




# Pretreatment High MCV as Adverse Prognostic Marker in Nonanemic Patients with Head and Neck Cancer

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**Objective:** Mean corpuscular volume (MCV) has been shown in to be a reliable prognostic marker in other cancers; however, no evidence exists on its use in head and neck squamous cell carcinoma (HNSCC). This study aimed to investigate the association between MCV, hemoglobin, platelet count and albumin concentration, and survival in stage III/IVA-B HNSCC treated with concurrent chemoradiotherapy.

**Study Design:** Retrospective cohort study.

**Methods:** In this multicenter retrospective study, we analyzed MCV, platelet count, hemoglobin concentration, and albumin concentration in peripheral blood samples from 260 patients with HNSCC undergoing organ preservation treatment with curative intent at the time of diagnosis. We then analyzed survival outcomes after accounting for confounders using multivariate analysis.

**Results:** After adjustment for potential confounders, patients with low hemoglobin had a 3.3-fold higher risk of death (95% confidence interval [CI]: 2.26-4.81) than those with normal hemoglobin. Patients with an elevated MCV had a 1.54-fold higher risk of death (95% CI: 1.06-2.24), independent of site, stage, and human papillomavirus status. Interestingly, the effect of MCV on overall and progression-free survival was limited to those with a normal pretreatment hemoglobin. We identified no associations between pretreatment platelet count or albumin concentration and survival.

**Conclusion:** These findings suggest that pretreatment anemia and macrocytosis are independent predictors of lower overall and progression-free survival in HNSCC patients undergoing organ preservation treatment.

**Key Words:** Mean corpuscular volume, Head and neck squamous cell carcinoma, anemia, platelets, HPV, prognosis.

**Level of Evidence:** III

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## INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is a potentially lethal cancer that develops in the mucosal epithelium of the oral cavity, hypopharynx, oropharynx, or larynx. It is estimated that HNSCC will affect 151 thousand new patients in Europe and approximately 833 thousand worldwide in 2020, thus representing 4% and

5% of all new cancers, respectively.<sup>1</sup> HNSCC is more common in men and in those aged over 60 years.<sup>1</sup> Tobacco and alcohol use represent the main risk factors for HNSCC, with a synergistic effect.<sup>2</sup> To date, traditional tumor-based histopathological risk factors such as tumor and lymph node staging, tumor differentiation, and status of resection margin remain the only clinical prognostic factors available.

Recent findings have shown hematological parameters to be predictive markers for patients with other types of cancer. Mean corpuscular volume (MCV) is a measure of the average volume of red blood cells, and as well as being an indicator of folate and vitamin B12 deficiency, elevated MCV has been linked to the incidence of several cancers. In addition, recent studies have found an association between high MCV and the prognosis of colorectal, esophageal, and liver cancers.<sup>3–5</sup>

In the last decade, platelet activation has been found to be an important step in the process of carcinogenesis and metastasis.<sup>6</sup> Platelet count (PLT) reflects platelet activation, and patients with thrombocytosis have been reported to have a worse prognosis in multiple solid tumors, such as ovarian cancer,<sup>7</sup> endometrial cancer,<sup>8</sup> gastric cancer,<sup>9</sup> and colorectal cancer.<sup>10</sup>

Furthermore, anemia, and particularly nonmicrocytic anemia, is associated with poorer cancer-specific and overall survival, independent of systemic

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TABLE I.  
Median Values and Interquartile Range (Q1-Q3) of Hemoglobin, Mean Corpuscular Value, Platelets, and Albumin According to Sociodemographic and Clinical Characteristics.\*

