A systematic review and meta-analysis of correlation between cough variant asthma and mycoplasma pneumonia in children

Type
Research paper

Keywords
children, Mycoplasma Pneumonia, cough variant asthma

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Multiple databases were searched for relevant studies, and the articles that eventually satisfied the inclusion criteria were included. All the meta-analyses were conducted with the Review Manager 5.2. To estimate the quality of each article, the risk of bias table was performed. Totally 1223 patients with CVA and 1437 patients with simple cough (SC) were included.

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Finally, 9 studies including 2660 patients were included, who eventually satisfied the eligibility criteria. The results of heterogeneity test suggested that the serum level of IgE (MD = 80.69, 95%CI [77.75, 83.62], P < 0.001; P for heterogeneity <0.001, I² = 95%), eosinophil count (MD= 2.93, 95%CI [2.72, 3.13], P < 0.001; P for heterogeneity < 0.001, I² = 71%) and the number of children with positive IgM (OR = 4.44, 95%CI [3.73, 5.29], P < 0.001; P for heterogeneity = 0.63, I² = 0%) were significantly different. The value of IgE in CVA was higher than that in SC, eosinophil count in CVA was higher than that in SC and the number of IgM positive children in the CVA group was higher than that in the SC group.

Conclusions
This study demonstrated a correlation between cough variant asthma and mycoplasma pneumonia.
A systematic review and meta-analysis of correlation between cough variant asthma and mycoplasma pneumonia in children

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Conclusion: This study demonstrated a correlation between cough variant asthma and mycoplasma pneumonia.

Key words cough variant asthma, mycoplasma pneumonia, children.
Introduction

Asthma is the most common chronic childhood disease in the world. According to the guidelines of the WHO on Integrated Management of Childhood Illness (IMCI), the diagnosis of asthma is based on the presence of wheezing[1-3]. It is closely related with childhood asthma and bronchiolitis. Cough variant asthma (CVA) mainly has features such as repeated episodes of coughing and prolonged treatment[4-6]. Long term irritant dry cough, less sputum and other severe symptoms would occur when cold irritation or pollen allergens get in. CVA is a special type of asthma and chronic cough (cough lasts for more than 8 weeks and chest X-ray shows no evidence of lung disease) is a main manifestation. In children with CVA, IgM antibody level, serum total IgE level and peripheral eosinophil count should be determined to rule out Mycoplasma pneumoniae. In a research based on 10000 samples, the highest age getting CVA among children is 3-6 years, the ratio of incidence between boys and girls is 1.42:1 and CVA accounts for 14%-33% of the causes of chronic cough [1].

In low-income countries, pneumonia is considered to be the main cause of respiratory disease and death among children under five years of age[7, 8]. Mycoplasma pneumoniae is a pathogenic microorganism, which is mainly adsorbed on the surface of the airway mucous membrane[9-11]. It will destroy the secretion of airway epithelial cells and glands, inhibiting cillum movement and so on. This can cause chronic inflammatory changes in the respiratory system. After long-term mycoplasma pneumonia, the airway smooth muscle becomes contracted, leading to dysfunction of non-cholinergic nerves, airway responsiveness, exacerbating inflammation and migrating disease [11].

The relationship between Mycoplasma pneumoniae infection and asthma has become a hot topic. Because of genetic differences among individuals, chronic and nonspecific airway inflammation leads to edema of the airway mucous membrane, increased mucus secretion and spasm of bronchial smooth muscles. Further studies on the etiology and determinants of asthma indicate that Mycoplasma pneumoniae is considered to be one of the pathogens associated with acute or chronic asthma [10].
Since association between CVA and *Mycoplasma pneumoniae* is still needed to be analyzed, exploration about the relationship is conducted. This study aimed to analyze the relationship between CVA and *Mycoplasma pneumoniae* infection in children.

**Materials and Methods**

**Search strategy**

The main aim of this article was to evaluate the relationship between CVA and *Mycoplasma pneumoniae*, to identify all of the relevant publications, multiple electronic databases including PubMed, Springer, EMBASE, OVID and China Journal Full-text Database were searched, without language restrictions. To increase search accuracy, the following MeSH (Medical Subject Headings) terms were assembled with the boolean operator “AND”: (1) Cough variant asthma OR CVA; (2) *Mycoplasma pneumoniae* OR MP; (3) Child OR Children related articles with all publication statuses (published, unpublished, in press and in progress) published from January 2000 to January 2020 were systematically searched and reviewed. Two authors in our team searched the literature independently and examined the reference lists to obtain additional relevant studies that were not identified.

