Plasma cell-free DNA and survival in non-small-cell lung cancer: A meta-analysis

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Abstract. In recent years, plasma cell-free DNA (cfDNA) has been attracting increasing attention as a potential tumor marker, as this method is easily applied and minimally invasive. A series of studies have confirmed the association between the level of cfDNA and overall survival (OS) in non-small-cell lung cancer (NSCLC), but the findings remain inconclusive. We herein conducted a meta-analysis of published articles evaluating the correlation between the level of cfDNA and OS. A total of 9 studies enrolling 1,170 patients were included. For the overall population, a high level of cfDNA was found to be significantly correlated with worse OS [hazard ratio (HR) = 1.57, 95% confidence interval (CI): 1.18-2.10] in NSCLC. The subgroup analysis suggested that a high cfDNA level was associated with worse outcome in stage III-IV patients (HR=1.53, 95% CI: 1.07-2.19). However, the level of cfDNA and OS were not found to be significantly associated in the subgroup of patients with tumor stage I-II. The present meta-analysis revealed that a high level of cfDNA may be correlated with poor OS in NSCLC.

Introduction

Biopsy is key to diagnosing cancer; however solid biopsies cannot always be successfully performed, due to their invasive nature. In addition, solid biopsies may not adequately reflect current tumor dynamics or sensitivity to treatment, and their diagnostic value may be limited by intratumoral heterogeneity as well (1). Since scientists first detected circulating cell-free DNA (cfDNA) in the plasma, which may be derived from cell necrosis, apoptosis, and/or digestion by macrophages, cfDNA has been extensively investigated to determine its possible use as a new tool for diagnosing cancer, monitoring treatment or even estimating prognosis (1-3). As opposed to solid biopsies, this method is referred to as 'liquid biopsy'.

The focus of the present study was non-small-cell lung cancer (NSCLC). NSCLC is the leading cause of cancer-related mortality worldwide. The 5-year survival rate is only ~16% for patients diagnosed with advanced lung cancer compared with 70-90% when the disease is diagnosed and treated at an earlier stage (4). Therefore, identifying a reliable prognostic factor may enable timely intervention, thus improving patient prognosis.

Although cfDNA has been known as a potential biomarker in cancer patients for >40 years (5), it has not attracted significant attention, possibly due to the limitations of detection technology and the lack of in-depth knowledge on the subject. With the maturation of cfDNA detection and extraction technology, cfDNA is attracting increasing attention, as this method is minimally invasive, convenient and easily applied. cfDNA detection is currently widely used in pancreatic, colorectal and lung cancer (6). At present, the application of cfDNA in clinical practice includes detecting changes in quality and quantity. As regards quantitative assessment, a number of studies have confirmed that cfDNA is definitely increased in the blood of NSCLC patients compared with the control group, indicating that the level of cfDNA may be an indicator of diagnosis (6-9). Certain studies report that a high plasma DNA concentration is associated with poor survival, whereas other studies did not report such a correlation. Therefore, the present meta-analysis was conducted to elucidate the association between cfDNA level and survival, and provide reliable evidence for application in the clinical setting.

Data collection methods

Search strategy. The present meta-analysis was performed according to the guidelines on diagnostic studies. MEDLINE (via PubMed), EMBASE (via OvidSP), the Cochrane Library, and ISI Web of Knowledge were searched for potentially relevant studies without restriction according to region or publication type. The search strategy included the combination of the following key words and medical subheadings: 'lung neoplasms' or 'lung cancer', 'circulating DNA' or 'ctDNA' or 'ct-DNA' or 'cfDNA' or 'cf-DNA' or 'circulating nucleic acid' or 'circulating tumor DNA', not 'RNA' and not 'microRNA'. The databases were searched from inception to January 23, 2016.

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The reference lists of the included studies and relevant reviews were also manually screened to identify related studies.

Inclusion and exclusion criteria

Inclusion criteria. Studies retrieved from the databases and reference lists were first screened by title and abstract and the full-text articles were further reviewed for eligibility. Eligible studies were selected according to the following inclusion criteria: i) Patients with NSCLC should be diagnosed histopathologically or cytologically, regardless of tumor stage, age and therapeutic method; ii) providing sufficient information on patient overall survival (OS) and hazard ratio (HR), 95% confidence interval (CI) or P-value; iii) cfDNA should be extracted from the plasma, regardless of the extraction method; iv) a cut-off value of cfDNA in the plasma divided patients into high and low cfDNA level groups, without limitation regarding the cut-off values; and v) cfDNA was evaluated prior to treatment.

