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Review Article

Meta-analysis of serum gastrin-releasing peptide precursor as a biomarker for prognostic evaluation of small cell lung cancer

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Abstract: Background and aims: The clinical value of gastrin-releasing peptide precursor (Pro-GRP) in small cell lung cancer (SCLC) remains controversial. This meta-analysis aimed to investigate the prognostic and recurrence-related significance of Pro-GRP in SCLC. Methods: The association between Pro-GRP expression and clinical significance was measured by odds ratios (ORs) using Review Manager. Pooled odds ratios (ORs) of Pro-GRP and survival time were calculated to measure the predictive value of the Pro-GRP level with respect to prognosis. Results: The serum levels of Pro-GRP were significantly associated with chemotherapy response, survival time, and the disease stage. After chemotherapy, patients with complete remission (CR), patients with partial remission (PR) and the effective group, which included CR+PR, PR+CR+SD, and PR+NC, showed a marked decrease in Pro-GRP concentrations ($P<0.05$). Compared with other chemotherapy responses, the levels of Pro-GRP in the above groups of patients showed significant changes before and after chemotherapy. The serum Pro-GRP or Pro-GRP levels in the progressive disease (PD) group were significantly lower than the post-treatment levels ($P<0.00001$), while the Pro-GRP concentration showed no significant difference in the stable disease (SD) group ($P=0.76$). In studies evaluating the survival time, Pro-GRP levels were associated with the survival time ($P<0.00001$). When the level of Pro-GRP was higher, the survival time was shorter, but there was no statistically significant difference for six months of survival time ($P=0.29$). The survival time for patients with low Pro-GRP levels was longer than for patients with elevated levels (12 months: $P=0.0003$; 24 months: $P<0.00001$). A significant difference between the limited disease (LD) group and the extensive disease (ED) group was found. The mean serum levels of Pro-GRP were significantly higher in ED patients than in LD patients ($P=0.02$). Conclusion: Pro-GRP has become a new blood biomarker and can be used to monitor the prognosis and progression of SCLC.

Keywords: Meta-analysis, prognosis, clinical value, SCLC, odds ratios

Introduction

Lung cancer is the leading cause of cancer death worldwide [1]. SCLC accounts for approximately 25% of all lung cancer [2]. SCLC exhibits rapid growth, invasive malignant tumors, sensitivity to chemotherapy and radiotherapy, and ease of relapse [3]. Patients with SCLC are prone to recurrence. In recent years, the 5-year survival rate has remained unchanged [4]. By the time many patients are diagnosed with SCLC, tumor metastasis to local lymph nodes or distant organs has already occurred. Therefore, early diagnosis is particularly important and will be expected to improve patient survival because SCLC is highly sensitive to chemotherapy and radiotherapy [5].

Most patients have been found in clinical practice to have advanced-SCLC. Serum tumor markers have been widely used by clinicians for the early screening of cancers, and studies have confirmed the role of serum tumor markers in the early diagnosis, prognosis and follow-up of lung cancer [6-9]. In recent years, studies on tumor markers in lung cancer have been widespread, but they lack specificity for SCLC malignancies [10-12]. This study demonstrated

that the serum levels of Pro-GRP reflect the disease course of SCLC patients most accurately. When more effective treatment programs for recurrent SCLC are available, serial Pro-GRP measurements may become more crucial [13]. Pro-GRP is a recently discovered blood marker in SCLC. It may also be useful as an SCLC biomarker for therapeutic monitoring [14]. In addition, Pro-GRP has a high degree of sensitivity and specificity, with values of 60-70% and 96%, respectively [15]. Furthermore, it has been widely used in clinical practice and significantly improved the early detection rate of SCLC. Therefore, it can be used as a potential tumor marker for SCLC [16].

