RESEARCH ARTICLE

Lack of Effects of HER-2/neu on Prognosis in Colorectal Cancer: a Meta-analysis

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Abstract

    <u>Background</u>: The prognostic value of human epidermal growth factor receptor-2 (HER-2/neu) for survival of patients with colorectal cancer (CRC) is still ambiguous. We therefore performed a meta-analysis to evaluate its prognostic significance. <u>Materials and Methods</u>: We searched the MEDLINE and EMBASE databases for published literature investigating associations between HER-2/neu status and overall survival of patients with CRC. A meta-analysis was performed using a DerSimonian-Laird model and publication bias was investigated by Begg's and Egger's tests. Subgroup analysis was also conducted according to the study design type, study quality score, cut-off value for HER-2/neu overexpression, publication region, patient number and publication year. <u>Results</u>: A total of 17 eligible studies involving 2,347 patients were identified for this meta-analysis. The combined hazard ratio (HR) was 1.31 (95% confidence interval (CI): 0.96-1.79), suggesting that HER-2/neu overexpression was not significantly associated with overall survival of patients with CRC. However, subgroup analysis revealed that HER-2/neu overexpression had an unfavorable impact on survival when the analysis was restricted to subgroups of study quality score ≤ 5 (HR=1.56, 95%CI: 1.17-2.10), Asian patients (HR=1.74, 95%CI: 1.22-2.49), patient number ≤ 106 (HR=1.57, 95%CI: 1.01-2.44), publication year before 2003 (HR=1.59, 95%CI: 1.02-2.49), and prospectively designed study (HR=3.62, 95%CI: 1.42-9.24). The effect disappeared in subgroups of study quality scores > 5 (HR=0.69, 95%CI: 0.33-1.44), non Asian patients (HR=1.14, 95%CI: 0.77-1.70), patients' number > 106 (HR=1.07, 95%CI: 0.67-1.72), publication year after 2003 (HR=1.13, 95%CI: 0.76-1.69), and retrospectively designed study (HR=1.22, 95%CI: 0.89-1.67). <u>Conclusions</u>: Our meta-analysis suggests that HER-2/neu overexpression might not be a significantly prognostic indicator for patients with CRC. Further studies are required to confirm these results.

    Keywords: Human epidermal growth factor receptor-2 - prognosis - overall survival - colorectal cancer - meta-analysis

Introduction

    Colorectal cancer (CRC) is the third most common cancer worldwide and its incidence has risen rapidly in Asian countries during the past few decades (Sung et al., 2005; Jemal et al., 2010). Despite improvements in diagnosis and treatment methods, the prognosis of patients with CRC is still very poor. This high mortality is related to the difficulty in detecting CRC at early stage, as well as the lack of effective prognostic factors for advanced stage cancer. Several independent clinical and biological prognostic factors have been identified for predicting the poor survival rate such as patients' age, TNM stage, tumor grade, tumor location and carcino-embryonicantigen (CEA) level (Mehrkhani et al., 2009; Park et al., 1999). Although these clinicopathological prognostic parameters reflect the biological features of CRC, however, they do not allow adequate prediction of outcomes for individual patient. Molecular biological prognostic parameters are more accurate in predicting clinical outcomes and may also serve as therapeutic targets (Oldenhuis et al., 2008). P53, HER-2/neu, KRAS and MMPs are molecular biological parameters being evaluated as potential prognostic factors for CRC (Huang et al., 2011). HER-2/neu is overexpressed in a wide variety of human malignancies, such as breast, ovary, prostate, lung, kidney and liver cancer, as well as osteosarcoma. Meta-analyses have shown that overexpression of HER-2/neu is associated with poor prognosis of breast, gastric, ovarian, prostate and lung cancer, but not osteosarcoma (Meert et al., 2003; de Graeff et al., 2009; Li and Geng, 2010; Neto et al., 2010; Harris et al., 2011; Wang et al., 2011). The prognostic value of HER-2/neu for CRC is still inconclusive. In some studies, the overexpression of HER-2 indicates worse or better clinical outcomes, whereas other studies report no correlation between HER-2 and prognosis. Therefore, we performed a meta-analysis to assess the prognostic value of HER-2/neu on survival of patients with CRC.