Exclusion criteria. i) SCLC or other histopathological subtypes; ii) the aim of the study was to determine whether cfDNA gene mutation affects prognosis; iii) no cut-off value (no control group); and iv) duplicate reports from the same center.

All the studies were reviewed by the authors independently and a consensus was reached on each eligible study.

Data extraction. Two authors (Z.Y. and B.L.) evaluated the eligible papers independently. The extracted data included HR and 95% CI of OS. For studies with insufficient information, the authors were contacted; if that was not possible, data provided in the study were used to calculate other required data (e.g., if 95% CI was missing, HR and P-value were used to calculate 95% CI via Stata/SE 11.0 software (StataCorp., College Station, TX, USA). Two authors (Z.Y. and B.L.) extracted these data independently and any discrepancies between the two authors was resolved by discussion or consensus with a third author (F.M.).

Quality assessment. The methodological quality of retrospective studies was assessed by the modified Newcastle-Ottawa Scale (NOS). This scale is based on three broad categories based on the selection of the study sample (4 points); the comparability of the sample groups (2 points); and the ascertainment of either the exposure for case-control (3 points) and cross-sectional studies (2 points), or the outcome for cohort studies (3 points). Achieving ≥7 points was considered as high methodological quality. The methodological quality of eligible studies was evaluated by two investigators.

Statistical analysis. The data analyses were performed using Stata/SE 11.0 software. The aggregated data of HRs and 95% CIs were analyzed using inverse-variance weighting. According to the level of cfDNA, the patients were divided into two groups to compare the OS. A Chi-squared-based Q statistic and inconsistency index (I^2) statistic were used to examine heterogeneity. A P-value of <0.1 and I^2 value of >50% indicated significant heterogeneity. A random-effects model was used if heterogeneity was significant; otherwise, the fixed-effects model was applied. If heterogeneity was significant, sensitivity analyses were conducted by deleting each study individually to evaluate the quality and consistency of the results, and subgroup analyses were conducted for TNM stage and treatment history. Publication bias was evaluated using Funnel plots and Begg's tests. Probable significant publication bias was considered for P-values <0.05. All the tests were performed using Stata/SE 11 software.

Results

Characteristics of studies. A total of 1,168 articles were initially identified using the abovementioned key words. Finally, 9 studies were included in the present meta-analysis. The 9 studies included a total of 1,170 patients with NSCLC (357 patients with stage I-III and 811 patients with stage IV disease; tumor stage was unknown in 2 patients; Fig. 1). A total of 5 studies only included NSCLC patients with stage III or IV disease, and 2 studies recruited NSCLC patients with stage I-III disease. A total of 5 studies included patients not receiving any treatment, 3 studies included patients who received adjuvant chemotherapy or radiotherapy after radical resection, and 1 study recruited NSCLC patients following palliative chemotherapy. The main characteristics of the 9 included studies are listed in Table I. The included studies were published between 2004 and 2015. Male patients comprised ~68.9% of the subjects in all the studies, and the median age of all patients was 63.7 years. Only 4 studies reported the median follow-up period, with a mean of 23.4 months. Circulating plasma DNA was quantified using quantitative polymerase chain reaction (PCR) in 7 studies, whereas 1 study used the PicoGreen dsDNA kit and another study used β-actin PCR to quantify the cfDNA. The cut-off value for high level of cfDNA differed among the included studies.

Association of the cfDNA level with OS. A total of 7 articles reported multivariable HRs and their respective 95% CIs, while 2 articles only reported HRs and P-values, and their 95% CIs were calculated using Stata/SE 11.0 software. Eventually, pooled analyses of the 9 studies reporting multivariable HRs demonstrated significant inter-study heterogeneity (I^2=71.7%). Thus, the random-effects model was used. The meta-analysis demonstrated that a high level of cfDNA was associated with worse OS compared with low cfDNA level (HR=1.57, 95% CI: 1.18-2.10, P=0.001) (Fig. 2).

