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REVIEW ARTICLE

Carbonic anhydrase-9 expression in head and neck cancer: a meta-analysis

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Abstract The purpose of the study was to compare CA-9 positivity versus CA-9 negativity in head and neck malignancies and to correlate levels of CA-9 with tumor grade, size, and nodal status. Overall and disease-free survival were also compared for CA-9 positive and negative tumors. A literature search was performed using Medline, Embase, Ovid and Cochrane databases for studies between 1990 and 2009. Carbonic anhydrase IX, CA IX, CA-9, head and neck, and survival were used as search terms. Random-effect meta-analytical techniques were conducted for outcome measures of overall survival and disease-free survival. Sixteen studies matched the selection criteria, reporting on 1,470 patients. Eight hundred and forty two specimens were reported as being CA-9 positive or negative: 512 (60.81%) were CA-9 positive and 330 (39.19%) were CA-9 negative. Nine hundred and eighty specimens had levels of CA-9 expression recorded: 547 (55.82%) had high levels of CA-9 and 433 (44.18%) had low CA-9 levels. Survival was significantly reduced if the tumor was positive for CA-9 ($P < 0.0001$). Disease-free survival is significantly reduced in patients with CA-9 positive ($P = 0.0008$) head and neck malignant tumors. The presence of CA-9 in head and neck malignant tumors is associated with reduced overall survival and disease-free survival.

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Introduction

Carbonic anhydrase-9 protein (CA-9), also known MN protein, is a glycoprotein belonging to a family of zinc-containing enzymes, involved in the catalytic hydration of carbon dioxide to carbonic acid at physiological pH ($CO_2 + H_2O \rightarrow HCO_3^- + H^+$) and, therefore, plays a role in pH regulation [1–5]. CA-9 is normally expressed in the alimentary tract and associated organs, but also can be expressed in various solid tumors, including renal cell carcinoma, non-small cell lung cancer, cervical squamous cell carcinoma, carcinoma of the tongue, colorectal cancer, breast cancer, ovarian cystadenocarcinoma, head and neck squamous cell cancer (HNSCC), and nasopharyngeal carcinoma [1, 2, 5–10]. Studies have identified that the expression of this biomarker is restricted to transformed, dysplastic, and malignant epithelial cells and thus is rarely expressed in benign tumors or normal tissue [6].

Tumor hypoxia induces the up-regulation of hypoxia-mediated gene expression. At low levels of oxygen, hypoxia inducing factor-1 (HIF-1) is activated and mediates the up-regulation and overexpression of a variety of genes, including those coding for vascular endothelial growth factor (VEGF) and CA-9 [11, 12].

We wanted to assess if there was any correlation between tumor characteristics [size (T), grade (G), and nodal status (N)] and level of CA-9 expression, and if CA-9 expression independently altered overall survival and the disease-free interval in otherwise matched tumors. Meta-analytical techniques and sensitivity analyses were used to assess the potential effect of CA-9 expression and explain

potential differences between the study methodologies and selection criteria.

Methods

Study selection

A literature search was performed using Medline, Embase, Ovid and Cochrane databases for studies between 1990 and 2009 that compared CA-9 positive (+) versus negative (−) tumors, and high tumor CA-9 levels versus low on overall and disease-free survival for any head and neck malignant tumor. The following MeSH search headings were used: carbonic anhydrase-9, head and neck malignant tumors, overall survival, disease-free survival, and comparative studies. The following text searches and search headings and their combinations were used: carbonic anhydrase IX, CA IX, CA-9, head and neck, and survival. The related articles function was used to broaden the search, and all abstracts, studies, and citations scanned were reviewed. No language restrictions were imposed. The references from articles also were used. The date of the most recent search was 10th April 2010.

Definitions

Mouse monoclonal antihuman CA9 antibody M75 was used for the detection of CA-9 by all investigators. Reactivity or absence of CA-9 immunohistochemical staining was considered as CA-9 (+) and CA-9 (−), respectively; the percentage of tumor cells positive for CA-9 in the whole tumor section was used to determine whether CA-9 levels were high or low. Each study determined their own cut-off value based on the mean staining percentage (for the cut-off value used by each author please refer to Table 1).

Data extraction

Two reviewers (SP and IA) independently performed the search as well as reviewing and extracting the following data according to the pre-specified protocol. Data were collected concerning first author, year of publication, study population characteristics, study design, inclusion and exclusion criteria, number of subjects, length of follow-up, and long-terms outcome. Areas of conflicts between the reviewers were subsequently discussed and there was 100% agreement on the final interpretation of the data.

Inclusion criteria

In order to be included in the analysis, studies had to compare CA-9 (+) versus (−), or high CA-9 levels versus low (different cut-offs were allowed) and report at least one of the outcomes of interest.

