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No association of CpG island methylator phenotype and colorectal cancer survival: population-based study

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Background: Previous studies have shown adverse effects of CpG island methylator phenotype (CIMP) on colorectal cancer (CRC) prognosis. However, sample sizes were often limited and only few studies were able to adjust for relevant molecular features associated with CIMP. The aim of this study was to investigate the impact of CIMP on CRC survival in a large population-based study with comprehensive adjustment.

Methods: The CIMP status and other molecular tumour features were analysed in 1385 CRC patients diagnosed between 2003 and 2010. Detailed information were obtained from standardised personal interviews and medical records. During follow-up (median: 4.9 years), we assessed vital status, cause of death and therapy details. Cox proportional hazard regression models were used to estimate adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) of survival after CRC.

Results: The CIMP-H occurred more frequently in patients with older age, female gender, cancer in the proximal colon, BRAF mutation and microsatellite instability-high (MSI-H). However, CIMP status was not associated with CRC prognosis in CRC patients (HR = 1.00; 95% CI = 0.72–1.40 for overall survival; HR = 0.96; 95% CI = 0.65–1.41 for disease-specific survival) or in any of the subgroups. Although CIMP status was associated with the presence of MSI-H and BRAF mutation, the prognostic effects of MSI-H (HR = 0.49; 95% CI = 0.27–0.90) and BRAF mutation (HR = 1.78; 95% CI = 1.10–2.84) were independent of CIMP status. Similar benefit of chemotherapy was found for CRC outcomes in both the CIMP-low/negative group and the CIMP-high group.

Conclusions: CpG island methylator phenotype was not associated with CRC prognosis after adjusting for other important clinical factors and associated mutations.

Colorectal cancer (CRC), one of the most common malignant diseases worldwide, is a genetically and epigenetically heterogeneous disease (Jass, 2007; Leggett and Whitehall, 2010). One of epigenetic alterations of CRC is the hypermethylation of CpG islands in the promoter region of tumour-suppressor genes that could physically inhibit the binding of transcription factors and silence the expression of these genes. This subgroup of CRC was firstly introduced by Toyota *et al* (1999)

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and defined as CpG island methylator phenotype (CIMP) (Kim and Deng, 2007).

Since then, CIMP was found to be associated with not only altered molecular characteristics (such as microsatellite instability (MSI) and BRAF mutation) and distinct clinical features such as older age, female gender, proximal location and poor differentiations of CRC (Weisenberger *et al*, 2006; Dahlin *et al*, 2010), but also with the prognosis of CRC. Findings for the latter are conflicting. Even though a meta-analysis was conducted on the prognostic value of CIMP among CRC patients concluding that CIMP positivity (CIMP+) or CIMP-high (CIMP-H) was an indicator for poor prognosis (Juo *et al*, 2014), the sample sizes of the included studies were limited (<500 for the majority of studies) and several important factors, such as MSI and BRAF mutation that are closely associated with both CIMP and CRC survival, were only adjusted for in a few included studies. So far, studies on the association between subtypes defined by CIMP and CRC prognosis considering other molecular tumour features were limited and the results were also conflicting (Kim *et al*, 2009; Ogino *et al*, 2009; Sanchez *et al*, 2009). Therefore, the aim of this study was to investigate the impact of CIMP on CRC survival in a large population-based study with comprehensive adjustment for clinical and pathological factors.

MATERIALS AND METHODS

Study population and follow-up. The patient cohort was derived from a large ongoing population-based case–control study on CRC conducted in southwestern Germany, with long-term follow-up of cases (DACHS: Darmkrebs: Chancen der Verhütung durch Screening). More details on the study design and participation rates have been described before (Brenner *et al*, 2014; Hoffmeister *et al*, 2015). In brief, patients were recruited in 22 hospitals of the Rhine–Neckar–Odenwald region and were eligible to participate if they had histologically confirmed CRC (ICD-10 codes C18-C20), were at least 30 years old and were physically and mentally able to participate in a personal interview of ~1 h in German. In this analysis, only patients recruited between 2003 and 2010 with complete information on important clinical and molecular factors (age, sex, tumour location, cancer stage, CIMP, MSI and BRAF mutation status) were included. The study was approved by the ethics committees of the Medical Faculty of the University of Heidelberg and of the Medical Chambers of Baden-Wuerttemberg and Rhineland-Palatinate.