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Materials and Methods

Search strategy and selection criteria

A MEDLINE and EMBASE search was performed exploring studies that investigated the prognostic significance of HER-2/neu in patient with CRC. We used mesh words and text words ("colorectal neoplasm (cancer)" or "colonic neoplasm (cancer)" or "rectal neoplasm (cancer)") and ("c-erbB-2" or "c-erbB2" or "neu" or "HER2" or "HER-2" or "erbB2") to identify relevant studies. The references of all identified publications and cross referenced studies were hand searched to identify missing relevant publications. When the same author reported, in several publications, the same patient population, only the most complete report was included in the analysis (to avoid overlap between cohorts). The search ended on November 12, 2013.

The eligibility criteria of the studies were as follows: (1) study included patients with primary CRC only; (2) study evaluated the correlation between HER-2/neu status and patients' overall survival; (3) study measured HER-2/neu expression in the primary tumor (not in metastatic tissue, tumor adjacent tissue or serum) using immunohistochemistry (IHC) methods; (4) study reported a hazard ratio (HR) and confidence interval (CI) or data sufficient to estimate the HR and CI; (5) study was published as a full paper in English; and (6) number of patients with HER-2/neu overexpression was at least three. Non original articles, reviews, non English articles and studies on non adenocarcinoma CRC were excluded.

Titles and abstracts of each article identified by computer were also independently examined by two of the investigators (Han J and Meng QY) to determine if full text articles should be obtained. Cases of disagreement were resolved by discussion with a third investigator (Xi QL). Full text articles were examined and excluded if they did not meet the selection criteria previously described.

Data extraction

Two investigators (Han J and Meng QY) independently extracted data from eligible studies by using a predefined form. Discrepancies were discussed with the third investigator (Xi QL). The following items of each article were recorded: author's last name, year of publication, regions, study design type (retrospective or prospective), tumor stage, tumor type, treatment method, number of analyzed patients, HER-2/neu evaluation method, cut-off value, result of univariate or multivariate survival analysis.

The study was called "positive" if HER-2/neu overexpression was identified to be a significant ($p<0.05$) favorable prognostic factor for overall survival. Conversely, if HER-2/neu overexpression was identified to be unfavorable, the study was called "negative". When no significant difference between two groups was detected, the study was called "not significant".

Assessment of study quality and publication bias

Study quality of each trial was evaluated independently by two investigators (Zhuang QL and Xi QL) using a predefined form adapted from the work of McShane et al (McShane et al., 2005), captured the following study parameters: (1) inclusion and exclusion criteria; (2) prospective or retrospective data; (3) patient and tumor characteristics sufficiently described; (4) HER-2/neu expression assay sufficiently described; (5) study endpoint definition provided; (6) patient follow-up time described; and (7) patients lost to follow-up or not available for statistical analysis identified. Studies with a total score of eight were considered to show the highest study quality, whereas one score indicated the lowest quality.

Statistical analysis

Statistical analysis was carried out using Stata version 11.0 (Stata Corporation, Collage Station, Texas, USA). The effect of HER-2/neu on overall survival between the two groups (with or without HER-2/neu overexpression) was measured by HR and 95%CI. The HR of each study was estimated by various methods depending on the data provided by the study (Parmar et al., 1998). Briefly, the most accurate method was to retrieve the HR from the reported results. If the study did not report the HR, it was calculated by two of the following parameters: the 95%CI: for the HR, the logrank statistic, its P-value or O-E statistic (difference between numbers of observed and expected events). If data for these calculations were unavailable, we used the total number of events, the number of patients at risk in each group and the logrank statistic or its P-value to calculate an approximate HR estimate. Finally, if the only available data provided in the study report were overall survival curves of the two groups, survival rates at specified times were extracted to reconstruct the HR estimate and its variance, with the assumption that the rate of patients was constant during the study follow-up. In addition, if studies reported survival of three or more groups using several cut-off values, the results were pooled, making a comparison between two groups feasible.

By convention, an observed HR>1 implied a worse survival for the group with HER-2/neu overexpression. Otherwise, an observed HR<1 implied a better survival for the group with HER-2/neu overexpression. The impact of HER-2/neu overexpression on survival was considered to be statistically significant if the 95%CI did not overlap with 1.

