Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis

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ABSTRACT

Background: Several studies have suggested that microsatellite instability (MSI) resulting from defective DNA mismatch repair confers a better prognosis in colorectal cancer (CRC). Recently, however, data have suggested this is secondary to the effects of ploidy/chromosomal instability (CIN). To estimate the prognostic significance of CIN for survival, data from published studies have been reviewed and pooled.

Methods: Studies stratifying survival in CRC by CIN status were identified by searching PubMed and hand-searching bibliographies of identified studies. Two reviewers confirmed study eligibility and extracted data independently, and data were pooled using a fixed-effects model. The principal outcome measure was the HR for death.

Results: 63 eligible studies reported outcome in 10 126 patients, 60.0% of whom had CIN+ (aneuploid/polyploid) tumours. The overall HR associated with CIN was 1.45 (95% CI 1.35 to 1.55, $p<0.001$). In patients with stage II–III CRCs, the HR was 1.45 (95% CI 1.27 to 1.65, $p<0.001$). The effect was similar for progression-free survival (HR = 1.71, 95% CI 1.51 to 1.94, $p<0.001$). There was no evidence of significant interstudy heterogeneity.

Conclusion: CIN is associated with a worse prognosis in CRC, and should be evaluated as a prognostic marker, together with MSI status, in all clinical trials, particularly those involving adjuvant therapies.

Colorectal cancer (CRC) remains a major health burden, with over 1 million cases worldwide, mostly in the developed world.[1] While treatment has advanced,[2] the disease-specific mortality remains about 40%,[3] and identifying patients who will benefit the most and least from therapy remains an important goal.

Two major types of genomic instability are recognised as alternative mechanisms of colorectal carcinogenesis. The more common, chromosomal instability (CIN), is present in about 65–70% of CRCs. CIN is poorly defined as the presence of multiple structural or numerical chromosome changes in tumour cells, and, in practice, often inferred from finding aneuploidy and/or polyploidy.[4]

Direct measurement of aneuploidy utilising flow cytometry is relatively crude, and CRCs thus assigned CIN+ status are likely to encompass a variety of chromosomal abnormalities. Generally, a more detailed assessment—for example, using array comparative genomic hybridisation (arrayCGH)—is impractical for large series. Nonetheless, many studies have reported that CIN+ measured by flow cytometry confers a worse prognosis; however, this observation is neither universal[5 6] nor always significant.[7–9] Consequently, it has been argued consistently that measuring CIN does not add further prognostic information to standard pathological and histological staging.[10–13] A recent meta-analysis assessing the prognostic importance of the other major type of genomic instability (microsatellite instability or MSI) in >7500 patients found that MSI+ tumours had a better prognosis than MSI− tumours, lending weight to the assertion that genomic prognostication by MSI status determination alone should be performed.[14]

However, CRCs are not always positive for only one of either CIN or MSI. In addition to rare MSI+/CIN+ tumours, about a quarter of CRCs display neither form of genomic instability.[15–18] It is therefore possible that determining MSI status alone does not capture all prognostic information, and it has recently been suggested that MSI-associated prognostic information is not independent of CIN status.[18]

The 2006 American Society of Clinical Oncology (ASCO) guidelines state that studies on CIN published since the last guidelines in 2000 are variable and advocate that measuring CIN in CRC is at best an experimental tool. The guidelines recommend that only MSI status should be investigated in large prospective series.[19] We have reviewed all the published studies on CIN and used standard techniques of meta-analysis to derive a summary estimate of the prognostic significance of the CIN phenotype for survival.

METHODS

Study eligibility

Peer-reviewed studies of CIN in CRC were eligible if they reported overall survival (OS) stratified by CIN or ploidy status, and a summary statistic could be extracted as described by Parmar *et al*.[20] Studies had to detail how CIN status was determined, and define aneuploidy/polyploidy as the presence of a second peak on the DNA histogram, the first peak corresponding to the diploid cell population. Studies had to be genetically non-selected, and could select patients only for stage or anatomical location (colon and rectum). Where data sets were overlapping or duplicated, only the most recent information was included. Studies only reporting progression-free survival (PFS) or equivalent were not included in the main analyses.

All identified studies were reviewed independently for eligibility by two authors ($\kappa = 0.96$). Studies not published in English were excluded after identification (see table 1).

Table 1 Study eligibility criteria

CIN definition	Presence of a second peak on the DNA histogram, the first peak corresponding to the diploid cell population
CIN measure	Flow cytometry
	Image cytometry
Study design	Genetically non-selected patient populations
Outcome measure	Overall survival
Anatomical site	Colon
	Rectum
	Colorectal
Stage	Any
Study size	Any
Ethnic background	Any
Therapy	Any
Length of follow-up	Any
Source	Peer-reviewed journals
Language	English

CIN, chromosomal instability.

Study identification

We followed MOOSE (Meta-analysis Of Observational Studies in Epidemiology) guidelines[21] to identify appropriate studies (see fig 1). A literature search of studies published up to September 2006 was performed using PubMed and Embase. The search terms were any of "colon cancer", "rectal cancer" or "colorectal cancer" combined with any of "chromosomal instability", "ploidy" or "aneuploidy", combined with either "outcome" or "prognosis", and the "all related articles" functionality of PubMed. Queries using equivalent terms in other languages did not add to the search in English. Studies thus identified, and all studies cited within, were examined for eligibility. We did not hand-search meeting abstracts, nor did we contact authors to identify unpublished data.

