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ORIGINAL ARTICLE

Meta-analysis of the predictive value of KRAS mutations in treatment response using cetuximab in colorectal cancer

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Summary

Purpose: *The monoclonal antibody cetuximab that targets epidermal growth factor receptor (EGFR) has been found effective in the treatment of colorectal cancer. However, mutations in exons 12 and 13 of KRAS oncogene have been reported as negative predictive factors for the treatment response using cetuximab.*

The purpose of this study was to conduct a meta-analysis of the published studies investigating the predictive value of KRAS mutations in the efficacy of cetuximab in patients suffering from colorectal cancer.

Methods: *A systematic search of the literature was performed in PubMed, Medline, and Cochrane databases. Sensitivities, specificities and predictive values (negative and positive) of KRAS mutations as regards treatment response were calculated.*

Results: *Twenty-six studies were initially found during the literature search. After thorough evaluation, 13 papers were excluded for various reasons. Therefore, 13 studies were included in the present meta-analysis. In these studies, specificities were found much higher than sensitivities. Combining the data from the 13 studies, it was found that KRAS mutations comprise a negative predictive biomarker for response to cetuximab with very high specificity (0.96; 95% CI 0.84-0.99), and low sensitivity (0.47; 95% CI 0.43-0.50). Finally, the publication bias was found statistically significant.*

Conclusion: *The results of the present meta-analysis suggest that cetuximab should be administered only to patients with colorectal cancer who have the wild type (KRASw) oncogene. Mutations in the KRAS gene are a negative predictive factor for response to cetuximab with very high specificity and low sensitivity. The latter may very well be attributed to additional mechanisms of resistance to anti-EGFR therapies such as mutations in BRAF.*

Key words: cetuximab, colorectal cancer, KRAS, K-RAS, meta-analysis, predictive value

Introduction

Colorectal cancer is a major public health problem and one of the main causes of death from cancer in the western world. It is the second most common malignancy in the developed countries. Approximately 700,000 new cases are being diagnosed and 400,000-500,000 deaths from colorectal cancer are being reported each year worldwide [1].

EGFR signal transduction pathway is frequently involved in colorectal cancer. This pathway has been thoroughly investigated as a target for anticancer therapy [2]. Treatment agents such as cetuximab and panitumumab are monoclonal antibodies that target EGFR. Cetuximab is a IgG1 chimeric monoclonal antibody that exhibits high affinity with EGFR and its heterodimers. When cetuximab locks onto EGFR it inhibits the attachment of other proteins and induces endocytosis of the receptor. This way, it inhibits the dimerisation of the receptor and, consequently, the signal transduction. As a result, the proliferation of tumor cells stops, as well as both angiogenesis and metastasis, and tumor cells are led to apoptosis [3]. Cetuximab has been shown to be effective in the treatment of colorectal cancer [4].

Point mutations in the codons 12 and 13 of KRAS are predictive of poor prognosis or of non-responsive-

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ness to anti-EGFR therapies [5]. KRAS encodes a small GTP-binding protein which acts as a signal transducer after bonding to its receptor and activating the EGFR-protein complex on the cell surface. When KRAS oncogene is mutated, its product (i.e. the KRAS protein) stays activated regardless of the activation of EGFR [6]. KRAS mutations can induce downstream signal transduction and cause resistance to the upstream inhibition of EGFR by the monoclonal antibodies. KRAS mutations are early events in the development of colorectal cancer and are present in about 40% of the patients [7].

The aim of this study was to perform a bivariate meta-analysis of the studies that examined the predictive value of KRAS mutations with regard to the effectiveness of cetuximab in colorectal cancer.

Methods

Initially, a systematic electronic search was conducted in the PubMed and Medline databases, as well as in the Cochrane library, to retrieve published papers that had studied patients with colorectal cancer treated with cetuximab and had evaluated the effectiveness of this agent in relation to KRAS condition (KRASw or mutant - KRASm). The limitation regarding the time of publication was until May 2009. The following combination of keywords has been used: "cetuximab", "anti-EGFR", "KRAS or K-RAS", and "colorectal cancer".

Statistical considerations

A valid meta-analysis of the studies that involve diagnostic examinations requires statistical techniques that analyse pairs of related cumulative statistical parameters (such as sensitivity and specificity) and not a simple statistical parameter. In the present meta-analysis, the sensitivity and specificity were analysed simultaneously, using a 2-dimensional random-effects model.