	n	Hb (g/dL)	MCV (fL)	Platelets (10 <sup>3</sup> /mm <sup>3</sup> )	Albumin (g/dL)
Overall	260	13.8 (12.6–14.8)	93.1 (89.8–98.7)	258 (209–317)	4.4 (4.1–4.7)
Gender					
Male	200	13.9 (12.7–15.0)	94.2 (90.7–99.5)	259 (210–308)	4.4 (4.1–4.7)
Female	60	13.4 (12.2–14.0)	91.6 (89.1–94.3)	256 (208–339)	4.5 (4.4–4.7)
		<i>P</i> = .016	<i>P</i> = .002	<i>P</i> = .560	<i>P</i> = .160
Age (yrs)					
< 60	119	13.8 (12.6–14.9)	93.8 (89.4–99.4)	275 (206–339)	4.4 (4.1–4.6)
60–69	96	13.8 (12.6–15.0)	93.2 (90.5–98.3)	247 (211–314)	4.5 (4.2–4.7)
≥ 70	45	13.5 (12.6–14.0)	92.3 (89.4–97.7)	240 (212–186)	4.4 (4.2–4.6)
		<i>P</i> = .429	<i>P</i> = .558	<i>P</i> = .176	<i>P</i> = .148
Tobacco smoking					
Never	29	14.1 (13.4–15.1)	89.1 (87.1–92.9)	220 (201–279)	4.6 (4.1–4.7)
Ever	209	13.6 (12.5–14.5)	94.2 (91.0–99.6)	262 (213–325)	4.4 (4.1–4.7)
Unknown	22	14.3 (12.6–15.4)	92.3 (89.1–93.9)	277 (181–315)	4.4 (4.0–4.7)
		<i>P</i> = .031	<i>P</i> < .001	<i>P</i> = .129	<i>P</i> = .215
Alcohol drinking					
Never	42	13.5 (12.2–14.6)	90.0 (86.4–94.7)	240 (207–299)	4.3 (3.9–4.5)
Ever	196	13.8 (12.7–14.8)	94.2 (91.1–99.6)	261 (211–325)	4.5 (4.2–4.7)
Unknown	22	14.3 (12.6–15.4)	92.3 (89.1–93.9)	277 (181–315)	4.4 (4.0–4.7)
		<i>P</i> = .430	<i>P</i> < .001	<i>P</i> = .682	<i>P</i> = .050
Period of cancer diagnosis (year)					
1997–2004	90	13.4 (12.2–14.4)	95.1 (91.2–98.8)	262 (212–315)	4.3 (4.0–4.7)
2005–2008	91	13.8 (12.7–14.9)	91.8 (89.0–96.2)	274 (228–343)	4.4 (4.1–4.6)
2009–2015	79	14.0 (13.1–15.0)	93.8 (89.7–99.8)	236 (199–297)	4.6 (4.4–4.7)
		<i>P</i> = .001†	<i>P</i> = .858†	<i>P</i> = .174†	<i>P</i> = .020†
Cancer site					
Oropharynx	153	13.8 (12.6–14.9)	92.5 (89.1–97.6)	264 (209–309)	4.4 (4.2–4.7)
Other	107	13.8 (12.6–14.5)	94.1 (91.6–99.5)	245 (206–325)	4.5 (4.1–4.7)
		<i>P</i> = .871	<i>P</i> = .019	<i>P</i> = .621	<i>P</i> = .528
T					
cT1-T2	61	14.0 (12.7–15.3)	92.4 (89.4–94.3)	257 (206–304)	4.5 (4.3–4.6)
cT3	88	14.0 (13.3–15.0)	94.2 (91.0–99.4)	253 (204–313)	4.5 (4.2–4.7)
cT4	111	13.2 (12.0–14.5)	93.9 (89.8–99.2)	263 (214–345)	4.4 (4.0–4.6)
		<i>P</i> < .001†	<i>P</i> = .178†	<i>P</i> = .138†	<i>P</i> = .127†
N					
cN0	53	14.0 (13.1–15.2)	93.3 (89.4–99.3)	257 (207–300)	4.6 (4.3–4.8)
cN1	45	13.9 (12.7–14.5)	92.4 (90.0–96.9)	256 (220–310)	4.4 (4.1–4.7)
cN2-N3	162	13.6 (12.4–14.7)	93.8 (89.8–99.0)	260 (209–335)	4.4 (4.1–4.6)
		<i>P</i> = .101†	<i>P</i> = .558†	<i>P</i> = .577†	<i>P</i> = .042†
Stage					
III	71	14.0 (13.1–15.0)	92.8 (89.9–99.3)	249 (207–301)	4.5 (4.2–4.7)
IVA-B	188	13.6 (12.4–14.7)	93.6 (89.8–98.7)	260 (210–336)	4.4 (4.1–4.6)
		<i>P</i> = .028	<i>P</i> = .959	<i>P</i> = .285	<i>P</i> = .113
HPV status‡					
Negative	63	13.3 (12.1–14.4)	92.6 (89.3–100.1)	260 (199–337)	4.4 (4.3–4.7)
Positive	54	14.5 (13.6–15.6)	90.9 (88.1–93.9)	235 (201–291)	4.6 (4.3–4.7)
Undetermined	36	12.9 (12.2–14.6)	95.8 (91.9–99.6)	288 (238–367)	4.1 (3.9–4.2)
		<i>P</i> < .001	<i>P</i> = .003	<i>P</i> = .031	<i>P</i> < .001