There are many articles on the meta-analysis of the diagnostic value of tumor markers for Pro-GRP in SCLC, as well as the meta-analysis of its sensitivity and specificity, but there are few articles on the meta-evaluation of its prognostic efficacy. The aim of this study is to provide a meta-analysis and systematic review of Pro-GRP in assessing the prognostic efficacy of SCLC.

Materials and methods

Literature search

The PRISMA statement (Supplementary Checklist 1) was used in our meta-analysis. We comprehensively searched the Cochrane Library, OVID, PubMed, Web of Science databases and China National Knowledge Infrastructure (CNKI) until May 10, 2016, without language and publication restrictions. The key words in the search were "Pro-gastrin-releasing peptide OR Pro-GRP OR pro gastrin releasing peptide OR Human Pro-GRP OR Human pro-gastrin-releasing peptide OR pro gastrin releasing peptide" and "Small lung tumor OR small lung neoplasm OR small lung cancer OR small lung carcinoma". In addition, we reviewed the relevant research articles and references to supplement our search. Searches were performed using Oncomine and The Cancer Genomic Atlas (TCGA) so that our data were sufficient. H. Xiang and S. Zhang searched the databases independently to obtain the raw data.

Selection criteria

Studies were included if they met the following criteria: (a) participants were diagnosed with SCLC, and the prognostic significance of Pro-GRP expression in SCLC was tested; (b) all participants were treated with chemotherapy, and the connection of Pro-GRP with survival outcomes or clinical parameters was analyzed; (c) at least 30 patients were registered, with non-small cell lung cancer patients or healthy individuals as controls; and (d) the Pro-GRP expression levels before and after chemotherapy were available. The titles and abstracts were independently read by two researchers (H. Xiang and S. Zhang), and unrelated studies were excluded; then our review team checked the full text and obtained the necessary data.

Data extraction

H. Xiang and S. Zhang independently extracted the following data: first author, year of publication, tumor stage, Pro-GRP detection method, number of patients with high and low Pro-GRP expression, and survival data types. Multivariate analysis was selected because it takes confounding factors into consideration and thus is more accurate [17]. If the article did not report the HR, we used Engauge Digitizer version 4.1 (free software download http://sourceforge.net) to read the Kaplan-Meier survival curve to obtain the HR and its 95% confidence interval (CI). Two independent authors (H. Xiang and S. Zhang) examined the curves to reduce reading variability. If the data in the article were insufficient, were disputed, or contained any other uncertainty that might be relevant to our meta-analysis, we provided more information to the relevant author.

Quality assessment

For NRCCTs, we used the Methodological Index for Non-Randomized Studies (MINORS) guidelines [18] to assess the methodological quality. These guidelines list 12 indices for comparative studies: (i) a clearly stated aim, (ii) inclusion of consecutive patients, (iii) prospective collection of data, (iv) endpoints appropriate to the aim of the study, (v) unbiased assessment of the study endpoint, (vi) a follow-up period appropriate to the aim of the study, (vii) <5% loss to follow-up, (viii) prospective calculation of the study size, (ix) an adequate control group, (x) contemporary groups, (xi) baseline equivalence of the groups, and (xii) adequate statistical analyses. The items are scored 0 (not reported), 1 (reported but inadequate), or 2 (reported

