

## RESEARCH ARTICLE

# Are p53 Antibodies a Diagnostic Indicator for Patients with Oral Squamous Cell Carcinoma? Systematic Review and Meta-Analysis

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## Abstract

**Background:** P53 has been reported to be involved with tumorigenesis and has also been implicated as a significant biomarker in oral squamous cell carcinoma (OSCC). However, the diagnostic value of p53 antibodies remains controversial; hence, we comprehensively and quantitatively assessed the potential in the present systematic review. **Materials and Methods:** A comprehensive search was performed using PubMed and Embase, up to October 31, 2014, without language restriction. Studies were assessed for quality using QUADAS (quality assessment of studies of diagnostic accuracy). The positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were pooled separately and compared with overall accuracy measures using diagnostic odds ratios (DORs) and symmetric summary receiver operating characteristic (SROC) curves. **Results:** Of 150 studies initially identified, 7 eligible regarding serum p53 antibodies met the inclusion criteria. Some 85.7% (6/7) were of relatively high quality (QUADAS score  $\geq 7$ ). The summary estimates for quantitative analysis of serum p53 antibody in the diagnosis of squamous cell carcinoma were: PLR 2.06 [95% confidence interval (CI) : 1.35-3.15], NLR 0.85 (95% CI: 0.80- 0.90) and DOR 2.47 (95% CI: 1.49- 4.12). **Conclusions:** This meta-analysis suggests that the use of s-p53-antibodies has potential diagnostic value with relatively high sensitivity and specificity for OSCC particularly with serum specimens for discrimination of OSCCs from healthy controls. However, its discrimination power is not perfect because of low sensitivity.

**Keywords:** OSCC - diagnosis - P53 antibodies - serum biomarker - meta-analysis

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## Introduction

OSCC is one of the most common cause of cancer-related death worldwide, with most patients dying within a year after diagnosis, which makes OSCC one of the most aggressive and malignant cancers. During the last several decades, the incidence of oral squamous cell carcinoma (OSCC) has been declining. However, OSCC remains the predominant carcinoma in many countries. Squamous cell carcinoma (OSCC) is a highly aggressive malignancy due to rapid progression, late diagnosis, and poor prognosis of survival, making the mortality rate of OSCC patients similar to the rate of the incidence (Crew and Neugut, 2004; Vaupel and Mayer, 2007). However, overall survival could be significantly improved by early diagnosis. The majority of patients with early OSCC are asymptomatic and without clinical manifestations. The usual methods of have limited usefulness in early detection because such procedures are often invasive, unpleasant, inconvenient and expensive. In addition, the optimal treatment strategy for advanced OSCC is still not well established.

To our knowledge, there are no suitable diagnostic biomarkers of OSCC, in contrast to other cancer. The spread of malignant tumors is a multistep process involving rapid growth and invasion into the lymph node and blood vessels (Samantaray et al., 2004). Therefore, a low cost, non-invasive, convenient method for routine OSCC diagnosis is necessary. The detection of biomarkers in serum currently plays an important role in the detection of certain tumors and in monitoring for recurrence or metastasis. Serum tumor markers can be operationally defined as serum molecules whose levels can be used in the diagnosis, prognosis, or clinical management of malignant diseases (Moskal et al., 1995). Therefore, the early diagnosis of OSCC clinically challenging and the development of valid, reliable biomarkers for the early detection and monitoring of OSCC is of great importance for clinical management of this malignancy.

To the best of our knowledge, there are no suitable biomarkers for diagnosis of OSCC. Therefore, we performed a systematic review and meta-analysis of published clinical studies in order to comprehensively and quantitatively summarize the diagnosis value of p53 antibody in OSCC.

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## Materials and Methods

### Search strategy and study selection

Potential relevant studies identified by a comprehensive literature up to October 31, 2014, which covered the following computerized bibliographic database: Pubmed and EMABASE, no start data limit was applied. We also identified the articles by use of the related articles function in PubMed and searched manually the references of identified articles. The search terms were 'oral cancer', 'blood OR serum', 'biomarker OR diagnostic marker', 'P53', without language restriction.

