**Diagnosis Role of APC Promoter Methylation in Non-Small Cell Lung Cancer: A Integrate-Analysis of Published Article and Microarray Data**

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Background: Adenomatous polyposis coli (APC) also known as deleted in polyposis 2.5 (DP2.5) is a protein that in humans is encoded by the APC gene. Mutations in the APC gene may result in colorctal cancer and has been reported to be a candidate tumor suppressor in many cancers. However, the association between APC promoter methylation and NSCLC cancer remains unclear.

Methods: We systematically reviewed studies of APC promoter methylation and Lung cancer published in English or Chinese from January 2001 to January 2012, and quantified the association between APC promoter methylation and Lung cancer using meta-analysis methods.

Results: A total of 1812 samples in 974 participants from seventeen studies were included in the meta-analysis. A significant association was observed between APC promoter methylation and Lung cancer, with an aggregated odds ratio (OR) of 3.79 (95%CI 2.22, 6.45) with random effect model. There was obvious heterogeneity among studies. Subgroup analyses (including by tissue origin, country and age, publish year, tumor stage proportion, gender proportion, Ad2Sc proportion, methylation analysis method, primer location, expriment aim et al ). Meta-regression which were performed to determine the source of the heterogeneity, showed that the trend in ORs was inversely correlated with age(beta=-0.28, P-value <0.001). And significant associated with expriment aim(P-value=0.0093) and gender proportion (P-value=0.0152) . No publication bias was detected with 4 different bias test method. Summary ROC curve was establishmented to determind the best performance of sensitivity and specificity is respectively. Meta-analysis results were validated in independent 957 samples extracted from TCGA and GEO datasets.

Conclusions: This meta-analysis identified a strong association between methylation of the APC promoter and Lung cancer, confirming the role of APC as a tumor suppressor gene.

**Introduction**

Non-small cell Lung cancer(NSCLC) , including adenocarcinoma(AD) and squamous cell carcinoma(SC), is the leading cause of cancer death in men and women in the United States[[1](#_ENREF_1)]. Over 159,480 Americans die of this disease every year in U.S.A[[1](#_ENREF_1)]. The 5-year relative survival rate varies markedly depending on the stage at diagnosis, from 49% to 16% to 2% for patients with local, regional, and distant stage disease, respectively (SEER Cancer Statistics Review 1975-2002). Early detection is a key bottleneck in increasing lung cancer patient survival. DNA hyper-methylation is recognized as an important mechanism for tumor suppressor gene inactivation in cancer and could yield powerful biomarkers for early detection of lung cancer and own incomparable advantage than other traditional diagnosis approaches (stable chemical property, detectable in remote patient media, quantitative signal, convenient low cost detection method) . Several revolutionary steps has been made to push methylation biomarker into cancer screening. However, more biomarkers should be identified to improve the sensitivity and specificity of screening or diagnosis so that it can be push into the reality application.

Along with P16INK4A and RASSF1A, the relationship between hypermethylation of APC with NSCLC was extensively estimated while the resluts were dramatic difference between individual researches which may be caused by the difference of gender proportion, age distribution, race source and some other epidemiology characteristic in samples, detection method etc.

APC gene encodes a tumor suppressor protein that acts as an antagonist of the Wnt signaling pathway. It is also involved in other processes including cell migration and adhesion, transcriptional activation, and apoptosis. Defects in this gene cause familial adenomatous polyposis (FAP), an autosomal dominant pre-malignant disease that usually progresses to malignancy which suggested it is a potential predictor for cancer initial or development. Promoter Methylation Inhibits APC Gene Expression mediated by changes of chromatin conformation and aberrant binding of CCAAT-box binding transcription factors[[2](#_ENREF_2)].