Exclusion criteria

Studies were excluded from the analysis if the outcomes of interest were not clearly reported, there were animal studies or it was impossible to extract or calculate the appropriate data from the published results. When the same institution reported two studies, either the one of better quality or the one most recent publication was included unless the study outcomes were mutually exclusive or measured at different intervals.

Outcomes of interest

We were interested in the following outcomes:

1. *CA-9 expression* CA-9 (+)/(−) and CA-9 high/low at different cut-offs for any head and neck malignant tumor.
2. *CA-9 expression* at tumor grade, T and N stages.
3. *Long-term outcomes* Impact of CA-9 expression on overall survival and disease-free survival of patients with any head and neck malignant tumor at maximal follow-up.

Statistical analysis

The meta-analysis was performed in line with recommendations from the Cochrane Collaboration and the Quality of Reporting of Meta-analyses (QUORUM) guidelines [13, 14]. Statistical analysis of dichotomous variables was carried out using odds ratio (OR) as the summary statistic, whereas continuous variables were analyzed using the weighted mean difference (WMD) [15], both were reported with 95% confidence intervals (CI). The odds ratio represented the odds of an adverse event occurring in the CA-9 (+) and CA-9 high compared with the CA-9 (−) and CA-9 low group, respectively; whereas WMD summarizes the differences between the two groups with respect to continuous variables, accounting for sample size. For studies that presented continuous data as means and range values, the standard deviations (SD) were calculated using statistical algorithms and checked using bootstrap resampling techniques. An OR < 1 favors the CA-9 (−) and CA-9 low group and the point estimate of the OR is considered to be statistically significant if the P value ≤ 0.05 and if the confidence interval does not include the value 1. In the tabulation of the results, squares (■) indicate the point estimates of the risk factor (OR) with 95% CI indicated by horizontal bars. The diamond (◆) represents the summary estimate from the pooled studies with 95% CI.

Table 1 Characteristics of included studies

Study	Year	Study type	Number of patients			Cut-off (%)	Type of cancer	Inclusion criteria	Exclusion criteria	Matching criteria	Follow-up, range (months)	Study quality (star rating) (Max 12)
			Carbonic anhydrase-9									
			Total	CA-9 (+)/(−)	CA-9 high/low							
Koukourakis [19]	2003	R	75	20/55	–	–	HNSCC	1,3,6	1	3,4,5	6–108	*****
Hui [20]	2002	RCT	90	55/35	–	5%	NPC	1,3,4,6	1	3,4,5,6,8	6.2–74.5	–
Koukourakis [21]	2006	R	198	115/83	115/83	10%	HNSCC	1,2	–	4,5,6	–	*****
Winter [22]	2005	R	151	–	94/57	n.s.	HNSCC	2,5	1,2	1,2,4,5,6	–	******
Beasley [23]	2001	R	79	69/8	71/8	25%	HNSCC	1,2,3,5,7	1,2	1,2,4,5,6	–	****
Hoogsteen [24]	2005	R	68	–	13/39	75%	HNSCC	2,4,8,9,10,11	3,4,5	1,2,3,4,5,6	–	*******
Kim [25]	2007	R	60	–	38/22	10%	Tongue cancer	2,5	1	1,2,3,4,5,6,7	4.1–117.1	**********
Jonathan [26]	2006	R	58	–	29/29	6%	HNSCC	2,4,9,10,11	3,4,5,6	1,2,3,4,5,6	–	********
Le [27]	2005	R	101	51/41	–	–	HNSCC	1,6,7	–	1,2,4,5,6,7	–	******
Eriksen [28]	2007	R	198	108/42	72/78	10%	HNSCC	2	–	3,4	–	****
Choi [29]	2007	R	117	68/49	–	5%	Oral squamous cancer	1	–	1,2,3,4,5,6,7	2–120	******
Nordsmark [30]	2007	P	57	–	31/26	10%	Head and neck cancer	2,3,8	1	1,2,3,4,5,6	4–120	–
Koukourakis [31]	2008	R	39	–	23/16	10%	Head and neck cancer	2,11	–	3,4,5,6	12–28	******
Schrijvers [32]	2008	R	91		39/52	12,5%	Laryngeal cancer	2,6	–	1,2,3,4,5,6	1–119	**********
Roh [33]	2008	R	43	26/17	19/24	10%	Tongue cancer	1,2,5	–	1,2,3,4,5,6,7	37–123	**********
Koukourakis [34]	2004	R	45	–	22/23	10%	Head and neck cancer	2	–	4,5	–	****