Two reviewers assessed the studies independently based on the inclusion criteria: *i*) the performance of biomarkers for the diagnosis of OSCC were evaluated using a prospective or retrospective design, *ii*) the gold standard should be pathologic examinations of biopsied specimens for all cases diagnosis, serum must have been collected before any treatment, e.g. chemotherapy or radiotherapy, and controls were without other cancers, and *iii*) positive values of the cases and controls were available, and the results of an individual study on diagnostic accuracy could be summarized in a 2x2 table. When the same author reported results obtained from the same patient population in several publications, only the most recent or the most complete report was included in the analysis to avoid overlap between cohorts.

### Assessment of methodological quality

Two reviewers systematically evaluated the quality of each study according to the critical review checklist of the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS), which is demonstrated to be an efficient tool for the quality assessment of diagnostic accuracy studies. The QUADAS system is comprised of four key domains: patient selection, index test, reference standard, and flow and timing. It uses 11 questions to evaluate the quality of diagnostic accuracy studies. The 11 items were recommended by the Cochrane Collaboration Methods group on screening and diagnostic tests (Smidt et al., 2011). The items got a "1" score if the item score was "yes", and aggregate scores totaled 11. Each questions is answered with "yes", "no" or "unclear". An answer of "yes" means that the risk of bias can be judged low, while an answer of "no" or "unclear" means that the risk of bias can be judged high.

### Data extraction and management

We used a standardized data form in duplicate to collect the following descriptive information: *i*) first author, year of publication, country of publication, *ii*) participants' inclusion/exclusion criteria, ethnicity, disease stage, histology stage, diagnostic guidelines, and type of control, *iii*) extraction time and storage temperature of the sample, assay method, cut-off value, blindness, and a detailed report of the assay procedure, *iv*) the positive value of the cases and controls, and other comparison data (e.g. mean age, sex ratio, smoking, drinking) between cases and controls. If data from any of the above categories were not reported in the primary article, items were treated as "not reported." Disagreement on the inclusion

of a single study was settled by discussion or a third investigator was consulted.

### Statistical analyses

We used standard methods recommended for meta-analysis of diagnostic test evaluations (Deville et al., 2002). The statistical analysis was based on the following steps (Deville et al., 2002): 1-presentation of the results of individual studies, 2-searching for the presence of heterogeneity, 3-testing of the presence of an (implicit) cut-point effect, 4-dealing with heterogeneity, 5-statistical pooling: positive likelihood ratio (PLR), negative likelihood ratio (NLR) and their 95% confidence interval (CI) were calculated using a fixed effects model according to the Mantel-Haensel method and random effects model based on the work of Der Simonian and Laird (DerSimonian and Laird, 1986), respectively. The accuracy measure used was the diagnostic odds ratio (DOR) computed by the Moses's constant of linear method, which indicates the change in diagnostic performance of the test under study per unit increase in the covariant (Gu et al., 2007). Summary receiver operating characteristic curves were used to summarize overall test performance, and the area under the SROC curve (AUC) was calculated. The potential problem associated with sensitivities and specificities of 100% are solved by adding 0.5 to all cells of the diagnostic 2x2 table (Deville et al., 2002).

We used a chi-squared test to detect statistically significant heterogeneity. Between-study heterogeneity was assessed using  $I^2$ , according to the formula:  $I^2 = 100\% \times (\text{Cochran } Q - \text{degrees of freedom}) / \text{Cochran } Q$  (Dinnes et al., 2005). To detect cut-off threshold effects, the relationship between sensitivity and specificity was evaluated by using the Spearman correlation coefficient  $r$ . Possible sources of heterogeneity were investigated by meta regression, which used a generalization of Littenberg and Moses linear model weighted by the inverse of the variance (Gu et al., 2007). Also, we conducted subgroup analysis. In order to evaluate the statistical outcome validity, we detected the pooled outcome by sensitivity analysis. Since publication bias is of concern for meta-analysis of diagnostic studies, we tested for the potential presence of this bias using funnel plots (Egger et al., 1997). All analyses were undertaken using Meta DiSc statistical software (version 1.4; Ramon y Cajal Hospital, Madrid, Spain) (Zamora et al., 2006) and STATA SE12.0 software (State Corporation).