However, there was still not yet any quantitative assessment of relationship between the hypermethylation in the promoter region of APC gene and NSCLC. In this article, we conducted a meta-analyses of the sensitivity and specificity of APC methylation on NSCLC diagnosis from tissues and serum. source of the factors which leaded heterogeneity to the sensitivity and specificity were discovered with meta-regression. In additional, the recent TCGA project provided with massive DNA methylation information for NSCLC which included (535 AD and 50 Control) and (385 SC and 67 control) with comprehensive clinical and demography information. Integrate analysis of all these existing data would make a unbiased conclusion on the relationship between APC methylation and NSCLC.

MATERIALS AND METHODS

**Search strategy, selection of studies and data extraction**

This pooled study involved searching a range of computerized databases, including Pubmed, Cochrane Library, OVID Medline and TMC ProSearch for articles published in English or Chinese to March 2013. The study used a subject and text word strategy with (APC OR BTPS2 OR DP2 OR DP2.5 OR DP3 OR PPP1R461) AND ((Lung OR NSCLC) AND (cancer OR neoplasm)) as the primary search terms. wildcard character of star, dollar or some other truncation were applied according to the rule of the databases to make effective collection of the articles.

Two independent reviewers (Guo, Tan) screened the titles and abstracts identified by the literature search to identify relevant studies. The following types of studies were excluded: animal experiments, case reports, reviews or meta-analyses and studies with non-case-control design or insufficient data or be inaccessible after the contact with the authors. The remained articles were further examined to see if they met the inclusion criteria: 1) the patients had to be diagnosed with NSCLC(AD and SC), 2) the studies had to have APC gene promoter methylation data from tissue,blood or serum, 3) case-control study, which included tissue-tissue, blood-blood or serum-serum in case and controls respectively. The reference sections of all retrieved articles were searched to identify further relevant articles. Potentially relevant papers were obtained and the full text articles were screened for inclusion by two independent reviewers (Guo, Tan). Disagreements were resolved by discussion with KX, JJW. Included studies were summarized in data extraction forms. Authors were contacted when relevant data were missing. The name of the first author, year of publication, origin of the study patients(Europe-America, China, Japan, and Korea), sample size, age(mean or median), gender proportion(male/female, M2F), the ratio of AS proportion(Ad/Sc,Ad2Sc), the proportion of TNM stage I/II samples(proportion of early stage of NSCLC samples), publication aim( for diagnosis or not), analyze multiple genes or not(one or more genes detected simultaneously), control type(autogenous or heterogenous counterpart) and methylation status of the APC promoter in human NSCLC and normal or control tissues were extracted.

**Data analysis and synthesis**

Data were analyzed and visualized mainly using R Software (R version 2.15.3) including meta, metefor and mada packages.[1] The strength of association was expressed as pooled odds ratio (OR) with corresponding 95% confidence intervals (90% CI). Data were extracted from the original studies and recalculated if necessary. The data were pooled using the DerSimonian and Laird random effects model (I2 >50%, P ≤ 0.05) or fixed effects model (I2<50%) according to heterogeneity statistic I2,[[3](#_ENREF_3)] A two-sided P ≤ 0.05 was considered significant without special annotation. Heterogeneity was tested using the I2 statistic with values over 50% and Chi-squared test with P ≤ 0.05 indicating strong heterogeneity between the studies[[4](#_ENREF_4)]. tau-squared(τ2) was used to determine how much heterogeneity was explained by subgroup differences. Meta-regression analyses were employed to analyze the sources of the heterogeneity when the heterogeneity was significant[[5](#_ENREF_5)]. Subgroup analyses of the ORs of APC promoter methylation in cancer tissue versus normal tissue were performed according to control types (autogenous and heterogeneous), patient origins (Europe-America, China, Japan, and Korea) and age. Sensitivity analyses were performed to assess the contributions of single studies to the final results with the abandon of one article each time. publication bias was analyzed by funnel plot. If bias was suspected, the conventional meta-trim method was used to re-estimate the effect size.