Information from patients was collected by trained interviewers during face-to-face interviews using a standardised questionnaire including questions on sociodemographic information, lifestyle factors and medical history. In addition, discharge letters, pathology reports and endoscopy reports were collected at baseline. At ~3 years after diagnosis, information on CRC therapy, recurrence and diagnosis of concomitant diseases was obtained from the physicians of the patients with a standardised questionnaire. At ~5 years after diagnosis, this information was updated and a questionnaire was requested from the survivors. In addition, data on vital status and death dates were collected from the population registration offices and causes of death were verified by death certificates from the health authorities. More information on data collection and follow-up has been reported previously (Jansen *et al*, 2014; Hoffmeister *et al*, 2015).

Tumour sample analyses. Routine formalin-fixed, paraffin-embedded (FFPE) tumour samples from the patients enrolled were requested and used for tumour tissue analyses. The DNA was isolated from tumour samples under microscopic control of unstained slides and was prepared using the DNeasy tissue kit (Qiagen, Hilden, Germany) (Warth *et al*, 2011). After bisulphite conversion using the EZ DNA methylation kit (Zymo Research, Orange, CA, USA), genomic DNA was analysed by methylation-specific PCR for the following CIMP loci: MLH1, MINT1, MINT2, MINT31 and MGMT. The PCR conditions and primers were used as previously described (Esteller *et al*, 1999; Chan *et al*, 2002; Park *et al*, 2003). The CIMP status was defined according to the number of hypermethylated loci: hypermethylation at 3 or more loci was classified as CIMP-high, methylation at 1 or 2 loci was classified as CIMP-low (CIMP-L) and if none of the loci was methylated, CIMP status was negative (CIMP-N). A mononucleotide marker panel (BAT25, BAT26 and CAT25) was used to screen for MSI-high (MSI-H) (Findeisen *et al*, 2005). In most tumour samples, KRAS mutation was determined by single-stranded conformational polymorphism technique (SSCP) using the same DNA sample, and expression of BRAF V600E was determined by immunohistochemical analyses in sections of tissue microarray blocks and evaluated by two pathologists independently (Blaker *et al*, 2004). In the remaining tumour samples, KRAS mutation ($N=178$) and BRAF mutation ($N=242$) were determined by Sanger sequencing: exon 2 of KRAS and exon 15 of BRAF were amplified by PCR using FideliTaq polymerase (Affymetrix, Santa Clara, CA, USA) and the following primers: KRAS fw: 5′-GTGTGACATGTTCTAATATAGTCA-3′ and KRAS rv: 5′-GAATGGTCCTGCACCAGTAA-3′; BRAF fw: 5′-TGCTTGCTCTGATAGGAAAATG-3′ and BRAF rv: 5′-AGCATCTCAGGGCCAAAAAT-3′. After purification, Sanger sequencing was performed using the BigDye Terminator v1.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA, USA) according to standard protocols. Samples were sequenced on an ABI 3500 Genetic Analyzer (Life Technologies).