## Results

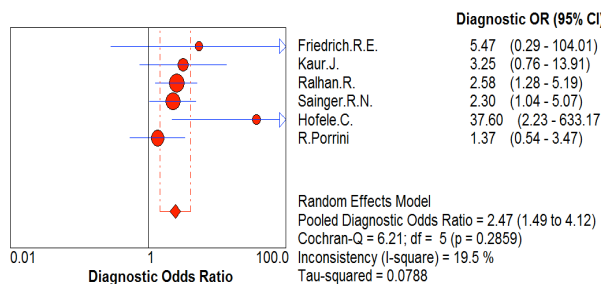
### Results of the search and characteristics of the studies

Using the search strategy above, 172 studies were retrieved initially. After reviewing the titles and abstracts, 12 of those studies were excluded because of duplication. Of the remaining studies, 52 studies were excluded for not about OSCC diagnosis, not about p53 antibody, and lacking necessary data. Finally, six studies were included according to our inclusion and exclusion criteria. (Hammel et al., 1997) Abstracts and titles of 150 primary studies were identified for initial review using the search strategies. Of the other 61 publications, 5 articles,

including a review and case report, were excluded because they provided insufficient information. An additional 38 were excluded because there was no control, and 17 studies were excluded because they focused on the p53 gene and p53 protein and did not detect s-p53 antibody. As a consequence, only 6 publications were considered to be eligible for inclusion in the analysis which allow the calculation of sensitivity or specificity.

The main characteristic of the included six studies are outlined in Table 1 (a, b). These studies followed several different characteristics. The studies included were conducted in different countries, such as German (Friedrich, 1997), Italy (1), India(3). The years of the publication ranged from 1997 to 2010. All of the six included studies did not report related information about the patient choose. All of the six studies were retrospective, 2 provided the TNM stage and 3 provided the histology stage. Three studies included health volunteers as a control, and the remaining three studies included health volunteers and patients with benign disease as controls.

We assessed the quality of all six studies included for systematic review the based on QUADAS guidelines. Of the four eligible studies had QUADAS score $\geq$ 8, one had a QUADAS score=7 and one had a QUADAS score=6. In total included studies.



**Figure 1. Forest Plot of Estimates of Diagnostic Odds Ratio (DOR) for s-p53-antibody in the Diagnosis of OSCC.** The point estimates of positive likelihood ratio from each study are shown as solid circles. Error bars are 95% confidence intervals

**Table 1. Main Characteristics of the 6 Eligible Studies**

| Author             | Country | Reference standard | Assay method  | Cut-off  | Histology Well/Moderate/poorly/other | Sample collection time | Stage I (%)  | QUADAS | TP* | FP* | FN* | TN* |
|--------------------|---------|--------------------|---------------|----------|--------------------------------------|------------------------|--------------|--------|-----|-----|-----|-----|
| Friedrich RE, 1997 | Germany | unknown            | ELISA         | unknown  | unknown                              | unknown                | 1/33. (22%)  | 6      | 8   | 0   | 29  | 9   |
| Kaur J, 1997       | India   | histology          | immunblotting | unknown  | 11/5/14                              | unknown                | unknown      | 9      | 7   | 3   | 23  | 32  |
| Ralhan R, 1998     | India   | unknown            | ELISA         | unknown  | unknown                              | unknown                | unknown      | 8      | 24  | 19  | 46  | 94  |
| Sainger RN, 2006   | India   | histology          | ELISA         | 0.85u/ml | 21/54                                | unknown                | 5/75 (6.67%) | 9      | 17  | 13  | 58  | 102 |
| Hofele C, 2002     | Germany | unknown            | ELISA         | unknown  | unknown                              | unknown                | unknown      | 7      | 19  | 0   | 83  | 80  |
| R.Porrini, 2010    | Italy   | unknown            | ELISA         | unknown  | 31/19/10                             | before diagnosis       | unknown      | 8      | 8   | 15  | 42  | 108 |

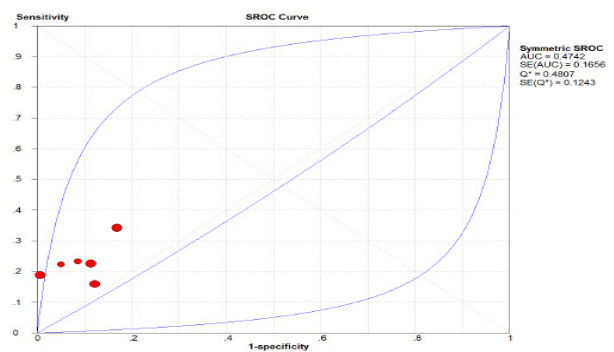
ELISA\*=Enzyme-linked immunosorbent assay; TP\*=true positives, FP\*=false positives, FN\*=false negatives, TN\*=true negatives; Abs\* = Antibody

### Threshold effect

The Spearman correction coefficient between the logit of sensitivity and logit of 1-specificity of s-p53 antibody was 0.371 (P=0.332), indicating no threshold effect, and the positive correlation had no statistical significance.