Compared with traditional meta-analysis, such as SNP association study associated meta-analysis in which we suppose diagnostic standard should be same or similar for each study, diagnostic associated meta-analysis might be involved with different diagnostic thresholds. Summary receiver operating characteristics (SROC) analysis was applied to meta-analysis of diagnostic tests[[6](#_ENREF_6)]. It was plotted to show the performance of the diagnosis ability of APC methylation to NSCLC. Each study produces values for sensitivity, specificity and therefore TPR and FPR. A graph is made from the (TPR, FPR) points. The SROC curve is placed over the points to form a smooth curve. Linear regression model were selected to fit the SROC curve where sensitivity and (1-specificity) are transformed into complex logarithmic variables. The exact area under the curve (AUC) for the SROC function was used to assess the accuracy of the test.

RESULTS

**Study characteristics**

The electronic search strategy identified 506 potentially relevant articles(Pubmed, 315; Scopus, 112, Cochrane Library,3; OVID Medline, 53, TMC ProSearch, 23), which were further screened for inclusion on the basis of their titles, abstracts, full texts, or a combination of these. The electronic search was supplemented from reference lists of relevant articles including reviews, and by discussion with experts. Finally, 17 studies included data on the relationship between APC gene promoter methylation and NSCLC and these studies were pooled for analysis (Table 1) [[7-23](#_ENREF_7)]. All the included articles were written in English. Totally, 1338 lung cancer tissues /serum and 913 normal counterpart tissues/ serum. Age range of subjects in the 17 studies was 25-86 years while mean or median age was 53-67. Among the 17 retrieved studies(13 articles were especially for diagnosis while 5 for other designs, such as prognosis or survival research), 7 observations used methylation-specific polymerase chain reaction(MSP) to explore APC promoter methylation while others used quantitative MSP such as Methylight, Prosequencing, etc. The proportions of samples of stage I and stage (I+II) were calculated for which the range were 32.1-100% and 70-100% respectively. Range of the ratio of male to female in the NSCLC samples was also estimated which was 53%-81%.

The ORs of random effect model pooled and fixed effect model for APC methylation in cancer tissues compared with normal tissues were 4.67 (95%CI, 2.66-8.22, z=5.3534, P < 0.0001), 2.74 (95%CI,1.99-3.23, z =8.1038, P < 0.0001), indicating an increased likelihood of methylation in Lung cancer tissue, compared with normal tissue **(Figure 2)**.

**Subgroup analysis and meta-regression**

Subgroup analysis were conducted under different subtype of the sample type( tissue or serum), counterparts category(autogenous and heterogeneous), proportion of stage I/II (less or more than median), aim of the study(for diagnosis or non-diagnosis) and other possible interference factors**(Table 2)**. Both tissue and serum groups had showed significant association between APC methylation and NSCLC(OR=11.54, 3.72 respectively) which suggested APC methylation can be taken as a potential biomarker for NSCLC diagnosis or screening as a noninvasive approach in remote patient media. Significant difference were found between the OR of heterogeneous(ORh=8.33, 95%CI, 3.77-18.39) and autogenous(ORa=2.25, 95%CI, 1.06-4.77) subgroup (P-value=0.0187), which might be caused by complex situation of the blood compositions and should be deeply considered in the application in clinical application. The OR in the young subgroup(OR=4.65) was greater than older subgroup(OR=2.24), in additional, subgroup of high proportion of stage I and stage (I+II) had a larger OR than that of subgroup of low proportion of stage I and stage (I+II) **(Table 2)**. These indicated methylation of APC might occurred or functioned at the early stage of the tumorigenesis, which had been founded in other independent studies[[24](#_ENREF_24)]. Differences of the ORs in diagnosis(OR=6.79) or non-diagnosis group(OR=2.59) was very large which supposed that there were some public bias in diagnosis subgroup and the truth of the methylation ratio might be lower than the reported data**(Table 2)**. No significant differences were found in subgroup of MSP and qMSP (P-value=0.7685), which suggested both these two methods were equivalent in methylation detection(Table 2) and the result was consistent with Wu’s conclusion[[25](#_ENREF_25)] .

