Liver	2
Pancreas	2
Gall bladder	1
Uterine cervix	1
Renal pelvis	1
Breast	1
Skin	1

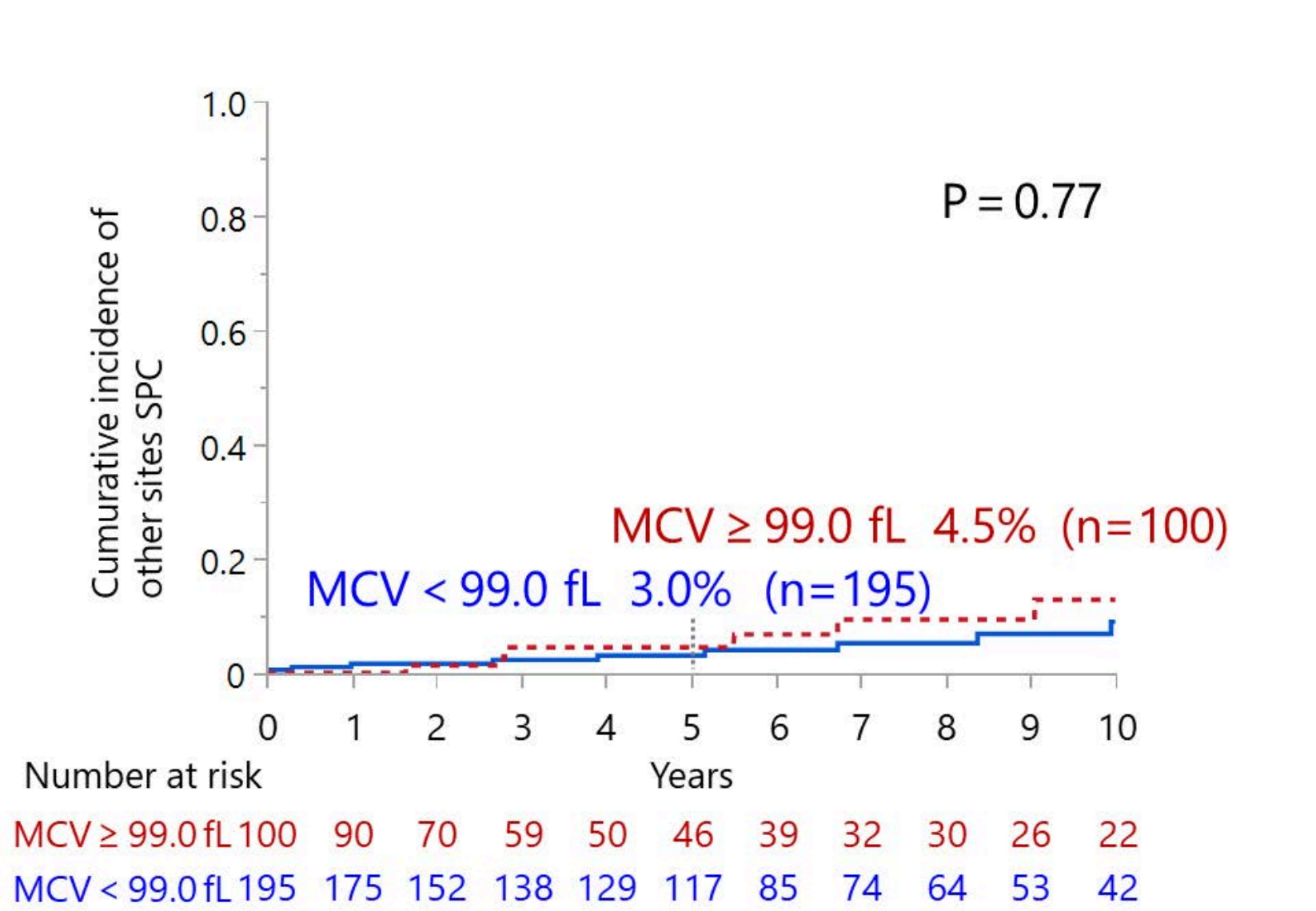

1.0
0.8
0.6
0.4
0.2
0
Cumurative incidence of esophageal SPC
14% (n=295)
0 1 2 3 4 5 6 7 8 9 10
Years
Number at risk
295 247 210 190 170 151 113 96 85 71 53


1.0
0.8
0.6
0.4
0.2
0
Cumurative incidence of esophageal SPC
P < 0.001
MCV ≥ 99.0 fL 27% (n=100)
MCV < 99.0 fL 8.7% (n=195)
0 1 2 3 4 5 6 7 8 9 10
Years
Number at risk
MCV ≥ 99.0 fL 100 78 64 56 45 39 33 28 26 23 17
MCV < 99.0 fL 195 169 147 134 125 112 80 68 59 48 36


1.0
0.8
0.6
0.4
0.2
0
Cumurative incidence of head and neck SPC
P = 0.020
MCV ≥ 99.0 fL 10% (n=100)
MCV < 99.0 fL 2.5% (n=195)
0 1 2 3 4 5 6 7 8 9 10
Years
Number at risk
MCV ≥ 99.0 fL 100 87 68 59 51 44 37 35 31 25 18
MCV < 99.0 fL 195 178 152 139 130 117 84 73 53 53 39


1.0
0.8
0.6
0.4
0.2
0
Cumurative incidence of lung SPC
P = 0.051
MCV ≥ 99.0 fL 6.1% (n=100)
MCV < 99.0 fL 3.3% (n=195)
0 1 2 3 4 5 6 7 8 9 10
Years
Number at risk
MCV ≥ 99.0 fL 100 89 70 59 52 46 37 31 28 24 19
MCV < 99.0 fL 195 179 154 141 132 119 87 76 66 56 40


1.0
0.8
0.6
0.4
0.2
0
Cumurative incidence of stomach SPC
P = 0.67
MCV ≥ 99.0 fL 1.0% (n=100)
MCV < 99.0 fL 0.51% (n=195)
0 1 2 3 4 5 6 7 8 9 10
Years
Number at risk
MCV ≥ 99.0 fL 100 90 71 62 53 48 40 34 29 26 21
MCV < 99.0 fL 195 178 154 142 133 121 87 75 64 55 41


1.0
0.8
0.6
0.4
0.2
0
Cumurative incidence of other sites SPC
P = 0.77
MCV ≥ 99.0 fL 4.5% (n=100)
MCV < 99.0 fL 3.0% (n=195)
0 1 2 3 4 5 6 7 8 9 10
Years
Number at risk
MCV ≥ 99.0 fL 100 90 70 59 50 46 39 32 30 26 22
MCV < 99.0 fL 195 175 152 138 129 117 85 74 64 53 42