### Diagnostic accuracy

For all studies, the pooled DOR was 2.47 (95%CI: 1.49-4.12), heterogeneity chi-squared = 16.22 (p = 0.30) and I<sup>2</sup>=13.7% (Figure 1). There did not appear to be any major qualitative evidence for heterogeneity between studies, as assessed by inspection of the forest plot. The DOR is a single indicator of test accuracy that combines the data from sensitivity and specificity into a single number (Glas et al., 2003a). The value of DOR ranges from 0 to infinity, with higher values indicating better discriminatory test performance (higher accuracy) (Glas et al., 2003a). A DOR of 1.0 indicates that a test does not discriminate between patients with the disorder and those without it (Glas et al., 2003a). The DOR value approximate to 10 indicated that the s-p53 antibody could be useful biomarker for OSCC patients diagnosis. Figure 2 presented the symmetrical SROC of s-p53 antibody,

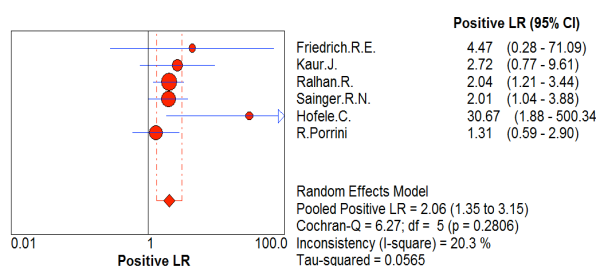


**Figure 2. Summary Receiver Operating Characteristic Curves for s-p53 Antibody in the Diagnosis of OSCC.** Each solid circle represents each study in the meta-analysis. The size of each is indicated by the size of the solid circle. The weighted (solid line) and unweighted (dashed line) regression summary receiver operating characteristic curves summarize the overall diagnostic accuracy

**Table 2. The Indicator of the Six Included Single Studies and the Results of the Meta-analysis**

| Studies            | sensitivity (95%CI) | specificity (95%CI) | PLR (95%CI)         | NLR (95%CI)               | DOR (95%CI)         | AUC (95%CI) |
|--------------------|---------------------|---------------------|---------------------|---------------------------|---------------------|-------------|
| Friedrich RE, 1997 | 0.22 (0.10-0.38)    | 1.00 (0.66-1.00)    | 4.47 (0.28-71.09)   | 0.82 (0.65-1.02)<br>7.13  | 5.47 (0.29-104.01)  |             |
| KaurJ, 1997        | 0.23 (0.10-0.42)    | 0.91 (0.77 -0.98)   | 2.72 (0.77-9.61)    | 0.84 (0.67-1.05)<br>7.14  | 3.25 (0.76-13.91)   |             |
| Ralhan R, 1998     | 0.34 (0.23-0.47)    | 0.83 (0.75-0.90 )   | 2.04 (1.21-3.44)    | 0.79 (0.65-0.95)<br>9.90  | 2.58 (1.28-5.19)    |             |
| Sainger RN, 2      | 0.23 (0.14-0.34)    | 0.89 (0.81-0.94)    | 2.01 (1.04-3.88)    | 0.87 (0.76-1.00)          | 2.30 (1.04-5.07)    |             |
| Hofele C, 2002     | 0.19 (0.12-0.28)    | 1.00 (0.95-1.00)    | 30.67 (1.88-500.34) | 0.82 (0.74-0.90)<br>39.05 | 37.60 (2.23-633.17) |             |
| R.Porrini, 2010    | 0.16 (0.07-0.29)    | 0.88 (0.81-0.93)    | 1.31 (0.59-2.90)    | 0.96 (0.83-1.10)<br>18.53 | 1.37 (0.54-3.47)    | 0.47        |
| Pooled indicator   | 0.23 (0.19-0.27)    | 0.89 (0.86-0.92)    | 2.06 (1.35-3.15)    | 0.85 (0.80-0.90)          | 2.47 (1.49-4.12)    |             |
| (I-square)         | 31.30%              | 79.60%              | 20.30%              | 0.00%                     | 19.50%              |             |