We performed further analyses using the meta-regression method with the Knapp-Hartung modification since heterogeneity significantly existed among all studies (I2 = 79.2%, Q = 52.78, P < 0.0001) (**Figure 1**). The results of meta-regression indicated that the trend in ORs was inversely correlated with age, which accounted for some of the heterogeneity (coefficient = -0.16, P = 0.042, adjusted R2 = 44.47%, Table 3). However, other factors such as sample size, year of publication, proportion of males, and the origin of the patients could not explain the heterogeneity (**Table 3**).

**Summary Receiver Operating Characteristic Curve for diagnosis capacity of APC methylation**

Pooled sensitivity and specificity were 0.548(95%CI,0.420,0.669,P<0.0001) and 0.776(95%CI, 0.623-0.879, P<0.0001) for the whole studies. the sensitivity of tissue subgroup was higher than that of serum subgroup, 0.609(0.453-0.745) versus 0.681(0.495-0.828), while specificity of serum subgroup was higher than that of tissue subgroup 0.396(95%CI,0.256-0.555) versus 0.92(0.855-0.957), which suggested the advantage of this biomarker for its high specificity, especially in remote non-invasive media.

Although sensitivity and specificity were two of most important feature of a diagnosis test, sometime, pooling sensitivity or specificity could be misleading event[[26](#_ENREF_26)]. we descripted the ability of the test with SROC curve, which could depict the stability and accuracy of the diagnosis test. In additional, Since the quantitative assess of the APC methylation in diagnosis of prostate cancer had been conducted by Yang Chen[[27](#_ENREF_27)], we compared the two SROCs to give a comprehensive understand of the diagnosis role of APC methylation**(Figure 3)**.The result showed that both sensitivity and specificity in diagnosis of prostate cancer(sen=0.75,spe=0.85) were higher than that in NSCLC (sen=0.55,spe=0.78). AUC for NSCLC was 0.671 while that for PCa was 0.82.

**Bias analysis and robust estimation of pooled OR**

A funnel plot of methylation status of Lung cancer tissue versus normal tissue showed that eight studies exceeded the 95% confidence limits **(Figure 3)**. In order to eliminate the effect of public bias, trim and fill analysis was performed using the random effects model. The adjusted pooled OR were 2.50 (95%CI, 1.43-4.38, P=0.0013) in random effect model and 2.19(95%CI, 1.74-2.77, P<0.0001) in fixed effect model, respectively, indicating a significantly positive association between APC methylation and Lung cancer.

Sensitivity analyses to determine the effects of omitting a single study on the overall effect showed the overall ORs were between 4.3(95%CI, 2.46-7.52) and 5.27 (95%CI, 2.92, 9.53) in the random effect method, which suggested that there was no single sensitive study **(Figure 4)**.

In spite of slight influence to combined effect size of OR, the influence to meta-regression were analyzed when omitting one study each time to explore heterogeneity sources. The sample type of tissue or serum would be one of the heterogeneity sources when Begum et al (2011, USA) were removed from the meta studies(P-value < 0.026), likewise, the proportion of stage I, stage II and aim of the study would become heterogeneity source when Lin et al (2009, China), Zhang et al (2011,China) and Yanagawa et al (2003, Japan) was removed ( P-value were 0.0046, 0.029 and 0.039 respectively), which suggest these factors should be considered in the future case-control association study.

**Validation by independent TCGA Lung cancer Dataset**

In order to make independent validation of the result above, we collected the methylation status of 6 CpG sites in 336 Ad, 409 Sc and its counterparts from TCGA Lung cancer project (<http://cancergenome.nih.gov/>). The information of these 6 CpG sites were descripted in Supplementary Table S1.

DISCUSSION

APC gene has been supposed as a important tumor suppressor gene in colorectal cancer[[28](#_ENREF_28)]. The role of the APC gene in Lung cancer is controversial[[29-31](#_ENREF_29)]. No matter what’s the biological function of APC in tumorigenesis, the diagnostic role in cancer screening or subgroup definition has been studied by numbers of research.

