Meta-analysis on the prognostic value of CpG Island Methylator Phenotype in gastric cancer

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Abstract

Background: CpG Island Methylator Phenotype (CIMP) has been identified as a distinct molecular subtype of gastric cancer, yet associations with survival are conflicting. A meta-analysis was performed to estimate CIMP's prognostic significance.

Methods: A systematic review of Embase, Medline, PubMed, PubMed Central and Cochrane databases on studies related to the association between CpG Island Methylator Phenotype and survival in patients undergoing potentially curative resection for gastric cancer was done.

Results: A total of 967 patients from 10 studies were included, and the median rate of tumour CIMP-H (high) was 40.9% (range 5.3 - 62.7%). Pooled analysis suggested that specimens exhibiting CIMP-H were associated with poorer 5-year survival (OR 1.49, 95% CI 1.11 - 2.01, $p<0.05$). Significant heterogeneity was observed between studies ($I^2 = 88\%$, $p<0.001$). Sub-analysis related to poor (5 studies) or improved outcomes (5 studies), revealed that CIMP was associated with both poor (OR 8.15, 95% CI 4.65 - 14.28, $p<0.05$, study heterogeneity $I^2 = 52\%$, $p=0.08$) and improved survival (OR 0.42, 95% CI 0.27 - 0.65, $p<0.05$, study heterogeneity $I^2 = 0\%$, $p=0.960$).

Conclusion: There was significant heterogeneity in the gene panels used to identify CIMP, which may explain the survival differences.

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Introduction

Gastric cancer is the second commonest cause of cancer related death worldwide accounting for some 740,000 deaths annually[1]. Surgery remains the only treatment modality with curative potential but some 40% of patients develop recurrence and die of their disease. Response rates to chemotherapy are poor, and prescribing adjuvant chemotherapy to all patients has no evidence base and is not recommended. Hence, one of the prime challenges is to identify biomarkers that may improve prognostic modeling, independent of the current AJCC TNM staging system, and which may promote new therapeutic targets.

The molecular mechanism underlying gastric cancer carcinogenesis remains unclear, however, genomic and epigenetic changes are important causes of activation of oncogenes and silencing of tumour suppression genes. Epigenetic silencing through hypermethylation of CpG islands of the genes promoter region plays an important role in silencing tumour related genes[2]. There is conflicting evidence reporting CpG Island Methylator Phenotype (CIMP) with survival[3-4]. The relatively small sample sizes reporting CIMP positivity with survival makes interpreting the true prognostic influence of this biomarker difficult. A possible solution is to perform a meta-analysis of published data. Unfortunately, the meta-analysis performed by Zong and Seto contained only 2 studies reporting the prognostic value of CIMP[5]. Therefore, the aim of this study was to perform a systematic review and meta-analysis of the prognostic value of CIMP status in gastric cancer using overall survival as the time-to-event endpoint.

Methods

Search protocol.

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Original studies were searched for those that documented patients with surgically resected primary gastric adenocarcinoma, where the specimens were assessed for the presence of CpG Island Methylator phenotype (CIMP). The outcome measure chosen was 5-year overall survival. Embase, Medline, PubMed, PubMed Central and Cochrane databases were searched using the following Boolean search term: CpG Island Methylator Phenotype AND (cancer OR carcinoma OR adenocarcinoma OR tumor OR tumour) AND (Gastric OR stomach) for articles published up to March 2017. All search results were combined in a reference manager database (Endnote) and duplicates removed. A grey search of reference lists of included studies was also undertaken.

Study selection

All types of original scientific reports were considered. Reviews and book chapters were excluded, as were texts written in languages other than English, and reports including survival analysis or patients who did not undergo surgery with curative intent. Only studies related to the association between CpG Island Methylator Phenotype and survival in patients undergoing potentially curative resection for gastric cancer were therefore included.