Statistical Analyses. The distribution of patient characteristics was compared between patients with CIMP-H and patients with CIMP-L/N by χ^2 test and *t*-test. Adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) of overall survival (OS) and disease-specific survival (DSS) were analysed by Cox regression. Potential covariates including age at diagnosis, sex, education level, family history of CRC, physical activity, body mass index, alcohol consumption, smoking status, ever use of nonsteroidal anti-inflammatory drugs (NSAIDs), ever use of statins, ever use of hormone replacement therapy, cancer stage, tumour location, tumour histologic grade, tumour resection, chemotherapy, MSI status and KRAS and BRAF mutation status were considered for inclusion in the final model. Covariates differentially distributed according to CIMP status with a *P*-value of <0.05 were chosen for the final model. According to this, clinical factors including age, sex, education level, alcohol consumption, tumour location, cancer stage, number of invaded lymph nodes, use of hormone replacement therapy, use of chemotherapy and molecular features including MSI status and BRAF mutation status were included in the final model. The proportional hazards assumption was tested via inclusion of time-dependent variables in the adjusted model that were kept in the final model if required for the proportional hazards assumption to hold. In addition, a correction for late entry that was defined as the potentially delayed time period between date of diagnosis and date of enrolment was also included in the final model. All analyses were performed with SAS, software version 9.4 (SAS Institute Inc., Cary, NC, USA). Tests of statistical significance were defined as two sided at an α-level of 0.05.

RESULTS

Study population. Of the 3146 patients diagnosed with CRC between 2003 and 2010 in the DACHS study, methylation data of all five CIMP markers were available for 1751 patients. Among them, tumour tissue samples of 1385 patients had complete information on BRAF mutation and MSI-H at the time of this analysis. Patients finally included in this analysis were on average

Table 1. Characteristics of the study population by CIMP status

Characteristics	Total ($n=1385$)	CIMP-low/negative ($n=1196$)	CIMP-high ($n=189$)	*P*-value[a]
Age, *n* (%)				
⩽65 Years	501 (36)	450 (38)	51 (27)	
66–74 Years	456 (33)	401 (34)	55 (29)	
75+ Years	428 (31)	345 (29)	83 (44)	0.0001
Sex, *n* (%)				
Female	599 (43)	495 (41)	104 (55)	
Male	786 (57)	701 (59)	85 (45)	0.0004
Education, *n* (%)[b]				
Low	890 (64)	753 (63)	137 (73)	
Medium	273 (20)	241 (20)	32 (17)	
High	221 (16)	202 (17)	19 (10)	0.0186
Family history of CRC, *n* (%)[c]	212 (15)	183 (15)	29 (15)	0.9940
Lifetime regular active smoking, *n* (%)[d]				
None	587 (42)	503 (42)	84 (44)	
<20 Pack-years	481 (35)	420 (35)	61 (32)	
20+ Pack-years	314 (23)	270 (23)	44 (23)	0.7288
Alcohol consumption, mean (g per day)[e]	18.3	18.8	14.9	0.0180
Body mass index, mean ($kg\,m^{-2}$)[f]	26.5	26.5	26.8	0.2868
Physical activity, mean (lifetime METs, h per week)[g]	235.5	237.8	220.9	0.1107
Regular use of NSAIDs, *n* (%)[h]	368 (27)	321 (27)	47 (25)	0.6310
Regular use of statins, *n* (%)[i]	174 (13)	152 (13)	22 (12)	0.6928
Regular use of hormone replacement therapy, *n* (%)[j]	181 (13)	144 (12)	37 (20)	0.0045
Tumour location, *n* (%)				
Proximal colon	492 (36)	370 (31)	122 (65)	
Distal colon	375 (27)	342 (29)	33 (17)	
Rectum	517 (37)	483 (40)	34 (18)	<0.0001
Cancer stage, *n* (%)				
I	255 (18)	220 (18)	35 (19)	
II	466 (34)	391 (33)	75 (40)	
III	467 (34)	411 (34)	56 (30)	
IV	197 (14)	174 (15)	23 (12)	0.2502
No. of invaded lymph node, *n* (%)				
⩽12	386 (28)	345 (29)	41 (22)	
12–20	653 (47)	572 (48)	81 (43)	
20+	346 (25)	279 (23)	67 (35)	0.0012
Surgery, *n* (%)	1378 (99)	1189 (99)	189 (100)	0.2917
Chemotherapy, *n* (%)[k]	626 (45)	555 (47)	71 (38)	0.0200
KRAS mutation, *n* (%)[l]				
Negative	859 (68)	731 (68)	128 (71)	
Positive	402 (32)	350 (32)	52 (29)	0.3524
BRAF mutation, *n* (%)				
Negative	1284 (93)	1149 (96)	135 (71)	
Positive	101 (7)	47 (4)	54 (29)	<0.0001
Microsatellite instability, *n* (%)				
MSS	1247 (90)	1137 (95)	110 (58)	
MSI-H	138 (10)	59 (5)	79 (42)	<0.0001