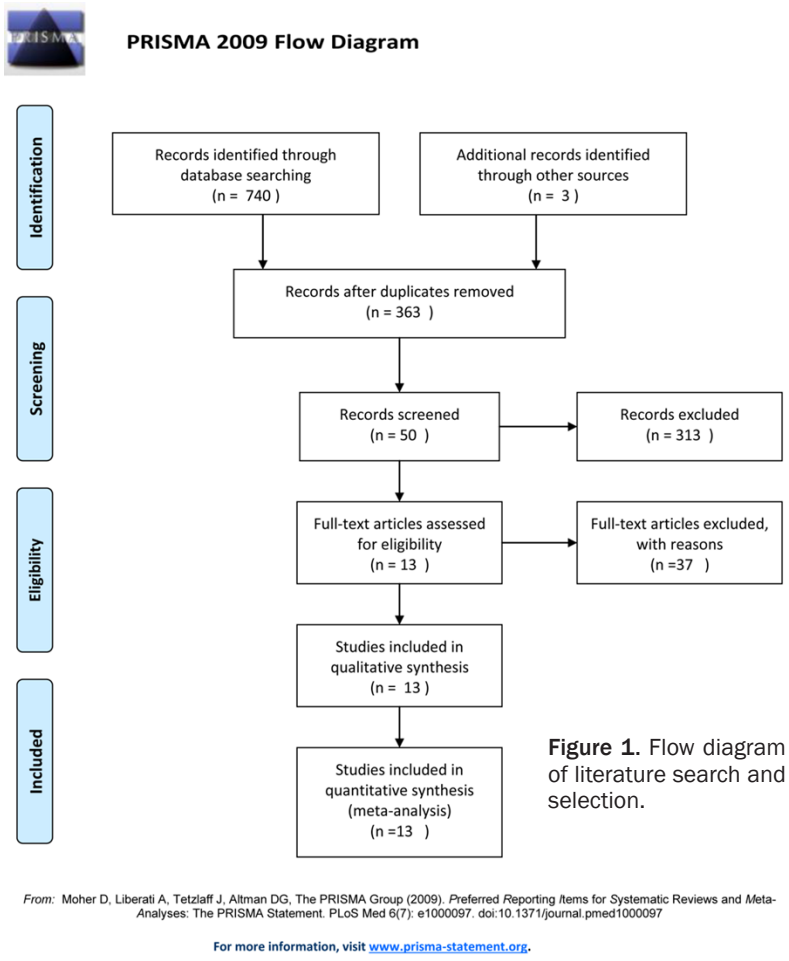

Figure 1. Flow diagram of literature search and selection.

and adequate). The global ideal score for comparative studies is 24. A score ≥16 points indicates high quality, whereas a score <16 points indicates low quality. Two authors (H. Xiang and S. Zhang) independently assessed the quality. Inconsistencies were discussed with two reviewers (Z. Fang and Y. Li), who acted as arbiters.

Statistical analysis

The relationship of Pro-GRP expression to the survival of patients with SCLC was measured by odds ratios (ORs) and 95% confidence interval (CI). We obtained data from the Kaplan-Meier survival curve using digitized version 7.2 if there was no direct data study [19]. In addition, *P* values <0.05 were considered statistically significant. Two authors (H. Xiang and S. Zhang) checked the curves independently to reduce reading variability. The heterogeneity among the studies was measured using Cochran's Q test and the Higgins I-squared statistic. Random-effects models were chosen to avoid the effects of heterogeneity. All statistical analysis was performed using the Review Manager Version 5.1 software (http://ims.cochrane.org/revman).

Results

Characteristics of included studies

After the primary literature search in the databases, 740 studies were reviewed. Moreover, there were 3 studies found when the authors examined additional records identified through other sources. A total of 363 studies remained after excluding duplicate studies. The investigators carefully read the titles and abstracts and then excluded 313 irrelevant studies. Next, the full texts of the remaining articles were reviewed in detail. There were 13 studies included in our meta-analysis (**Figure 1**, **Table 1**) [5, 20-31]. The studies were published from 2003 to 2016. There were 836 participants from China, Japan, and Europe. All patients showed high expression of gastrin-release peptide precursor. Ten studies measured the concentration of Pro-GRP by immunosorbent assay (ELISA) [5, 20-24, 27, 29-31], two studies used an electro-chemiluminescence immunoassay (ECLI) [25, 28], and only one study used ARCHITECT [26]. According to the MINORS quality assessment method, all studies included in this meta-analysis failed to meet the requirements in indices (vi), (vii), (viii), and (xi). **Table 1** shows that all 12 studies were of high quality based on MINORS. The basic characteristics (e.g., age, assay, treatment response, cut-off and follow-up) of each study in our meta-analysis are described in **Table 1**.