**Study selection**

Two authors selected the citations independently in this study with the following inclusion criteria: (1) pediatric patients with CVA; (2) sample size more than 10; (3) randomized control trial or controlled clinical trial; (4) availability of full text; (5) since clinical laboratories can provide diagnostic testing for *Mycoplasma pneumoniae* infections using culture, serology, or nucleic acid amplification methods, blood test results should be reported. The exclusion criteria were: (1) patients without CVA or adult patients; (2) sample size was less than 10; (3) case report or meta-analysis; (4) results about patients were ineligible. They screened the titles and
abstracts of the articles identified, and subsequently, the full text of the studies that potentially met the criteria was obtained. These two investigators determined the included articles together, and disagreements were resolved by consultation with a third investigator if necessary.

**Data extraction**

After reading the full text of the articles, we extracted the characteristics from each study using a standard data extraction method: the first author’s name, year of publication, nation, age range of patients, sex (male/female), sample size (cough variant asthma (CVA)/ simple cough (SC)), follow-up duration, and other parameters. SC patients only had cough without other symptoms.

**Statistical analysis**

The meta-analyses were conducted with the software Review Manager 5.2 (The Cochrane Collaboration, 2011) to estimate the association between cough variant asthma and *Mycoplasma pneumonia* in child patients. As continuous outcomes, standard mean difference (SMD) with 95% confidence intervals (CIs) was performed. In discontinuous outcomes, odds ratio (OR) with 95% confidence intervals (CIs) was conducted. A P value <0.05 was considered to be statistically significant. Heterogeneities in this study were assessed using the I^2 index. We chose the random-effects model when the heterogeneity I^2 statistic was > 50%; otherwise, the fixed-effects model was applied.

In addition, the quality of the studies was assessed with sensitivity analysis and bias analysis. Risk of bias table of the included studies was independently assessed according to the Cochrane Handbook for Systematic Reviews of Interventions by two members of our team. In addition, if there was poor agreement and no consensus could be achieved, a third investigator was the adjudicator.
Results

Search results

As shown in the flow diagram Figure 1, 1031 relevant studies were initially identified, and 1022 articles were excluded due to duplication, irrelevant studies, incomplete data, incomplete comparison, other diseases and lack of full text. Finally, 9 articles[12-20] satisfied the inclusion criteria. Among these 9 articles, 7 articles were published in English and 2 articles in Chinese. The study selection process is shown in Fig. 1.

Characteristics of the included studies

The detailed characteristics of the included studies, including first author’s name, year of publication, nation, recruitment time, age range of patients, sex (male/female), sample size (CVA/SC), follow-up period, and other parameters are presented in Table 1. All these studies were published from 2000 to 2018. The sample size ranged from 53 to 600. Totally 1223 patients with CVA were included in these studies, and 1437 were patients with simple cough (SC).

Quality assessment

The bias table in the Review Manager 5.2 Tutorial was used to evaluate the risk of each study by applying the criteria for evaluating design-related bias. The evaluation in this study is shown in Figure 2 and 3. In the 9 articles, 1 trial showed problem in patient selection and 1 showed problem in attrition bias. Overall, all the trials were free of risk.

Results of meta-analysis

1. Meta-analysis of serum IgE level in CVA and SC patients

Nine studies compared serum IgE level in CVA and SC patients. The forest plot for serum IgE level in different groups is shown in Figure 4. Total IgE was detected by immunoturbidimetry. All these studies reported that serum IgE levels between
CVA and SC patients were statistically significant. The value of IgE in CVA patients (MD=167.85) was higher than that in SC patients (MD=90.29). The overall result is shown in Figure 4 (MD = 80.69, 95%CI [77.75, 83.62], P <0.001; P for heterogeneity <0.001, I² = 95%).

2. Meta-analysis of eosinophil count in CVA and SC patients

Nine studies comparing eosinophil count showed statistical difference (Figure 5). Eosinophil count was detected by blood cell counter. The meta-analysis indicated that eosinophil count in patients with CVA (MD=6.56) was higher than patients with SC (MD=3.53) (MD= 2.93, 95%CI [2.72, 3.13], P< 0.00001; P for heterogeneity< 0.001, I² = 71%).