Statistical analysis

Survival data from eligible studies were summarised using a log hazard ratio ($\ln HR_i$) for comparison between CIN+ and CIN− groups. Data from individual studies were extracted by two independent reviewers, and pooled to generate the summary statistic $\ln HR$ and var($\ln HR$) using a fixed-effects model with inverse variance weighting.

If a trial reported observed and expected events in each group, the $\ln HR_i$ and variance var($\ln HR_i$) were calculated directly. If a trial reported hazards ratio (HR) and CI, these were converted to $\ln HR_i$ and variance var($\ln HR_i$). Where a direct calculation of $\ln HR_i$ and var($\ln HR_i$) was not possible, estimates were derived indirectly from other numerical data presented using the methods described by Parmar *et al*.[20]

If no numerical data for the estimation of summary statistics were given, data were extracted manually from Kaplan–Meier survival curves: survival rates were estimated at constant time points to reconstruct the $\ln HR_i$ and var($\ln HR$), and patient censoring was assumed to be constant during follow-up, starting from the minimal follow-up period.[20] If censoring data were presented, censored patients were allocated to the appropriate time interval. Survival curves were magnified to improve the accuracy of the reading.

In one study with 248 patients,[22] no deaths occurred in the CIN− group, and 0.5 death was arbitrarily allocated in the last time interval as the resultant $\ln HR_i$ and var($\ln HR_i$) would otherwise have been uninterpretable. This had no effect on the overall HR and CI.

In six studies representing 674 patients,[23–28] it was not possible to extract data by any of the methods described above. None reported a significant difference in outcome between CIN− and CIN+ CRC, and we assigned an $\ln HR_i$ of 0 (corresponding to $HR_i = 1$), and a var($\ln HR_i$) of a similar sized study to avoid selection bias.[29]

Subgroup data were extracted as above.

Bias was assessed using the I^2 and Q estimates. For values $I^2 \geqslant 50\%$ (considered moderate heterogeneity[30]), a random-effects model would have been used. Heterogeneity was assessed with Egger's bias coefficient[31] and by funnel plot.[32] Sensitivity analysis by meta-regression (empirical Bayes model) was performed to exclude a significant influence of other trial characteristics.[33]

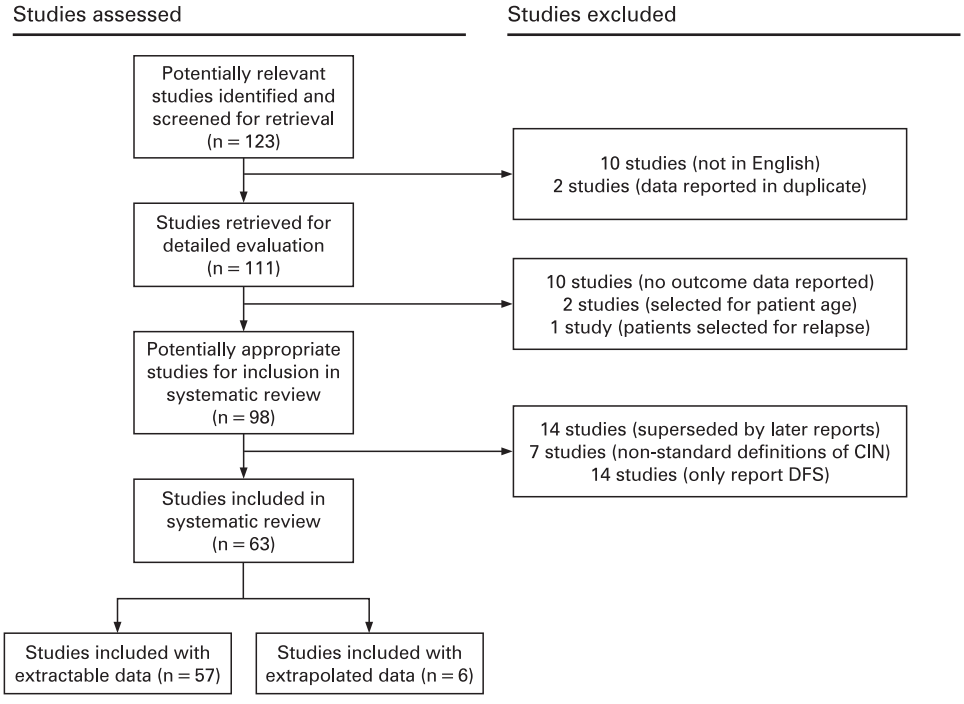

Figure 1 Flow chart of study identification, rejection and selection according to MOOSE (Meta-analysis Of Observational Studies in Epidemiology) guidelines.[21] CIN, chromosomal instability; DFS, disease-free survival.

All statistical analyses were conducted using Stata 9.2 statistical software (Stata Corp, College Station, Texas, USA)

RESULTS

Eligible studies

We identified 123 potential studies: 10 were excluded as they were not in English,[84–93] two as they were duplicates,[94 95] 10 as they did not report outcome data in CIN+ patients,[96–105] two as they selected patients for age[106 107] and one as it selected patients for relapse.[108] Fourteen were subsequently superseded by other reports,[109–122] seven were excluded as they used non-standard definitions of aneuploidy.[123–129] Fourteen studies solely reporting PFS[130–143] were only included in the PFS analysis.