R retrospective, *RCT* randomized control trial, *P* prospective, *n.s.* not specified, *HNSCC* head and neck squamous cell carcinoma, *NPC* nasopharyngeal carcinoma

Inclusion criteria: 1, CA-9 (+)/(−); 2, CA-9 high/low; 3, no previous treatment; 4, no distant metastasis (M0); 5, surgical excision of tumor as primary surgery; 6, postoperative radiation therapy; 7, postoperative chemotherapy; 8, age over 18; 9, no severe lung or heart disease; 10, no severe liver or kidney disease; 11, WHO performance status 0–2

Exclusion criteria: 1, previous treatment for any head and neck cancer; 2, radiotherapy before surgery; 3, distant metastasis; 4, severe heart or lung disease; 5, severe stridor; 6, concurrent malignancy

Matching criteria: 1, age; 2, sex; 3, follow-up; 4, M75 immunohistochemistry for CA-9 detection; 5, tumor site; 6, tumor stage; 7, American Joint Committee on Cancer staging (AJCC); 8, Ho staging

The Mantel–Haenszel method was used to combine the OR for the outcomes of interest using a "random effect" meta-analytical technique [15, 16]. Yate correction was used for those studies that contained a zero in one cell for the number of events of interest in 1 of the 2 groups [17]. If there were no events for both groups, the study was discarded from the meta-analysis of that outcome.

Sensitivity analysis was performed to quantitatively assess heterogeneity (HG). Sensitivity analysis was undertaken using the following groups: (1) papers reporting more than 100 patients, (2) studies of higher quality with six or more stars (as assessed by the Newcastle–Ottawa scale) and (3) those published in or after 2006. Analysis was conducted using the statistical software Review Manager Version 5.0.24 for Macintosh (Review Manager (RevMan), Version 5.0. Copenhagen: The Nordic Centre, The Cochrane Collaboration, 2008).

Quality of the study

The quality of the studies was assessed using the Newcastle–Ottawa Scale with some modifications to match the needs of this meta-analysis [18]. This was done by assessing patient's selection criteria, group comparability, and the outcome in the individual studies. A star rating of 0–12 was allocated to each retrospective study based on these parameters. Two reviewers (SP and IA) assessed the quality of the studies. Where discrepancies arose, papers were re-examined and consensus was reached by discussion. Studies were considered high quality if they received six or more stars.

Results

Studies selected

We identified 35 studies as potentially satisfying the inclusion criteria, which required comparison of outcomes between CA-9 (+) versus CA-9 (−) or CA-9 high level versus CA-9 low level for any head and neck malignant tumor. Of these, nine studies were excluded after the abstracts were reviewed and further ten studies were excluded after the full text was reviewed. A total of 19 studies were excluded because they did not meet the inclusion criteria. Consequently, 16 studies published between 2001 and 2009 were included in the analysis (Fig. 1) [19–34].

Eight hundred and forty two specimens were reported as being CA-9 positive or negative: 512 (60.81%) were CA-9 positive and 330 (39.19%) were CA-9 negative [19–21, 23, 27–29, 33]. 1,023 specimens had levels of CA-9 expression