The data from each study were inserted in 2×2 tables. From these tables, the sensitivity, specificity, and predictive (negative and positive) value were calculated. Sensitivity and specificity are statistical measures of the performance of a binary classification test. Sensitivity measures the proportion of the true positive values that are correctly identified as such (i.e. the percentage of patients who are correctly identified as having the disease), whereas specificity measures the proportion of the true negative values that are correctly identified as such (i.e. the percentage of disease-free people who are correctly identified as not having the specific disease). Defining as *tp* the true positives, as *fp* the false positives, as *fn* the false negatives, and as *tn* the true negatives, sensitivity can be calculated as:

$$sensitivity = \frac{tp}{tp + fn},$$

and specificity as:

$$specificity = \frac{tn}{fp + tn}$$

Usually, sensitivity and specificity are transformed using the logit transformation in order to be analysed properly in a meta-analysis using standard fixed or random effects methods [8,9]. Since they are independent in each study (i.e. sensitivity is calculated from tp and fn, whereas specificity from fp and tn), two separate meta-analyses could be performed using standard methods [8,9]. However, these measures are likely to be correlated in the various studies, that is their random effects can be correlated. Hence, a bivariate approach for meta-analysis is more appropriate [10,11]. Several alternative methods have been proposed [12-14] but recent studies [15,16] have found them to be equivalent statistical-wise. In the present meta-analysis, the bivariate approach based on the binary data was used [17]. Separate diagnostic likelihood ratios (LR^+ or LR^-) were not calculated as the modern approaches do not suggest it [18]. However, we calculated the diagnostic odds ratio (dOR) that compares the odds for sensitivity to the odds for specificity [19] as:

$$dOR = \frac{sens/(1-sens)}{(1-spec)/spec} = \frac{(tp)(tn)}{(fp)(fn)} = \frac{LR^+}{LR^-}$$

The *dOR* can be pooled in a meta-analysis using standard methods [8,9]. However, the bivariate approach offers the additional possibility of obtaining estimates by appropriately transforming the output estimates [15,16]. As *dOR* measures the discrimination capability of the test, since it combines the values of sensitivity and specificity in a single measure, it should exhibit less heterogeneity in case some studies are optimized towards higher sensitivity whereas others towards higher specificity. A summary Receiver's Operator Characteristic (SROC) curve was also calculated from the estimates of the bivariate model as suggested by Littenberg and Moses [20,21].

The bivariate (HSROC) method is, statistically, the most rigorous and can be used to provide areas of acceptance and prediction, and a cumulative ROC curve in addition to the cumulative sensitivity and specificity.

Publication bias and other small-study related bias were initially evaluated using the rank correlation method of Begg and Mazumdar [22], the Egger's et al. fixed effect regression method [23], and the random effects analogue [24] using the logarithm of *dOR* as the appropriate effect size. The most important bias that has to be assessed in a meta-analysis is the publication bias, which is attributed to the difficulties in finding all related studies. The publication bias emerges because, firstly, most meta-analyses are based on published data and studies and, secondly, because most scientists believe that it is easier to publish studies that have a significant conclusion in comparison to those that have not. The results of the estimations of the possible publication bias are very important for the integrity of the conclusions of a meta-analysis.

The statistical analysis in the present study used the statistical package Stata 10 (StataCorp) and the statistical significance was set to $p<0.05$.

Results

Twenty-six studies were initially found in the literature search that had been published before May 2009. After thorough evaluation, 13 papers were excluded for the following reasons: 5 were reviews, 5 just referred to EGFR and its polymorphisms, 2 referred to PIK3CA/PTEN mutations, and 1 paper had assessed patients' quality of life. Therefore, 13 studies were finally included in the present meta-analysis. In Table 1, the abbreviations tp, fp, tn, and fn are defined for the two conditions of the KRAS gene in relation to the response to cetuximab, whereas in Table 2 the main characteristics and results of the studies included in this meta-analysis are illustrated.

Table 1. Definitions of true positive (tp), false positive (fp), true negative (tn), and false negative (fn) for both conditions of KRAS gene in relation to response to cetuximab

KRAS condition	*No response to cetuximab*	*Response to cetuximab*
Mutant KRAS	tp	fp
Wild type KRAS	fn	tn

The results of the meta-analysis regarding sensitivity and specificity are illustrated in Figures 1 and 2, respectively. It is worth mentioning that, in the papers included in the present meta-analysis, specificity was much higher than sensitivity. Combining the data of the 13 papers, sensitivity was found to be 0.47 (95%CI 0.43-0.50) and specificity 0.96 (95%CI 0.84-0.99). In the forest plot of Figure 2, the 3 last studies on the left in the plot [25,27,29], have lower specificities and confidence intervals. Due to their size these 3 studies significantly affected the results of the present meta-analysis (studies 1, 2, and 3 in Table 2).