The 63 studies included are summarised in table 2. The table does include six studies which did not report outcome data other than indicating that there was a non-significant trend towards worse survival in the CIN+ group.[23–28]

Study characteristics

The 63 included studies analysed 10 126 patients for CIN status and OS. The mean number of patients was 161 per study, with a median number of 138 (range 24–565). Eight studies (1045 patients) were solely based on patients with colonic (non-rectal) carcinoma,[7 24 54 61 71 75 79 81] and seven (968 patients) on those with rectal carcinoma.[39 41 48 55 56 65 73] Most studies examined a mixture of tumour stages. Seven studies were, however, based solely on single-stage disease: two (273 patients) with stage II;[59 66] two (140 patients) with stage III;[54 83] and three (123 patients) with stage IV.[27 47 52] Other studies also reported details for individual stages.[9 18 34 37 40 43 46 57 60 61 64 67 72]

Fifty-six studies were conducted in patients of Caucasian origin, six in patients of East Asian origin[8 9 42 77 78 82] and one in Indian patients.[58] Of the patients, 53.3% were male, based on 39 studies which provided this information.[7 8 18 23 26 28 34–39 41 42 46–51 53 55 58–62 66–70 72 73 75–78 81]

Determination of CIN status

CIN (aneuploidy/polyploidy) status of CRCs was determined using flow cytometry in 59 studies (9526 patients) and image analysis in four studies (600 patients).[27 35 40 59] In addition to the standard definition of aneuploidy, the DNA index, defined as the modal channel position of the G0/G1 peak of the aneuploid cell population divided by the modal channel position of the G0/G1 peak of the diploid reference cells, needed to be above a cut-off point (range >1.0–1.2) in 35 studies.[5 9 22 24 25 28 34–37 40–43 46–49 51 52 55 57–59 61 68 69 71–75 77 80 82] The frequency of CIN was 60.0%, the remaining CRCs being classified as CIN− (diploid/near-diploid).

Survival analysis

Nineteen studies provided data for a direct estimation of $\ln HR_i$ and $\mathrm{var}(\ln HR_i)$.[7 18 34 38 44 49 51 53 56 59 61 68 70 74–77 80 83] In eight studies, other numerical data were used for an indirect estimation.[5 8 36 42 43 55 57 79] Six studies had an HR_i of 1.00 and a $\mathrm{var}(\ln HR_i)$ of a similar sized study allocated (see Methods).[23–28] For all other studies, $\ln HR_i$ and $\mathrm{var}(\ln HR_i)$ were estimated from Kaplan–Meier curves.

Except for nine studies, all HR_i values were >1.0, indicating that patients with CIN+ cancers had a worse prognosis. Of these, 20 had a lower 95% CI >1, suggesting a significant effect.[9 18 36 38 40 43 55–57 59 61 65 68 71–73 76 78 81 83] Of the nine studies where HR_i was <1.0, seven presented Kaplan–Meier curves suggesting a worse prognosis for CIN+ tumours.[37 39 46 52 60 63 67] These paradoxical findings were due to few remaining patients at the end of the study,[39 46] low patient numbers,[52 63] low event rate in the CIN− group[37 60] and an unmatched drop in survival in the CIN− group during one time interval, skewing the resulting $\ln HR_i$.[67] Two studies[5 6] reported that CIN− tumours fared worse even by Kaplan–Meier analysis. All nine studies had an upper 95% CI >1.

The forest plot in fig 2 shows the HR_i and 95% CI for all studies, and the summary HR of 1.45 (95% CI of 1.35 to 1.55, p<0.001). There was no evidence of heterogeneity between studies ($Q = 69.10$, $I^2 = 10.3\%$, $p = 0.250$). If the six studies[23–28] which had an $\ln HR_i$ of 1 allocated were excluded, the overall effect remained virtually unchanged (HR = 1.47, 95% CI 1.37 to 1.58, p<0.001), with no evidence of heterogeneity ($Q = 64.12$, $I^2 = 12.7\%$, $p = 0.213$). Analysis of Caucasian patients only gave HR = 1.45 (95% CI 1.34 to 1.56, p<0.001; $Q = 63.21$, $I^2 = 13.0\%$, $p = 0.209$).

A similar outcome was found for the colonic[7 24 54 59 61 66 71 75 79 81] and rectal subgroups[39 41 48 55 56 59 65 66 73]: for colonic disease, HR = 1.67 (95% CI 1.32 to 2.11, p<0.001; $Q = 12.93$, $I^2 = 30.4$, $p = 0.166$); for rectal disease, HR = 1.63 (95% CI 1.33 to 1.99, p<0.001; $Q = 12.32$, $I^2 = 35.0\%$, $p = 0.138$).

Impact of CIN on PFS

Assessing whether the above estimates were realistic, we analysed 2100 patients in 14 studies[130–143] that only reported PFS (see table 3). Most patients who relapse will eventually die from CRC,[144 145] and therefore the summary statistic for PFS studies should be similar to those of OS studies. As expected, this was the case (PFS HR = 1.56; 95% CI 1.30 to 1.87, p<0.001; $Q = 13.62$, $I^2 = 4.6\%$, $p = 0.401$).

Taking all reported PFS outcome in 4026 patients, including studies which also reported OS included above,[18 35 38 41 42 60–62 68 74 75 81] the HR was 1.71 (95% CI 1.51 to 1.94, p<0.001, $Q = 32.21$, $I^2 = 22.4\%$, $p = 0.152$).

Survival by stage

CIN conferred a worse prognosis in 1179 stage II patients,[9 18 34 37 43 57 59–61 64 66 67] HR = 1.68 (95% CI 1.25 to 2.25, $p = 0.001$; $Q = 6.12$, $I^2 = 0\%$, $p = 0.865$), and in 1177 stage III patients,[9 18 34 40 43 54 57 60 61 67 72 83] HR = 1.38 (95% CI 1.14–1.67, $p = 0.001$; $Q = 11.16$, $I^2 = 1.4\%$, $p = 0.430$).