Meta-analysis for prognostic value

Overall, eight studies showed that Pro-GRP levels decreased significantly after treatment [20,

Table 1. Studies included in meta-analysis

Study	Year	Region	Total subjects (Male/Female)	Age	Assay	Treatment Response	LD Subjects	ED Subjects	Cut-off (value)	Follow-up	MINORS scores
Joachim Schneider	2003	*Germany*	34 (26/8)	61±5.1	ELISA	CR/PR/SD/PD	13	21	46 pg/ml	20 weeks	18
Y Li	2015	China	48 (31/17)	57±6.7	ECLI	CR+PR/SD+PD	10	38	30.7 ng/ml	NR	16
Ewa Wojcik	2008	*Poland*	64 (NR)	NR	ELISA	CR+PR/PD	64	NR	49.0 ng/l	3-6 month	18
Takashi Hirose	2011	Japan	103 (86/17)	NR	ELISA	CR/PR	14	89	50 ng/ml	Death/2 years	16
Marina Petrovic	2014	Serbia	97 (62/35)	62±6.8	ELISA	NR	50	47	58 pg/ml	13 months	18
Z. Huang	2016	China	122 (92/30)	NR	ELISA	CR+PR/SD/PD	54	68	43 ng/l	12 months	18
Y Lin	2016	China	45 (31/14)	41.5±3.1	ECLI	CR/PR/NC/PD	NR	NR	69.2 pg/ml	NR	14
X Qian	2016	China	97 (65/32)	61±5.3	ELISA	CP/PR/SD/PD	NR	NR	NR	NR	16
D Li	2016	China	60 (41/19)	63±10	ELISA	CR+PR+SD/PD	38	22	64.68 pg/ml	NR	16
Naumnik W	2004	*Poland*	39 (35/4)	64±12.3	ELISA	PR+NC/PD	9	10	NR	NR	16
Benjamin Nisman	2016	Israel	52 (NR)	NR	ARCHITECT	CR+PR/SD+PD	14	38	140 pg/ml	NR	16
Takuo Shibayama	2001	Japan	47 (41/6)	63±9.75	ELISA	CR+PR/ CR	26	21	49 pg/ml	NR	16
Takuji Okusaka	1997	Tokyo	44 (36/8)	65.5±10.25	ELISA	CR/PR/NC/PD	20	24	NR	11.4 months	18

NR, not reported; ELISA: enzyme-linked immunosorbent assay; ECLI: electro chemiluminescence immunoassay; ARCHITECT: chemiluminescent microparticle immunoassay; CR: complete remission; PR: partial remission; PD: progressive disease; SD: stable disease; NC: no change.