Abbreviations: CIMP = CpG island methylator phenotype; CRC = colorectal cancer; MET = metabolic equivalent task; MSI-H = microsatellite instability-high; MSS = microsatellite stable; NSAID = nonsteroidal anti-inflammatory drug.

[a]Derived from Pearson's χ^2 test of independence between covariables and CIMP status.

[b]Missing data for 1 patient.

[c]Missing data for 5 patients.

[d]Missing data for 3 patients

[e]Missing data for 9 patients.

[f]Missing data for 8 patients.

[g]Missing data for 31 patients..

[h]Missing data for 15 patients.

[i]Missing data for 3 patients.

[j]Missing data for 3 patients.

[k]Missing data for 6 patients.

[l]Missing data for 124 patients.

69 years old and 43% were female. Almost all participants had surgery after diagnosis (99%) and 45% of the participants had chemotherapy before or after surgery. Median follow-up time was 4.9 years (interquartile range = 3.6–5.1 years).

Of the 1385 patients, 189 (13.6%) showed simultaneous hypermethylation at three or more of the five CIMP loci (Table 1). CIMP-H occurred more frequently in patients with older age, female gender, cancer in the proximal colon, BRAF mutation and MSI-H, consistent with the results reported previously (Issa, 2004; Samowitz *et al*, 2005a). Besides, CIMP-H was found associated with lower education level, lower alcohol consumption and more frequent lymph node invasion in this study (Table 1).

CIMP and CRC prognosis. After adjusting for major confounders, CIMP status was associated with neither OS (HR = 1.00; 95% CI = 0.72–1.40) nor DSS (HR = 0.96; 95% CI = 0.65–1.41) in CRC patients (Table 2). No differences were also observed for CIMP-H patients compared with CIMP-L or with CIMP-N patients, or for CIMP-L patients compared with CIMP-N patients (data not shown). In addition, analyses for hypermethylation in each of the CIMP loci did not show any association with CRC survival either, except for MINT 31 (Supplementary Table S1). No meaningful association between CIMP status and CRC prognosis was found in analyses further stratified by age, sex, tumour location and cancer stage (Table 2).

Combinations of other molecular features and CIMP status were assessed using the same multivariable Cox regression model (Table 3). Regardless of CIMP status, MSI-H was associated with significantly longer DSS (HR = 0.49; 95% CI = 0.27–0.90). Additional consideration of CIMP status yielded very similar HRs. Contrarily, BRAF mutation was similarly associated with poorer DSS (HR = 1.78; 95% CI = 1.10–2.84) with and without consideration of CIMP status. The KRAS mutation and subtypes of CIMP combined with KRAS were not associated with CRC prognosis.

CIMP, chemotherapy and CRC prognosis. As chemotherapy is generally not administrated to patients with stage I CRC, analyses on the prognostic value of chemotherapy among CRC patients with different CIMP status were only conducted among stage II to IV CRC patients (Table 4). For CRC patients with CIMP-L/N, chemotherapy was strongly associated with better OS (HR = 0.59; 95% CI = 0.43–0.79) and DSS (HR = 0.57; 95% CI = 0.40–0.80). Although not statistically significant, a similar benefit was observed for CRC patients with CIMP-H (HR = 0.66; 95% CI = 0.25–1.78 for OS and HR = 0.54; 95% CI = 0.15–1.88 for DSS). In analyses further stratified by stage, similar positive effects of chemotherapy were observed in both CIMP-L/N patients and CIMP-H patients.