Considering those who might be offered adjuvant therapy, data on a further 738 stage II–III patients were available (outcome pooled in individual studies)[7 48 51 63 68]: in 3094 stage II–III patients the HR was 1.45 (95% CI 1.27 to 1.65, p<0.001; $Q = 23.75$, $I^2 = 0\%$, $p = 0.750$).

There were insufficient data for stage I patients to reach a meaningful estimate[9 43 67]; likewise in stage IV, where the HR_i was also unreliable and not suitable for pooling, depending on data extracted from Kaplan–Meier curves in seven small cohorts.[9 27 46 47 52 67 72]

CIN and the effectiveness of therapy

To assess whether CIN+ tumours have inherently different outcomes from CIN− tumours, we analysed all studies in which patients did not receive systemic therapy,[23 59 75 81] or which only included non-metastatic disease and enrolled before 1987, when patients rarely received adjuvant chemotherapy.[8 54 56 57 66 76 78 79] This provided an indication of the underlying differences in tumour biology affecting outcome. Again,

Table 2 Summary of studies of CIN status and colorectal cancer overall survival

Reference	Ethnic origin	Study size	Stage	Clinical trial	Site	Ploidy test	DNA index cut-off
Ahnen *et al*, 1998[34]	White Caucasian	224	II–III	y	CRC	fc	1.2
Albe *et al*, 1990[35]	White Caucasian	210	I–IV	n	CRC	ia	1.1
Armitage *et al*, 1990[36]	White Caucasian	416	I–IV	n	CRC	fc	1.0
Baretton *et al*, 1991[37]	White Caucasian	72	I–III	n	CRC	fc	1.1
Baretton *et al*, 1996[23]	White Caucasian	86	I–II	n	CRC	fc	n/s
Barratt *et al*, 2002[7]	White Caucasian	340	II–III	y	Colon	fc	n/s
Bauer *et al*, 1987[24]	White Caucasian	97	I–IV	n	Colon	fc	1.2
Bazan *et al*, 2002[38]	White Caucasian	160	I–IV	n	CRC	fc	n/s
Bendardaf *et al*, 2004[27]	White Caucasian	53	IV	n	CRC	ia	n/s
Berczi *et al*, 2002[39]	White Caucasian	52	I–III	n	Rectum	fc	n/s
Bosari *et al*, 1992[40]	White Caucasian	169	I–IV	n	CRC	ia	1.1
Chang *et al*, 1987[41]	White Caucasian	30	I–II	n	Rectum	fc	1.1
Chang *et al*, 2006[42]	Asian	194	I–IV	n	CRC	fc	1.0
Chapman *et al*, 1995[43]	White Caucasian	346	I–III	n	CRC	fc	1.1
Deans *et al*, 1993[44]	White Caucasian	275	I–IV	n	CRC	fc	n/s
Emdin *et al*, 1987[45]	White Caucasian	34	I–III	n	CRC	fc	n/s
Enker *et al*, 1991[46]	White Caucasian	176	I–IV	n	CRC	fc	1.1
Fausel *et al*, 1990[25]	White Caucasian	27	I–IV	n	CRC	fc	1.2
Finan *et al*, 1986[47]	White Caucasian	46	IV	n	CRC	fc	1.0
Fisher *et al*, 1989[48]	White Caucasian	232	I–IV	y	Rectum	fc	1.2
Flyger *et al*, 1999[49]	White Caucasian	163	I–IV	y	CRC	fc	1.03
Foggi *et al*, 1993[50]	White Caucasian	150	I–IV	n	CRC	fc	n/s
Geido *et al*, 2002[51]	White Caucasian	110	II–III	n	CRC	fc	1.0
Graham *et al*, 1992[52]	White Caucasian	24	IV	n	CRC	fc	1.0
Halvorsen *et al*, 1990[53]	White Caucasian	149	I–IV	n	CRC	fc	n/s
Harlow *et al*, 1991[54]	White Caucasian	69	III	n	Colon	fc	n/s
Heiman *et al*, 1990[55]	White Caucasian	39	I–III	n	Rectum	fc	1.1
Jass *et al*, 1989[56]	White Caucasian	369	I–III	n	Rectum	fc	n/s
Jones *et al*, 1988[57]	White Caucasian	119	I–III	n	CRC	fc	1.1
Karelia *et al*, 2001[58]	Indian	79	I–III	n	CRC	fc	1.0
Kay *et al*, 1996[59]	White Caucasian	168	II	n	CRC	ia	1.15
Kokal *et al*, 1989[60]	White Caucasian	138	I–III	n	CRC	fc	n/s
Lanza *et al*, 1998[61]	White Caucasian	191	II–III	n	Colon	fc	1.0
Lichtman *et al*, 1994[26]	White Caucasian	138	I–IV	n	CRC	fc	n/s
Mazzei *et al*, 1995[62]	White Caucasian	45	II–IV	n	CRC	fc	n/s
Melamed *et al*, 1986[6]	White Caucasian	33	I–IV	n	CRC	fc	n/s
Offerhaus *et al*, 1992[63]	White Caucasian	26	II–III	n	CRC	fc	n/s
Purdie *et al*, 2000[64]	White Caucasian	210	I–III	n	CRC	fc	n/s
Quirke *et al*, 1987[65]	White Caucasian	125	I–IV	n	Rectum	fc	n/s
Risques *et al*, 2001[5]	White Caucasian	108	I–III	n	CRC	fc	1.1
Robey-Cafferty *et al*, 1990[66]	White Caucasian	105	II	n	CRC	fc	n/s
Rognum *et al*, 1987[67]	White Caucasian	100	I–IV	n	CRC	fc	n/s
Salud *et al*, 1999[68]	White Caucasian	107	II–IV	n	CRC	fc	1.2
Schillaci *et al*, 1990[69]	White Caucasian	66	I–IV	n	CRC	fc	1.0
Sciallero *et al*, 1994[70]	White Caucasian	119	I–IV	n	CRC	fc	n/s
Scivetti *et al*, 1989[71]	White Caucasian	44	I–IV	n	Colon	fc	1.1
Scott *et al*, 1987[72]	White Caucasian	258	I–IV	n	CRC	fc	1.1
Scott *et al*, 1987[73]	White Caucasian	121	I–IV	n	Rectum	fc	1.1
Silvestrini *et al*, 1993[74]	White Caucasian	181	I–IV	n	CRC	fc	1.0
Sinicrope *et al*, 1999[75]	White Caucasian	150	I–II	n	Colon	fc	1.18
Sinicrope *et al*, 2006[18]	White Caucasian	528	II - III	y	CRC	fc	n/s
Sun *et al*, 1993[76]	White Caucasian	228	I–III	n	CRC	fc	n/s
Tang *et al*, 1995[8]	Asian	565	I–III	n	CRC	fc	n/s
Tonouchi *et al*, 1998[77]	Asian	140	I–III	n	CRC	fc	1.0
Tsuchiya *et al*, 1992[78]	Asian	137	I–III	n	CRC	fc	n/s
Venkatesh *et al*, 1994[22]	White Caucasian	248	I–III	n	CRC	fc	1.1
Visscher *et al*, 1990[79]	White Caucasian	121	I–II	n	Colon	fc	n/s
Wiggers *et al*, 1988[80]	White Caucasian	279	I–IV	y	CRC	fc	1.0
Wolley *et al*, 1982[81]	White Caucasian	33	I–IV	n	Colon	fc	n/s
Yamamoto *et al*, 1998[82]	Asian	230	I–IV	n	CRC	fc	1.0
Yamazoe *et al*, 1994[9]	Asian	330	I–IV	n	CRC	fc	1.0
Zarbo *et al*, 1997[28]	White Caucasian	273	I–IV	n	CRC	fc	1.2
Zoras *et al*, 1994[83]	White Caucasian	71	III	n	CRC	fc	n/s