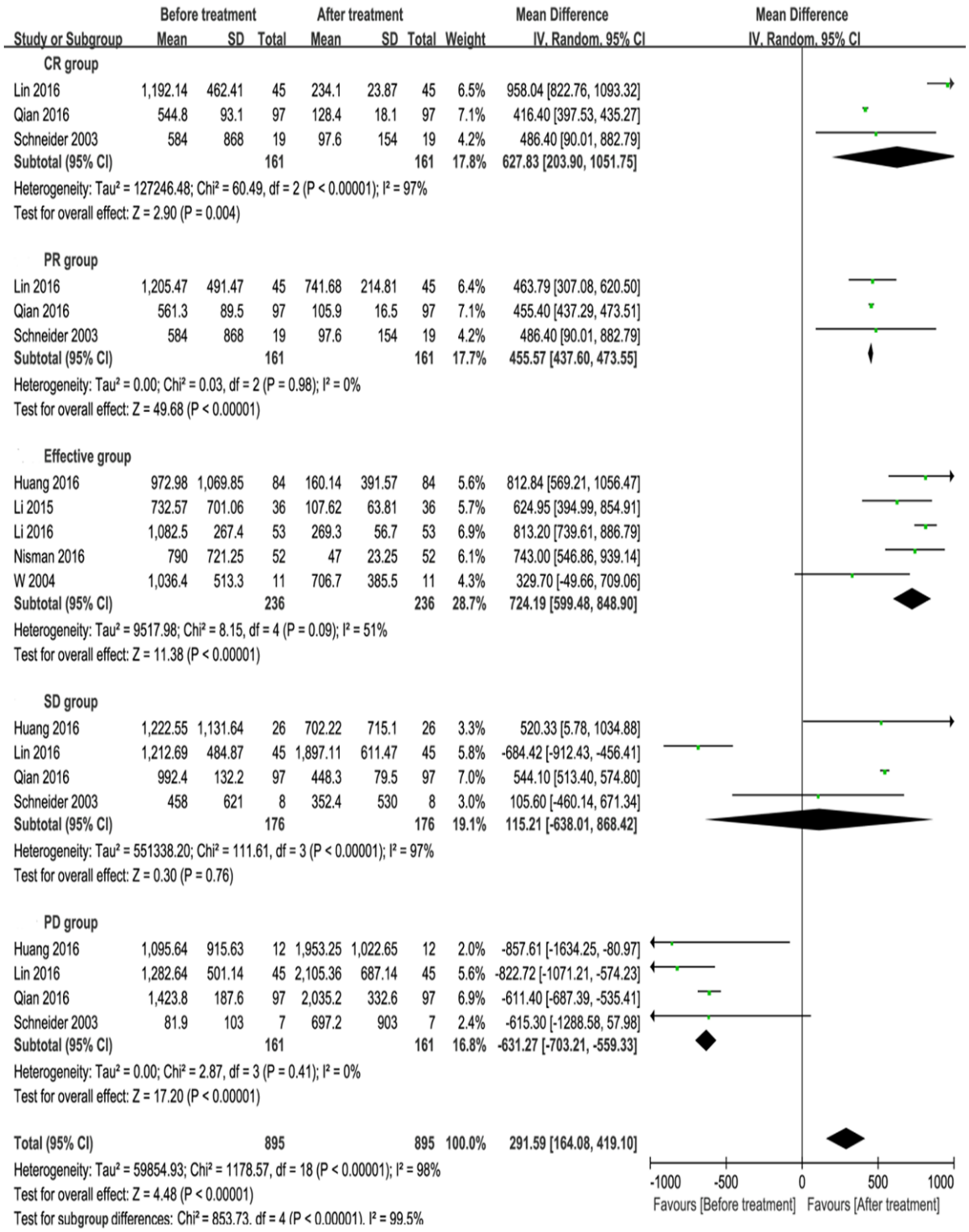

Figure 2. The serum levels of Pro-GRP in different groups before and after treatment.

21, 25-30] (MD: 291.59, 95% CI: 164.08-419.10, P<0.00001). For patients in the CR group, PR group, and effective group, the Pro-GRP concentration after chemotherapy was significantly decreased from the value before chemotherapy, so Pro-GRP can become a reliable tumor marker to evaluate the effectiveness of treatment in disease monitoring. However, there was obvious heterogeneity (I^2=98%, P<0.00001) (**Figure 2**). The combined analysis