Subgroup analysis. The 9 articles were classified into two subgroups by treatment history and three subgroups by tumor stage. A total of 5 studies were included in the no treatment group and the 4 remaining studies were included the group that received adjuvant therapies or palliative therapies. The combined HRs of the group including stage III-IV patients suggested that the high level of cfDNA was associated with worse outcome (HR=1.53, 95% CI: 1.07-2.19). However, the level of cfDNA and OS were not found to be significantly associated in the subgroup of patients with tumor stage I-II (Fig. 3).

Publication bias. The publication bias was examined using funnel plots and Egger's regression test. There was no evidence of publication bias, as shown by inspection of a funnel plot for OS (Fig. 4). The Egger's test demonstrated no significant bias in the meta-analysis of cfDNA concentration and OS (Z=0.94, P=0.348, Fig. 5).

Table I. Characteristics of the eligible studies included in the meta-analysis.

First author	Year	Country	Tumor type	Tumor stage (IIII/IV)	Treatment history	Median age (y)	Patient no.	Sex (F/M)	Median follow-up (m)	Cut-off (ng/ml)	Assay method	NOS	(Refs.)
Gautschi	2004	Switzerland	NSCLC	81/104	Surgery and adjuvant therapies	63.0	185	55/130	NR	10	qPCR	6	(10)
Ludovini	2008	Italy	NSCLC	76/0	No treatment	68.0	76	11/65	23	3.25	qPCR	6	(11)
van der Drift	2010	The Netherlands	NSCLC	29/15	No treatment	66.1	46	16/30	NR	67	qPCR	6	(12)
Kumar	2010	India	NSCLC	61/39 (III/IV)	No treatment	56.0	100	8/92	NR	98	PicoGreen dsDNA kit	5	(13)
Lee	2011	Korea	Lung adenoCa	12/122 (IIIb/IV)	Surgery and adjuvant therapies	56.0	134	121/13	36	5.26	qPCR	8	(14)
Sirera	2011	Spain	NSCLC	70/376 (IIIb/IV)	No treatment	60.0	446	71/375	9.7	49.8	qPCR	8	(15)
Vinayanuwattikun	2013	Thailand	NSCLC	6/52 (IIIb/IV)	Palliative chemotherapy	63.0	58	19/39	NR	4.5	qPCR	6	(16)
Bortolin	2015	Italy	NSCLC	22/0	Radiotherapy	76.4	22	11/11	24.7	10	qPCR	8	(17)
Li	2015	USA	NSCLC	0/103	No treatment	65.0	103	53/50	NR	7	PCR (β-actin)	6	(3)

NSCLC, nonsmallcell lung cancer; adenoCa, adenocarcinoma; M, male; F, female; y, years; m, months; NR, not reported; qPCR, quantitative polymerase chain reaction; NOS, Newcastle-Ottawa Scale.

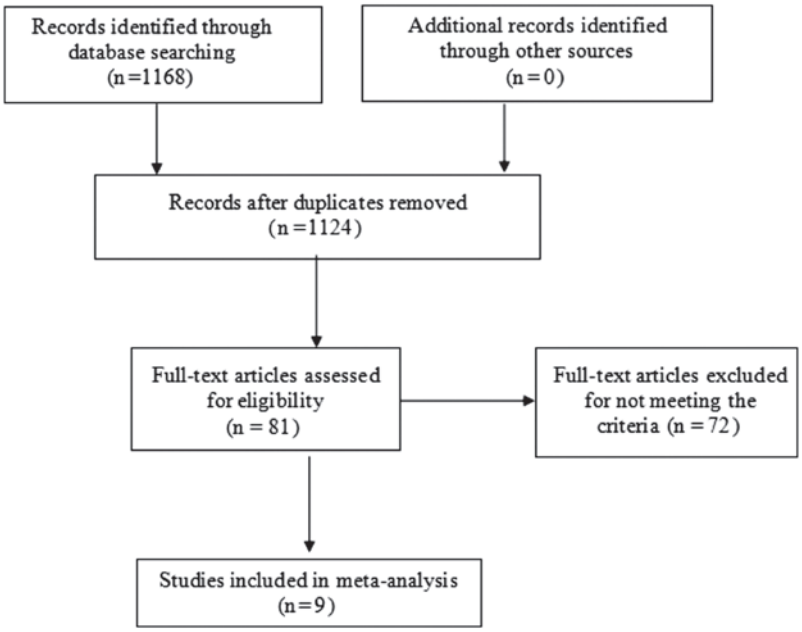

Figure 1. Flow chart of the literature search and study selection process.