CIN, chromosomal abnormality; CRC, combined analysis for colorectal cancers; fc, flow cytometry; ia, image analysis; n, no; n/s, not stated in report; y, yes.

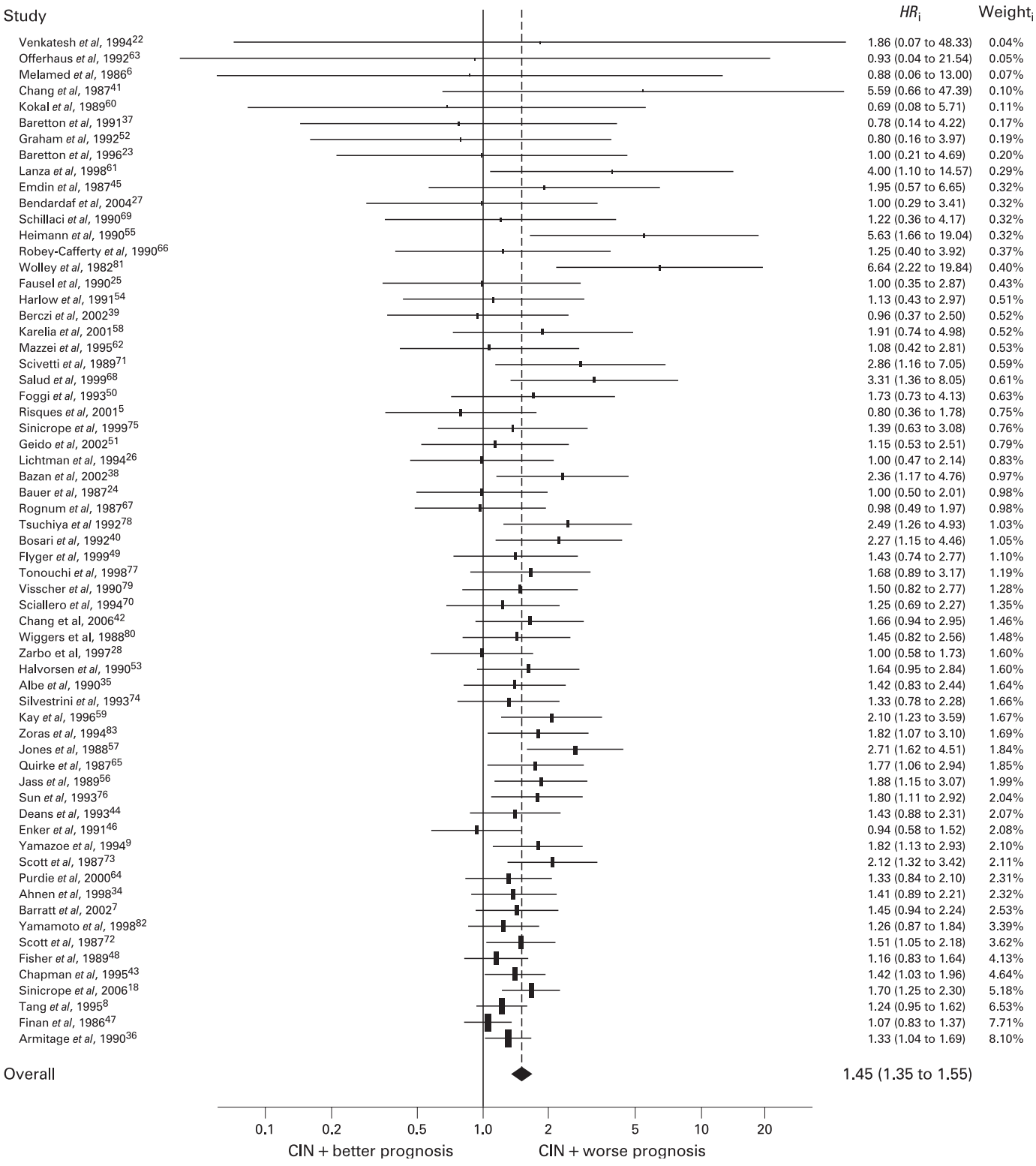

Figure 2 Forest plot of the HR for overall survival from colorectal cancer associated with chromosomal instability (CIN) in 63 studies. Studies are plotted in order of decreasing variance of lnHR_i. Horizontal lines represent 95% CI. Each box represents the HR_i point estimate, and its area is proportional to the weight of the study, determined by inverse variance weighting. The diamond (and broken line) represents the overall summary estimate, with the 95% CI given by its width. The unbroken vertical line is at the null value (HR = 1.0).

CIN+ patients fared worse (HR = 1.66; 95% CI 1.41 to 1.95, p<0.001; $Q = 17.34$, $I^2 = 36.6\%$, p = 0.098).

To determine if 5-fluorouracil (5-FU)-based adjuvant chemotherapy can modify the worse outcome of CIN+ patients with stage II–III CRC, we pooled the data from the only two studies reporting outcome in this setting.[7 18] All patients received adjuvant 5-FU-based chemotherapy, and CIN+ patients had worse outcome compared with diploid patients (HR = 1.85; 95% CI 1.21 to 2.82, p = 0.004; $Q = 0.19$, $I^2 = 0\%$, p = 0.662). It was not possible to draw conclusions regarding the differences

Table 3 Summary of studies of chromosomal instability status and colorectal cancer progression-free survival

Reference	Ethnic origin	Study size	Stage	Clinical trial	Site	Ploidy test	DNA index cut-off
Armitage *et al*, 1991[130]	White Caucasian	236	I–IV	n	CRC	fc	1.1
Bottger *et al*, 1992[131]	White Caucasian	68	I–III	n	Rectum	fc	n/s
Chen *et al*, 2002[133]	Asian	666	I–III	n	CRC	fc	n/s
Cosimelli *et al*, 1998[132]	White Caucasian	120	I–III	n	CRC	fc	n/s