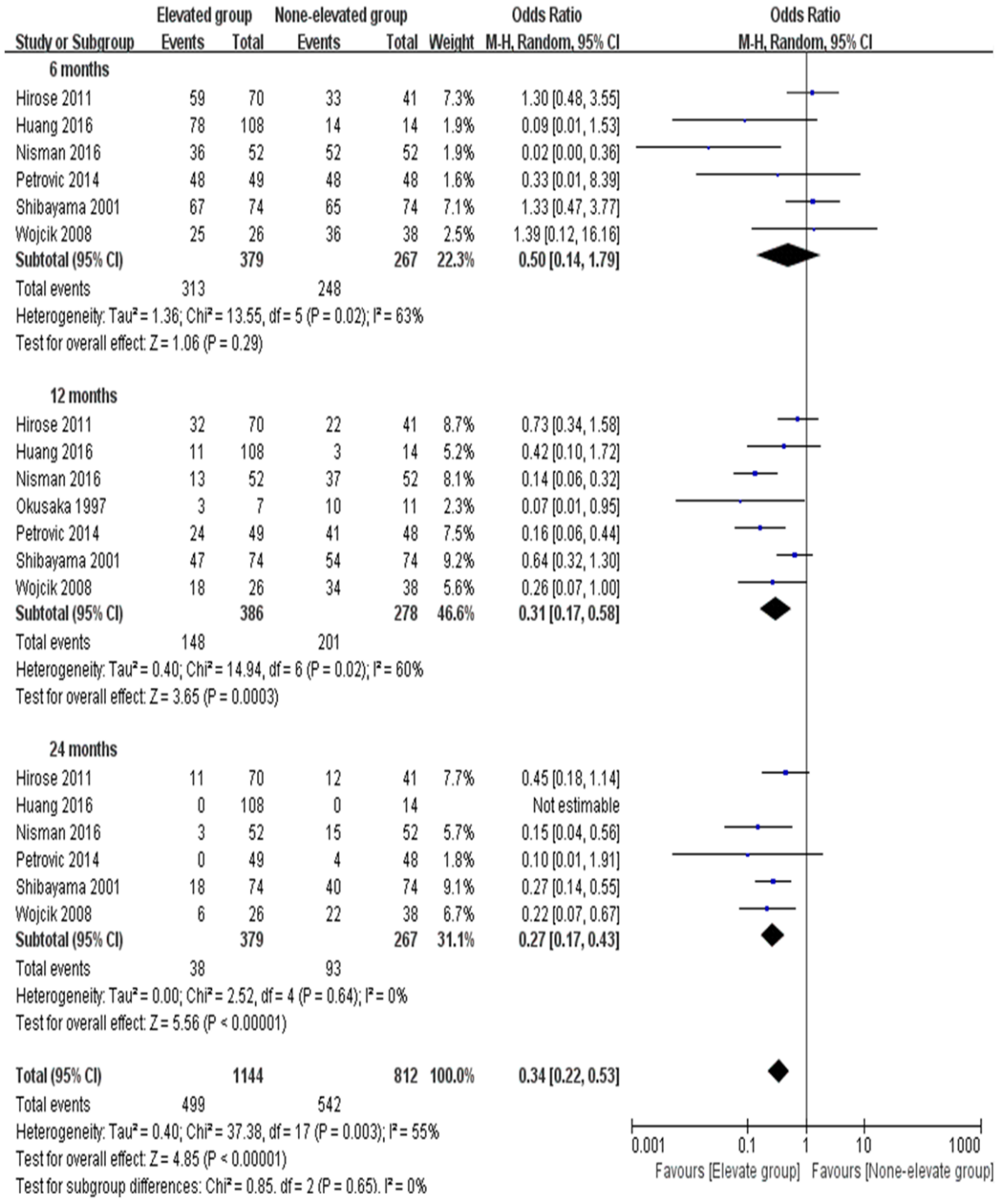

Figure 3. Meta-analysis evaluating Pro-GRP levels and survival time in SCLC.

of the seven studies [5, 22-24, 26, 27, 31] showed that the serum levels of Pro-GRP were associated with poor prognosis in OS survival time in SCLC patients (OR=0.34, 95% CI: 0.22-0.53, P<0.00001) with significant heterogeneity (I^2=55%, P=0.003) (**Figure 3**). The Pro-GRP levels provided additional information about survival. The survival time for patients with low Pro-GRP levels was longer than for patients with elevated levels at 12 months and 24 months (P<0.05), but there was no statistically significant difference at six months (P=0.29). Five studies reported [21, 24, 25, 27, 30] that the level of Pro-GRP was related to the disease