Data extraction

Two independent reviewers applied the inclusion criteria to study abstracts and selected full papers for data analysis. Data from full text papers were extracted by a single reviewer, with 50% undergoing independent review. Discrepancies were

verified by consensus. If multiple publications reported results in the same population, the most comprehensive data were chosen. For each study, baseline data (author, institution, country, study period, total number of patients, gender, TNM stage, CIMP definition and methodology) were extracted. The number of patients exhibiting CIMP, and 5-year overall survival death rates were obtained where available. Outcomes were described as odds ratios with 95% confidence intervals. Where these were not reported, the methods described by Parmar and Rogers were used to extract data from Kaplan–Meier curves, or percentage survival[6-7]. Authors were contacted if data was not presented in a useable form.

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Definition of CpG Island Methylator Phenotype

No consensus on the most accurate method of assessing CIMP exists; with variation in the cut-off for gene promoter methylation and the number/type of genes studied. For this reason, the defined term was catalogued from each included paper and displayed in the results. For the analysis, CIMP was determined to be either present (positive) or absent (negative). Where CIMP was graded into groups (e.g., high (H) /low (L) /none (N)), the results for the low/none groups were combined (CIMP-negative) and compared with the CIMP-H (CIMP-positive) group.

Quality of studies.

This meta-analysis was conducted in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines. The quality of the studies was measured using the Newcastle–Ottawa Scale which assesses the methodological quality of non-randomised cohort studies for meta-analysis. The studies were judged by two independent assessors using a nine-point scale comprising

analysis on the selection of the study group, the comparability of cohorts and the ascertainment of outcome. Scores above 6 points were taken to denote studies of high methodological quality and were included in the meta-analysis.

Meta-analysis of CIMP status, clinicopathological factors and survival

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Methylation of the promoter region of a gene results in epigenetic silencing and a subsequent loss of expression of the target protein. There are two possible explanations for potentially conflicting survival results; first, the observed prognostic association between CIMP status and survival is influenced by the choice of gene panel; second, the clinicopathological make-up of the cohort identifies different molecular subtypes of CIMP tumours. To test the first hypothesis, studies and genes were grouped by survival and oncogene/tumour suppressor genes (TSG). To test the second hypothesis, comparisons were made between the clinicopathological factors and the CIMP status of the meta-cohorts, when studies were dichotomised based on the reported survival observed.

Statistical analysis

All analyses were conducted with the RevMan statistical package (Review Manager (RevMan) Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Heterogeneity between studies was tested using Cochran's G test. The I^2 statistic was calculated for an objective measure of heterogeneity. A fixed-effects meta-analysis was performed in all cases, and where there was appreciable heterogeneity ($I^2 > 50\%$ or chi-squared p-values<0.10), a random-effects model was used. Corresponding funnel plots of Ln standard error as a function of effect size were used to examine the effect of publication bias visually, and were statistically tested

using Eggers test. P-values >0.05 were indicative of no publication bias. For meta-analysis, Mantel–Haenszel Odds Ratios for CIMP status and 5-year death rate was extracted and described with 95% confidence intervals. Sensitivity analysis was performed to identify if any methodological features were indicative of heterogeneity among studies. Studies were excluded if they had poor methodological quality (Newcastle - Ottawa scores <7).

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Results

The electronic search of the literature yielded 110 potential studies. A grey search through cross-referencing did not yield any additional manuscripts. Of the 110 studies, 96 were excluded based on the contents of the abstract (figure 1). Forty-four studies concerned non-gastric cancers, 4 looked at single gene methylations, and 46 did not include survival information. Of the 14 studies undergoing full text evaluation, 4 did not include survival information and therefore 10 studies were retained for final analysis (table 1) [3-4, 8-15]. The median quality score for studies was 9 (range 8-9). All studies were retrospective cohort studies of one or regional institutions and therefore constitute level IV evidence. All studies reporting methylation of CpG Islands on promoter regions of genes were based on resected specimens.