DISCUSSION

In this population-based study, CIMP status was associated with all expected patient and tumour characteristics among CRC patients such as older age, female gender, proximal colon location, MSI-H

Table 2. Association of CIMP status and survival among CRC patients

		Overall survival[a]			Disease-specific survival[a]		
Factor	***N***	**Deaths (%)**	**HR**	**95% CI**	**Deaths (%)**	**HR**	**95% CI**
CIMP-L/N	1170	330 (28)	1.00	Reference	254 (22)	1.00	Reference
CIMP-H	187	52 (28)	1.00	0.72–1.40	35 (19)	0.96	0.65–1.41
Female							
CIMP-L/N	482	142 (29)	1.00	Reference	109 (23)	1.00	Reference
CIMP-H	104	29 (28)	1.22	0.77–1.95	24 (23)	1.24	0.74–2.08
Male							
CIMP-L/N	688	188 (27)	1.00	Reference	145 (21)	1.00	Reference
CIMP-H	83	23 (28)	0.88	0.54–1.44	11 (13)	0.65	0.34–1.27
Age ⩽68 years							
CIMP-L/N	584	132 (23)	1.00	Reference	115 (20)	1.00	Reference
CIMP-H	68	15 (22)	0.93	0.53–1.66	13 (19)	0.96	0.52–1.76
Age 69+ years							
CIMP-L/N	586	198 (34)	1.00	Reference	139 (24)	1.00	Reference
CIMP-H	119	37 (31)	1.15	0.75–1.75	22 (18)	1.09	0.65–1.86
Proximal location							
CIMP-L/N	360	105 (29)	1.00	Reference	78 (20)	1.00	Reference
CIMP-H	121	34 (28)	1.33	0.83–2.15	20 (17)	1.03	0.57–1.86
Distal location[b]							
CIMP-L/N	810	225 (28)	1.00	Reference	176 (22)	1.00	Reference
CIMP-H	66	18 (27)	0.77	0.47–1.27	15 (23)	0.81	0.47–1.41
Stages I and II							
CIMP-L/N	600	94 (16)	1.00	Reference	49 (8)	1.00	Reference
CIMP-H	108	16 (15)	0.71	0.37–1.36	6 (6)	0.41	0.14–1.20
Stage III							
CIMP-L/N	405	107 (26)	1.00	Reference	83 (20)	1.00	Reference
CIMP-H	56	16 (29)	1.30	0.70–2.40	10 (18)	1.09	0.51–2.30
Stage IV							
CIMP-L/N	165	129 (78)	1.00	Reference	122 (74)	1.00	Reference
CIMP-H	23	20 (87)	1.15	0.69–1.94	19 (83)	1.18	0.69–2.00

Abbreviations: CI = confidence interval; CIMP = CpG island methylator phenotype; CIMP-H = CIMP-high; CIMP-L/N = CIMP-low/negative; CRC = colorectal cancer; HR = hazard ratio.
[a]Adjusted for age, sex, education level, alcohol, tumour location, cancer stage, number of invaded lymph nodes, use of hormone replacement therapy, chemotherapy, microsatellite instability and BRAF mutation; additional adjustment for time-dependent effects of age and chemotherapy.
[b]From the splenic flexure, including the rectum.