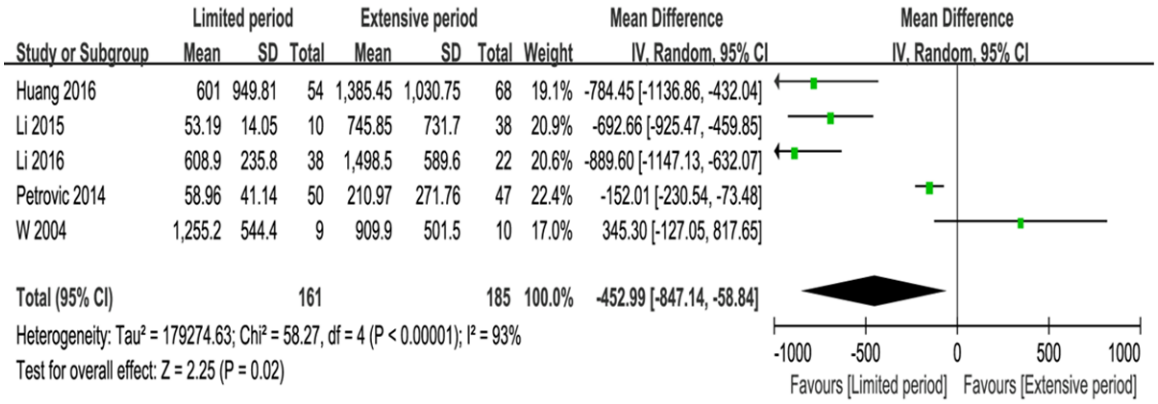

Figure 4. Differences in serum levels of Pro-GRP according to disease stage.

stage (95% CI: -847.14 - -58.84, P=0.02) with significant heterogeneity (I^2=93%, P<0.00001) (**Figure 4**). The mean serum levels of Pro-GRP were significantly greater in ED patients than in LD patients.

Subgroup analysis

Pro-GRP is useful tumor markers in evaluating response to therapy and predicting survival in patients with SCLC. The subgroup analysis was performed according to the chemotherapy response, survival time, and disease stage according to the Veterans Administration Lung Study Group (VALG) staging system. We divided the patients into four groups according to their chemotherapy responses, namely, complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD) groups. The grouping in different articles was not consistent, so we added the effective group (CR+PR or CR+PR+SD or PR+NC). There was a significant difference in serum Pro-GRP before and after chemotherapy. Comparisons were made with regard to the group pairing: in each paired group among the complete remission (CR) group (MD: 627.83, 95% CI: 203.90-1051.75, P=0.004) [20, 28, 29], the partial remission (PR) group (MD: 455.57, 95% CI: 437.60-473.55, P<0.00001) [20, 28, 29], and the effective group (MD: 724.19, 95% CI: 599.48-848.90, P<0.00001) [21, 25-27, 30], the serum Pro-GRP levels were significantly decreased after chemotherapy from their values before the treatment. For patients in the PD group, the Pro-GRP concentration measured after chemotherapy was significantly increased compared to the value measured before chemotherapy (MD: -631.27, 95% CI: -703.21 - -559.33, P<0.00001) [20, 27-29], but there was no significant difference in serum Pro-GRP levels before and after chemotherapy in the SD group (MD: 115.21, 95% CI: -638.01-868.42, P=0.76) [20, 27-29]. A significant difference between subgroups was found (MD: 291.59, 95% CI: 164.08-419.10, P<0.00001). Based on the cut-off of different Pro-GRP levels for each of the articles screened, we calculated the survival time, which was associated with the level of Pro-GRP, remarkable differences between subgroups were discovered (OR=0.34, 95% CI: 0.22-0.53, P<0.00001) [5, 22-24, 26, 27, 31]. The survival time for patients with low Pro-GRP levels was longer than for patients with elevated levels (12 months: OR=0.31, 95% CI: 0.17-0.58, P=0.0003; 24 months: OR=0.27, 95% CI: 0.17-0.43, P<0.00001), but there was no significant difference at 6 months (OR=0.50, 95% CI: 0.14-1.79, P=0.29). So elevation of Pro-GRP was a poor prognostic factor, and patients with elevated levels of Pro-GRP showed shorter survival than those without. Significant differences between the LD patients and the ED patients were found. The mean serum levels of Pro-GRP were significantly greater in ED patients than in LD patients (MD: -452.99, 95% CI: -847.14 - -58.84, P=0.02) [21, 24, 25, 27, 30].