In Figure 3 the SROC curves of the meta-analysis are illustrated. The curves have been calculated from the estimates of the 2-dimensional model [20,21]. As illus-

Table 2. Studies included in the present meta-analysis that examine the predictive value of KRAS gene in the treatment of colorectal cancer using cetuximab

No	*Study*	*Journal*	*Pts*	*KRASw*	*w_RR %*	*fn*	*tn*	*KRASm*	*m_RR %*	*tp*	*fp*
1	Van Cutsem et al (2009) [25]	NEJM	277	172	59.30	70	102	105	36.20	67	38
2	Bokemeyer et al (2009) [26]	JCO	113	61	61	24	37	52	33	35	17
3	Tol et al (2009)[27]	NEJM	256	158	61.40	61	97	98	45.90	53	45
4	Bibeau et al (2009) [28]	JCO	64	37	27	27	10	27	3.70	26	1
5	GarmSpindler et al (2009) [29]	Ann Oncol	64	42	40	25	17	22	0	22	0
6	Perrone et al (2009) [30]	Ann Oncol	29	22	45.45	12	10	7	0	7	0
7	Karapetis et al (2008) [31]	NEJM	198	117	12.80	102	15	81	1.20	80	1
8	Lievre et al (2008) [32]	JCO	89	65	40	39	26	24	0	24	0
9	De Roock et al (2008) [33]	Ann Oncol	108	66	41	39	27	42	0	42	0
10	Di Fiore et al (2007) [34]	BJC	59	37	32	25	12	22	0	22	0
11	Khambata-Ford et al (2007) [7]	JCO	80	50	10	45	5	30	0	30	0
12	Frattini et al (2007) [35]	BJC	27	17	53	8	9	10	10	9	1
13	Lievre et al (2006) [5]	Cancer Res	30	17	64.70	6	11	13	0	13	0

KRASw: the number of patients with wild type KRAS, KRASm: the number of patients with mutant KRAS, w_RR: the percentage of patients with wild type KRAS who responded to cetuximab, m_RR: the percentage of patients with mutant KRAS who responded to cetuximab. For other abbreviations see Table 1.

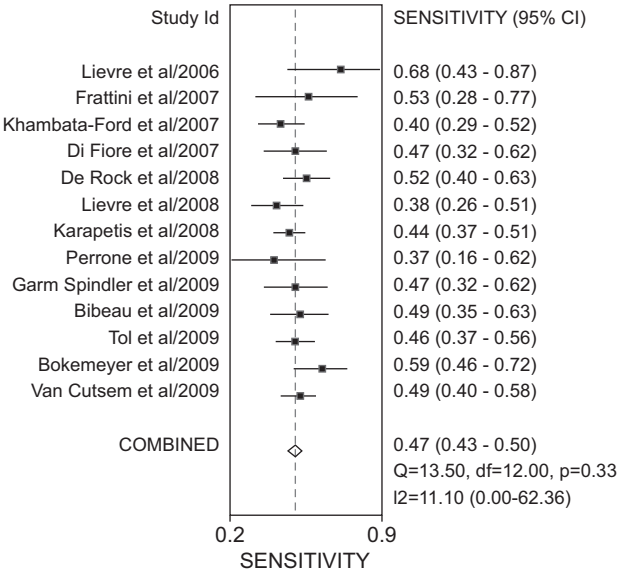

Figure 1. Forest plot illustrating the sensitivities of the predictive value of KRAS condition in relation to the effectiveness of cetuximab in colorectal cancer. The sensitivity in each study is reported, as well as the overall pooled sensitivity.

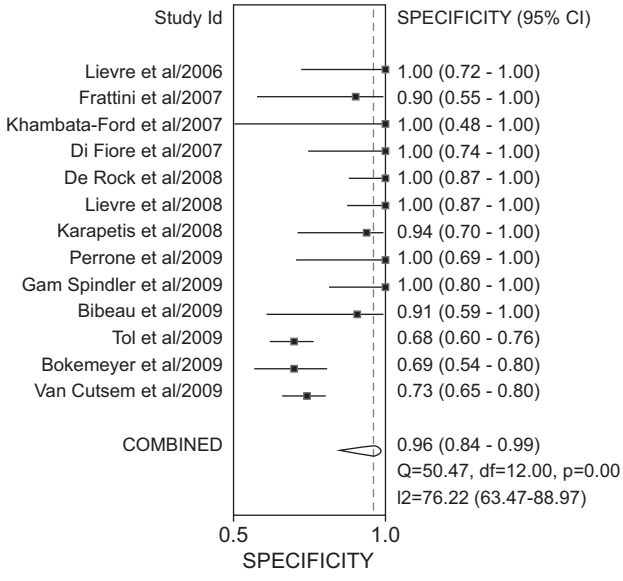

Figure 2. Forest plot illustrating the specificities of the predictive value of KRAS condition in relation to the effectiveness of cetuximab in colorectal cancer. The specificity in each study is reported, as well as the overall pooled specificity.

