Expression of p63 in Squamous Cell Carcinoma of the Lung and its Diagnostic Significance: A Meta-Analysis

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Abstract: *Introduction*: The expression of p63 has been studied in various tumor types, including squamous cell carcinoma (SCC).

Methods and Results: Twenty-five trials met the inclusion criteria with a total of 1,193 patients. The overall positive proportion of p63 was 91.5% (95% CI, 86.3–94.8). Both histological and cytological methods of obtaining specimens showed a high expression of p63 in SCC at 89.8% (95% CI, 81.9–94.5) and 88.7% (95% CI, 80.9–93.6). The p63 positive proportion of the well or moderately differentiated subgroups was 92.7% (95% CI, 77.9–97.9) compared to the poorly differentiated subgroup at 86.9% (95% CI, 61.6–96.5). When using >1% of p63 immunoreactive cells as the positive standard, both sensitivity and specificity at 0.91 (95% CI, 0.86–0.94) and 0.80 (95% CI, 0.75–0.85), respectively, were acceptable. When using >10% and >50% standards, sensitivities of 0.92 (95% CI, 0.90–0.94) and 0.82 (95% CI, 0.78–0.85) and specificities of 0.84 (95% CI, 0.82–0.86) and 0.92(95% CI, 0.90–0.94) were shown.

Conclusions: In SCC, there is a high expression of p63, which has no association with the histological or cytological methods used to obtain specimens or the degree of differentiation of the specimens. Even when only a small amount of cells were stained (>1%) as the positive standard, the sensitivity and specificity of p63 were maintained at a high level. We suggest that >50% of immunoreactive cells be used as the positive standard to achieve proper sensitivity and specificity.

Keywords: p63, squamous cell carcinoma, lung cancer, meta-analysis, diagnose.

1. INTRODUCTION

Lung cancer is the most commonly diagnosed cancer worldwide, with an estimated 1.5 million new cases detected annually. It continues to be the most lethal human malignancy, accounting for 1.1 million deaths annually worldwide [1]. Squamous cell carcinoma (SCC) of the lung accounts for 40~50% of all lung cancer cases [1]. Pathological classification and staging provide important prognostic information about lung cancer [2]. It is important to differentiate between SCC and adenocarcinomas due to distinct management options and a significantly better prognosis for SCC [4]. Most primary lung carcinomas can be classified based on their histological features. However, histological characterization fails to classify 30% to 40% of all tumors, especially when the tumor is poorly differentiated or the biopsy specimen is inadequate [3].

Immunohistochemistry (IHC) can help resolve the diagnostic dilemma, since the nuclear antigen p63 has been shown to be a good marker for SCC [5]. p63 is a characterized member of the p53 family, and its expression has been studied in various tumor types. p63 is normally expressed in squamous and urothelial cells, basal layers of prostatic glands, breast, and bronchial epithelia [6].

To the best of our knowledge, the association between p63 expression and SCC has not been clearly defined. Therefore, we conducted a meta-analysis to investigate the expression of p63 among the specimens histologically diagnosed as SCC.

2. METHODS

2.1. Search Strategy

We searched PubMed (from 1966 to April 2012), Embase (from 1980 to April 2012), and the Cochrane Library. In addition, abstracts published in the proceedings of the American Society of Clinical Oncology (ASCO), European Society for Medical Oncology (ESMO), and International Association for the Study of Lung Cancer (IASLC) were also searched. Keywords included in the text word search were “p63”, “squamous cell carcinoma”, and “diagnosis”. The search was restricted to articles published in English.

The inclusion criteria were as follows:

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(1) Expression of p63 was evaluated in primary lung carcinoma tissues or cytologic specimens;

(2) IHC was used to evaluate p63 expression;

(3) The histological type of the tumors included SCC;

(4) The authors must offer the number of enrolled specimens and the outcome of p63 IHC in SCC; and

(5) When multiple articles were published by the same authors or group, the newest or most informative single article was selected.

2.2. STUDY SELECTION AND DATA COLLECTION

Using a standardized approach, 2 reviewers independently conducted data abstraction and a quality assessment. The 2 reviewers adjudicated disagreements together. The 2 reviewers then extracted data, including the first author's name, year of publication, methods used to obtain the specimens, histological type of the tumors, degree of their differentiation (if available), method of diagnosis, number of enrolled specimens, and adverse outcomes of interest (p63 positive number of the target specimens and p63 reactivity intension).

2.3. Quality Assessment

The quality assessment for diagnostic accuracy studies (QUADAS) [7] is a professional tool used to assess the accuracy of diagnostic trials. The QUADAS tool contains 14 items, which can be answered as yes, no, or unclear. Here, we applied QUADAS to assess the quality of the included trials.

2.4. Statistical Methods

Data were analyzed using the Comprehensive Meta Analysis 2 software for calculating p63 positive proportion along with 95% confidence interval (CI) of SCC specimens. Meta-Disc 1.4 software was used to pool the sensitivity, specificity (negative control to adenocarcinoma), and area under curve (AUC) of summary receiver operating characteristic. Subgroup analysis was performed according to the included articles' characteristics such as tumor differentiation and method of obtaining the specimens. Interaction text was calculated if 2 parallel groups were compared [8], or if $p < 0.05$ suggested 2 groups were different statistically. We used both the fixed-effects and random-effects models in the meta-analysis. In each meta-analysis, I^2 values were first calculated to assess the heterogeneity of the included trials [9]; $I^2 < 25\%$ were interpreted as signifying low-level heterogeneity. When there was no statistically significant heterogeneity, a pooled effect was calculated using a fixed-effects model; otherwise, a random-effects model was employed. Funnel plots [10] were also employed to assess the probability of publication bias.