A meta-analysis was performed using the DerSimonian-Laird random effects model (DerSimonian and Laird, 1986), applying the inverse of variance as a weighing factor. Heterogeneity was investigated by using the I^2 statistic, which takes values from 0 to 100% (Higgins and Thompson, 2002). If I^2 value was>50%, it was considered to represent substantial heterogeneity between studies. Sources of heterogeneity were analyzed by subgroup analysis (Thompson and Higgins, 2002). The following potential sources of heterogeneity were explored: study design type, study quality score, cut-off value for HER-2/neu overexpression, publication region, patients' number and publication year. Publication bias was assessed by Egger's and Begg's test (Begg and Mazumdar, 1994; Egger et al., 1997).

Results

Study selection and characteristics

Our literature search identified 1138 articles, including 465 from MEDLINE and 673 from EMBASE, of which 827 were excluded from analysis due to duplication of articles, non original articles, non human articles, or non English articles. The 311 remaining articles were first screened based on titles and abstracts, of which 265 were excluded from further analysis due to no available survival data. Upon reviewing the full text of the 46 remaining articles, we excluded another 29 articles because we could not calculate HR estimate due to incomplete information. Out of these excluded studies, one shared the same population with another study (Sun et al., 1995), two analyzed HER-2/neu expression in serum (Tsigris et al., 2002; Kovacevic et al., 2007), and patients number of HER-2/neu overexpression of one was less than three (Pappas et al., 2013). Finally, 17 eligible studies were included into this meta-analysis (Kay et al., 1994; Sun et al., 1995; Kapitanovic et al., 1997; Osako et al., 1998; Knosel et al., 2002; McKay et al., 2002; Rossi et al., 2002; Sanz-Casla et al., 2004; Essapen et al., 2004; Jesus et al., 2005; Uner et al., 2005; Schuell et al., 2006; Park et al., 2007; Kavanagh et al., 2009; Li et al., 2011; Conradi et al., 2013; Lim et al., 2013). Figure 1 shows a flowchart of the literature selection process.
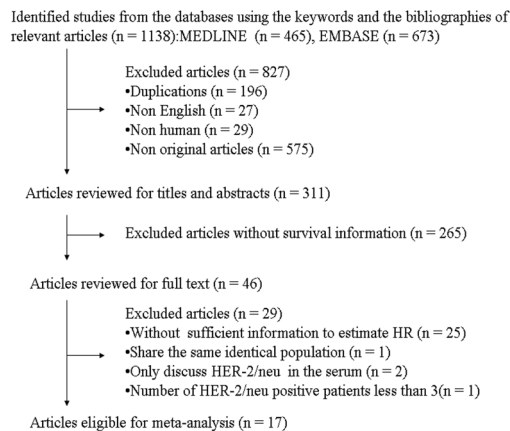
The main characteristics of the 17 eligible studies are reported in Table 1. The total number of patients from all studies was 2347 (range 45-317; median 106). Among these 17 studies, only two were prospective, the other 15 were retrospective studies. All of these studies used immunohisto-chemistry (IHC) method to determine HER-2/neu status in primary tumor, with five studies additionally performing silver in situ hybridization (SISH), fluorescent in situ hybridization (FISH) or comparative genomic hybridization (CGH). Various antibodies and different cut-off values were used for HER-2/neu overexpression. We divided the different cut-off values to "1+" and "2+" groups (10% was considered to be "1+" and 20%, 25%, 50% were considered to be "2+"). Five studies were conducted in Asian countries and 12 studies conducted in non Asian countries. As shown in Table 1, six of these 17 studies (35%) were identified as "negative"; nine (53%) were identified to be "not significant"; and only two (12%) were identified as "positive".

Quality assessment and publication bias

The median quality score of studies was five (range 2-8). We defined quality score more than six as high quality studies. As shown in Table 1, 12 out of 17 studies (71%) quality score was less than six, suggesting most studies were not well designed. Investigation of publication bias by a funnel plot showed funnel plots were symmetric (Figure 2). Egger's test and Begg's test suggests that
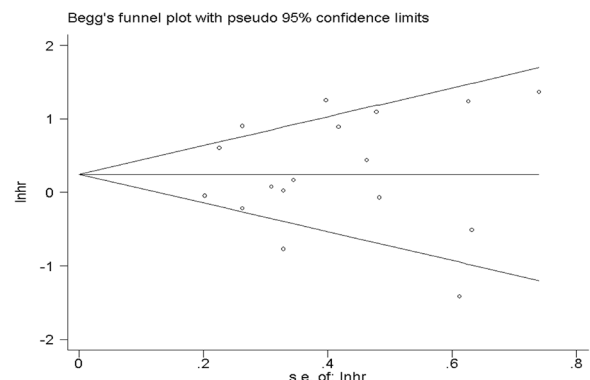