Costa *et al*, 1997[134]	White Caucasian	104	IV	n	CRC	fc	1.0
Hixon *et al*, 1995[135]	White Caucasian	52	I–III	n	CRC	fc	n/s
Kouri *et al*, 1990[136]	White Caucasian	143	I–IV	n	CRC	fc	1.0
Lammering *et al*, 2000[141]	White Caucasian	103	I–III	n	Rectum	fc	n/s
Michel *et al*, 2000[137]	White Caucasian	38	II–III	n	CRC	fc	n/s
Moran *et al*, 1993[138]	White Caucasian	138	I–III	n	Rectum	fc	n/s
Pietra *et al*, 1998[139]	White Caucasian	98	I–IV	n	CRC	fc	1.0
Lin *et al*, 2003[142]	Asian	146	I–IV	n	CRC	fc	1.0
Sampedro *et al*, 1999[143]	White Caucasian	88	I–III	n	CRC	fc	1.1
Tomoda *et al*, 1993[140]	Asian	100	I–III	n	CRC	fc	n/s

CRC, combined analysis for colorectal cancers; fc, flow cytometry; n, no; n/s, not stated in report; y, yes.

in outcome of receiving versus not receiving adjuvant chemotherapy therapy within the groups of diploid and aneuploid patients, respectively.

There were no studies that commented on the combined impact of therapy and CIN status on outcome in stage IV disease.

Publication bias and heterogeneity

Visual assessment of a funnel plot of studies provided no evidence of overt publication bias towards studies reporting a poorer OS associated with CIN (fig 3), nor did formal evaluation of publication bias using Begg's and Egger's tests (p = 0.735 and p = 0.101 respectively). On the assumption that significant heterogeneity might have been missed, all analyses were repeated using a random-effects model; this changed neither the direction nor the significance of our findings (overall HR = 1.47, 95% CI 1.36 to 1.58, p<0.001; $Q = 69.10$, $I^2 = 10.3\%$, p = 0.250). An influence analysis in which one study at a time was omitted from the summary estimate confirmed that no study significantly influenced the overall summary statistic (data not shown).