3. RESULTS

3.1. Flow of Included Studies

Of a total 136 potentially relevant trials identified using the search strategy, 111 were excluded for the

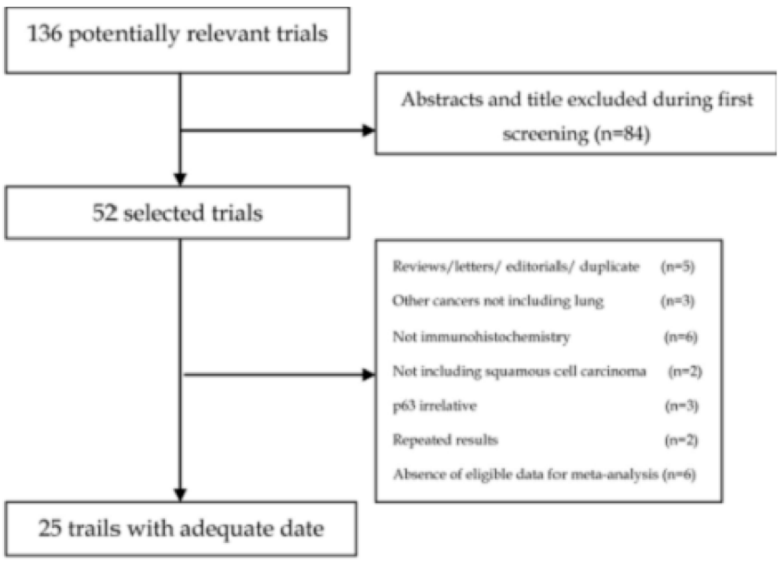

Figure 1: Outline of the search-flow diagram.

reasons shown in Figure **1**. Twenty-five [6, 11-34] trials met the inclusion criteria with a total of 1,193 patients available for the meta-analysis.

3.2. Study Characteristics

Table **1** shows the characteristics of the individual trials. In the 25 [6, 11-34] trials, the total number of SCC specimens was 1,193, ranging from 3 to 162 per trial. The specimens were obtained *via* histological methods such as surgery or bronchoscopic biopsy or cytological methods, such as fine needle aspirations (FNA), bronchial washings, bronchial brushings, and bronchioloalveolar lavages. The method used affected the specimen size, hence determining p63 expression in SCC. The degree of differentiation of SCC specimens ranged from poorly differentiated to well differentiated. Twenty-two of the 25 trials used monoclonal 4A4 antibody to determine p63's ability to detect antibodies in IHC. The other 3 [18, 23, 28] trials used Clone 7JUL, Ab-1, and Clone 70 instead. Among the 22 4A4 trials, 11 of the 4A4 antibodies were from Dako, 5 from Santa Cruz Biotechnology, 3 from Neomarkers, 2 from other corporations, and only 1 did not name its source.

3.3. Quality Assessment of the Included Trials

The quality assessment of the included trials using QUADAS is shown in Figure **2**. The value "Yes" in spectrum of disease (item 1) had a percentage of 88%, confirming a good representation; in clear selection

Table 1: Characteristics of Trials Included in the Meta-Analysis

Trials	SCC Specimen number	Method of obtaining Specimens	Differentiation	Monoclonal antibody (Source; Dilution)
Pelosi G. 2002 [11]	118	Sur.	W:1; M:86; P:29	4A4 (Dako; 1:500)
Charles J. 2002 [12]	4	Sur.	mix	4A4 (Santa Cruz Biotechnology; 1:200)
Beverly Y. 2002 [7]	30	Sur.	mix	4A4 (Santa Cruz Biotechnology; UK)
Jorge S. 2003 [13]	30	UK.	W &M:27; P:3	4A4 (Neomarkers; 1:200)
Pierre P. 2003 [14]	94	Sur.	mix	4A4 (Oncogene Research Products; 1:500)
Hina A 2004 [15]	3	Sur.	mix	4A4 (Dako; 1:300)
Au N. 2004 [16]	97	Sur.	mix	4A4 (Neomarkers; 1:1000)
Gown A. 2004 [16]	104	Sur.	mix	4A4 (Neomarkers; 1:1000)
Camilo R. 2005 [17]	18	Sur.	mix	4A4 (Dako; 1:3000)
Zhang H. 2005 [18]	28	Sur.; BsB	P:28	Clone 7JUL (Novocastra Laboratories Ltd; 1:100)
Shtilbans V. 2005 [19]	4	FNA	P:4	4A4 (Santa Cruz Biotechnology; 1: 500–750)
Wu M. 2005 [20]	11	BB; FNA	mix	4A4 (Santa Cruz Biotechnology; 1:800)
Nelson G. 2006 [21]	30	UK.	mix	4A4 (Santa Cruz Biotechnology; 1:200)
Kargi A. 2007 [22]	39	BsB	W:39	4A4 (Neomarkers; 1:100)
Robert T. 2007 [23]	15	Sur.	mix	Ab-1 (Lab Vision; 1:100)
Merce J. 2009 [24]	26	FNA; BW; BB; BlL	mix	4A4 (Dako; 1:50)
Shimada Y. 2009 [25]	162	Sur.	mix	4A4 (Dako; 1:200)
Uke M. 2009 [26]	28	Bll	mix	4A4 (Dako; 1:20)
Khayyata S. 2009 [27]	12	FNA	mix	4A4 (Dako; 1:60)
Kim D.H. 2010 [28]	13	FNA	W:2; M:10; P:1	Clone 70 (Transduction Laboratories; 1:400)
Moreira A. 2010 [29]	11	Sur.	mix	4A4 (Dako; 1:800)
Conde E. 2010 [30]	91	SUR.	mix	4A4 (Dako; 1:50)
Pereira T.C. 2011 [31]	32	UK.	mix	4A4 (Biocare; UK)
Rekhtman N. 2011 [32]	115	Sur.	W:2; M:53; P:59	4A4 (Dako; 1:700)
Noh S. 2011 [33]	38	Sur.	P:38	4A4 (Dako; 1:50)
Ocque R. 2011 [34]	30	FNA; BB; BW	mix	4A4 (UK; UK)