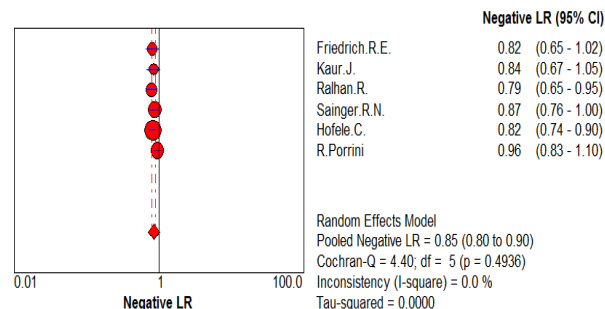
PLR: positive likelihood ratio, NLR: negative likelihood ratio, DOR: diagnostic odds ratio, AUC: the area under the SROC curve; PLR (95% CI)\*, DOR (95% CI)\* and NLR (95% CI)\* was calculated using random effect model



**Figure 3. Forest Plot of Estimates of Positive Likelihood Ratio (PLR) for s-p53-antibody in the Diagnosis of OSCC.** The point estimates of positive likelihood ratio from each study are shown as solid circles. Error bars are 95% confidence intervals

and the AUC was 0.47. The SROC curve has been recommended to represent the performance of a diagnostic test, based on data from meta-analysis, and the area under the SROC curve (AUC) is not only useful to summarize the curve, but also quite robust to heterogeneity (Lijmer et al., 2002; Walter, 2002). A prior study (Jones and Athanasiou, 2005) showed that to demonstrate excellent accuracy, the AUC should be in the region of 0.97 or above. An AUC of 0.93 to 0.96 is very good; 0.75 to 0.92 is good. An AUC less than 0.75 can still be reasonable, but the test has obvious deficiencies in its diagnostic accuracy. In our study, the AUC of s-p53 antibody was 0.74, close to 0.75. Thus s-p53 antibody had reasonable accuracy in terms of differential diagnosis in cases of OSCC.

According to Honest H, Khan KS (Honest and Khan, 2002), sensitivity and specificity are considered inappropriate for meta-analyses, as they do not behave independently when they are pooled from various primary studies to generate separate averages. The range of the sensitivity and specificity were 15%-60% and 91%-



**Figure 4. Forest Plot of Estimates of Negative Likelihood Ratio (NLR) for s-p53-antibody in the Diagnosis of OSCC.** The point estimates of positive likelihood ratio from each study are shown as solid circles. Error bars are 95% confidence intervals

100%, respectively (please see additional file 1: appendix Figure.2). The likelihood ratio incorporates both the sensitivity and specificity of the test, and provides a direct estimate of how much a test result will change the odds of having a disease (Gallagher, 1998). The PLR indicates how much the odds of the disease increase when a test is positive (Gallagher, 1998), and the NLR indicates how much the odds of the disease decrease when a test is negative. Likelihood ratios of >10 or <0.1 generate large and often conclusive shifts from pretest to posttest probability (indicating high accuracy) (Gallagher, 1998).

In the present study, a pooled PLR of 2.06 (95% CI: 1.35-3.15) suggests that patients with OSCC have a nearly 7-fold higher chance of being s-p53 antibody test-positive compared with patients without OSCC (Figure 3). Also, there were no heterogeneity between PLRs, heterogeneity chi-squared = 15.27 (p = 0.36) and I<sup>2</sup> = 8.3%. Regarding NLR, we found significant heterogeneity for all of the



eligible studies, heterogeneity chi-squared = 72.93 ( $p = 0.00$ ) and  $I^2 = 80.8\%$ . The pooled negative likelihood ratio was 0.85 (95% CI: 0.80-0.90) (Figure 4).

#### *Possible sources of heterogeneity*

The meta-regression and sub-group analyses were used to explore the overall heterogeneity and the possible sources of heterogeneity, which may include variation in method quality of the studies (QUADAS), assay method, the representation of the participants (stage I%), negative control, sample collection time among each study. Meta-regression indicated that above variables were not the sources of heterogeneity for s-p53-antibody (data not shown). The subgroup analysis results were shown in Table 2, and the main source may be from assay method, the percentage of the stage I, negative control, sample collection time.