Figure 1. Flowchart Identification of Eligible Studies

Figure 2. Begg's Funnel Plots with Pseudo 95% Confidence Limits for Studies Evaluating HER-2/neu Status in Colorectal Cancer

Table 1. Main Characteristics and Results of the Eligible Studies

Author (year)	Country	P/R	Histology	Stage	Treatment method	N pts	Technique	Cut-off value	HR estimation	Results	Score
Conradi et al., (2013)	Germany	R	RC	I-IV	Surgery	264	IHC(+SISH)	2+	HR+CI	Positive	6
Lim et al., (2013)	South-Korea	P	CRC	I-IV	Surgery Serum	95	IHC(+FISH)	2+	Survival curves	Negative	5
Li et al., (2011)	China	R	CC	I-IV	Surgery	317	IHC	2+	Logrank+n events	NS	5
Kavanagh et al., (2009)	Ireland	R	CRC	I-IV	Surgery	106	IHC(+FISH)	2+	Survival curves	NS	3
Park et al., (2007)	South-Korea	R	CRC	I-IV	Surgery	137	IHC(+FISH)	2+	HR+CI	Negative	4
Schuell et al., (2006)	Austria	R	CRC	I-IV	Surgery	77	IHC	1+	Survival curves	NS	2
Jesus et al., (2005)	Brazil	R	CRC	I-IV	Surgery	108	IHC	1+	Survival curves	NS	4
Uner et al., (2005)	Turkey	R	CRC	I-IV	Surgery	69	IHC	20%	HR+CI	NS	4
Essapen et al., (2004)	UK	R	CRC	III	Surgery	105	IHC	20%	HR+CI	Positive	6
SanzCasla et al., (2004)	Spain	P	CRC	I-IV	Surgery	106	IHC	20%	HR+CI	NS	8
Knosel et al., (2002)	Germany	R	CRC	I-IV	Surgery	45	IHC(+CGH)	2+	Survival curves	Negative	4
Rossi et al., (2002)	USA	R	CRC	I-III	Surgery	155	IHC	25%	HR+CI	NS	7
Mckay et al., (2002)	UK	R	CRC	I-IV	Surgery	248	IHC	2+	Logrank+n events	NS	7
Osako et al., (1998)	Japan	R	CRC	I-IV	Surgery	102	IHC	20%	HR+CI	Negative	5
Kapitanovic et al., (1997)	Croatia	R	CRC	I-IV	Surgery	80	IHC	1+	Logrank+n events	Negative	3
Sun et al., (1995)	Sweden	R	CRC	I-IV	Surgery	169	IHC	1+	Survival curves	NS	3
Kay et al., (1994)	Ireland	R	CRC	II	Surgery	164	IHC	1+	HR+CI	Negative	5

*P: prospectively; R: retrospectively; CC: colon cancer; RC: rectal cancer; CRC: colorectal cancer; N pts: number of patients. IHC: immunohisto-chemistry; SISH: silver in situ hybridization; FISH: fluorescent in situ hybridization; CGH: comparative genomic hybridization; HR: hazard ratio; CI: confidence interval; NS: not significant

publication bias was not significant (*p*=0.70 for Egger's test, *p*=0.90 for Begg's test).

Meta-analysis

Using the DerSimonian-Laird random model, a meta-analysis of 17 studies showed that overexpression of HER-2/neu in primary tumor was not significantly associated with poor overall survival (pooled HR 1.31, 95%CI: 0.96-1.79) (Figure 3). Heterogeneity analysis showed significant heterogeneity between studies (I^2=68.4%, *p*=0.000). We performed subgroup analysis according to the potential sources of heterogeneity (Table 2). When we restricted the meta-analysis to study quality score≤5 (HR=1.56, 95%CI: 1.17-2.10), Asian patients (HR=1.74, 95%CI: 1.22-2.49), patients' number≤106 (HR=1.57, 95%CI: 1.01-2.44), publication year before 2003 (HR=1.59, 95%CI: 1.02-2.49), and prospectively designed study (HR=3.62, 95%CI: 1.42-9.24), the prognostic values of HER-2/neu for survival were statistically significant, showing a worse survival when HER-2/neu overexpressed. But there were still no significant prognostic values of HER-2/neu for survival when the analysis was limited to study quality scores>5