Abbreviation: SCC: squamous cell carcinoma; Sur : Surgery; FNA: fine-needle aspirations; BW: bronchial washings; BB: bronchial brushings; BlL: bronchioloalveolar lavages; BsB: bronchoscopic biopsy; UK: unknown; W: well differentiated; M: moderately differentiated; P: poorly differentiated.

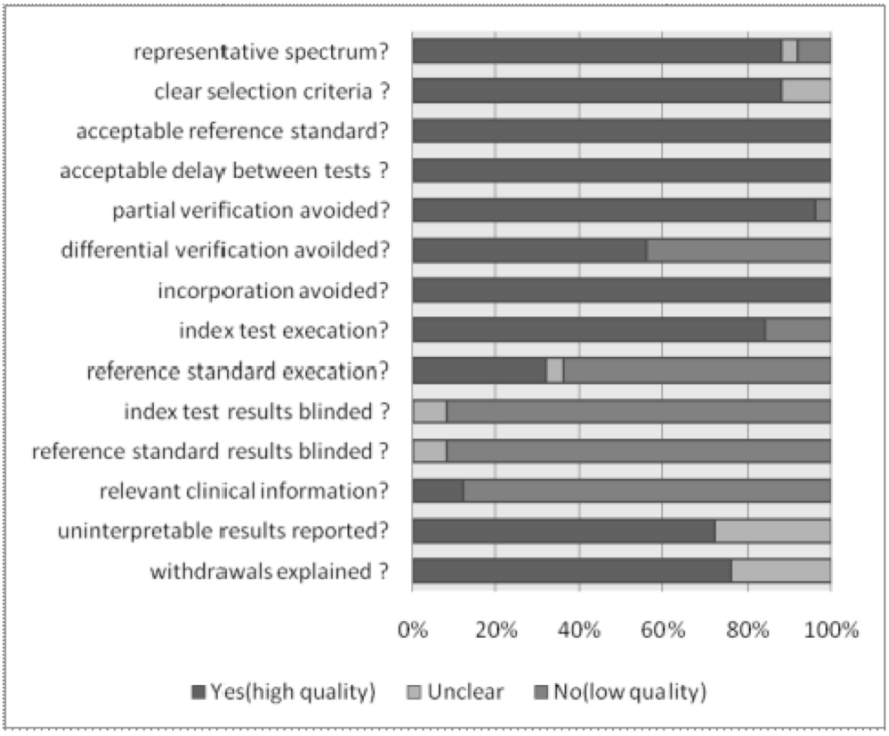

Figure 2: Quality assessment of included trials.

criteria (item 2), the percentage of "Yes" was also 88%, denoting high quality; and both acceptable reference standard (item 3) and acceptable delay (item 4) were "Yes" at 100%, which was fundamental for a correct analysis. The partial verification avoided (item 5), differential verification avoided (item 6), incorporation avoided (item 7), index test execution (item 8), uninterpretable results reported (item 13), and withdrawals explained (item 14) valued as "Yes" and index test results blinded (item 10) and reference standard results blinded (item 11) valued as "No" had percentages of 96%, 56%, 100%, 84%, 72%, 76%, 92%, 92%, which meant a low possibility of bias; while the reference standard execution (item 9) and relevant clinical information (item 12) valued as "No" and "Unclear," respectively, had a percentage of 68% and 88%, which meant a high possibility of bias.

3.4. Meta-Analysis

In all of the 25 trials, useful data for calculation were obtained directly from the original articles without indirect estimation. One [16] trial consisted of 2 data sets from 2 different laboratories, so there were 26 data sets included in total. The positive proportion of p63 in the included trials (Figure **3**) ranged from 50.0% to 99.6% with high heterogeneity (I^2 = 78.75%). A random-effects model revealed an overall positive proportion of 91.5% (95% CI, 86.3–94.8%). In order to explore the affected factors of high heterogeneity and p63 expression, we conducted subgroup analyses according to study characteristics.

First, we examined the methods used to obtain SCC specimens. The histological analysis group (Figure **4A**) including surgery or bronchoscopic biopsy comprised 967 specimens enrolled in 15 trials. The positive proportion ranged from 50.0% to 99.6%, with a high heterogeneity (I^2 = 85.47%). The overall positive proportion of the histological group was 89.8% (95% CI, 81.9–94.5). The cytological analysis group (Figure **4B**) including FNA, bronchial washings, bronchial brushings, and bronchioloalveolar lavages, consisted of 124 specimens from 7 trials. The positive proportion ranged from 82.1% to 98.4% with a total proportion 88.7% (95% CI, 80.9–93.6, I^2 for heterogeneity = 0.00%). No statistical difference was found between the 2 groups according to interaction text (p = 0.81).