#### *Sensitivity analysis and publication bias*

A sensitivity analysis was also performed to determine whether review conclusion were affected by the choice of a single study; the finding revealed that no single study had the effect on the pooled DORs in the current meta-analysis.

Sensitivity analysis was conducted in terms of statistical analysis methods, sample size, and study design. We used a random effect model to analyze the data again to replace the fixed effect model, however, the results produced no obvious changes. When we excluded the studies without matched cases and control sample size, the results were similar to the original results. In addition, we excluded the studies which studied various cancers that included OSCC and did not provide the detailed information of the participants, but this did not change the results. Publication bias is assessed visually by using a scatter plot of the inverse of the square root of the effective sample size ( $1/ESS^{1/2}$ ) versus the diagnostic log odds ratio ( $\ln DOR$ ) which should have a symmetrical funnel shape when publication bias is absent (Deeks et al., 2005). Formal testing for publication bias may be conducted by a regression of  $\ln DOR$  against  $1/ESS^{1/2}$ , weighting by ESS (Deeks et al., 2005), with  $P < 0.05$  for the slope coefficient indicating significant asymmetry. Although meta-analysis itself has some bias, the results showed no publication bias in this meta-analysis ( $p = 0.305$ ). The funnel plots for publication bias also showed symmetry.

## **Discussion**

This meta-analysis of seven relevant randomized controlled studies has shown consistently that significantly positive of s-p53-antibody. Our meta-analysis allow some conclusions based on available evidence: *i*) patients with OSCC have higher chance of being s-p53 antibody test-positive compared with patients without OSCC; *ii*) the ratio of the odds of a positive test result among OSCC was approximate 10-folders to the odds of a positive test result among the non-OSCC; *iii*) we believe that s-p53-antibody may be useful for monitoring residual tumor cells and for aiding in the selection of candidates for less invasive treatment procedures because of the high specificity of s-p53-antibody. In brief, s-p53-antibody could be useful

for the detection and diagnosis of OSCC, whereas it is imperfect. (Hammel et al., 1997)

Early detection of OSCC is still a common problem in clinical practice. To our knowledge, there is no diagnostic biomarker for OSCC. Usually, histological examination is used to diagnose OSCC. More and more studies have been focused on the detection of s-p53 antibody in OSCC to evaluate the diagnostic and clinical usefulness of the anti-p53 antibody response as a serological marker. Several studies have reported that serum p53 antibodies (s-p53 Abs) are detected in different populations that are at increased risk for developing malignant disease (Lubin et al., 1995; Trivers et al., 1995; Kaur et al., 1997). S-p53 Abs can be used to follow the response of patients with malignant tumors during treatment (Hammel et al., 1997). Because the ELISA assay is a quick and convenient assay for detecting p53 genetic alterations, s-p53 Abs may serve as a useful marker for routine screening in OSCC patient groups. This is the first meta-analysis about s-p53 antibody and esophageal cancer screening. In the present study, 6 studies which including 1079 serum samples from OSCC patients and 2260 serum samples from controls without OSCC were eligible according to our inclusion criteria. Although all of the 6 eligible studies aimed to ensure the diagnostic accuracy of the s-p53 antibody, those studies could be only regarded as being in the early stage of diagnostic testing. In all 6 studies, OSCC patients diagnosed by histology were regarded as positive. However, the negative controls without OSCC who were healthy or had benign disease were not diagnosed by histology. In addition, the 6 studies did not report whether the investigators were blinded. Therefore, such non-strict designs could exaggerate the diagnostic accuracy and lead to bias due to unfavorable representation of the participants. Simultaneously, QUADAS, recommended by Cochrane, which can be used in systematic reviews of diagnostic accuracy studies, was used to evaluate the methodological quality of the included studies. Our meta-analysis showed that methodological quality of reports on diagnostic research of s-p53-antibody is moderate, as expressed by the QUADAS tool. Systematic reviewers are advised to use comprehensive searches to attempt to locate all relevant studies (Dickersin et al., 1994; Khan et al., 2001; Clarke and Oxman, 2003). In our study, we did not find any publication bias ( $p = 0.31$ ).