Figure 3 Forrest Plots of Hazard Ratio (HR) for the Association of HER-2/neu with Overall Survival. HR>1 implies a worse survival for the group with HER-2/neu overexpression. Figure symbols include : hazard ratio value of each study, proportion of each study is calculated from random effect model. ■: combined hazard ratio of the meta-analysis of the studied group; ◆the centre of the diamond gives the combined hazard ratio and its extremities the 95% confidence interval

(HR=0.69, 95%CI: 0.33-1.44), non Asian patients (HR=1.14, 95%CI: 0.77-1.70), patients' number>106 (HR=1.07, 95%CI: 0.67-1.72), publication year after 2003 (HR=1.13, 95%CI: 0.76-1.69), and retrospectively designed study (HR=1.22, 95%CI: 0.89-1.67), cut-off value as "1+" (HR=1.17, 95%CI: 0.69-1.97), cut-off value as "2+" (HR=1.43, 95%CI: 0.96-2.14). There was still significant heterogeneity (I^2>50%, *p*<0.10) between studies in most subgroups, indicating that not all sources of heterogeneity were identified (Table 2). However, in subgroups of prospectively designed study (I^2=0.00%, *p*=0.90) and study of Asian patients (I^2=19%, *p*=0.29), heterogeneity significantly decreased, which suggested that heterogeneity between studies might be partly explained by patient ethnicity and study design type.

Discussion

We performed this meta-analysis to determine the prognostic effect of HER-2/neu for patients with CRC. Our results suggest that overexpression of HER-2/neu in primary CRC tumor was not a statistically significant prognostic factor for survival. However, in subgroup analysis, there is a significant prognostic value of HER-2/neu on CRC, indicating the true prognostic value of HER-2/neu for survival needs further analysis. Adequately designed prospective studies, with large sample sizes and an appropriate statistical methodology, including multivariate analysis, are urgently needed in order to demonstrate the true effect of HER-2/neu on CRC prognosis.

Subgroup analysis of study quality scores≤5, Asian patients, patients' number≤106, prospectively designed study, and publication year before 2003 showed the prognostic value of HER-2/neu was significant, making the result of our meta-analysis limited. It is well known that studies with statistically significant results are more likely to be published than those not showing such an effect, resulting in fewer studies with "not significant" results being published. In addition, during our publication selection, several "not significant" studies with large samples were excluded due to lack of data to estimate the HR value (Lazaris et al., 1995; Ochs et al., 2004; Kountourakis et al., 2006; Molaei et al., 2009; Molaei et
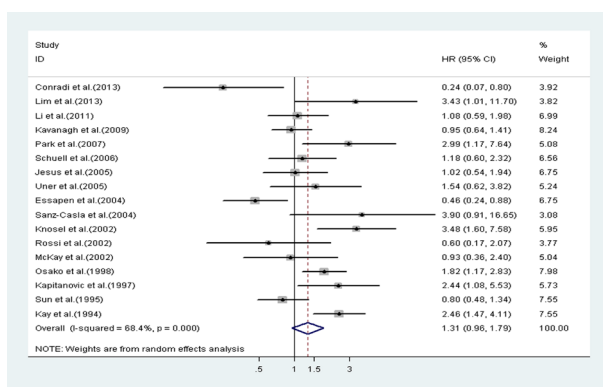
Table 2. Results of Subgroup Analysis

Subgroup factor	Divided standard	Number	Pooled HR (95%CI)[a]	I^2 value (%)	*p* value[b]
Study quality scores	Scores >5	5	0.69 (0.33-1.44)	60.30%	0.039
	Scores ≤5	12	1.56 (1.17-2.10)	60.10%	0.004
Cut-off values	1+	6	1.17 (0.69-1.97)	64.50%	0.002
	2+	11	1.43 (0.96-2.14)	71.30%	0.001
Regions	Asian	5	1.74 (1.22-2.49)	19%	0.294
	Non Asian	12	1.14(0.77-1.70)	72.90%	0
Patients' number	Number > 106	8	1.07 (0.67-1.72)	68.40%	0.002
	Number ≤ 106	9	1.57(1.01-2.44)	71.10%	0.001
Study design type	Prospective	2	3.62 (1.42-9.24)	0.00%	0.895
	Retrospective	15	1.22 (0.89-1.67)	69.40%	0
Publication year	Before 2003	7	1.59 (1.02-2.49)	67.50%	0.005
	After 2003	10	1.13 (0.76-1.69)	63.40%	0.003