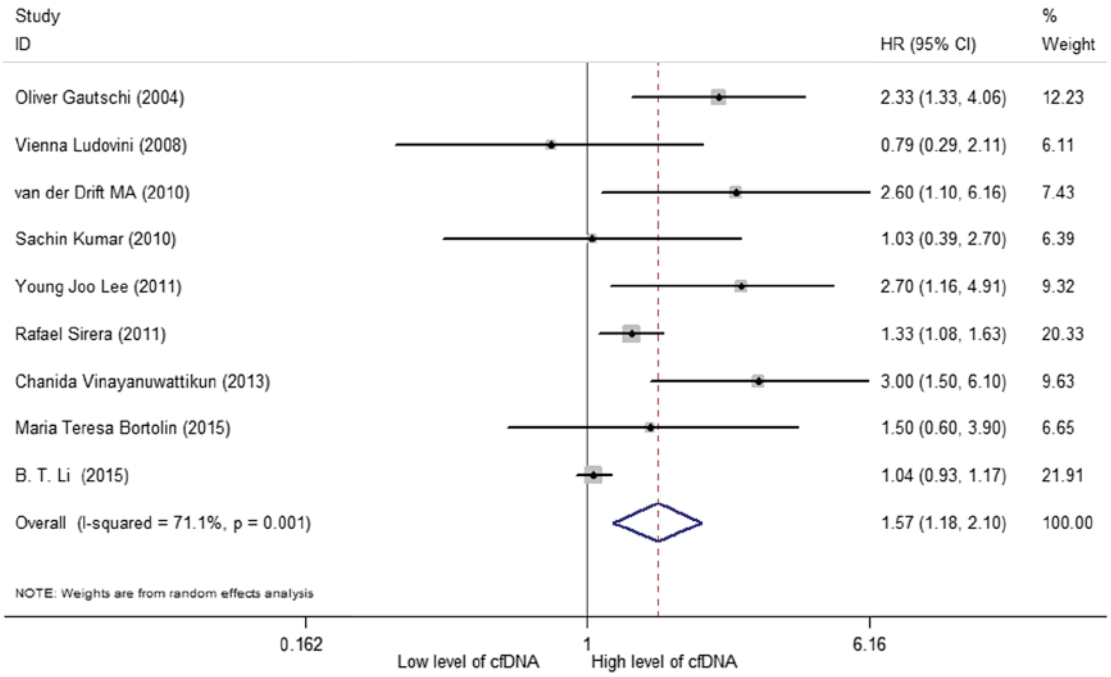

Figure 2. Forest plot and meta-analysis of studies evaluating the hazard ratio (HR) of high level of cell-free DNA (cfDNA) compared with low level of cfDNA for overall survival in non-small-cell lung cancer patients.

Quality assessment. A total of 3 studies scored 8 points and 6 studies scored <7 points (5 studies scored 6 points and 1 study scored 5 points).

Discussion

In recent years, circulating DNA as a potential tumor marker has been attracting increasing attention. A number of primary studies have investigated the potential association between cfDNA and the prognosis of NSCLC patients, and the importance of cfDNA for survival was found to be both significant as well as non-significant. The reported results differed among studies. Thus, it was necessary to analyze the variability of the survival results and evaluate the prognostic value of cfDNA by quantitative aggregation of survival statistics. The overall meta-analysis suggested that a high level of cfDNA was correlated with a poor OS.