We therefore performed a meta-analysis to quantify the ability for APC promoter methylation test in NSCLC diagnosis. The overall OR for methylation status in NSCLC versus normal Lung tissue was 5.63 (3.15, 10.07) in the random effects model on pooled data from 16 observations in 15 studies. Subgroup analysis showed an OR in the heterogeneous tissue-origin subgroup of 4.44 (0.35, 56.70), which was significant in the fixed, but not in the random effects model. This discrepancy might be a result of the smaller number of studies analyzed. The ORs also differed in subgroups with different ethnic origins: the OR in the Chinese subgroup was 26.19 (95%CI: 8.04, 85.33), followed by the

Trim and fill tests were performed using the random effects model, and two virtual studies were filled. The overall OR of the trim and fill method was 4.67, which was slightly smaller than that of the crude meta-analysis, but it was still significant, indicating a strong association between APC promoter methylation and Lung cancer.

Meta-analysis has been widely applied in SNP-disease risk association studies that is because SNPs had specific genome location, meanwhile, it is also booming in DNA methylation realm gradually. The primers for methylation detection have been considered when extracting information from studies, however, this information was difficult to be analyzed in the following subgroup or meta-regression analysis, since the high diversity of the primers used in each individual articles. we observed there were at least 6 different primers used in 18 studies. In order to expatiate on the divergence of different CpG sites, we collected the methylation signals of 6 CpGs from methylation 27K and 450K microarray dataset from TCGA project(LUAD and LUSC). we found the methylation status of six different CpG sites were dramatically different which methylation ratio in cancers were from ? to ? (Table ). this phenomenon reminded that future DNA methylation detection in case-control studies should be design more accurately and comprehensively to some certain CpG site or blocks.

European-American subgroup (OR = 5.15, 95%CI: 0.23, 113.17), the Korean subgroup (OR = 4.96, 95%CI: 2.14, 11.47), and the Japanese subgroup (OR = 3.20, 95%CI: 1.60, 6.39). The ORs for the different age subgroups were 15.00 (95%CI: 5.21, 43.15) for age <60, 6.31 (95%CI: 3.19, 12.46) for age 60-65, and 3.75 (95% CI: 1.29, 10.89) for age ≥ 65. Heterogeneity within the studies was demonstrated by c2and I2 tests and meta-regression was therefore used to determine the sources of the heterogeneity. This showed that the trend in ORs was inversely correlated with age, which accounted for at least some of the heterogeneity (coefficient = -0.16, P = 0.042, adjusted R2 = 44.47%). These results were consistent with the results of subgroup analysis according to age. The incidence of age-related methylation in most organs was in accordance with the reported incidences of methylation in their malignant counterparts [45]. APC methylation was reported to occur preferentially in the lower third portion of the stomach in individuals older than 70 years [45]. We found that the ORs for APC methylation decreased from 15.00 in the younger age group, through 6.31, to 3.75 in the oldest age group. The coefficient for age was calculated to be -0.16 by meta-regression analysis, indicating that the tendency for APC methylation decreased with advancing age. To the best of our knowledge, the current study is the first to report this finding. However, there was no significant difference in APC promoter methylation status of cancer tissues between older (more than 60) and younger (less than 60) individuals. This suggests that APC methylation was not correlated with age in Lung cancer tissue. However, in accordance with the previous study, APC methylation did decrease with advancing age in normal Lung mucosa [45]. The negative correlation between APC methylation and age suggests that the influence of APC methylation on Lung cancer is reduced in older individuals. Other factors including sample size, year of publication, proportion of males, and the origins of the patients were not identified as sources of heterogeneity by meta regression analysis. Meta-analysis of small studies might result in biased results. The funnel plot showed that the standard errors in four of the studies exceeded the 95% confidence limits; furthermore, the asymmetry test of the funnel plot by Egger’s regression method showed that the smaller studies reported more positive results, raising the suspicion of bias among the studies. To produce a more robust estimation, we performed sensitivity analysis using the random effects model and were unable to identify any individual sensitive study with a strong influence on the pooled results.