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The 10 studies contained 967 patients with a median sample size of 81 (range 68 - 196). Only three studies contained more than 100 patients. Eight studies included patients with TNM stage I-IV disease with only Ayed-Guerfali and Liu et al including patients with stage I-III disease. The approximate median age of the studied patients was 60 years with most being male (range 59% - 82%). Nine studies gave no information on the use of chemotherapy with only An et al. reporting that neoadjuvant chemotherapy was not prescribed.

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CpG Island methylation was quantified on a median of 5.5 genes (5 - 28). The range of genes used are shown in table 2. The CIMP categorisation thresholds varied however, and the most common groupings were a trichotomy of CIMP-N (normal), CIMP-L(low) and CIMP-H(high). The prevalence of CIMP-H ranged from 5.3% to 62.7% with a median of 40.8%. Five studies reported an association between CIMP-H

and improved survival, four studies reported an association with poorer survival, and a single study reported no statistically significant association with survival (table 2).

Meta-analysis of CIMP status, clinicopathological factors and survival

For the purposes of pooled analysis, CIMP-H (CIMP positive) was compared with a combined grouping of CIMP-L and CIMP-N (CIMP negative). The pooled Odds Ratio for CIMP positive and death was 1.49 (95% Confidence Interval (CI) 1.11 - 2.01). Significant study heterogeneity was noted $\chi^2 = 75.66$, 9 d.f., $p < 0.001$, $I^2 = 88\%$ (figure 2).

Studies and genes were grouped related to survival and oncogene/tumour suppressor genes (TSG) respectively (supplementary table 1). Studies demonstrating an association between CIMP positivity and improved survival had gene panels consisting of TSGs and oncogenes. In the studies demonstrating an association between CIMP positivity and poor outcome, apart from Park et al, all of the studies included tumour suppressor genes predominantly in their gene panels. Comparisons were made between the clinicopathological factors and CIMP status of the meta-cohorts when studies were dichotomised based on the reported survival. The only extractable data related to clinicopathological factors were gender and TNM stage, which were classified as early (stage I and II), or advanced (stage III and IV). The frequency of male patients in studies reporting improved survival was 66.7%, compared with 69.6% in studies reporting poor survival (p=0.440). The proportion of patients with advanced disease in studies reporting improved survival was 53.5%, compared with 68.0% in the studies reporting poor survival (p<0.001). The ratio of CIMP negativity to positivity was 2.3 in the poor survival cohort, and 2.1 in the improved survival cohort. Despite this, CIMP positivity was associated with advanced

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TNM stage ($p<0.001$) in the poor survival cohort (supplementary table 2). The association between CIMP positivity and early stage in the improved survival cohort was not statistically significant ($p=0.061$, supplementary table 2).

Discussion

This study found marked variability in the genes employed in the selection panel for determining CIMP status, with clear heterogeneity related to survival. Five studies showed associations with improved, and 4 studies associations with poor survival. The 5-year survival for CIMP positivity ranged from 68% in studies reporting improved survival, to 14.3% in those reporting poor survival. The causes of these observations were unclear, but likely reflect the make up of the individual patient cohorts and gene selection panel, as the poor survival meta-cohort had a higher proportion of advanced disease (53.5% vs. 68.0%, $p<0.001$), and was predominantly composed of tumour suppressor genes. The lack of a consensus regarding CIMP status methodology in gastric cancer makes translating this potential biomarker into clinical practice challenging.

Heterogeneity in the methodology for determining CIMP status was a major finding, with the number, type, and identity of genes employed in the selection panel different in every study. Such findings have also been report in colorectal cancer by Jia et al, who reported that in 16 studies the number of markers ranged from 5 to 15, and different critical values were used[16]. The prevalence of CIMP ranged from 6.4% to 48.5% in colorectal cancer, compared with 5.3% to 94.1% in gastric cancer. It is possible that methylation occurs in a number of CpG islands, which has little influence on the phenotype of the cancer, but it is unknown to what extent these methylated genes are passengers, rather than drivers, and composing the CIMP panel with cancer drivers may provide a better picture of CIMP's pathogenesis and prognostic impact.