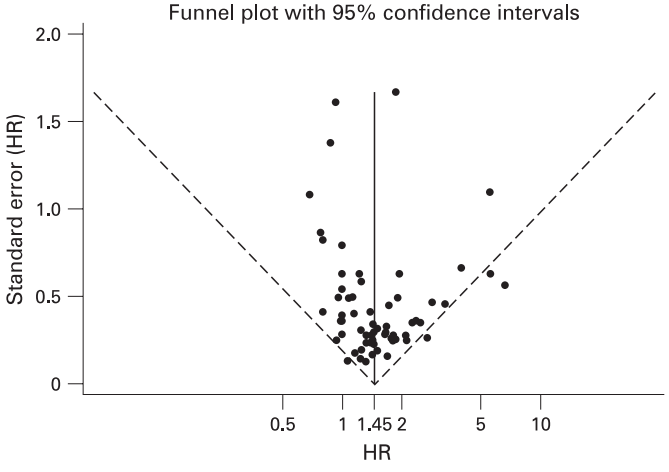

Figure 3 Detecting publication bias using the Begg funnel plot. The funnel plot displays HR_i (the HR associated with chromosomal instability in an individual study) on a log scale against its standard error (SE_i) for each study included in the meta-analysis. The vertical line indicates the pooled estimate of the overall HR, with the sloping lines representing the expected 95% CI for a given SE. Under the assumption of no heterogeneity between studies, 95% of studies lie between or on these two lines.

Publication bias introduced by researchers only reporting significant positive findings was a concern, even if over half of the studies included reported non-significant findings. We performed an analysis restricted to studies based on trial patients: four studies reported non-significant HR_i[7 34 48 49]; two reported significant survival differences between CIN+ and CIN– patients.[18 80] The summary statistic was very similar to that if all studies were considered (HR = 1.43, 95% CI 1.21 to 1.70, p<0.001, $Q = 2.61$, $I^2 = 0\%$, p = 0.759).

Other potential, non-quantitative sources of heterogeneity (different methods for determining ploidy, use of DNA index, ethnic background, variation in stage and anatomical location) were formally assessed by meta-regression and subgroup analysis: neither revealed any significant associations; study size, length of follow-up, method of data presentation and extraction (direct numerical vs indirect numerical vs graphic) and year of publication were also included and not found to be associated with outcome (see table 4). Ploidy measurement and definition varied very little between studies, and studies with non-standard definitions were excluded[123–129]; four studies used cytometric image analysis [27 35 40 59] and there is good concordance between this and flow cytometry.[146] Exclusion of seven studies in Asian and Indian patients[8 9 42 58 77 78 82] did not alter the overall finding; the summary HR and 95% CI of these seven studies alone were similar to the overall summary statistics for all studies (data not shown). PFS was analysed separately from OS.

DISCUSSION

We have shown that the published data support the view that CIN (ie, aneuploidy/polyploidy) is associated with a worse prognosis in CRC, and, it appears, can stratify CRC patients further after standard pathological staging. Patients with CIN+ CRC appear to have a poorer survival irrespective of ethnic background, anatomical location and treatment with 5-FU. The poorer outcome is found in terms of OS and PFS. CIN influences outcome in patients with stage II–III CRC, irrespective of whether these receive adjuvant therapy (see table 5). It was difficult to determine whether CIN in stage I and IV has prognostic value from the studies included as only around 8% of

Table 4 Sensitivity analysis

Trial characteristic	Coefficient	SE	p Value
Trial vs observational study	0.10	0.13	0.430
Flow vs image cytometry	0.25	0.22	0.253
Use of DNA index	0.03	0.10	0.739
Ethnicity (white Caucasian, Asian, Indian)	0.06	0.12	0.625
Single stage vs multiple stage analysis	0.22	0.17	0.195
Site of disease (colorectal, colon, rectum)	0.08	0.08	0.306
Study size (<100, 100–400, >400 patients)	−0.06	0.12	0.628
Length of follow-up	0.00	0.00	0.804
Method of data extraction*	–0.08	0.07	0.254
Year of publication	0.00	0.01	0.960

*Direct numerical estimation, indirect numerical estimation, data extraction from a graph.

patients had either stage I or IV disease. While in stage I the data were consistent with an effect of the same direction and magnitude to those in stage II and III (data not shown), in stage IV the problem of low patient numbers was compounded by the highest heterogeneity encountered in our analyses, based on the unreliable data extraction in this subset. It is still possible that CIN has prognostic value in these stages, but requires further study.

Our findings are likely to be robust: they include large numbers of patients and have no evidence of statistical heterogeneity or bias. There was little evidence of qualitative heterogeneity, although some studies did come from different ethnic backgrounds or utilised different methods to detect CIN. For both, the number of studies which did not conform to the majority was small, and, importantly, their exclusion did not significantly alter the summary statistic. Further, the very similar HR for PFS and OS in non-overlapping sets of patients suggests that the finding of worse prognosis in CIN+ CRC is qualitatively and quantitatively correct.

The method of data extraction did not significantly influence the overall HR, as indirect numerical data extraction correlated well with direct methods; analysing outcome by method of data extraction produced very similar significant results (data not shown) and the sensitivity analysis was not significant (table 4).