There were no significant differences in APC methylation in cancer tissues in relation to gender, TNM stage, invasion of tumors into vessels or lymphatic ducts, or tumor stage. Although some studies have reported significant differences in methylation status and protein expression of APC in relation to tumor invasion depth [46], the overall results of the current study failed to support the existence of such a relationship. Previous studies have also reported increased methylation of APC in stage I and II Lung cancers, suggesting that the APC gene contributes to Lung cancer development [38]. However, the results of the current meta-analysis did not support this assumption. Other factors, such as tumor invasion of blood vessels, lymph nodes or lymphatic ducts, and tumor metastases, also demonstrated no relation with APC methylation. The aggregated results found that APC methylation was more frequent in intestinal-type compared with diffuse- type Lung carcinomas, suggesting that inactivation of APC might play a more significant role in the development of intestinal-type Lung carcinomas. Analysis of the pooled data also showed that undifferentiated Lung cancers had a higher methylation OR than well-differentiated cancer tissues. This suggests that APC promoter methylation or down-regulation of the APC gene might be related to poor prognosis, as suggested in previous studies [47,48].

**Conclusions**

In conclusion, this meta-analysis of pooled data provides additional evidence to support a strong association between methylation of the APC promoter and NSCLC. This association depended on patient origin and the controls used, and further studies are needed to explore these aspects. Younger individuals had higher APC methylation rates than older individuals. APC methylation was also associated with histological type and differentiation state of the Lung cancer. However, gender, TNM stage, invasion of tumors into blood vessels or lymphatic ducts, and tumor stage showed no significant associations with APC methylation in Lung cancer tissues.

When we evaluating the specificity of the methylation test, we should promise all the controls are healthy or non-cancer, However, neither of the adjacent tissues nor random healthy serum were taken rigorously biosy to assure this precondition.

**Authors’ contributions**

All authors have made substantial contributions to this article: contributed to the conception, design and final approval of the submitted version. contributed to the analysis and interpretation of data, and drafting of the article. contributed to the acquisition of data and revision of the article. contributed to the acquisition of data and discussion of the article design. All authors read and approved the final manuscript.

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Table 1, Basic characteristics of studies included

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Author (Published Year) | Sample Type | Agea (years) | Stages I % | StagesI+II % | Gender (M/F) | Patients(M+/M-) | Control (M+/M-) | Methods | Aim | Multiple Targe |
| **Zhanget al (2011,China)b** | tissue | 59 | 32.05 | 74.36 | 29/39 | 44/34 | 10/68 | MSP | Diagnose | Yes |
| Wang et al (2008, China) | tissue | NA | NA | NA | 17/28 | 19/9 | 1/11 | 3-D PCR | Diagnose | Yes |
| Jin et al (2009, Japan) | tissue | 66.7 | NA | NA | 17/24 | 27/45 | 22/41 | MethyLight | Non-diagnose | Yes |
| Feng et al (2008, USA) | tissue | 64.3 | 42.86 | 78.00 | 26/49 | 26/23 | 21/28 | MethyLight | Diagnose | Yes |
| Brabender et al (2001, USA) | tissue | 63.3 | 49.45 | 70 | 69/91 | 86/5 | 80/11 | qRTPCR | Non-diagnose | SIngle |
| Virmani et al (2001, USA) | tissue | NA | NA | NA | NA | 22/26 | 0/18 | MSP | Diagnose | Yes |
| Yanagawa et al (2003, Japan) | tissue | 67.3 | 66.67 | 74.67 | 18/25 | 28/47 | 36/39 | MSP | Diagnose | Yes |
| Topaloglu et al (2004, USA) | tissue | NA | 54.84 | 83.87 | NA | 17/14 | 5/17 | qRTMSP | Diagnose | Yes |
| Kim et al (2007, Korea) | tissue | 63 | 56.57 | 74.00 | 64/79 | 48/41 | 33/66 | MSP | Non-diagnose | Yes |
| Vallbohmer et al (2006, USA) | tissue | 63 | 49.45 | 70.00 | 69/91 | 86/5 | 80/3 | PCR | Non-diagnose | Yes |
| Lin et al (2009, China) | tissue | 61.1 | 100.00 | 100.00 | 20/31 | 49/75 | 2/24 | MSP | Diagnose | Yes |
| Shivapurkar et al (2007, USA) | tissue | NA | NA | NA | NA | 35/5 | 23/17 | semiq RTPCR | Diagnose | Yes |
| Suzuki et al (2006, Japan) | tissue | 64 | 34.00 | NA | 33/49 | 53/97 | 3/57 | MSP | Non-diagnose | Yes |
| **Zhang et al (2011, China) b** | serum | NA | NA | 100 | NA | 54/56 | 5/45 | MSP | Diagnose | Yes |
| Pan et al (2009,China) | serum | 53 | NA | NA | 17/26 | 40/38 | 0/31 | RT-qMSP | Diagnose | Single |
| Begum et al (2011, USA) | serum | 65 | NA | 76 | 10/19 | 12/64 | 3/27 | qMSP | Diagnose | Yes |
| Rykova et al (2004, Russia) | serum | NA | NA | NA | NA | 3/6 | 0/16 | MSP | Diagnose | Yes |
| Usadel et al (2002, USA) | serum | 64.2+-9.6 | NA | NA | NA | 42/47 | 0/50 | RT-qMSP | Diagnose | Single |