Meta-analysis of cohorts associating CIMP with poor outcomes revealed that CIMP was associated with a more advanced TNM stage, yet a meta-analysis of

cohorts associated with CIMP improved outcomes, revealed that CIMP positivity was associated with earlier TNM stage (supplementary table 2). The reason for this is unclear, but it emphasises the importance of using study cohorts that reflect the population being treated. Standardised biomarker reporting, including the cohort composition, adds to result reliability, which is particularly important given the variability in stage and survival observed between eastern and western populations. It is possible that cancers arising from these cohorts are phenotypically different but could only be evaluated once consensus regarding the optimum methodology has been agreed and validated.

The studies contained in this systematic review used 59 different genes across 10 studies. This was not a comprehensive analysis of cancer related genes, with less than 1% of the genome studied. It is now becoming clearer that cancer related genes may be described as 'drivers' or 'passengers' depending on their influence on carcinogenesis, growth and metastasis. It possible that tumours with large numbers of methylated 'passenger' genes are identified as CIMP despite these 'passenger' genes having little influence on the final phenotype and subsequent prognosis. Epigenetic silencing of the hMLH1 gene, which leads to loss of the mismatch repair protein expression and the microsatellite instability (MSI) phenotype, has been associated with improved survival in both colorectal and gastric cancer [2, 12]. Furthermore, in this systematic review, studies using hMLH1 in their gene panel were all associated with improved survival. In colorectal cancer the CIMP+/MSI+ phenotype is a recognised entity associated with improved survival. hMLH1 can be confidently identified as a cancer driver and therefore the CIMP+/hMLH1 subtype likely explains the observed improved survival in some of the CIMP studies, although it remains unclear why

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CIMP was associated with poor survival in a subset of studies which deserves further evaluation.

The Cancer Genome Atlas Network identified 4 molecularly distinct subtypes of gastric cancer, based on Epstein-Barr virus (EBV), MSI, chromosomal instability (CIN) and genomic stability (GS)[17]. In particular, EBV and MSI gastric cancer were reported to be associated with hypermethylation of promoter regions of up to 526 genes. Based on molecular associations, four cluster patterns of hypermethylation have been reported, with two attributed to EBV and MSI gastric cancer, but unfortunately, neither survival analysis, nor a defined classification for CIMP was given. Nevertheless, it is clear that even within a hypermethylation subgroup, there is heterogeneity, which is likely to exhibit different associations with survival. Therefore, the different gene panels employed in this systematic review may identify subtypes of CIMP, and consensus regarding methodology is desirable.

CIMP's value as a predictive biomarker to guide whether or not to prescribe neoadjuvant or adjuvant chemotherapy is uncertain. Shiovitz et al. reported that in patients with stage III colorectal cancer undergoing Fluorouracil/Leucovorin therapy, CIMP positivity was associated with poorer survival compared with CIMP negativity, consistent with chemotherapy resistance [18]. The proportion of gastric cancer patients responding to neoadjuvant chemotherapy is reported to be in the order of 21%, and although performing CIMP analysis on diagnostic biopsies is possible, [19] this strategy might not be pragmatic, because of the variable amount of cancer genomic material available within any given biopsy, and any such approach would require validation. Nevertheless, CIMP is a promising biomarker for the management of patients with gastric cancer and further work to quantify and validate this technique to determine its

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relationship with responses to contemporary chemotherapeutic algorithms may support its integration into clinical practice.

This study has a number of inherent limitations, in the main related to the spectrum of gene panel markers utilized for CIMP. Unfortunately, this is a common finding pervading CIMP studies, and other systematic reviews and meta-analyses in colorectal cancer[16] and gastric cancer[5] have accepted this relative limitation when performing pooled analyses. In contrast, the study has significant strength in that it is the first systematic review and meta-analysis of prognostic studies relating to CIMP in gastric cancer, and the studies included were methodologically sound with Newcastle-Ottawa scores of >7.