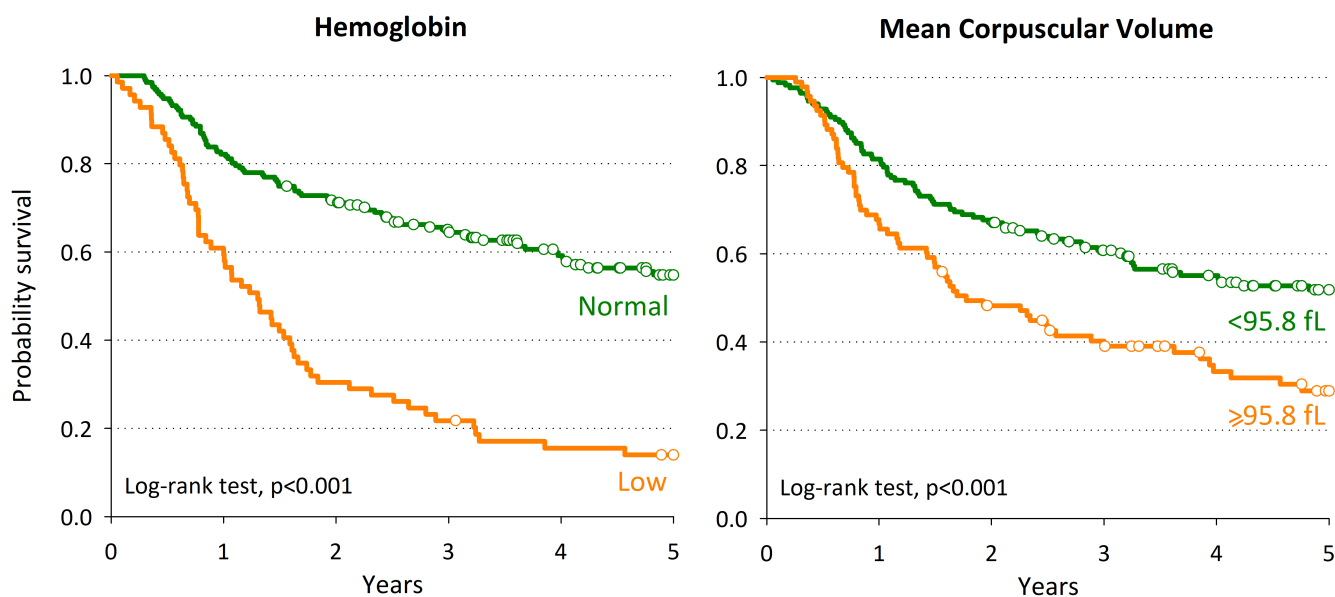
\*Differences across strata were evaluated through Kruskal-Wallis test;

†Test for trend using Jonckhere-Terpstra test;

‡On oropharyngeal squamous cell carcinomas only, based on p16 immunostaining.

c = clinical staging; Hb = hemoglobin; HPV = human papillomavirus; MCV = mean corpuscular volume; N = node; Q1-Q3 = interquartile ranges; T = tumor.