Second, we considered the degree of differentiation of the tissue. Since moderately and well-differentiated SCC are usually easily diagnosed, we grouped them into 1 group to compare to the poorly differentiated group. The moderately and well-differentiated group (Figure **5A**) consisted 190 specimens from 4 trials. The positive proportion ranged from 82.1% to 99.6%, with the overall positive proportion being 92.7% (95% CI, 77.9–97.9, I^2 for heterogeneity = 61.45%). In

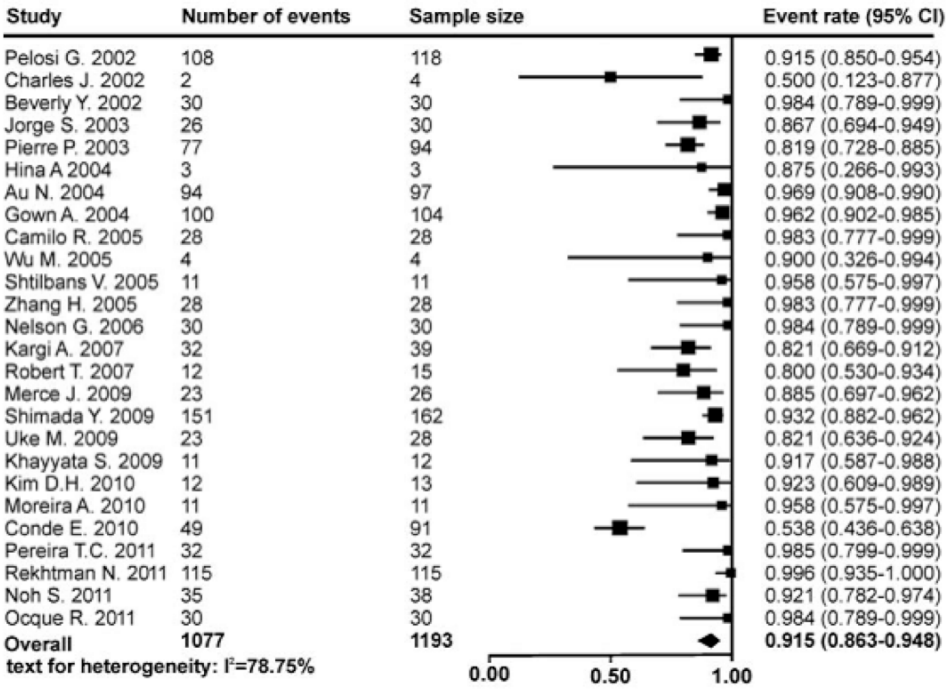

Figure 3: Forest plot for meta-analysis of p63 positive proportion of included trials in specimens histological diagnosed as squamous cell carcinoma of the lung.

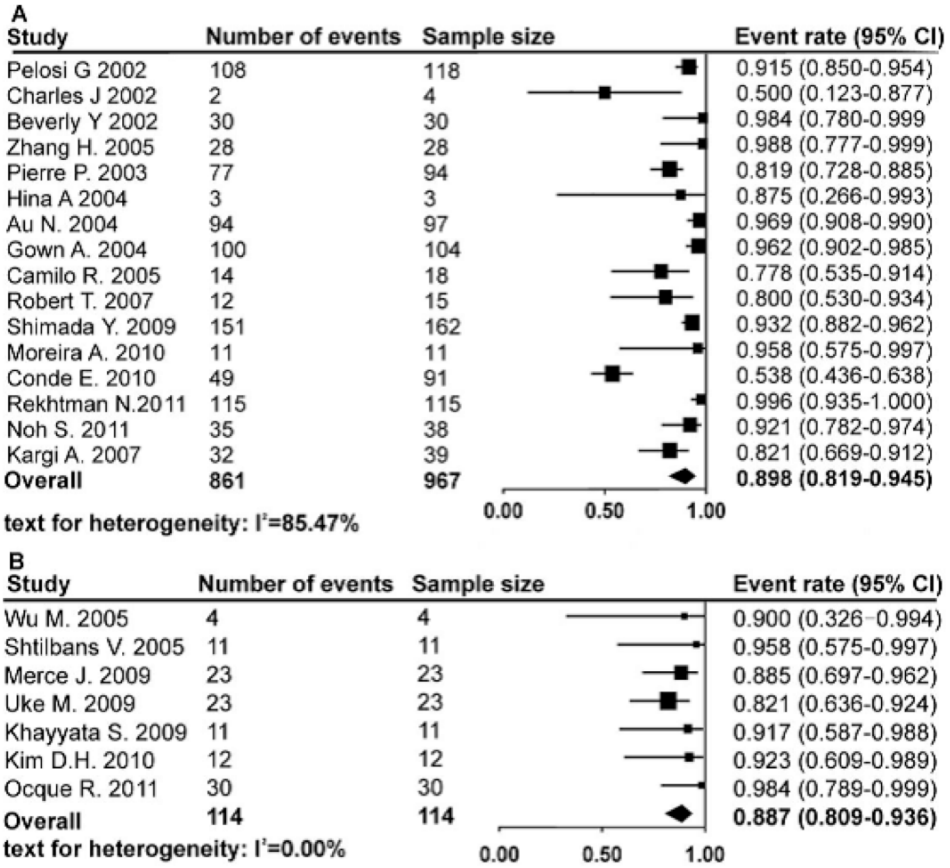

Figure 4: Forest plot for meta-analysis of p63 positive proportion of histological (**A**) or cytological (**B**) methods used to obtain SCC specimens.