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Table 1. Baseline data on included studies

First Author	Study period	Number of patients	Age	Gender	AJCC stage	Surgery	Evidence level	Newcastle-Ottawa Score
An[8*]	1986 - 1998	83	Data not given	65% male	I-IV	Yes	IV	9
Ayed-Guerfali[9]	2000 - 2008	79	Mean 57 years	59% male	I-III	Yes	IV	9
Chang[10]	1996 - 1998	106	Median >60 years	76% male	I-IV	Yes	IV	9
Chen[11]	2003 - 2009	120	Mean 58 years	67% male	I-IV	Yes	IV	9
He[3]	2000 - 2006	94	Median <60 years	82% male	I-IV	Yes	IV	8
Ksiaa[12]	1998 - 2002	68	Mean 61 years	59% male	I-IV	Yes	IV	8
Kusano[13]	Data not given	78	Mean 65 years	67% male	I-IV	Yes	IV	8
Liu[14]	2008 - 2009	75	Mean 52 years	71% male	I-III	Yes	IV	8
Park[4]	2002 - 2003	196	Mean 59 years	68% male	I-IV	Yes	IV	9
Shigeyasu1[5]	1998 - 2004	68	Median <70 years	68% male	I-IV	Yes	IV	9

* The status of neoadjuvant +/- adjuvant chemotherapy was unknown apart from one study (An et al), where the patients did not receive neoadjuvant chemotherapy.

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Table 2. Baseline data on included studies

First author	CIMP markers	CIMP cut-off value	CIMP distribution	Association with survival
An[8*]	p16, hMLH1, MINT1, MINT2, MINT25 and MINT31	CIMP-H (> 50%) CIMP-L (< 50%) CIMP-N	26 (31.3%) 46 (55.4%) 11 (13.3%)	CIMP-H improved survival (p=0.040)
Ayed-Guerfali[9]	RARb2, DAPK, RASSFIA, CDH1, p16INK4a	CIMP-H ≥ 3 CIMP-N < 3	40 (50.6%) 39 (49.4%)	CIMP-H poorer survival (p=0.003)
Chang[10]	LOX, HRASLS, FLNc, HAND1, TM, p14, p15, p16, p73, GPSTP1, MGMT, hMLH1, TIMP-3, E-cadherin and DAPK. (Indicator genes - LOX, HRASLS, FLNc, HAND1 and TM)	CIMP-H = 4-5 CIMP-L = 1-3 CIMP-N = 0	40 (37.7%) 41 (38.7%) 25 (23.6%)	CIMP-H improved survival (p=0.031)
Chen[11]	ALX4, TMEFF2, CHCHD10, IGFBP3 and NPR1	CIMP-H = 4-5 CIMP-L = 1-3 CIMP-N = 0	18 (15.0%) 94 (78.3%) 8 (6.7%)	CIMP-H poorer survival (p<0.001)
He[3]	p16, FHIT, CRBP1, WWOX and DLC-1	CIMP-H = 4-5 CIMP-L = 1-3 CIMP-N = 0	53 (56.4%) 25 (26.6%) 16 (17.0%)	CIMP-H poorer survival (p=0.003)
Ksiaa[12]	RASSFIA, APC, hMLH1, MGMT, GSTP1, p14, p16, DAPK, SHP1, RAR-b2 and TIMP3	CIMP-H ≥ 3 CIMP-L = 1-2 CIMP-N = 0	41 (60.3%) 23 (33.8%) 4 (5.9%)	CIMP-H improved survival (p=0.075)
Kusano[13]	MINT1, MINT2, MINT12, MINT25, MINT32	CIMP-H = 4-5 CIMP-L = 1-3 CIMP-N = 0	19 (24.4%) 39 (50.0%) 20 (25.6%)	CIMP-H improved survival (p=0.004)
Liu[14]	APC, WIF-1, RUNx£, DLC-1, SFRP-1, DKK and E-cad	CIMP-H ≥ 3 CIMP-N < 3	47 (62.7%) 28 (37.3%)	No statistical difference (p > 0.05)
Park[4]	BCL2, BDNF, CACNA1G, CALCA, CHFR, CYP1B1, DLEC1, GRIN2B, RUNX3, SEZ6L, SFRP4, TERT, THBS1, TIMP3, TP73, TWIST1	CIMP-H ≥14 CIMP-L <14	9 (5.3%) 187 (94.7%)	CIMP-H poor survival (p=0.012)
Shigeyasu1[5]	APC, CACNA1G, CHFR, COX2, DAPK, DCC, HPP1, MGMT-Mp region, MGMT-Eh region, MINT1, MINT2, MINT31, MLH1 5',	CIMP-H ≥10 CIMP-L <10	30 (44.1%) 38 (55.9%)	CIMP-H improved survival (p=0.069)