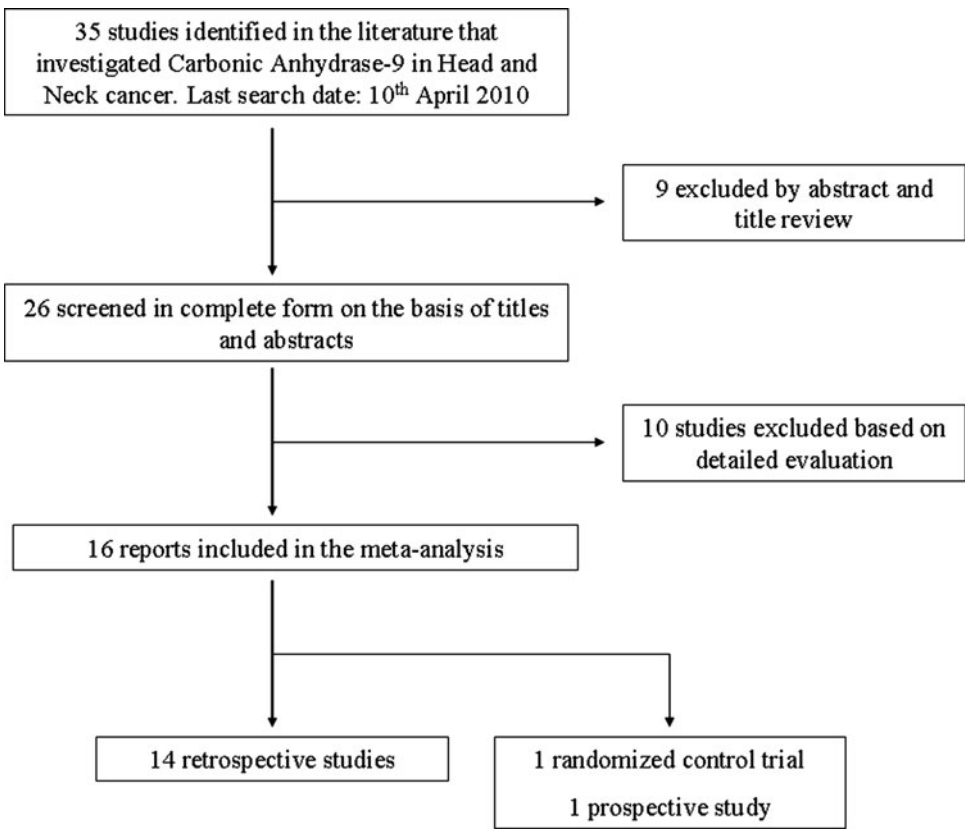

Fig. 1 Study selection flow chart

recorded: 566 (55.33%) had high levels of CA-9 and 457 (44.67%) had low CA-9 levels. Different thresholds for high and low expression were used by different authors, ranging from 5 to 75% [20–26, 28, 30–34]. The population demographics, inclusion and exclusion criteria of the eligible studies are summarized in Table 1. Where stated, follow-up ranged from 1 to 123 months. Nine studies [22, 24–27, 29, 31–33] scored six or more stars on the modified Newcastle–Ottawa scale, nine were published from 2006 onward [21, 25, 26, 28–33], and five studies reported more than 100 patients [21, 22, 27–29]. All papers used mouse monoclonal antihuman CA-9 antibody M75. CA-9 (+) is considered cases with positive reactivity to immunohistochemistry staining; whereas CA-9 high/low was identified by the intensity of staining and by the use of cut-offs. The outcomes of interest reported by each study are summarized in Table 2.

Meta-analysis of CA-9 expression (Fig. 2a, b)

CA-9 (+)/(−) expression was reported in eight studies [19–21, 23, 27–29, 33]. It was found that CA-9 (+) is expressed in a significant proportion of resected head and neck malignant tumor specimens ($P = 0.04$; OR 0.40; 95% CI 0.16–0.96). CA-9 levels did not vary significantly when using variable author specified cut-offs for high versus low levels of expression [21–26, 28, 30–34], ($P = 0.23$; OR 0.66; 95% CI 0.34–1.30). When using a fixed threshold (<30% [21, 23, 25, 26, 28, 30–34], and ≤10% [21, 25, 26, 28, 30–34] to symbolize a low level of expression), there was still no significant difference in the frequency of high versus low levels of CA-9 expression in the tumor specimens. ($P = 0.07$; OR 0.52; 95% CI 0.26–1.04 and $P = 0.14$; OR 0.76; 95% CI 0.53–1.10, respectively).

Meta-analysis of CA-9 on tumor size (T), nodal status (N), and tumor grade (G)

When comparing level of CA-9 expression and tumor size, low levels of CA-9 are more likely to be found in smaller tumors (T1/2, $P = 0.01$; OR 1.91; 95% CI 1.41–3.22) [25, 26, 28], whereas high levels of CA-9 are more likely to be found in larger tumors (T3/4, $P = 0.01$; OR 0.52; 95% CI 0.31–0.88) [25, 26, 28]. There was no significant difference between high and low levels of CA-9 either in tumors that did not have nodal spread ($N = 0$, $P = 0.82$; OR 0.92; 95% CI 0.44-1.93) [25, 26], or in tumors with associated lymph node involvement ($N > 1$, $P = 0.28$; OR 1.31; 95% CI 0.80–2.14) [25, 26, 28]. Equal results were achieved for CA-9 high/low expression on low (G1/G2) and high (G3/G4) tumor grades. There was no significant difference in levels of CA-9 expression according to the tumor grade [low tumor grade (G1/G2, $P = 0.07$; OR 1.99; 95% CI 0.94–4.20) and high tumor grade (G3/G4, $P = 0.49$; OR 2.54; 95% CI 0.18–35.25)].