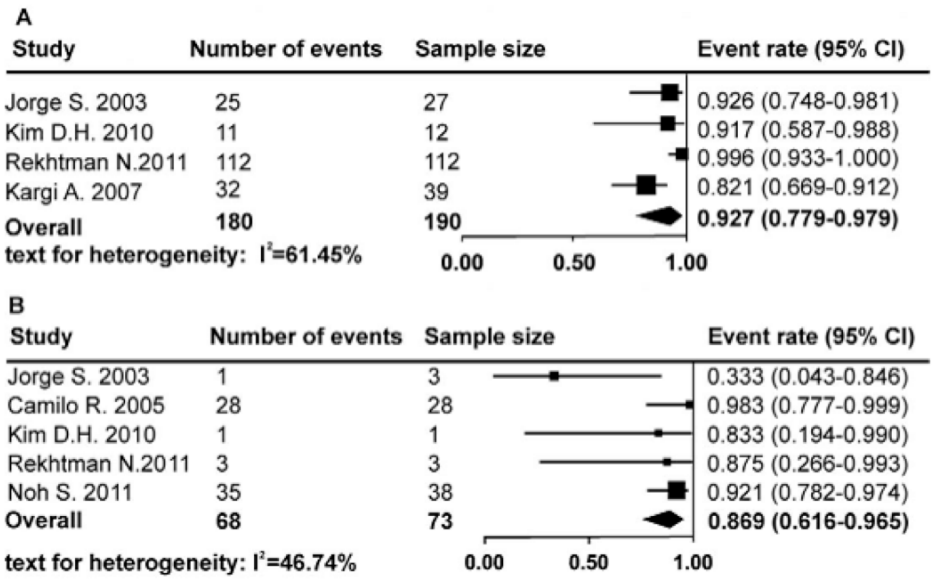

Figure 5: Forest plot for meta-analysis of p63 positive proportion of moderate or well-differentiated (**A**) and poorly differentiated (**B**) subgroups in SCC specimens.

comparison, the positive proportion of the poorly differentiated group (Figure **5B**) ranged from 33.3% to 98.3%, based on 73 specimens from 5 trials with moderate heterogeneity (I^2 = 46.74%). Its overall proportion was 86.9% (95% CI, 61.6–96.5). The 2 groups shared the same meaning in statistical analysis (interaction text p = 0.92).

Third, we evaluated each type of monoclonal antibody according to their source. The 4A4 group (Figure **6**) included 1097 samples from 22 trials. The overall positive proportion was 90.3% (95% CI, 84.2–94.2, I^2 for heterogeneity = 80.73%). The Dako, Santa, Neomarkers groups had an overall positive proportion of 0.81 (95% CI, 0.76–0.85, I^2 for heterogeneity = 86.5%), 0.93 (95% CI, 0.69–0.99, I^2 for heterogeneity = 54.8%), and 0.92 (95% CI, 0.82–0.97, I^2 for heterogeneity = 73.1%), respectively. The interaction text showed no statistical difference between Dako and Santa (p = 0.15) or between Santa and Neomarkers (p = 0.96). However, no statistical difference was found between the Dako and Neomarkers group (p = 0.008).

Finally, we analyzed the positive standard (i.e., the percentages of p63 immunoreactive cells as positive standard may have the proper sensitivity and specificity), which can affect the diagnostic accuracy when using p63 in SCC. Of 25 trials, 13 provided the

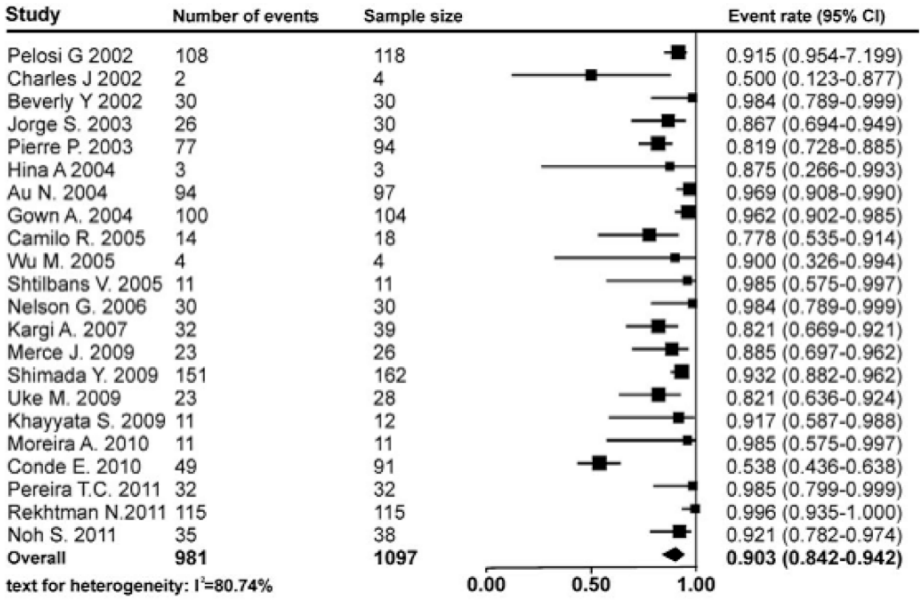

Figure 6: Forest plot for meta-analysis of p63 positive proportion of monoclonal antibody 4A4 subgroup in SCC specimens.

percentage of p63 immunoreactive cells in SCC or the accuracy of its positive standard. Three standards were applied: >1%, >10%, and >50% of p63 immunoreactive cells. The >1% standard (Figure **7**) showed a combined sensitivity of 0.91 (95% CI, 0.86–0.94, I^2 for heterogeneity = 0.0%), specificity of 0.80 (95% CI, 0.75–0.85, I^2 for heterogeneity = 79.4%), and an AUC of 0.9509. The >10% standard (Figure **8**) carried a combined sensitivity of 0.92 (95% CI, 0.90–0.94, I^2 for heterogeneity = 82.7%), specificity of 0.84 (95% CI, 0.82–0.86, I^2 for heterogeneity = 86.9%), and an AUC of 0.9609. In comparison, the >50% standard showed a slight decrease in sensitivity at 0.82 (95% CI, 0.78–0.85, I^2 for heterogeneity = 94.2%), slight increase in specificity at 0.92 (95% CI, 0.90–0.94, I^2 for heterogeneity = 82.1%), and the highest AUC at 0.9771 (Figure **9**).