Table 3. Associations of CIMP in combination with microsatellite instability, BRAF mutation and KRAS mutation with survival among CRC patients

		Overall survival[a]			Disease-specific survival[a]		
Factor	***N***	**Deaths (%)**	**HR**	**95% CI**	**Deaths (%)**	**HR**	**95% CI**
CIMP-L/N and MSS	1113	320 (29)	1.00	Reference	250 (22)	1.00	Reference
CIMP-H and MSS	108	35 (32)	1.02	0.71–1.46	26 (24)	0.92	0.61–1.40
CIMP-L/N and MSI-H	57	10 (18)	0.80	0.42–1.53	4 (7)	0.42	0.15–1.14
CIMP-H and MSI-H	79	17 (22)	0.75	0.43–1.32	9 (11)	0.54	0.26–1.12
Any CIMP and MSI-H	136	27 (20)	0.77	0.49–1.21	13 (10)	0.49	0.27–0.90
CIMP-L/N and BRAF −	1125	313 (28)	1.00	Reference	239 (21)	1.00	Reference
CIMP-H and BRAF −	133	40 (30)	1.08	0.77–1.53	25 (19)	0.96	0.62–1.46
CIMP-L/N and BRAF +	45	17 (38)	1.50	0.90–2.49	15 (33)	1.80	1.04–3.11
CIMP-H and BRAF +	54	12 (22)	0.99	0.51–1.94	10 (19)	1.73	0.82–3.66
Any CIMP and BRAF +	99	29 (29)	1.28	0.82–1.98	25 (25)	1.78	1.10–2.84
CIMP-L/N and KRAS −	712	191 (27)	1.00	Reference	147 (21)	1.00	Reference
CIMP-H and KRAS −	128	41 (32)	1.08	0.73–1.59	28 (22)	0.99	0.62–1.56
CIMP-L/N and KRAS +	346	106 (31)	1.12	0.88–1.43	82 (24)	1.08	0.82–1.43
CIMP-H and KRAS +	50	9 (18)	0.81	0.41–1.43	5 (10)	0.61	0.24–1.53
Any CIMP and KRAS +	396	115 (29)	1.09	0.86–1.38	87 (22)	1.04	0.79–1.36

Abbreviations: BRAF + = BRAF mutation; BRAF − = BRAF wild type; CI = confidence interval; CIMP = CpG island methylator phenotype; CIMP-H = CIMP-high; CIMP-L/N = CIMP-low/negative; CRC = colorectal cancer; HR = hazard ratio; KRAS + = KRAS mutation; KRAS − = KRAS wild type; MSI-H = microsatellite instability-high; MSS = microsatellite stability.
[a]Adjusted for age, sex, education level, alcohol consumption, tumour location, cancer stage, number of invaded lymph nodes, usage of hormone replacement therapy, chemotherapy, microsatellite instability and BRAF mutation; additional adjustment for time-dependent effects of age and chemotherapy.

and BRAF mutated tumour. However we found no association of CIMP status with OS or DSS. Even when stratified by major clinical factors, no significantly meaningful association or suggestions of an effect was found between CIMP and CRC survival. However, combinations of CIMP with MSI-H or BRAF mutation were associated with CRC survival, but these associations were observed regardless of CIMP status.

The MSI-H is often a sequence of defects in the DNA mismatch repair (MMR) system, consisting of the genes *MLH1*, *MSH2*, *MSH6* and *PMS2*, resulting in the accumulation of nucleotide mutations and an alteration in microsatellite length (Strand *et al*, 1993). Independent of CIMP, MSI-H was found significantly associated with improved CRC survival, and this finding was in agreement with the results of previous studies (Guastadisegni *et al*, 2010; Nash *et al*, 2010). BRAF, a proto-oncogene involved in the RAS/RAF/MAPK pathway, is found mutated in nearly 10% of CRC patients in our study, as in most previous studies, it was found to be an independent indicator for poorer prognosis of CRC (Samowitz *et al*, 2005b; Ogino *et al*, 2009; Hughes *et al*, 2012; Phipps *et al*, 2012; Barras, 2015). Previous studies demonstrated that BRAF mutation is strongly associated with MSI-H that may be mediated by the relationship between BRAF mutation and CIMP (Nosho *et al*, 2008; Tran *et al*, 2011). Although KRAS mutation is an important factor for the onset and progression of CRC, the effect of KRAS mutation on CRC survival was inconsistent in previous studies (Phipps *et al*, 2013; Kim *et al*, 2016). In our study KRAS mutation was neither associated with CIMP status nor with CRC survival.