Meta-analysis of long-term outcomes

Four studies [20, 21, 27, 29] reported on overall survival comparing CA-9 (+) versus (−) tumors, which was found

Table 2 Outcomes of interest of included studies comparing CA-9 (+)/(−) and CA-9 high/low

Outcome of interest	No of studies	No of patients	OR/WMD	95% CI	*P* value	HG *P* value
CA-9 expression						
CA-9 (+)/(−) expression	8	842	0.40	0.16–0.96	**0.04**	**<0.00001**
CA-9 high/low expression (all cut-offs)	11	978	0.66	0.34–1.30	0.23	**<0.00001**
CA-9 high/low expression (cut-offs < 30%)	9	777	0.52	0.26–1.04	0.07	**<0.00001**
CA-9 high/low expression (cut-offs ≤ 10%)	8	650	0.76	0.53–1.10	0.14	**0.02**
CA-9 expression T, N and G stages						
CA-9 high/low—low T stage expression	3	268	1.91	1.41–3.22	**0.01**	0.37
CA-9 high/low—high T stage expression	3	268	0.52	0.31–0.88	**0.01**	0.37
CA-9 high/low $N = 0$	2	118	0.92	0.44–1.93	0.82	0.37
CA-9 high/low $N > 1$	3	268	1.31	0.80–2.14	0.28	0.54
CA-9 high/low—low G grade expression	2	118	1.99	0.94–4.20	0.07	0.44
CA-9 high/low—high G grade expression	2	118	2.54	0.18–35.25	0.49	**0.001**
CA-9 long-term outcomes						
CA-9 (+)/(−) overall survival	4	497	1.93	1.41–2.64	**<0.0001**	0.67
CA-9 (+)/(−) disease-free survival	3	380	1.77	1.27–2.48	**0.0008**	0.48
CA-9 high/low disease-free survival	5	270	1.84	1.17–2.90	**0.009**	0.96

Statistically significant values are marked in bold

OR odds ratio, *WMD* weighted mean difference, *CI* confidence interval, *HG* heterogeneity

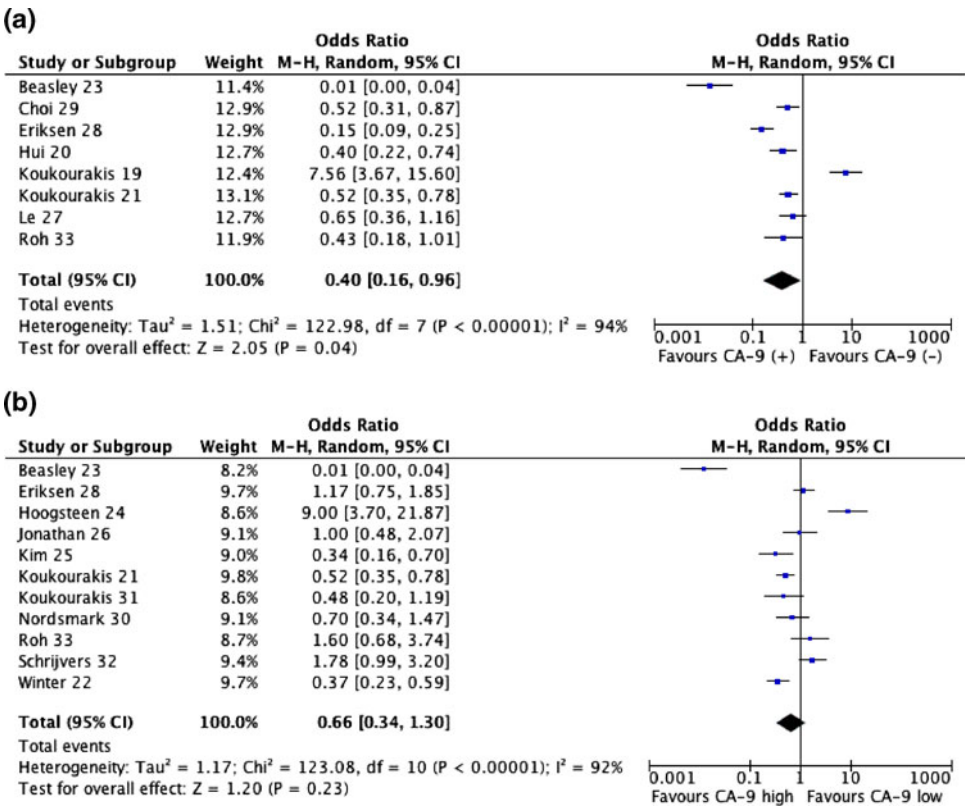

Fig. 2 Forest plots of comparison. CA-9 expression: **a** CA-9 (+)/(−), **b** CA-9 high/low (using variable cut-offs)