Discussion

SCLC differs clinically and biologically from non-small cell lung cancer (NSCLC). The incidence of distant metastases of SCLC at the time of primary diagnosis is very high, and therefore, early diagnosis, more effective treatment, more accurate evaluation of treatment

and early detection of progression are needed to improve the survival of patients suffering from SCLC. Reliable tumor markers are beneficial for checking the effectiveness of therapy.

Gastrin-releasing peptide (GRP) is a 27-amino-acid peptide that is homologous to the carboxy terminus of porcine stomach protein [32] and is a member of the bombesin family of peptides that has been shown to be produced by SCLC in an autocrine fashion [33]. Pro-GRP is a neuropeptide hormone that was initially isolated from porcine stomach tissue and is a precursor form of GRP (or mammalian bombesin) [34]. Recently, the determination of serum Pro-GRP levels has come to play an important role in the diagnosis, treatment, and detection of relapse in patients with SCLC [15, 31, 35, 36]. Some studies have found that Pro-GRP is the most useful tumor marker to detect SCLC recurrence [13]. In particular, for the diagnosis of SCLC, studies have reported that the sensitivity and specificity of serum Pro-GRP are 0.716 and 0.921, respectively [16]. Therefore, Pro-GRP is a useful biomarker in SCLC management and may be a potential therapeutic target [37].

In our meta-analysis, there was no significant difference in serum Pro-GRP before and after chemotherapy in the SD group (P=0.76), while the Pro-GRP levels in the other three subgroups (CR, PR and effective group) had changed significantly post-treatment in comparison with the pretreatment concentrations (P<0.05). However, significant differences in the Pro-GRP concentration were only observed in the PD group, and the concentrations were higher before chemotherapy than after the treatment (P<0.05). Therefore, it can be said that Pro-GRP could be used as an indicator of patient response after chemotherapy. The patients' Pro-GRP levels became significant as a factor affecting survival time. The survival time for patients with low Pro-GRP levels was longer than for patients with elevated levels at 12 months and 24 months (P<0.05), but there was no statistically significant difference at six months (P=0.29). The Pro-GRP levels were also significantly higher in patients with ED than with LD. These data suggested that Pro-GRP was a useful tumor markers in evaluating the response to therapy and predicting survival in patients with SCLC.

Although we have comprehensively analyzed the prognostic value of Pro-GRP in SCLC, there were some limitations in our meta-analysis. First, in the treatment response grouping, there were few literatures reports in each group, and the groupings were not consistent. Second, the extraction of data from the survival curves to calculate some ORs might have introduced multiple tiny errors. Third, the cut-off values among these studies were disparate; therefore, we could not set up a baseline referring to high Pro-GRP expression, and inconsistency might be observed. Fourth, each subgroup included too few articles in the literature, which could affect the statistical results. More studies were needed in the meta-analysis to evaluate the Pro-GRP levels associated with clinical prognosis and recurrence.

In summary, our study demonstrated that Pro-GRP expression was significantly correlated with SCLC prognosis. Pro-GRP expression in the blood was significantly associated with prognosis, survival time, and VALG stage. More studies are needed to confirm the relationship between the expression of Pro-GRP and the prognosis of SCLC patients.

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Disclosure of conflict of interest

None.

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Supplementary Checklist 1. PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

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