In accordance with previous studies, age, sex, location and number of invaded lymph node were strongly associated with CIMP status (Issa, 2004; Samowitz *et al*, 2005a). However, some of the clinical characteristics such as the number of invaded lymph nodes were no longer associated with CIMP status when cases of MSI-H were excluded, and this may indicate that the association between these characteristics and CIMP status arise as a consequence of CIMP-related hypermethylation of MLH1. We observed lower mean intake of alcohol among CIMP-H patients compared with CIMP-L/N patients. To our knowledge, this is the first study reporting an association between alcohol and CIMP status. We have no explanation for such an association that could just be a chance finding.

The CIMP status did not show any relationship with CRC prognosis. This finding is not in line with the conclusion of a previous systematic review and meta-analysis (Juo *et al*, 2014). However, only three studies included in the meta-analysis took both BRAF mutation and MSI into account as confounders in their multivariable analyses (Ogino *et al*, 2009; Samowitz *et al*, 2009; Donada *et al*, 2013). Only in the study by Ogino *et al* (2009) that used three levels of CIMP (CIMP-H, CIMP-L and CIMP-N), CIMP-H was found to be associated with better DSS compared with CIMP-N CRC. The two other studies did not observe significant associations between CIMP (CIMP-H compared with CIMP-L/N) and CRC survival. In addition, Donada *et al* (2013) only analysed stage II colon cancer patients ($N = 120$), and Samowitz *et al* (2009) investigated the association between CIMP and rectal cancer only among 990 patients. With a much bigger sample of unselected CRC patients ($N = 1385$) and consideration of many potential confounders, our study did not find an association with CRC survival either. Although CIMP was still not associated with CRC survival even without adjustment for BRAF mutation and MSI-H in our study (data not shown), BRAF mutation and MSI-H should be considered as confounders in future CRC survival analyses.

Regarding chemotherapy, no obvious difference was found for the survival benefit associated with chemotherapy in the different CIMP groups, although the protective effect of chemotherapy was significant in patients with CIMP-L/N CRC only. A very similar effect was estimated for patients with CIMP-H CRC, although results were not statistically significant given the much lower number of patients. Results of previous studies on the role of chemotherapy in CIMP status subgroups were inconsistent. Li *et al* (2014) did not find an association between chemotherapy and OS for both CIMP-H and CIMP-L/N patients. Jover *et al* (2011) found that among stage II and III CRC patients chemotherapy was associated with significantly better DSS among CIMP-L/N patients (HR = 0.4; 95% CI = 0.2–0.6) but not among CIMP-H CRC patients (HR = 0.8; 95% CI = 0.3–2.0). However, the sample size with only 89 CIMP-H patients was rather small for the latter analysis. In addition, none of the associated molecular tumour features were adjusted for in either one of these studies. We found no evidence that chemotherapy could be differentially associated with CRC prognosis according to CIMP status, but this finding should be confirmed in other large studies.

Table 4. Association of chemotherapy with survival among CRC patients with different CIMP status