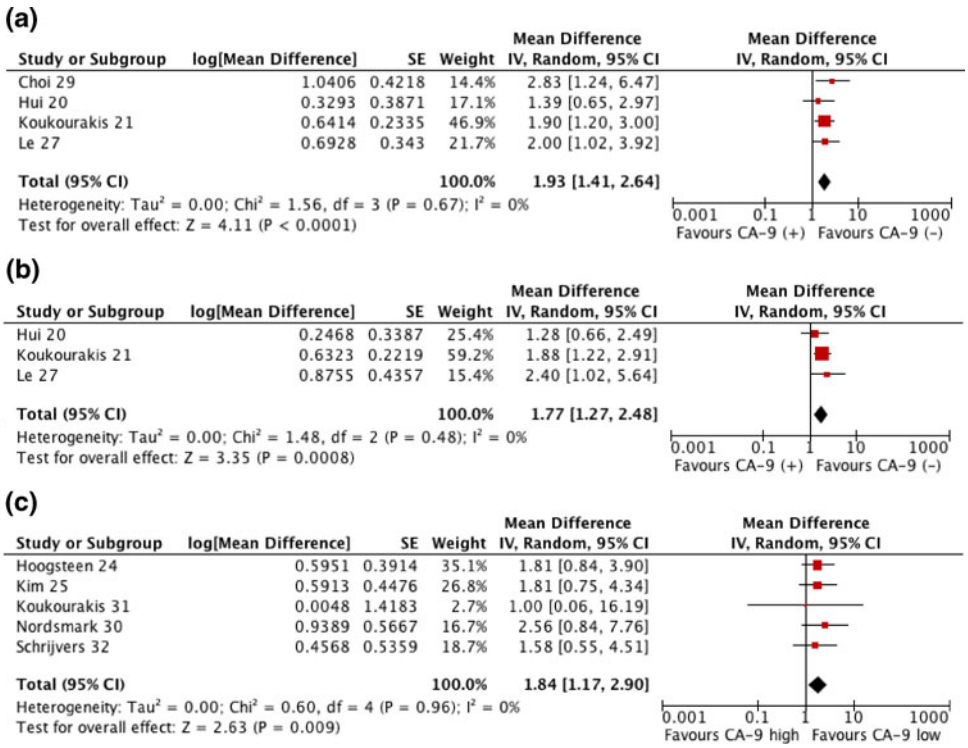

Fig. 3 Forest plots of comparison. Long-term outcomes: **a** overall survival, **b** disease-free survival CA-9 (+)/(−), and **c** disease-free survival CA-9 high/low

to be significantly reduced when CA-9 is positive ($P < 0.0001$; OR 1.93; 95% CI 1.41–2.64). Disease-free survival is significantly reduced in patients with tumors expressing CA-9 ($P = 0.0008$; OR 1.77; 95% CI 1.27–2.48) [19–21] and if the level of CA-9 is high [24, 25, 30–32] ($P = 0.009$; OR 1.84; 95% CI 1.17–2.90).

Forest plots on long-term outcomes are shown in Fig. 3a–c.

Sensitivity analysis (Table 3)

Meta-analysis on papers reporting >100 patients

Five studies comparing CA-9 (+)/(−) reported more than 100 patients [21, 22, 27–29]. CA-9 was significantly expressed on resected head and neck malignant tumors ($P = 0.001$; OR 0.41; 95% CI 0.24−0.70). Overall survival [21, 27, 29] and disease-free survival [21, 27] are significantly reduced when CA-9 is positive ($P < 0.0001$; OR 2.06; 95% CI 1.46−2.91) and ($P = 0.0006$; OR 1.98; 95% CI 1.34–2.92).

Meta-analysis of high-quality studies

Nine retrospective studies [22, 24–27, 29, 31–33] scoring six or more stars on the modified Newcastle–Ottawa scale were analyzed. CA-9 was significantly expressed in a significant number of resected head and neck malignant tumors ($P = 0.0008$; OR 0.55; 95% CI 0.38–0.78) [27, 29, 33]. Levels of CA-9 expression (high/low) did not reach a significantly differ when using variable [22, 24–26, 31–33] and <30% [25, 26, 31–33] cut-offs, however, when a threshold of ≤10% [25, 26, 31] is chosen, CA-9 is significantly expressed ($P = 0.03$; OR 0.59; 95% CI 0.36–0.95). Overall survival does not improve in patients with head and neck malignant tumors containing low levels of CA-9 compared to those containing higher levels ($P = 0.98$; OR