**A Progression-Free Survival**



**B Overall Survival**

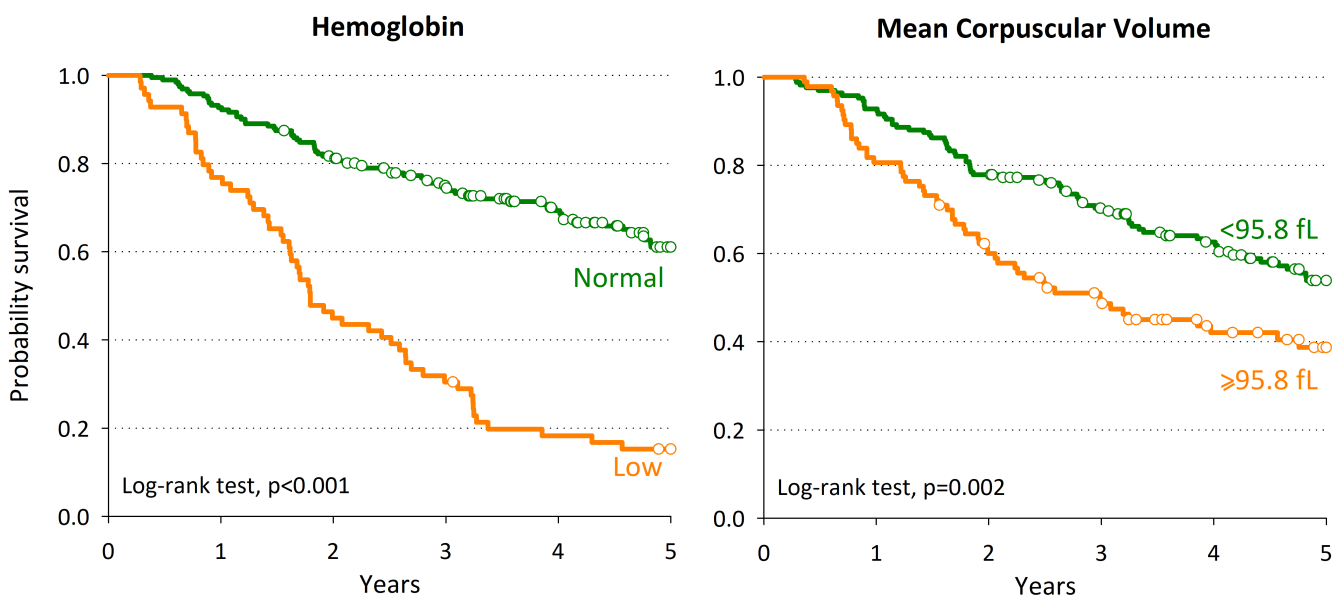


Fig. 1 Kaplan–Meier estimates of progression-free survival and overall survival according to Hb and MCV levels. Normal Hb was defined as  $\geq 12$  g/dL for women and  $\geq 13$  g/dL for men. Hb = hemoglobin; MCV = mean corpuscular volume.

inflammation in colorectal cancers.<sup>11</sup> The presence of a host systemic inflammatory response, and red cell markers of iron status are well documented to have a negative prognostic impact.<sup>12</sup>

Serum albumin, one of the most commonly used biomarkers to assess nutritional status, is usually unchanged in the early stages of cancer. However as the disease progresses, malnutrition and inflammation lead to suppression of albumin synthesis and increased albumin degradation, which may result in a significant

reduction in albumin level.<sup>13</sup> Several albumin-based markers, such as the Glasgow Prognostic Score, Advanced Lung Cancer Inflammation Index,<sup>14</sup> and Prognostic Nutritional Index,<sup>15</sup> have also been shown to correlate with survival in various cancers, with decreased albumin levels associated with shorter survival and increased cancer-related mortality.<sup>16</sup>

To date, the combined prognostic value of MCV, hemoglobin (Hb), PLT, and albumin in HNSCC patients has not been reported. The aim of this study

was to evaluate the prognostic value of preoperative MCV, Hb, PLT and albumin for patients who received organ preservation treatment with curable intent for HNSCC.

## MATERIAL AND METHODS

### Study Design

This is an international, multicenter retrospective cohort study of patients with locoregionally advanced HNSCC (stage III/IVA-B). Data for the present analysis were derived from two cohorts: The first cohort included 170 patients diagnosed between 1997 and 2014 at the University Hospital Cancer Centre of Treviso, Italy. The second cohort included 90 patients diagnosed and treated between 2008 and 2014 at the Queen Elizabeth Hospital Birmingham, a large tertiary head and neck oncology center in the United Kingdom. Consecutive patients were included if they had stage III/ IVA-B disease at presentation and had completed organ preservation treatment with concurrent platinum-based chemoradiotherapy with curative intent at the time of data collection. In both cohorts, diagnosis was confirmed histologically by tissue biopsy, and anatomical (computed tomography/magnetic resonance imaging) and functional (fluorodeoxyglucose/positron electron tomography) imaging were obtained as dictated by the clinical circumstances in order to stage the cancer in accordance with the American Joint Committee on Cancer (7th edition) staging classification. As a matter of course, all efforts are made to limit treatment interruptions in order to complete treatment within 7 weeks. Before treatment, all patients were discussed at the head and neck multidisciplinary team meeting of each institute. Patients with previous malignancy, distant

metastasis, additional synchronous primary tumors, or who were treated with up-front surgery were excluded, as were those with a history of hematological conditions and those for whom pretreatment blood test results were unavailable.

Data were retrospectively collected from case notes by the clinical team on the following clinical parameters: age at diagnosis, gender, smoking status, alcohol assumption, cancer site (oral cavity, oropharynx, hypopharynx and larynx), clinical tumor-node-metastasis (cTNM) stage, human papillomavirus (HPV) status (where relevant), treatment protocol, recurrent tumor, and survival.

In both centers, HPV-status was determined by p16 immunostaining. p16 was scored positive if 70% or more of malignant cells showed strong and diffuse nuclear and cytoplasmic staining.

Pretreatment serum biochemical and hematological indices (Hb, MCV, PLT and albumin), which were measured using standard protocols, were recorded.