In meta-analysis, pooled indicators were usually used in the homogeneity study. In the present study, however, there were significant heterogeneity between NLRs, so it is not suitable to pool NLR ( $I^2 = 80.8\%$ ). Therefore, the DOR and AUC were calculated for evaluating the potential diagnostic values of s-p53 antibody. DOR is difficult to be clinically interpreted, but useful from the statistical point of view in the assessment of the overall test accuracy in meta-analysis (Glas et al., 2003b; Martin and HTA, 2006; Sousa and Ribeiro, 2009). It is very important to note that the point estimates of PLR and DOR must evaluate carefully and the heterogeneity between NLRs should be searched and explained. As different cut-off values were used among the 6 included studies, we used the Spearman correlation coefficient to analyze the threshold effect. The result had no statistical significance ( $p = 0.66 > 0.05$ ),

indicating that a threshold effect was not the source of the heterogeneity. Nonetheless, the validation assay of s-p53 antibody used in each study was different; some used ELISA, others used immunoblot or both, adding additional heterogeneity. The spectrum of patients refers not only to the severity of the underlying target condition, but also to demographic features and to the presence of differential diagnosis and/or co-morbidity. It is therefore important that diagnostic test evaluations include an appropriate spectrum of patients for the test under investigation and also that a clear description is provided of the population actually included in the study (Whiting et al., 2003). The difference of the percentage of stage I patients between studies brought about spectrum bias and heterogeneity. Studies including healthy controls tend to show higher specificity than those recruiting patients with clinically suspected disease consecutively and prospectively in a representative clinical setting. Therefore, the distinct type of negative control may be a main source of heterogeneity. The sample collection time varied widely among the studies. Four studies (Hagiwara et al., 2000; Ralhan et al., 2000; Shimada et al., 2000; Wang et al., 2004) collected serum before treatment, seven studies (Cawley et al., 1998; Sobti and Parashar, 1998; Shimada et al., 2002; Shimada et al., 2003; Kato et al., 2005; Muller et al., 2006; Wu et al., 2010) did not report, two studies (Meghiorino et al., 2005; Cai et al., 2008) collected serum before chemotherapy and two studies (Kozlowski et al., 2001; Looi et al., 2006) collected serum before diagnosis, respectively. The differentials of DOR between sample collection time subgroups indicated that different collection times also led to significant heterogeneity.

Although we tried to avoid the bias in the process of identifying studies, screening, assessing, data extraction, data analyses, etc; the present study has several limitations: First, we did not calculate the diagnostic accuracy for the early stage (stage I-II), in that sufficient raw data was not provided. Although we aimed to determine the screening power of the s-p53 antibody for the early diagnosis of the OSCC, OSCC patients regardless of disease stage were used to evaluate the diagnostic power because of the limitation of the information. There were also not available primary data to investigate the elevated or decreased s-p53 antibody values as a function of tumor type, histology, age, or degree. Second, all of the 6 included studies used healthy controls and only two studies (2/6) added benign disease, which strongly exaggerated the diagnostic accuracy. Nevertheless, the evidence is compelling in that s-p53 antibody assay specificity were higher than 0.9 in all of the 6 included studies, ranging from 0.91 to 1.00. Third, although we did not observe significant publication bias between studies, it is uncertain whether some data were missed because of unpublished studies. Missing information may report lower diagnostic of s-p53-antibody.

Our study represents a new trend in diagnosis of the cancer: convenient, noninvasive, low costs biomarkers will play a significant role in screening cancer. Future studies should focus on the following tasks: *i*) improve the sensitivity and specificity of the detection method, *ii*) use blood, serum or other convenient samples, *iii*) standardize

the detection method and cut-off, and *iv*) conduct normative diagnostic tests or collect samples from cases before biopsies or at least before treatment to improve sensitivity. These tasks will reduce the heterogeneity among studies. Furthermore, more studies are greatly needed to examine the association between s-p53 antibody and the stage and the prognosis of the OSCC. This will help avoid the unnecessary treatment, as OSCC therapies are associated with significant adverse effects that impact patient health and quality of life enabling us to conduct an accurate meta-analysis to find the diagnostic value of the s-p53 antibody.

In conclusion, the current evidence suggests that s-p53 antibody has potential diagnostic value though currently provides low sensitivity. S-p53-antibody may serve as a useful marker for routine screening in asymptomatic high-risk patient groups because the ELISA assay is a quick and convenient assay for detecting p53 genetic alterations. Further studies may need to identify patterns of multiple biomarkers to further increase the power of OSCC detection.

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