Table 3 Sensitive analysis comparing CA-9 (+)/(−) and CA-9 high/low

Outcome of interest	No of studies	No of patients	OR/WMD	95% CI	*P* value	HG *P* value
Studies reporting on more than 100 patients						
CA-9 (+)/(−) expression	5	765	0.41	0.24–0.70	**0.001**	**0.0006**
CA-9 (+)/(−) overall survival	3	407	2.06	1.46–2.91	**<0.0001**	0.71
CA-9 (+)/(−) disease-free survival	2	290	1.98	1.34–2.92	**0.0006**	0.62
High-quality studies						
CA-9 (+)/(−) expression	3	252	0.55	0.38–0.78	**0.0008**	0.72
CA-9 high/low expression (all cut-offs)	7	494	0.92	0.46–2.34	0.92	**<0.00001**
CA-9 high/low expression (cut-offs < 30%)	5	291	0.87	0.45–1.69	0.69	**0.004**
CA-9 high/low expression (cut-offs ≤ 10%)	3	157	0.55	0.28–1.08	0.08	0.11
CA-9 (+)/(−) overall survival	2	218	2.30	1.36–3.87	**0.002**	0.52
CA-9 high/low overall survival	2	99	0.01	–0.92–0.95	0.98	0.78
CA-9 high/low disease-free survival	4	258	1.72	1.05–2.83	**0.03**	0.98
CA-9 high/low—low T stage expression	2	118	1.99	0.60–6.57	0.26	0.16
CA-9 high/low—high T stage expression	2	118	0.50	0.15–1.66	0.26	0.16
CA-9 high/low $N = 0$	2	118	0.92	0.44–1.93	0.82	0.37
CA-9 high/low $N > 1$	2	118	1.09	0.82–2.30	0.82	0.37
CA-9 high/low—low G grade expression	2	118	1.99	0.94–4.20	0.07	0.44
CA-9 high/low–high G grade expression	2	118	2.54	0.18–35.25	0.49	**0.001**
Studies published in or after 2006						
CA-9 (+)/(−) expression	4	508	0.36	0.19–0.68	**0.002**	**0.0008**
CA-9 high/low expression (cut-offs < 30%)	7	653	0.76	0.50–1.18	0.22	**0.002**
CA-9 high/low expression (cut-offs ≤ 10%)	6	562	0.66	0.45–0.99	**0.04**	**0.03**
CA-9 (+)/(−) overall survival	2	315	2.09	1.40–3.11	**0.0003**	0.41
CA-9 high/low overall survival	2	99	1.01	0.40–2.59	0.98	0.78
CA-9 high/low disease-free survival	4	247	1.86	1.06–3.26	**0.03**	0.90
CA-9 high/low—low T stage expression	3	268	1.91	1.41–3.22	**0.01**	0.37
CA-9 high/low—high T stage expression	3	268	0.52	0.31–0.88	**0.01**	0.37
CA-9 high/low $N = 0$	2	118	0.92	0.44–1.93	0.82	0.37
CA-9 high/low $N > 1$	3	268	1.31	0.80–2.14	0.28	0.54
CA-9 high/low–low G grade expression	3	118	1.99	0.94–4.20	0.07	0.44
CA-9 high/low—high G grade expression	3	118	2.54	0.18–35.25	0.49	**0.001**

Statistically significant values are marked in bold

OR odds ratio, *WMD* weighted mean difference, *CI* confidence interval, *HG* heterogeneity

0.01; 95% CI −0.92 to 0.95) [25, 31]. Disease-free survival is significantly reduced for patients with high levels of intra-tumoral CA-9 ($P = 0.03$; OR 1.72; 95% CI 1.05–2.83) [24, 25, 31, 32]. There was no significant difference in the levels of CA-9 expression when subdividing according to the tumor size (low/high), nodal status (no involvement/involvement), and tumor grade (low/high) ($P = 0.26$, $P = 0.26$, $P = 0.82$, $P = 0.28$, $P = 0.07$, $P = 0.49$) [25, 26].

Meta-analysis of studies in or after 2006

Higher levels of CA-9 are expressed in head and neck tumors when using a cut-off equal or less than 10% ($P = 0.04$; OR 0.66; 95% CI 0.45–0.99) [21, 25, 26, 28, 30, 31]; otherwise similar results to the original analysis were obtained.

Discussion

CA-9 catalyzes the reversible hydration of carbon dioxide to carbonic acid. This leads to intracellular alkalosis and extracellular acidosis in the tumor microenvironment, which allows tumors to survive under hypoxic conditions. Furthermore, extracellular acidosis facilitates the breakdown of the extracellular matrix promoting local invasion and metastasis [25]. Thus, the expression of CA-9 in tumors may indicate the presence of hypoxic cells with more aggressive behavior and treatment resistance due to the hypoxia-induced cellular changes [25, 35].

This meta-analysis of 16 studies demonstrated that CA-9 is present in the majority of head and neck cancer specimens: 60.83% are CA-9 positive and 56.15% contain high levels of CA-9. This result was statistically significant in studies that measured absolute presence of CA-9, but not for those studies that looked at percentage of tumor cells staining for CA-9 unless a cut-off value of 10% was used. Thus, statistical analysis of CA-9 expression was better achieved either using reactivity or absence of staining (±), or by intensity staining with a low threshold of claiming high uptake.