### Statistical Analyses

The study was designed to compare survival curves through Cox-proportional hazard model. Considering the retrospective nature of the study, sample size was determined by available data ( $n = 260$ ), and a power analysis was conducted. Assuming that 50% of patients die during the study period, and fixing the a priori probabilities  $\alpha = 0.05$  and  $\beta = 0.20$  (power = 80%), the present sample size allowed the detection of a hazard ratio (HR)  $\geq 1.55$  when the study population is split into two groups of equal size, and a HR  $\geq 1.62$  when the study population is split into two groups containing one-third and two-thirds of the study population, respectively.

Hb, MCV, PLT, and albumin were reported as median values and interquartile ranges (Q1-Q3).

TABLE II.  
Hazard Ratios\* of PFS Event and Death, and Corresponding Confidence Intervals, According to Hemoglobin, MCV, Platelets and Albumin Levels in the Discovery Cohort, in the Validation Cohort, and in Both Cohorts Combined.

	Patients	Progression-Free Survival			Overall Survival		
		Events	HR (95% CI)	Bootstrap HR (95% CI)	Events	HR (95% CI)	Bootstrap HR (95% CI)
Hemoglobin (g/dL)†							
Normal	191	81	Reference	Reference	67	Reference	Reference
Low	69	59	2.86 (1.99–4.10)	3.08 (2.11–4.51)	58	3.29 (2.26–4.81)	3.62 (2.50–5.98)
MCV (fL)							
<95.8	167	77	Reference	Reference	71	Reference	Reference
≥95.8	93	93	1.57 (1.10–2.24)	1.67 (1.18–2.55)	54	1.54 (1.06–2.24)	1.70 (1.11–2.66)
Platelets ( $10^3/\text{mm}^3$ )							
≥263	125	64	Reference	Reference	59	Reference	Reference
<263	135	76	1.18 (0.84–1.67)	1.10 (0.79–1.62)	66	1.05 (0.72–1.51)	0.97 (0.64–1.42)
Albumin (g/dL)							
<4.4	76	42	Reference	Reference	37	Reference	Reference
≥4.4	114	52	0.91 (0.58–1.41)	0.86 (0.55–1.41)	47	1.06 (0.66–1.70)	0.94 (0.55–1.53)

\*Estimated by Cox proportional hazard model conditioned on cohort and adjusted for gender, age, cancer site, stage and HPV status for oropharyngeal cancer.

†Normal Hb was defined as  $\geq 12$  g/dL for women and  $\geq 13$  g/dL for men.

CI = confidence intervals; Hb = hemoglobin; HPV = human papillomavirus; HR = hazard ratios; MCV = mean corpuscular volume; PFS = progression-free survival.

Differences in biochemical values according to sociodemographic and clinical characteristics were evaluated using the Kruskal-Wallis test. Trends in median values across calendar periods were tested using the Jonckheere-Terpstra test. The impact of biochemical measurements on cancer outcomes was investigated through survival analysis. For each patient, time at risk was calculated from the date of cancer diagnosis to death, cancer recurrence/progression (for progression-free survival only), or last follow-up information, truncating at 5 years. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan–Meier method, and differences in survival probabilities between levels of biochemical measurements were evaluated with the log-rank test.

To account for possible confounding factors, multivariable HRs and corresponding 95% confidence intervals were calculated according to the Cox proportional hazards model; conditioning on cohort; and adjusting for gender, age, and covariates significantly associated with OS in a multivariate model (i.e., cancer site, stage, and HPV status for oropharyngeal cancer). Anemia was defined based on World Health Organization guidelines<sup>17</sup> as Hb < 12 g/dL for women and Hb < 13 g/dL for men. For MCV, platelets, and albumin, optimal cutoffs were defined as the value that maximizes the predictive value in overall survival, as measured by Harrell's c-index. These were 95.8 fL for MCV,  $263 \times 10^3/\text{mm}^3$  for platelets, and 4.4 g/dL for albumin.

To avoid sparse data due to small sample size, the analyses were conducted on both cohorts combined. Internal validation was conducted using the bootstrap method with a total re-sampling number of 1 thousand.

### Role of the Funding Source

There were no contributions to study design, data collection, analysis and interpretation of data, writing of the report, or the decision to submit the article for publication. There was no funding obtained for this study.