Several studies reported that there was no association between cfDNA concentration and tumor stage (12), whereas Gautschi *et al* reported that the plasma cfDNA concentration at advanced tumor stages was higher compared with that at early tumor stages (10). Our meta-analysis included 9 studies

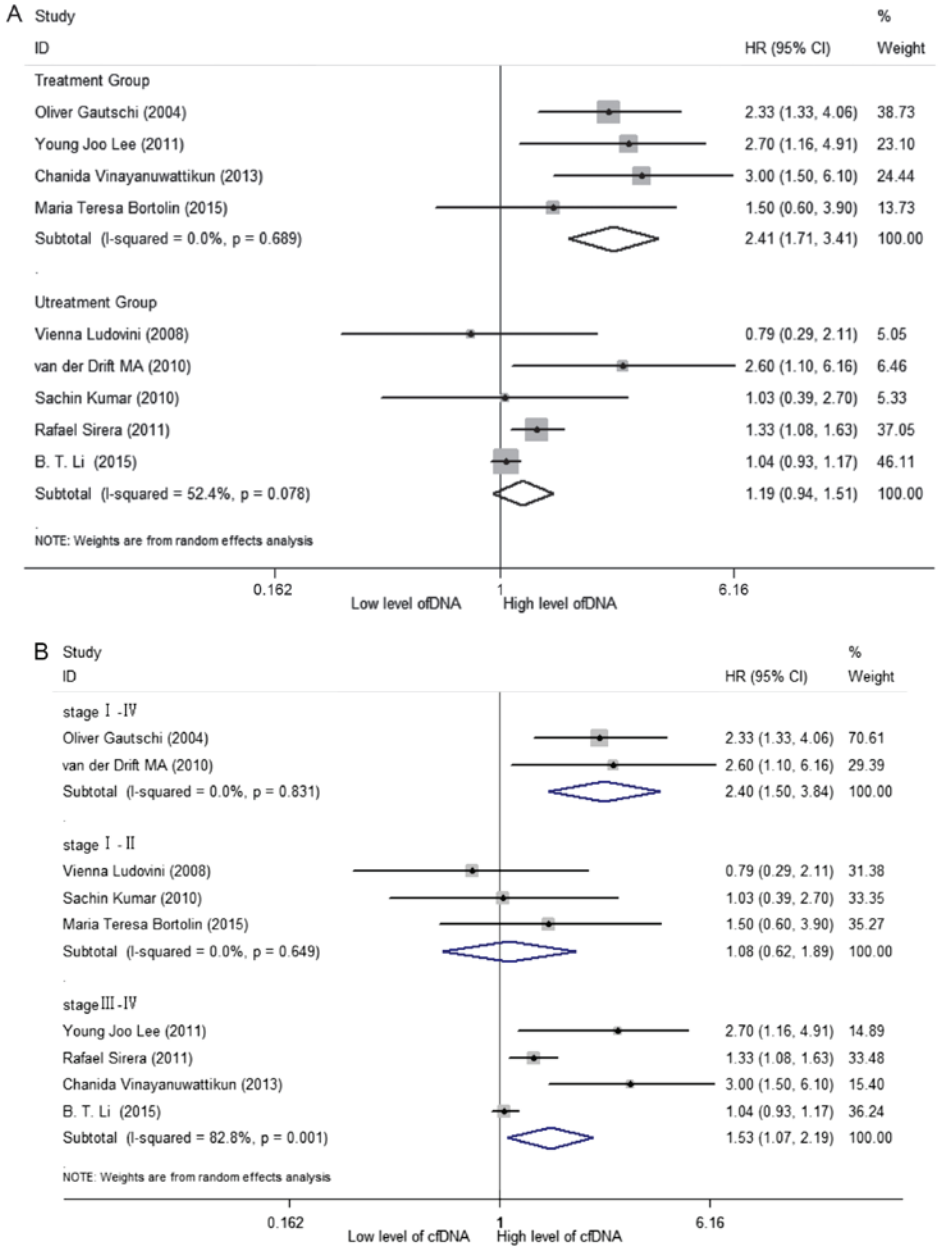

Figure 3. Forest plots and subgroup meta-analysis of studies evaluating the hazard ratio (HR) of high level of cell-free DNA (cfDNA) compared with low level of cfDNA. The combined HRs of high level of cfDNA for overall survival were aggregated in subgroups according to (A) treatment history and (B) tumor stage.