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	MLH1 3', p14, p16, RASSF1A, RASSF2A-region1, RASSF2A-region2, RASSF3, RASSF5, RASSF6, RUNX3, SFRP2-region1, SFRP2-region2, UNC5C, 3OST2, FOXL2			

Figure 1. Flowchart of literature selection

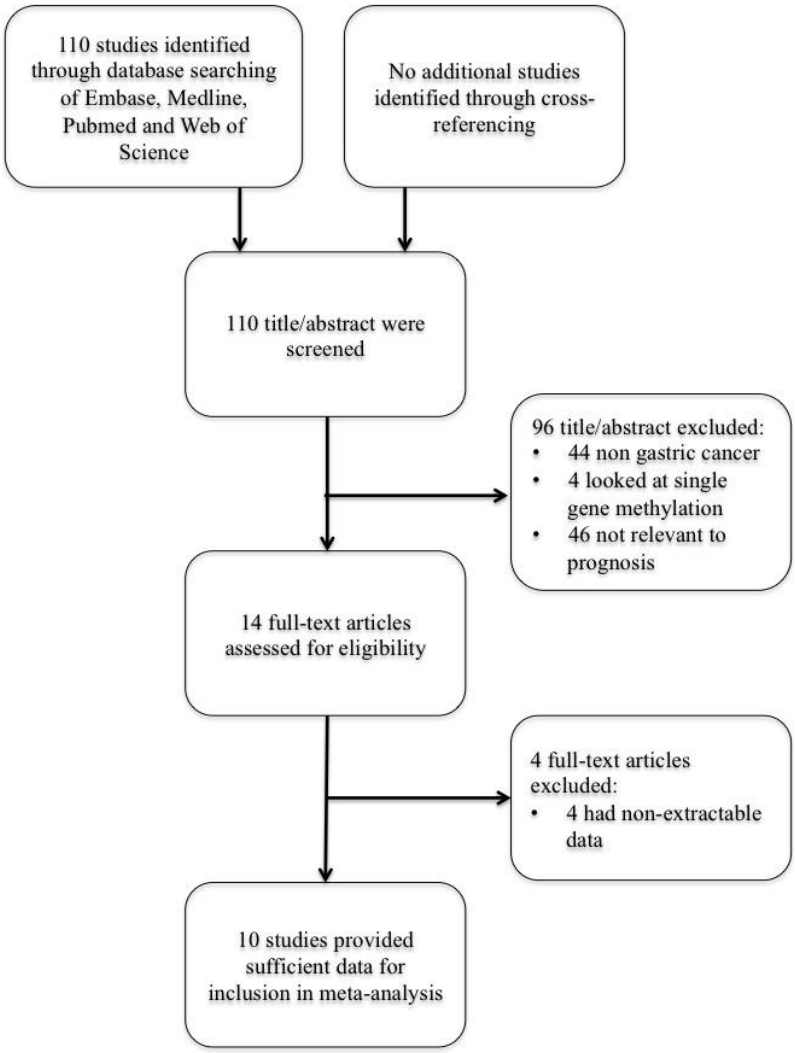

Figure 2. Association between CIMP positivity and overall survival (pooled analysis)