		Overall survival[a]			Disease-specific survival[a]		
Factor	***N***	**Deaths (%)**	**HR**	**95% CI**	**Deaths (%)**	**HR**	**95% CI**
Stages II–IV							
CIMP-H							
Chemotherapy −	82	20 (24)	1.00	Reference	12 (15)	1.00	Reference
Chemotherapy +	71	28 (39)	0.66	0.25–1.78	23 (32)	0.54	0.15–1.88
CIMP-L/N							
Chemotherapy −	415	120 (29)	1.00	Reference	85 (20)	1.00	Reference
Chemotherapy +	537	185 (34)	0.58	0.43–0.79	161 (30)	0.57	0.40–0.80
Stage II							
CIMP-H							
Chemotherapy −	67	12 (18)	1.00	Reference	6 (9)	1.00	Reference
Chemotherapy +	7	0 (0)	NA	NA	0 (0)	NA	NA
CIMP-L/N							
Chemotherapy −	305	58 (19)	1.00	Reference	34 (11)	1.00	Reference
Chemotherapy +	77	11 (14)	1.10	0.55–2.20	7 (9)	1.17	0.48–2.85
Stage III							
CIMP-H							
Chemotherapy −	13	6 (46)	1.00	Reference	4 (31)	1.00	Reference
Chemotherapy +	43	10 (23)	0.64	0.11–3.62	6 (14)	0.82	0.07–9.46
CIMP-L/N							
Chemotherapy −	85	38 (45)	1.00	Reference	29 (34)	1.00	Reference
Chemotherapy +	320	69 (22)	0.67	0.42–1.07	54 (17)	0.63	0.37–1.07
Stage IV							
CIMP-H							
Chemotherapy −	2	2 (100)	1.00	Reference	2 (100)	1.00	Reference
Chemotherapy +	21	18 (86)	NA	NA	17 (81)	NA	NA
CIMP-L/N							
Chemotherapy −	25	24 (96)	1.00	Reference	22 (88)	1.00	Reference
Chemotherapy +	140	105 (75)	0.32	0.18–0.55	100 (71)	0.34	0.19–0.60

Abbreviations: CI = confidence interval; CIMP = CpG island methylator phenotype; CIMP-H = CIMP-high; CIMP-L/N = CIMP-low/negative; CRC = colorectal cancer; HR = hazard ratio; NA = not available.
[a]Adjusted for age, sex, education level, alcohol consumption, tumour location, cancer stage, number of invaded lymph nodes, chemotherapy, microsatellite instability and BRAF mutation; additional adjustment for time-dependent effects of age and chemotherapy.

The current cohort was derived from a large population-based study including 1385 unselected patients with information on molecular tumour characteristics that is, to our knowledge, the so far largest study on this topic. Furthermore, detailed information including sociodemographic data, lifestyle factors, clinical pathological and molecular factors were collected and relevant confounding factors were adjusted for in the multivariable analyses. Despite the large size of the total study population, the statistical power was still very limited in some of the subgroup analyses owing to the low prevalence of CIMP-H. Accordingly, even larger or pooled studies with detailed adjustment are needed.

In this study, the CIMP panel consisted of five markers and was different from the panels used in previous studies on CRC survival analyses. However, CIMP in general is only defined as a subgroup of CRC characterised by simultaneous hypermethylation of numerous CpG islands surrounding the promoter regions of several genes (Jover *et al*, 2011), and no specific markers or panels have been confirmed yet to be superior to the others. In fact, in previous studies there have been >50 genes used as CIMP markers and 16 CIMP panels that have been used to investigate the association between CIMP and CRC, and all of the five markers used in our CIMP definition were among the most commonly used markers (Ashktorab *et al*, 2013; Jia *et al*, 2016). In addition, with this CIMP definition, CIMP-H was found to be associated with all known patient and tumour characteristics of CIMP, such as older age, female gender, proximal colon location, BRAF mutation and MSI-H.

In conclusion, CIMP was not associated with CRC survival after adjusting for relevant clinical factors and prognostically relevant tumour characteristics that were associated with CIMP in this study. Associations found between chemotherapy and improved survival in CIMP-L/N CRC demand further research with larger sample sizes to also elucidate potential relationships in CIMP-H patients. Lack of adjustment may have contributed to findings of adverse effects of CIMP-H in CRC on CRC survival suggested by previous studies. Additional large cohort studies with comprehensive adjustment are required to investigate the prognostic value of CIMP using current or new definitions among CRC patients.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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