### Ethical Considerations

The research protocol was conducted in compliance with the Declaration of Helsinki (2008). This study was observational and did not affect patient care in any way.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## RESULTS

### Patient Characteristics and Pretreatment MCV

The sociodemographic and clinical characteristics of the 260 patients enrolled for the study are summarized in Table I, together with median values of biochemical measurements. Overall, median preoperative Hb was 13.8 g/dL, which was significantly lower in ever than in never smokers ( $P = .031$ ), in stage IV tumors than in stage III

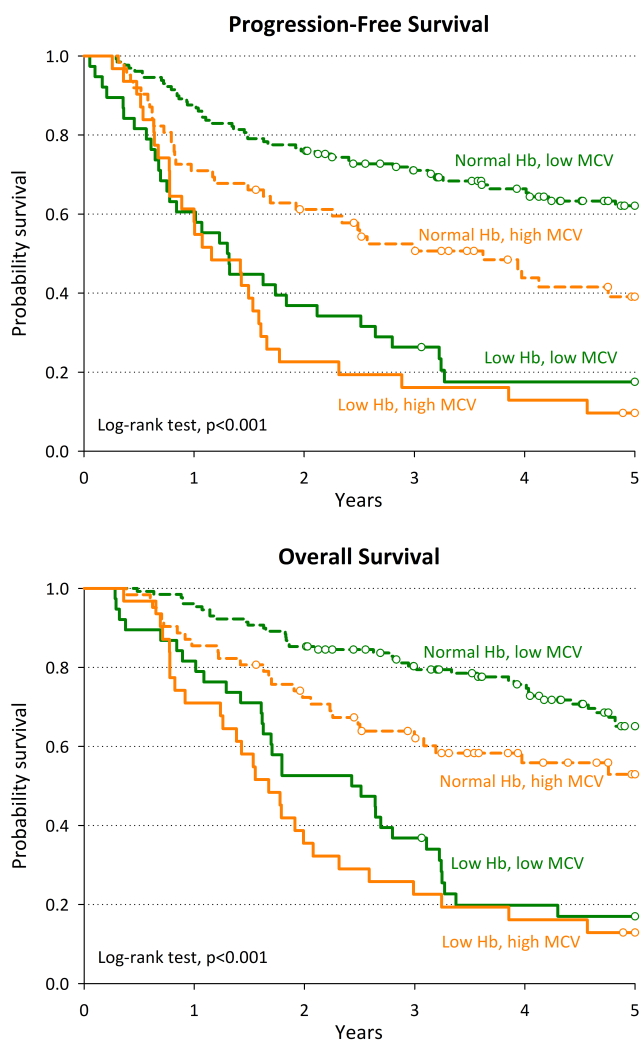


Fig. 2. Kaplan–Meier estimates of progression-free survival and overall survival according to combined levels of Hb and MCV. Normal Hb was defined as  $\geq 12$  g/dL for women and  $\geq 13$  g/dL for men. Follow-up was truncated at 5 years. Hb = hemoglobin; MCV = mean corpuscular volume.

( $P = .028$ ), and in HPV-positive than in HPV-negative oropharyngeal cancers ( $P < .001$ ). The median MCV was 93.1 fL, which was significantly lower in female than male patients ( $P = .002$ ), in never than ever smokers ( $P < .001$ ), in never than ever drinkers ( $P < .001$ ), in oropharyngeal cancer than other sites ( $P = .019$ ), and in HPV-positive than in HPV-negative oropharyngeal cancers ( $P = .003$ ). No association emerged for PLT or albumin with any of the reported sociodemographic and clinical characteristics.

Patients with normal pretreatment Hb levels had better PFS and OS than anemic patients (Fig. 1), with a 5-year OS of 61.0% and 15.2%, respectively ( $P < .001$ ). Similarly, patients with MCV < 95.8 fL had better oncological outcomes than patients with MCV  $\geq 95.8$  fL, with a 5-year OS of 53.9% and 38.9%, respectively ( $P = .002$ ). After adjustment for potential confounders, patients with low Hb had a 3.3-fold higher risk of death (95% CI: 2.26–