On the basis of funnel plots, no evidence of publication bias in the positive proportion of p63 was found.

4. DISCUSSION

To the best of our knowledge, this is the first meta-analysis to investigate the positive proportion of p63 in SCC patients, examining possible factors that influence p63 expression. The meta-analysis was based on 25 trials and 1,193 patients.

Considering all specimens in the literature, the total positive proportion of p63 in SCC was 91.5% (95% CI, 86.3–94.8%), ranging from 50% to 99.6%. In contrast, adenocarcinoma and large cell lung cancer, the other common tissue types of lung cancer, have only 30% and 40% positive proportions of p63, respectively [11]. The positive proportion of p63 in the SCC specimen is far greater than these non-small cell carcinomas. Thus, we conclude that p63 in SCC of lung is highly expressed.

To further investigate factors that may influence p63 expression in SCC, we conducted 4 subgroup analyses. First, we evaluated the total positive proportion of p63 in the methods used to obtain specimens. The positive proportion of p63 in histological methods, such as surgery or bronchoscopic biopsy, and cytological methods, including FNA, bronchial washings, bronchial brushings, bronchioloalveolar lavages, were 89.8% (95% CI,81.9–94.5) and 88.7% (95% CI, 80.9–93.6), respectively. No statistically significant difference was found between the 2 groups (interaction text, p = 0.81). Hence, the sampling methods do not affect p63 detection in SCC.

Second, we determined the effect of the degree of differentiation of tumor on p63 expression. In the moderately or well-differentiated group, the positive proportion of p63 ranged from 82.1% to 99.6%, with the

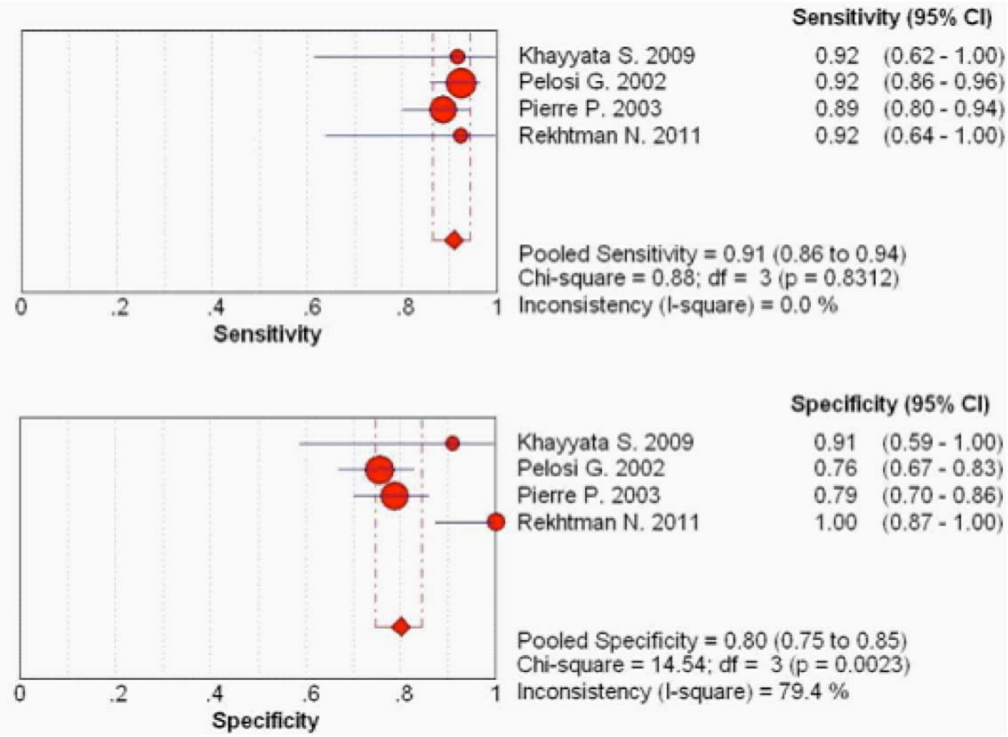

Figure 7: The pooled sensitivity and specificity of >1% immunoreactive cells test for diagnosing p63 positive.