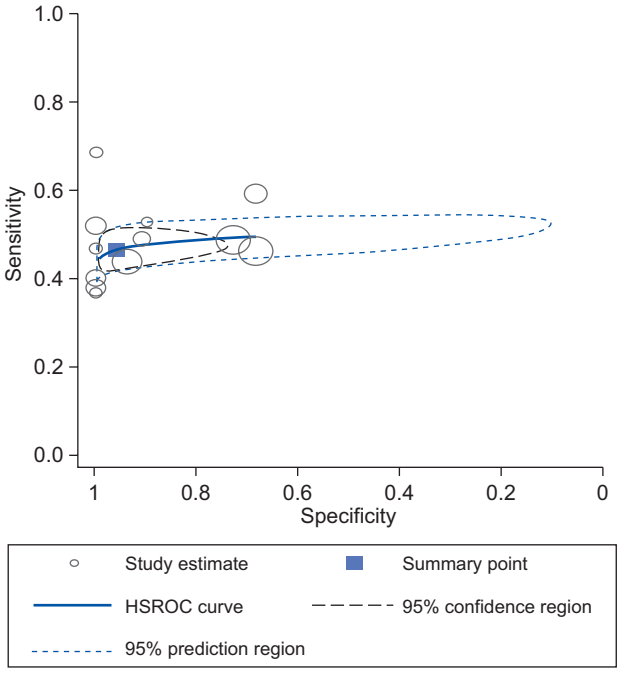

Figure 3. SROC curves illustrate that sensitivity exhibits small heterogeneity, while specificity exhibits large heterogeneity. The findings above are evident in the 3 studies already mentioned (studies in Table 2: 1, 2 and 3).

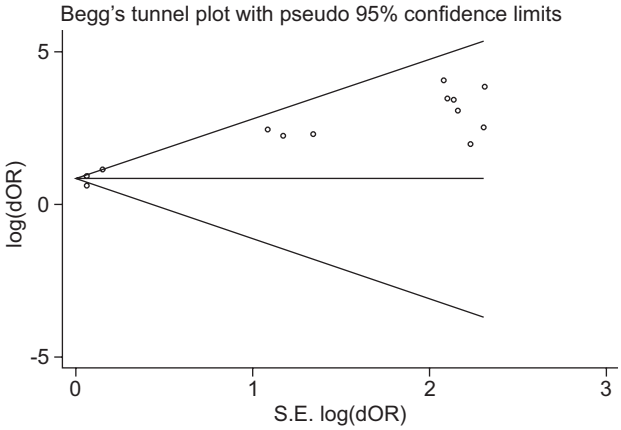

Figure 4. The funnel plot for the combined sample of the 13 studies using the Begg's method. There is an asymmetry in the graph as almost all studies (except one) are in the upper half of the graph.

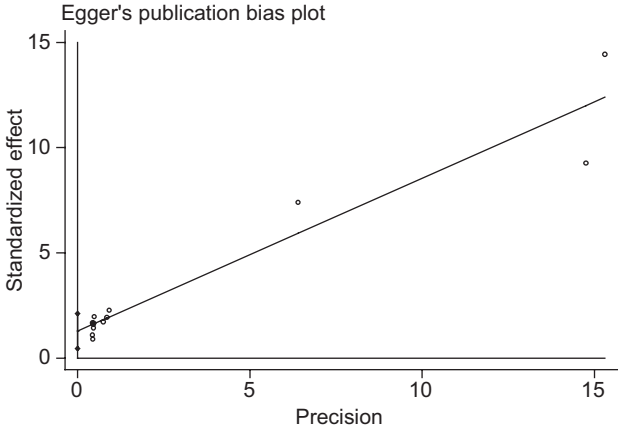

Figure 5. The plot for the publication bias from the cumulative sample of the 13 studies using Egger's method.

trated in the SROC curves, all studies exhibit low sensitivity and high specificity. In brown presented is the cumulative SROC curve with 95%CI. In green presented is the cumulative predictive SROC curve, usually found under the studies of the meta-analysis.