All but two identified foreign language studies reported a significant decrease in survival in the CIN group in their English abstracts, and exclusion probably makes our estimate conservative. Non-significant findings may be more commonly reported in abstracts, and their exclusion may inflate our estimate. However, Egger *et al* found that omission of either has only small effects on the HR and CI, while the inability to assess study quality increases heterogeneity.[147]

It is not clear how CIN status relates to more sophisticated pathological staging beyond the AJCC (American Joint Committee on Cancer) staging employed in the studies analysed. This higher standard may capture some of the information contained in molecular staging, and may even complement it. However, uniform molecular staging should be relatively easy to achieve, while achieving the equivalent pathological staging uniformly may prove more difficult.[148 149]

Our findings raise several questions: first, how is CIN measured by flow cytometry related to cancer biology? Assigning CIN+ status based on flow cytometry is a relatively blunt tool for assessing chromosomal changes, and does not distinguish stable and unstable chromosomal abnormalities, nor differentiate simple from complex changes. CRCs which constantly acquire new complex chromosomal abnormalities (unstable CIN) can thus be grouped with tumours which carry the same relatively minor changes in each cell. Disruptions to cell biology are likely to be varied depending on the level of CIN. However, all CIN+ CRCs must have abnormalities which impair faithful replication or segregation of sister chromatids, driving aneuploidy. As such, CIN status by flow cytometry is likely to assess accurately at least one aspect of tumour biology. Whether more sophisticated measures of global CIN, such as numerical and structural complexity and heterogeneity,[150] or arrayCGH can refine and add to the CIN concept is not clear at present.

Kern *et al*[151] found that increasing numbers of chromosomes showing loss of heterozygosity (LoH) correlated inversely with prognosis. Whilst we expect overall LoH to co-vary with CIN, LoH can result from several causes and is, at best, an indirect and time-consuming measure of CIN. An analogous analysis regarding the impact of levels of CIN on prognosis was not possible from the published data. Individual chromosomal abnormalities could act as markers for CIN,[15] but how the prognostic information of, say, loss of chromosome 18q relates to that of CIN is poorly defined,[63 152] even if it could be that 18q loss is the defining abnormality of the CIN−/MSI−group.[15]

Secondly, what is the prognostic relationship of CIN and MSI? Within individual CRCs, CIN and MSI status are not mutually exclusive: about a quarter of CRCs display neither, and there are rare cases of CIN+/MSI+ tumours.[15–18] In line with this report on CIN, in a previous report on the prognostic value of MSI status, we have found that MSI is associated with outcome in stage II–IV disease.[14] Unfortunately, neither data set allowed us to relate CIN to MSI status and to tease apart their relative contributions to prognosis. Only one published study has stratified survival by both CIN and MSI status, concluding that the univariate survival benefit in stage II–III CRC

Table 5 Summary of hazard ratios

Analysis	HR (95% CI)	Significance	Heterogeneity (I^2)	Total patients
Overall survival	1.45 (1.35 to 1.55)	<0.001	10.3%	10 126
Progression-free survival (all patients)	1.71 (1.51 to 1.94)	<0.001	22.4%	4026
Anatomical location				
Colon (all patients)	1.67 (1.32 to 2.11)	<0.001	30.4%	1213
Rectum (all patients)	1.63 (1.33 to 1.99)	<0.001	35.0%	1073
Stage				
Stage II	1.68 (1.25 to 2.25)	0.001	0%	1179
Stage III	1.38 (1.14 to 1.67)	0.001	1.4%	1177
Stage II–III (combined, all patients)	1.45 (1.27 to 1.65)	<0.001	0%	3094
Adjuvant therapy				
Treatment (CIN vs diploid)	1.85 (1.21 to 2.82)	0.004	0%	868

CIN, chromosomal instability.

Conclusions

- Chromosomal instability (CIN) confers worse survival in stage II and III colorectal cancer
- No clear relationship could be established in stage I and IV colorectal cancer
- The predictive value of CIN for 5-fluorouracil-based chemotherapy could not be determined from the published data
- While other publications have suggested that increasing chromosomal changes worsen prognosis, the data analysed were insufficient to establish CIN levels as a continuous prognostic variable

associated with MSI+ status was not independent of CIN status in multivariate analysis.[18] It remains possible, however, that CIN−/MSI− CRCs differ from CIN−/MSI+ CRCs, with MSI+ affording a better prognosis independent of CIN−. Likewise, a third form of genomic instability, the CpG island methylator phenotype (CIMP), may carry prognostic information—and explain the existence of the CIN−/MSI− group—but its association with MSI may not render it an independent marker.[153] There were insufficient data in the literature to try and assess the relationship of CIN and CIMP, but, if future studies assess all three forms of genomic instability, then this relationship may become clearer and lead to prospective trials including CIMP.

Lastly, should CIN status influence the type of chemotherapy given? No study consistently investigated the effectiveness of drugs other than 5-FU, and we cannot comment on these. It is conceivable that diploid patients in the adjuvant setting could be treated less aggressively than CIN+ patients. There is evidence that abnormalities of the spindle checkpoint drive CIN, and in turn promote taxane resistance.[154] Given that most CRCs are CIN+, this may explain the poor response of CRC to taxanes observed in phase 1 trials,[155] and diploid CRCs could show a better response to taxanes.

In the absence of clinical trials that address different treatment strategies, our findings should drive molecular stratification of patients within clinical trials to determine the contributions of CIN to treatment sensitivity and resistance. In stage II–III, where the published literature allows a firm conclusion regarding the prognostic value of CIN, it should be investigated as a predictive marker.

The association between genomic instability, outcome and benefit from systemic therapy makes it likely that determining the type(s) of genomic instability in CRCs is important. Contrary to current guidelines on prognostic markers in CRC,[19] our systematic review of published data suggests that there is likely to be value in determining CIN prospectively, using flow cytometry in conjunction with more sensitive but prognostically less well defined methods. It remains possible that MSI+ status affords a better prognosis independently, and we favour MSI testing until the relationship between CIN and MSI is understood more fully. The precise contribution of each type of genomic instability to prognosis should be evaluated in clinical trials, particularly those involving adjuvant therapy, with an expectation that routine testing for one or both types of instability will be of benefit in clinical practice.

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