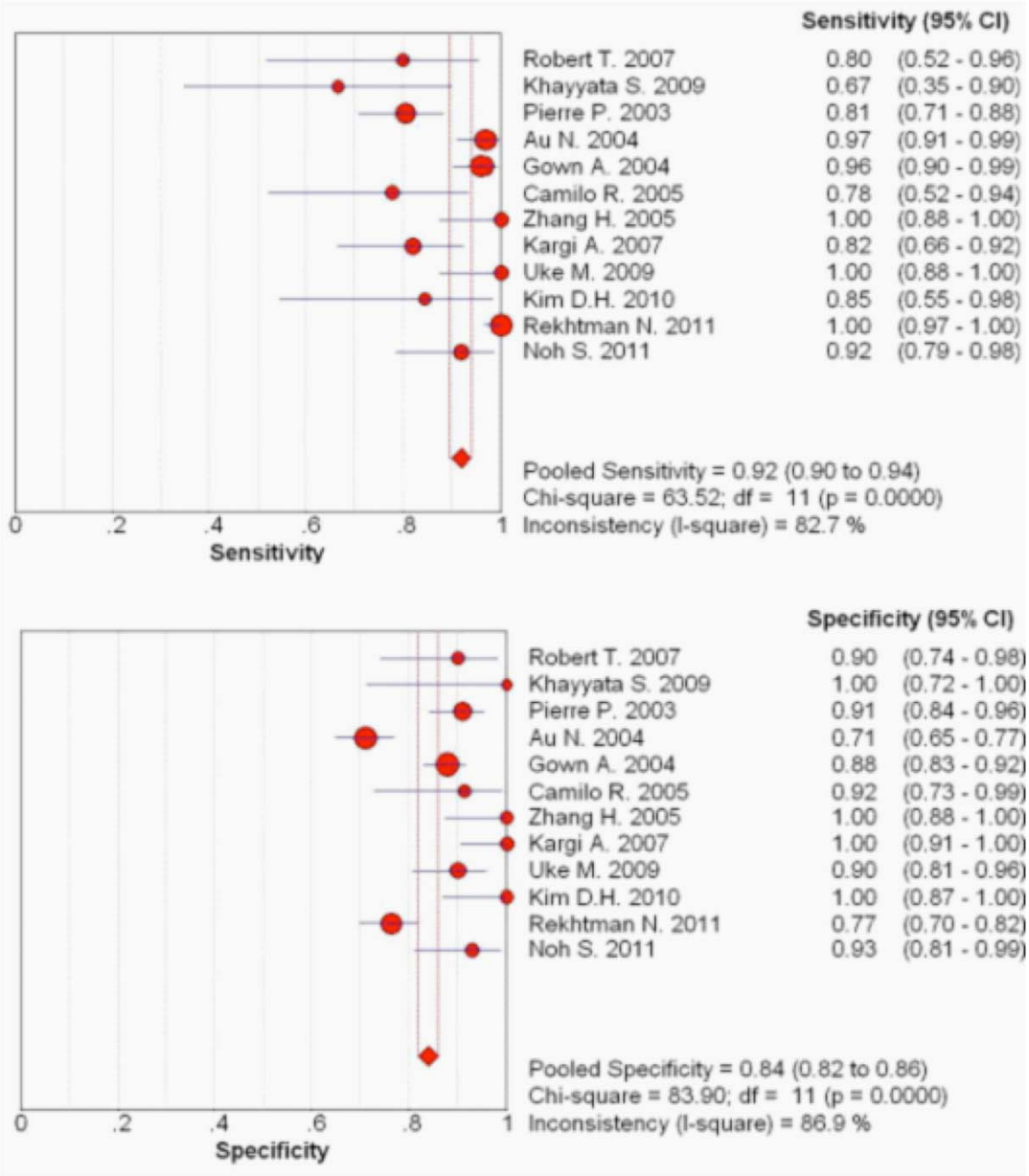

Figure 8: The pooled sensitivity and specificity of >10% immunoreactive cells test for diagnosing p63 positive.

total positive proportion maintained at 92.7% (95% CI, 77.9–97.9%). In comparison, the positive proportion of the poorly differentiated group ranged from 33.3% to 98.3%, with the total positive proportion maintained at 86.9% (95% CI, 61.6–96.5). On closer examination, the study describing a 33.3% positive proportion involved only 3 cases, which may explain why their result was markedly different from the other 4 studies reporting a greater than 80% positive proportion. The range in the poorly-developed is really spreading out, suggesting some complications in defining positive proportion or SCC development. Comparing these 2 groups (interaction text, p = 0.92), we concluded that p63 expression does not associated with SCC differentiation stages.

Third, we examined the effect of monoclonal antibody types used in IHC on p63 expression. The 4A4 is typically the main monoclonal antibody used to determine p63 expression in the IHC, and its total positive proportion reached 90.3% (95% CI, 84.2–94.2). As the numbers of the other antibodies used were too small, we could not compare 4A4 with the other antibodies. Nevertheless, we calculated the total positive proportion of each group according to their source of 4A4. The interaction text among the Dako, Santa, Neomarkers groups showed that the source of the 4A4 antibody could affect p63 detection, possibly due to dilution or a difference in manipulation. Thus, although monoclonal antibody 4A4 clearly does not