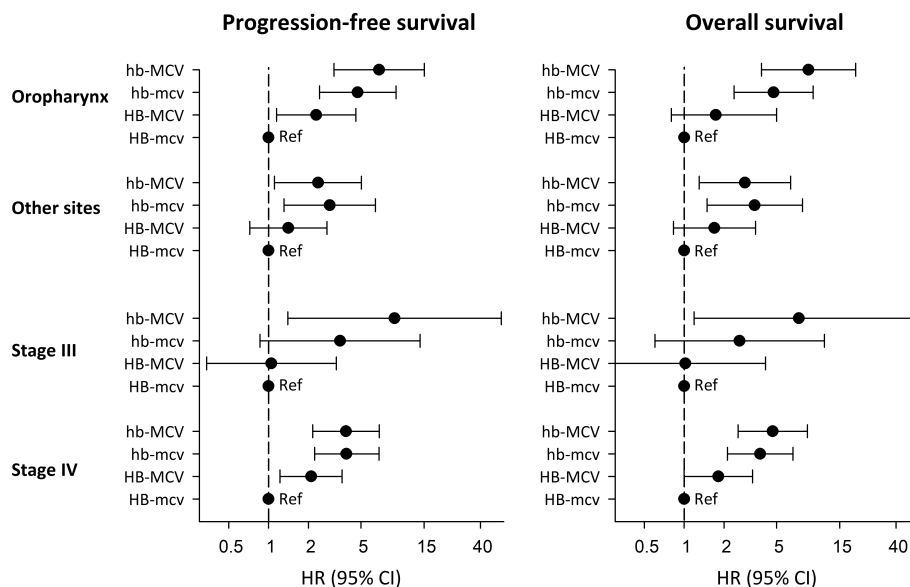


Fig. 3. Hazard ratios and corresponding 95% confidence intervals for progression-free and overall survival according to combined levels of Hb and MCV. HRs were adjusted for gender, age, cancer site, HPV status, and stage when appropriate. hb = Low Hb; HB = Normal Hb; mcv = MCV < 95.8 fL; MCV = MCV ≥ 95.8 fL. Low Hb was defined as <12 g/dL for women and <13 g/dL for men. CI = confidence intervals; Hb = hemoglobin; HPV = human papillomavirus; HR = hazard ratios; MCV = mean corpuscular volume.

4.81) than those with normal Hb (Table II). A similar association was found between low Hb and PFS (HR = 2.86; 95% CI: 1.99–4.10). Elevated MCV was also associated with worse PFS (HR = 1.57; 95% CI: 1.10–2.24) and OS (HR = 1.54; 95% CI: 1.06–2.24). No associations emerged for PLT or albumin. Further adjustment for calendar period of cancer diagnosis or drinking habits did not substantially change the results. All results were confirmed by bootstrap validation.

We further investigated the interaction between Hb and MCV levels. Interestingly, MCV seemed to play a role in survival outcomes only in patients normal Hb (Fig. 2). Indeed, in patients with normal Hb, high MCV reduced 5-year PFS to 39.1% compared to 62.1% in patients with low MCV (HR = 2.06; 95% CI: 1.27–3.34). Similarly, in patients with normal Hb, 5-year OS was 65.1% and 52.9% in those with low and high MCV level, respectively (HR = 1.78; 95% CI: 1.05–3.02). Conversely, in patients with anemia, no significant differences in survival outcomes emerged according to MCV levels (Fig. 2).

The interaction between Hb and MCV was then analyzed separately by cancer site and stage (Fig. 3). Although results should be interpreted with caution due to small sample sizes in some subgroups, this analysis suggests that elevated MCV did not have any impact on survival outcomes in patients with anemia. Conversely, among patients with normal Hb, elevated MCV level significantly increased the HRs of both PFS events and death in stage IV HNSCCs, but not in stage III cancers. Furthermore, the increase in HRs for PFS and OS due to elevated MCV was similar for all cancer sites, although the HR was significant only among oropharyngeal cancers.

## DISCUSSION

This represents the first study to investigate the combined role of pretreatment MCV with Hb as prognostic markers in HNSCC. Both anemia and macrocytosis were associated with worse OS and PFS, with anemia predicting a 3.3-fold higher risk of death than those with normal Hb levels, and increased MCV predicting a 1.54-fold higher risk of death than those with normal MCV. Interestingly, elevated MCV was a prognostic maker of poor PFS and OS in patients with normal Hb levels, who are expected to have good prognosis according their Hb level.

One possible explanation for these findings could be that folate deficiency and macrocytosis are induced by alcohol abuse or smoking, which themselves are notorious risk factors for HNSCC. As previously shown in esophageal squamous cell carcinoma, the risk of developing this cancer was increased in parallel with raised MCV; and tobacco, alcohol, dietary habits, and aldehyde dehydrogenase 2 were major determinants of MCV changes.<sup>5</sup> The association between higher MCV and poor outcome may therefore be indicative that patients with HNSCC who are exposed to ethanol have a poorer prognosis than other HNSCC.<sup>18</sup> Recently, alcohol consumption was found to be strongly associated with poor prognosis of HNSCC overall, with its impact being higher in patients submitted to radiation treatment compared to those receiving surgery.<sup>19</sup> However, this would not explain why the combination of macrocytosis and anemia does not have an impact on survival.<sup>11</sup> Moreover, in our study, the estimated HRs are independent from tobacco smoking. If further adjusted for alcohol consumption, the magnitude of the effect was similar. Therefore, we can conclude that the prognostic

effect of MCV is independent from tobacco and alcohol use.