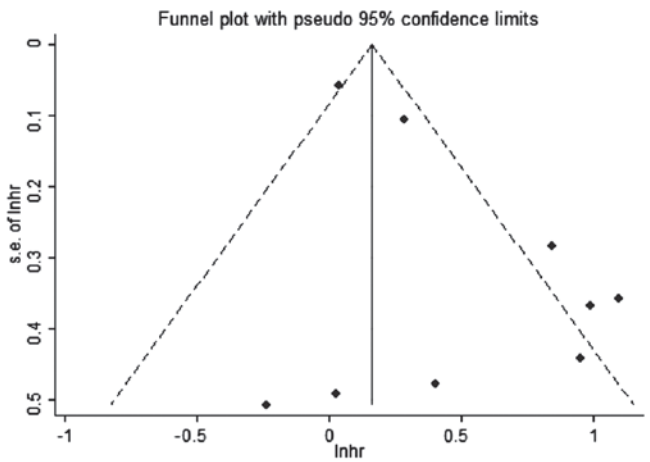

Figure 4. Funnel plots assessing potential publication bias in studies evaluating the level of cfDNA in patients with non-small-cell lung cancer.

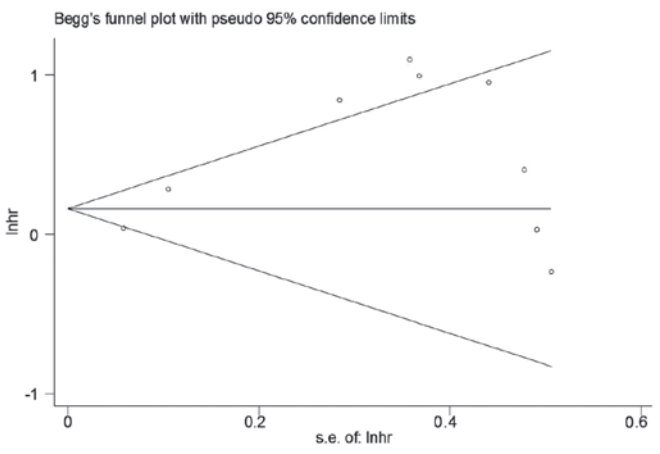

Figure 5. Begg's test assessing potential publication bias in studies evaluating the level of cfDNA in patients with non-small-cell lung cancer.

investigating newly diagnosed as well as relapsed NSCLC patients with any-stage disease. The subgroup analysis suggested that a high level of cfDNA was correlated with poor OS in the group of NSCLC patients with stage III or IV disease, while there was no correlation between the level of cfDNA and OS in the group of patients with stage I-II disease. Furthermore, it was reported that there was no association between the level of cfDNA and clinical parameters such as age, gender, histology, smoking or pulmonary inflammatory conditions (12).

Treatment may affect the concentration of cfDNA. Szpechcinski *et al* (18) reported that the level of cfDNA in the plasma increased within a week following primary tumor resection. The level of cfDNA was reduced in patients without disease recurrence during the next 3-6 months of follow-up, whereas in relapsed patients the plasma cfDNA concentration was significantly higher compared with the baseline. The changing trend of the cfDNA following surgery may be associated with the prognosis. However, Gautschi *et al* did not observe a decrease in plasma cfDNA levels after chemotherapy for NSCLC (10). Pan *et al* (19) reported that patients with high levels of plasma cfDNA after the third cycle of chemotherapy had a poor survival time, but the cfDNA at baseline and after the first and second cycles of chemotherapy was not associated with OS. In the present meta-analysis, 5 studies included patients who did not receive any treatment, 3 studies were performed on patients who received adjuvant chemotherapy or radiotherapy following radical resection, and 1 study recruited NSCLC patients following palliative chemotherapy. However, no association was observed between the level of cfDNA and OS in the treatment or no treatment groups on subgroup analysis. Further studies are required to verify the value of cfDNA as a prognostic factor during different treatment periods of NSLC.

There were certain limitations to the present meta-analysis. First, the studies included in this analysis were insufficient, particularly in terms of a subgroup analysis. Thus, potential publication bias is highly likely, despite the lack of such evidence in our statistical tests. Second, a cut-off value for the high level of cfDNA was not specified, as different assay methods were used by the included studies. An additional limitation resulted from the fact that the treatment method in the treatment group could not be unified and a further subgroup analysis could not be conducted due to the limited number of included studies. As the median follow-up duration was not mentioned in most included studies, the follow-up period was not limited.

In conclusion, a high level of cfDNA in the plasma is associated with worse outcome for NSCLC patients. However, studies with less heterogeneity are required to improve the accuracy of the estimation of the clinical impact of cfDNA in NSCLC.

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