In the present meta-analysis the results of the estimations of the publication bias are illustrated in Figures 4,5 and in Tables 3,4. The results, using both methods, are statistically significant for the presence of publication bias.

Discussion

Colorectal cancer is one of the most common cancers worldwide with KRAS mutations also commonly observed (30-40%) [36,37]. Changes in the codons 12 and 13 of KRAS are predictive markers of anti-EGFR treatments. When KRAS is mutated, its product, KRAS protein, remains constantly activated, independently of the activation of EGFR [38]. KRAS mutations can activate a downstream transporting signal (downstream signal transportation) and by this way attack the upstream inhibition of EGFR from monoclonal antibodies.

Table 3. Begg's test results

Adj. Kendall's score (P-Q) =	–28
SD of score =	16.39
Number of studies =	13
z =	–1.71
Pr> |z| =	0.088
z =	1.65 (continuity corrected)
Pr> |z| =	0.100 (continuity corrected)

SD: standard deviation

Table 4. Egger's test results

Std Eff	*Coef*	*SE*	*t*	*P>|t|*	*95% CI*	
slope	0.7262736	0.0612556	11.86	0.000	0.591451	0.8610962
bias	1283776	0.3785615	3.39	0.006	0.4505681	2.116.985

Std Eff: standard effect, SE: standard error, CI: confidence interval, Coef: co-effect

The first outcome-conclusion of this meta-analysis is that KRAS mutations constitute a negative predictive marker/bio-indicator in relation to the anti-EGFR monoclonal antibody cetuximab when given separately or when combined with chemotherapy in patients with colorectal cancer. The specificity of this negative predictive factor is very high (0.96; 95% CI 0.84-0.99), whereas the sensitivity is rather low (0.47; 95%CI 0.43-0.50). The most likely interpretation of this low sensitivity is an additional resistance mechanism in anti-EGFR therapeutic approaches. Today, there are some alternative research targets exploring the potential resistance mechanisms to these treatments apart from KRAS mutations. Regarding cetuximab, these potential biomarkers include mutations of BRAF, PIK3CA or PTEN and the expression of ligands of EGFR epiregulin and/or amphiregulin [38,39]. However, these mutations may become important within the framework of mutations that affect the EGFR pathway. Therefore, future approaches attempting to predict the sensitivity to anti-EGFR treatment should include the analysis of the whole network of the signal transduction rather than isolated mutations.

The second most important conclusion of the present meta-analysis is the high probability of publication bias occurrence. This was initially shown by the lack of symmetry in the funnel plots of the 13 studies. Moreover, the results of the publication bias assessment, based on the Begg and Egger method, were positive and statistically significant. In a survey, in which 28 meta-analyses of diagnostic examinations were evaluated with regard to publication bias using different statistical methods, it was found that smaller studies were related with results of higher diagnostic accuracy concerning the test under investigation [40]. Further analysis revealed that the smaller the literature search and the number of initial studies, the larger the asymmetry of the funnel plots in meta-analyses.

The publication bias appears because the published studies do not sufficiently represent all the studies conducted on a specific topic. Many factors may cause this bias, but the most well-known is the tendency to publish only studies that have statistically significant ($p<0.05$) or clinically important results. Other contributing factors are the size of the sample, the design of the study, the funding, the potential conflict of interest, and finally any prejudice by the researchers for an observed correlation. In case the effect of the publication bias cannot be clarified, the results of the literature review should be interpreted with caution.

A previous meta-analysis of KRAS mutations and the resistance to anti-EGFR treatment methods (monoclonal antibodies and tyrosine kinase inhibitors) in advanced non small-cell lung cancer and metastatic colorectal cancer found similar results [41]. The results of this meta-analysis were that the overall sensitivity was rather low (0.47; 95%CI 0.43-0.52), whereas the specificity quite high (0.93; 95%CI 0.83-0.87), with regard to the prediction of resistance to anti-EGFR monoclonal antibodies.

KRAS mutations comprise a negative predictive factor for the response to cetuximab with very high specificity and low sensitivity. The clinical importance of these findings is that cetuximab should be administered only to patients who have KRASw. As a result, KRAS testing is now mandatory when colorectal cancer patients are examined for the presence of metastatic disease. The low value of sensitivity is probably due to the existence of additional mechanisms of resistance to anti-EGFR therapies, such as mutations in BRAF (possibly in PIK3CA, EGFR and PTEN) and the expression of the EGFR ligands epiregulin and amphiregulin.

In conclusion KRAS mutational status represents a paradigm of a predictive biomarker. However, the low sensitivity shows that a long way is still ahead in the era of targeted therapies and personalized cancer treatment.

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