This could support the theory of the association between anemia, systemic inflammation, and survival, already widely studied in colorectal cancers.<sup>20</sup> One mechanism linking systemic inflammation with anemia is the action of proinflammatory cytokines, including IL-6 stimulating the hepatic expression of hepcidin, which inhibits the absorption of iron in the duodenum. Furthermore, inflammation results in a limited availability of iron for erythroid cells, leading to macrophage activation and phagocytosis of erythrocytes.<sup>21</sup> Finally, several cytokines produced by the inflammatory process, including TNF- $\alpha$  and IFN $\gamma$  inhibit the synthesis of erythropoietin in the kidney, leading to diminished erythropoiesis and anemia.<sup>22</sup>

A recent study on colorectal cancer patients has found that anemia is associated with poor cancer-specific and overall survival regardless of MCV (and independent of systemic inflammation).<sup>11</sup> Our data on HNSCC support this finding, with pretreatment anemia predicting a poorer PFS and OS ( $P < .001$ ).

MCV is not only an indicator of anemia but also a marker of inflammation and endothelial function.<sup>23</sup> Interestingly, MCV may predict the efficacy of some chemotherapeutic agents such as 5-Fluorouracil (5-FU).<sup>3</sup> This is particularly interesting considering that our cohorts included only patients who underwent organ preservation protocol treatment. 5-FU inhibits tumor growth by blocking thymidylate synthase (TS), which is involved in DNA synthesis. The high tumor expression of TS is generally associated with a poor prognosis and with favorable responses to 5-FU.<sup>24</sup> Accordingly, a relatively high preoperative MCV might be associated with the corresponding folate deficiency due to increased DNA synthesis in cases in which the tumor expression of TS is high.

No association emerged for PLT or albumin with any of the measured sociodemographic and clinical characteristics or with survival. Nevertheless, recent studies have proposed the hypothesis that activated platelets contribute to tumorigenesis and metastasis through direct cell-cell interactions and the release of various lipid and protein mediators. Antiplatelet agents (aspirin and possibly other antiplatelet agents) may slow down and/or prevent the development and progression of cancer.<sup>25</sup>

Despite our negative findings for albumin, an association between hypoalbuminemia and poor prognosis in patients with cancer is well recognized.<sup>16</sup> This could be due to our choice of definition for normal albumin, with the aim of maximizing the predictive value of overall survival (4.4 g/dL). If we had chosen the widely used definition used by other authors ( $< 3.5$  g/dL), only three patients would have met this criterion thus limiting the consistency of the analysis. It is of interest that hypoalbuminemia does not just reflect the nutritional status but also the inflammatory response in cancer patients. This reduction in circulating albumin concentration reflects the increased demand for specific amino acids for mediator and acute-phase protein synthesis and immune and antioxidant defenses. Inflammation also promotes the degradation of available body protein, including

albumin, and body cell mass.<sup>13</sup> It is however important to acknowledge that the low study power to detect small associations should be noted when interpreting these results.

This is the first report in the contemporary literature investigating MCV with Hb as potential prognostic factors in curable HNSCC. The use of two cohorts from separate centers improves the applicability of the findings to the general population. The main limitation is determined by the fact that HPV-related HNSCC may have very different biology to non-HPV related tumors, and the MCV was lower in HPV-positive oropharyngeal carcinoma than HPV-negative ones. Furthermore, patients with HPV-related oropharyngeal carcinoma tend to be younger than other HNSCC patients, with fewer comorbidities and other risk factors. Nonetheless, the inclusion of HPV status as a covariate in the regression model did not substantially modify the risk estimates, supporting the fact that MCV is prognostic factor independent from HPV status. A further limitation is the lack of data on treatment interruption and thus treatment duration. There is some evidence that in patients receiving concurrent chemoradiotherapy for HNSCC, prolonged duration of treatment increases the risk of local failure.<sup>26</sup> This is therefore a potential source of bias in the current study; however, standard practice in both centers during the study period was to limit pauses in treatment as much as possible. Therefore, it is likely that the number of patients with prolonged treatment times, and thus the effect on the outcome, was negligible.

## CONCLUSION

Pretreatment anemia is confirmed to adversely affect OS and PFS in patients receiving concurrent chemoradiotherapy with curable intent for locoregional advanced HNSCC. In addition, our findings suggest that high MCV could be used as a predictive marker of poor prognosis in patients with normal Hb levels, which in turn could facilitate the tailoring of frequency and intensity of follow-up after treatment. Further studies are required to validate these results and to clarify the mechanism underlying the association between anemia, macrocytosis, and the prognosis of patients with HNSCC.

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