Age, mean or median age from articles; Zhang et al (2011, China) b with two records since there are tissue and serum data simultaneously in this article.

Table 2 subgroup analysis for the main potential interference factors with random effect model

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Study | OR | 95%CI | Q | I2 | P-value |
| All | 12 | 3.28 | 1.74-6.17 | 52.78 | 79.2% |  |
| Age≤ 64 | 6 | 4.65 | 2.17-9.93 | 15.42 | 67.60% |  |
| Age＞64 | 6 | 2.24 | 0.89-5.56 | 24.89 | 79.90% | 0.22 |
| Stage I≥49.45% | 5 | 4.11 | 1.90-8.91 | 12.76 | 68.60% |  |
| Stage I＜49.45% | 4 | 2.81 | 0.87-9.09 | 19.42 | 84.60% | 0.5944 |
| Stage(I+II) ≥75.33% | 5 | 2.45 | 0.98-6.12 | 26.69 | 85% |  |
| Stage(I+II)＜75.33% | 5 | 4.4 | 1.70-11.39 | 12.77 | 68.70% | 0.38 |
| M2F≤ 69.1% | 6 | 5.98 | 2.04-17.53 | 16.66 | 70% |  |
| M2F＞69.1% | 6 | 2.13 | 0.99-4.55 | 29.05 | 82.80% | 0.1246 |
| MSP | 8 | 5.16 | 2.01-13.26 | 44.61 | 84.30% |  |
| qMSP | 10 | 4.32 | 2.08-8.94 | 29.28 | 69.30% | 0.7685 |
| Diagnose | 13 | 6.79 | 2.99-15.44 | 59.54 | 79.80% |  |
| Non-diagnose | 5 | 2.59 | 1.33-5.05 | 11.56 | 65.40% | 0.0745 |
| Multiple | 15 | 4.08 | 2.28-7.34 | 62.99 | 77.80% |  |
| Single | 3 | 18.72 | 1.23-283 | 9.03 | 77.80% | 0.2836 |
| heterogeneous | **12** | **8.33** | **3.77-18.39** | **35.71** | **69.2%** |  |
| autogenous | **6** | **2.25** | **1.06-4.77** | **27.19** | **81.6%** | **0.0187** |
| Serum | 5 | 11.54 | 2.87-46.40 | 10.4 | 61.50% |  |
| Tissue | 13 | 3.72 | 2.03-6.78 | 55.18 | 78.30% | 0.14 |

K represent the number of the study in each subgroup; P-value shows the significance of the difference between groups