*HR: hazard ratio; 95%CI: 95% confidence interval; [a]Pooled hazards ratios were obtained from using [a]DerSimonian-Laird random effects model. [b]*P* values obtained from χ^2-test for heterogeneity

al., 2009; Kruszewski et al., 2010). In our meta-analysis, two prospective studies and five studies of Asian patients revealed HER-2/neu to be a worse prognostic factor for CRC without significant heterogeneity. Therefore, the heterogeneity between studies might be partly explained by patient ethnicity and study design type. Based on our available data, HER-2/neu cannot be used as an independent prognostic factor for survival in clinical practice.

The techniques used to detect HER-2/neu status might also be potential sources of heterogeneity. In this meta-analysis, all of the included studies used IHC staining methods to determine HER-2/neu status in primary tumor with five studies additionally performed other methods. In order to reduce the potential heterogeneity, we only used the IHC staining results to determine HER-2/neu status. Although IHC staining is simple and cost-effective to perform, its results are susceptible to different tissue fixation methods and antibodies (Seidal et al., 2001). Various antibodies including CB11, 3B5 and AO 485 were used to assess HER-2/neu status in different studies. The different cut-off values determining the HER-2/neu overexpression might also be a potential source of heterogeneity. Six different cut-off values were used in various studies. We combined different cut-off values into two groups ("1+", "2+") and investigated the possibility of cut-off values serving as main source of heterogeneity. However, heterogeneities of both groups remained, indicating that it might not be a source of heterogeneity. A standardized method to determine HER-2/neu status is essential for future studies.

Although the significance of the HER-2/neu prognostic value is in dispute, overexpression of HER-2/neu in CRC may predict relatively worse clinical outcomes. The possible molecular biological mechanisms underlying this phenomenon have been well studied. For example, the HER-2/neu gene codes for a 185-kDa receptor type tyrosine protein kinesis similar to epidermal growth factor receptor (EGFR). HER-2/neu has been demonstrated to be associated with multiple biological processes, including regulation of normal cell growth and differentiation, signal transduction pathways, tumorigenesis and tumor progression (Iivanainen et al., 2003; Wingens et al., 2003; Xiong et al., 2011). Studies have shown that HER-2/neu can induce normalization and regression of the vasculature by modulating the effects of different anti-angiogenic factors (Izumi et al., 2002; Kara et al., 2012Ren et al., 2012;). Clinical researches have shown that expression of HER-2/neu correlates with Dukes stage of disease, liver metastasis and lymph node metastasis (Lazaris et al., 1995; Jesus et al., 2005). Patients with late Dukes stage disease, liver and lymph node metastasis are known to have a poor prognosis, which may partially explain the poor effect of HER-2/neu on survival.

Limitations of our meta-analysis include the considerable heterogeneity observed even in subgroup analysis. This indicates that not all sources of heterogeneity could be accounted for in our meta-analysis, and results should be interpreted with caution. We restricted our meta-analysis to published studies written in English only, which may result in language bias. In addition, many "not

significant" studies were excluded from our meta-analysis, leading to an overestimation of effect size. Other possible limitations include differences in calculation of HR and CI values: only eight of 17 articles provide HR and CI values, the other studies' HR and CI values were calculated as part of our meta-analysis. Finally, our meta-analysis was based on unadjusted estimates; a more precise estimate could be obtained using a multivariate analysis adjusting for clinicopathological variables.

In conclusion, overexpression of HER-2/neu in CRC may not predict a shorter overall survival, and cannot be used in clinical practice currently. An adequately designed prospective study in an appropriate multivariate analysis setting, taking into account the classical well defined prognostic factors, is urgently needed to confirm our meta-analysis result.

Acknowledgements

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100.0

6.3

75.0

56.3

50.0

25.0

31.3

0

Newly diagnosed without treatment