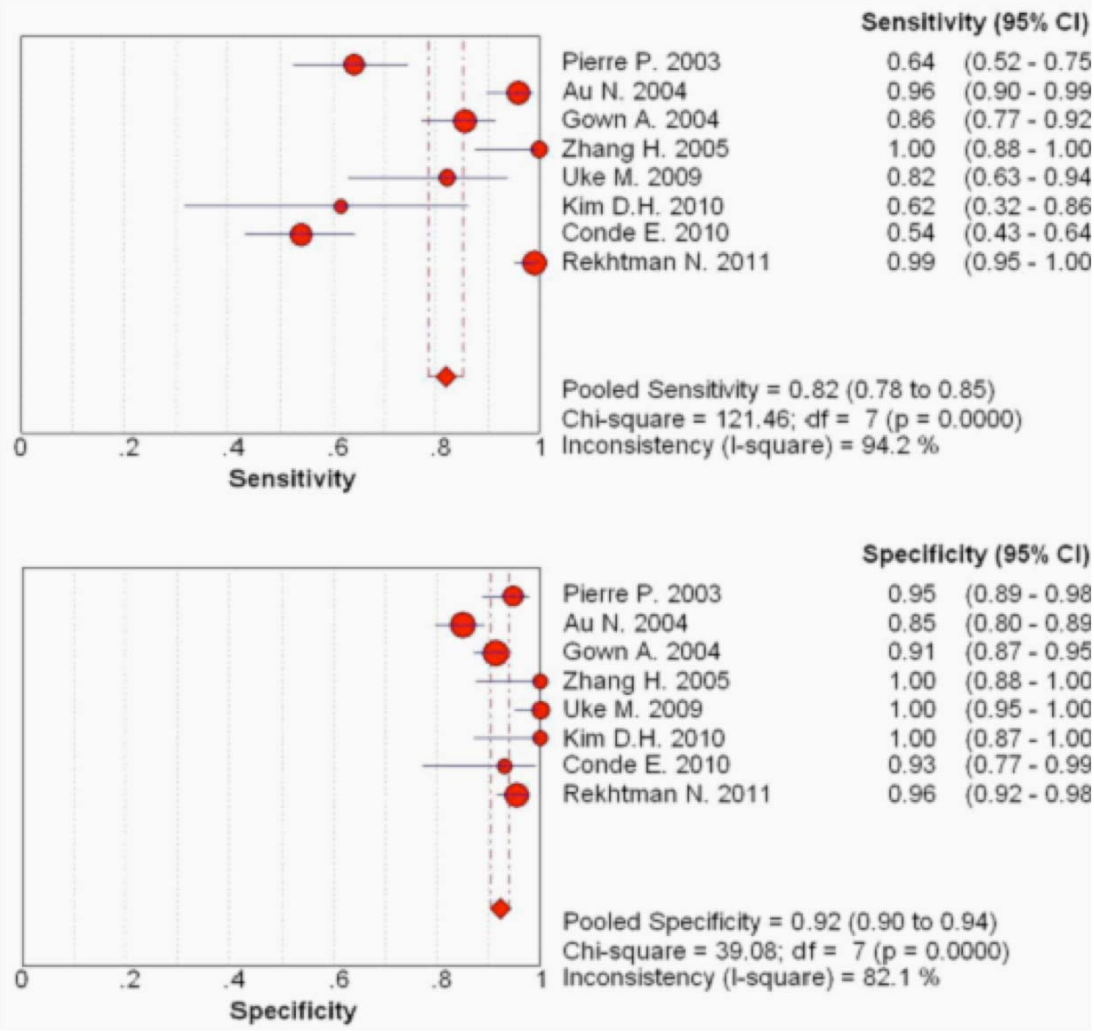

Figure 9: The pooled sensitivity and specificity of >50% immunoreactive cells test for diagnosing p63 positive.

affect p63 expression in SCC, its source may influence the expression of p63.

We observed that a certain randomness existed when each laboratory identified p63 as positive. There were no uniform rules to defining how many percentages of p63 immunoreactive cells can be considered positive. Furthermore, relative analysis considering positive standard as an influencing factor of sensitivity and specificity was limited. Hence, in a fourth subgroup analysis, we artificially divided positive criteria into 3 groups: > 1%; > 10%; and > 50% of the immunoreactive cells as positive. In the >1% standard, the group had a sensitivity of 0.91 (95% CI, 0.86–0.94), specificity of 0.80 (95% CI, 0.85–0.85), and an AUC of 0.9509. Despite only a small amount of cells being stained, the sensitivity and specificity of p63 were maintained at a high level. At the >10% and >50% standard, sensitivity decreased while specificity continued to rise. The >10% standard had a sensitivity of 0.92 (95% CI, 0.90–0.94), specificity of 0.84 (95% CI, 0.82–0.86, I^2 for heterogeneity = 86.9%), and an AUC of 0.9609 whereas the >50% standard had a sensitivity of 0.82 (95% CI, 0.78–0.85), specificity of 0.92 (95% CI, 0.90–0.94), and an AUC of 0.9771. Using AUC as a comprehensive measure of sensitivity and specificity, we could consider that >50% of immunoreactive cells were used as positive indicators, which maximized the effect of combining sensitivity and specificity.

The heterogeneity in our analysis was maintained at a high level, but in the subgroup analysis, the heterogeneity was reduced. The heterogeneity of the overall positive proportion was I^2 = 78.75%, but I^2 ranged from 0% to 85.47% in subgroup analyses. We believed that the high heterogeneity was generated by a variety of factors; not only was the factor incorporated into the subgroup analysis, but also, other factors not incorporated into the analysis, such as the

methodology differences in IHC (such as monoclonal antibody dilution ratio), subjective interpretations of investigators in identifying immunoreactive cells, etc.

Although our study evaluate the association between p63 expression and survival rate, Pelosi [11] reported that p63 immunoreactivity did not affect either overall survival or disease-free survival. However, Pierre [13] observed a strong association between the intensity of p63 staining in SCC and increased the odds of surviving. Hence, the association between p63 expression and overall survival or disease-free survival remains unclear.

Our meta-analysis has several limitations. First, our analysis only focused on SCC of the lung, and did not consider other tissue types, such as adenocarcinoma. Second, despite a broad literature search, a relatively small number of patients, especially in each subgroup analysis, were selected. Finally, we did not consider survival data, which may be clinically relevant when considering p63 expression in SCC.

In conclusion, we found that p63 is highly expressed in SCC and its high expression is not associated with histological or cytological methods of obtaining specimens or the degree of differentiation of the specimens. Even when only a small amount of cells was stained (>1%) as positive standard, the sensitivity and specificity of p63 were maintained at a high level. We recommend >50% of immunoreactive cells as a positive standard in order to achieve proper sensitivity and specificity.

CONFLICT OF INTEREST STATEMENT

Bibo Wang made no disclosures.

Yiping Han made no disclosures.

Jiajie Zang made no disclosures.

ABBREVIATIONS

SCC = squamous cell carcinoma

IHC = immunohistochemistry

ASCO = American Society of Clinical Oncology

ESMO = European Society for Medical Oncology

IASLC = International Association for the Study of Lung Cancer

QUADAS = diagnostic accuracy studies

CI = confidence interval

AUC = area under curve

FNA = fine needle aspirations

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