It may be a law of "all or nothing" when considering CA-9 expression in head and neck malignancy, as has been shown that presence or absence of CA-9 expression was correlated significantly with overall survival and disease-free survival. On the other hand, subdividing further into high or low levels of CA-9 expression did not further affect survival. The only exception was a cut-off of ≤10% on sensitivity analysis. This discrepancy may be correlated with the fact that the cut-offs should be less than 5%, confirming the "all or nothing hypothesis". Unfortunately, there were no studies using those cut-offs, and hence such sub-analysis could not be performed.

It is known that tumors with CA-9 expression have a significant poorer complete response rate to chemoradiotherapy. This suggests that the levels of hypoxia necessary for the induction of CA-9 are clinically relevant to resistance to radiotherapy [19]. Assessment of endogenous hypoxia markers, apart from being a simpler method associated with clinically relevant hypoxia, also reflects metabolic and biochemical conditions, which can be involved in radiation resistance [21].

An initial outcome of interest and purpose of this project were to perform a meta-analysis on a co-expression of CA-9 and microvessel density (MVD), and to correlate these results with recurrence and disease-specific survival. MVD has been associated with an increased incidence of hematogeneous metastasis and poor prognosis [8]. The expression of CA-9 is mainly confined in tumors with very low MVD. The poor blood supply in these tumors probably accounts for hypoxia-mediated induction of CA-9 [19]. A limitation of this study was that only two of the included papers [19, 23] reported on these markers. There was a non-significant incidence of co-expression of CA-9 and low MVD ($P = 0.06$). A larger number of studies would be required to demonstrate if there was a significant association. However, immunohistochemical staining of tumor sections for several hypoxic markers might be a useful approach to identify prognostically distinct subgroups of patients with advanced head and neck cancer [36].

We have demonstrated that low levels of CA-9 is more likely to be expressed at lower T (T1/T2) stages ($P = 0.01$) and high levels of CA-9 at higher T (T3/T4) stages ($P = 0.01$). Therefore, we might suggest that CA-9 assists in tumor growth, such that tumors with a higher level of CA-9 can attain a larger size that those with less CA-9 expression. Analysis of nodal stages and tumor grades with respect to high or low levels of CA-9 did not reach statistical significance. Unfortunately, only one paper [28] compared CA-9 presence or absence at T and N stages, therefore, no meta-analysis could be performed.

Despite the strength of our study, there are some limitations we should discuss. First, 14 out of 16 studies included are retrospective [19, 21–29, 31–34]. In this case, there is a practical consideration that limits the number of cases that can be collected and analyzed. The use of meta-analytic techniques allowed the inclusion of 1,470 patients. A sample of this size is substantial because to accumulate such data in a randomized, controlled trial setting would take considerable time and cost.

It is important to note that in a meta-analysis data from a wide variety of sources are combined. As illustrated in Table 1, there is a degree of heterogeneity in the threshold different centers used for designating a sample's intensity as high or low in its positivity for CA-9. This means that in the watershed areas some samples with the same intensity

staining could have been analyzed in either low or high intensity group depending on which center they originated from. This could potentially have artificially reduced the extent of the correlation found between CA-9 positivity and reduced survival.

This study looked at the effect of CA-9 positivity on pooled data from all squamous cell carcinomas of the head and neck as well as nasopharyngeal cancer. It is possible that outcomes from certain cancer subtypes have a stronger association with CA-9 than others, although the fact that a highly significant result was found with the pooled data suggests that it is an important marker in head and neck cancers per se. However, more work focusing on specific tumor types needs to be done to confirm or refute this hypothesis.

Despite these limitations, our study provides significant information concerning the impact of CA-9 expression on survival of head and neck cancer. The results may be used in the future for designing prospective studies which take into account the tumor aggressiveness, as depicted by the expression of hypoxia induced marker CA-9.

All of the cancers studied were histologically defined as squamous cell carcinoma [19, 21–34], except one [20], which was nasopharyngeal carcinoma. In the West, nasopharyngeal carcinoma usually belongs to the WHO type I squamous cell carcinoma, which is associated with alcohol and smoking. In many parts of Asia, nasopharyngeal carcinoma histologically belongs to WHO types II and III, nonkeratinizing and undifferentiated carcinoma. Unlike WHO type I, types II and III have no association with alcohol and smoking but are strongly associated with the Ebstein-Barr virus [20, 37]. We could suggest that the possibility of further studies, evaluating the significance of CA-9 as a prognostic marker of survival in nasopharyngeal carcinoma.

Conflict of Interest None declared.

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