3. Meta-analysis of the number of IgM positive CVA and SC patients

Nine studies compared the number of IgM (anti IgM for mycoplasma pneumonia) positive CVA and SC patients. The determination of serum IgM was as follows, blood was extracted from 2 mL in children and centrifuged at 2000 rpm. Serum IgM was extracted by passive agglutination, and then frozen in the refrigerator at 15 ℃ and IgM > 1:80 was considered positive [7].

The meta-analysis showed that CVA and SC patients showed statistically significant difference in the number of positive IgM cases (OR = 4.44, 95%CI [3.73, 5.29], P < 0.001; P for heterogeneity =0.63, I² = 0%) (Figure 6). The number of IgM positive patients in the CVA group (MD=73.67) was higher than that in the SC group (MD=33.12).

Sensitivity Analysis

According to the results of meta-analyses, the heterogeneity of serum IgE level was very high (I²>=95%). As shown in Figure 7, the high heterogeneity of serum IgE level was probably due to differences in the studies. When the study in 2008 by Juhn was excluded, I² changed from 95% to 92%, which suggested that the sensitivity of the article was stable.
Bias Analysis

A funnel plot for serum IgE level in CVA and SC patients was performed. All the studies were included in the plot. The results showed some publication bias since the symmetrical characteristic of the funnel plot was not good (Figure 8).

Discussion

Asthma affects almost 30 million Americans and 300 million persons worldwide. The prevalence of asthma has increased over the past 2 decades in both children and adults[21-23]. CVA is a common cause of emergency hospital visits in low and medium income countries[24, 25]. Outcomes of children with CVA are influenced by age of the child, peripheral oxygen saturation on admission and adherence to asthma therapy.

Pneumonia and CVA are different disease entities with similar clinical presentations among young children. The gold diagnostic standards for pneumonia and CVA in young children are quite sensitive[26, 27]. Pneumonia is considered the major cause of morbidity and mortality among children. Diagnosis is usually based on the presence of cough and/or difficulty in breathing, rapid breathing with/without chest in drawing.

In the comparison of CVA and SC, IgE level in the CVA group was higher than that of the SC group. Since the infection of Mycoplasma pneumoniae was highly related with IgE level, it could be interpreted that CVA was associated with mycoplasma pneumonia. Koh YY[28] reported that CVA can be secondary to mycoplasma pneumonia which indicated that CVA and mycoplasma pneumonia had a strong relationship. When patients developed CVA, the possibility of mycoplasma pneumonia should be considered. Laboratory examinations should be carried out as soon as possible, and Mycoplasma pneumoniae should be removed in time.

The eosinophil count in the CVA group was more than that in the SC group, which is also an indicator of mycoplasma pneumonia. This supports the association between CVA and mycoplasma pneumonia.
The number of IgM positive patients was higher in the CVA group than that in the SC group. *Mycoplasma pneumoniae* would lead to a rise in the number of IgM positive patients. The number of IgM positive patients was not statistically significantly different between the CVA group and the SC group. Therefore, the association between CVA and mycoplasma pneumonia had another proof. Gui[29] reported that IgM can be used as a test for children with CVA. For screening CVA in children, eosinophil count and IgE level have high predictive value. Therefore, eosinophil count, IgE and IgM can be combined for early diagnosis of CVA. These articles further support the conclusion in this study.

This study had some limitations. First, more indicators such as gender and stratification of age in both the CVA and SC group should be analyzed since gender and age might influence the results, and will be evaluated in the future. Second, more indicators should be included to comprehensively analyze the results, which could be conducted in future studies. Third, articles from more countries could be included in future studies to guarantee the universality of the association. Fourth, all reported studies used serology to identify mycoplasma pneumoniae infection, and none used culture or PCR; more identification methods could be included in the future.

In conclusion, based on the results of blood test including IgE, eosinophil count and positive IgM between the CVA group and the SC group, there may be a correlation between CVA and mycoplasma pneumonia, although the causation of mycoplasma pneumonia need to be further studied. Therefore, comprehensive prevention like staying away from smoke and air pollution should be taken for CVA among children.

References
1. Chaves LE, Nascimento LFC, Rizol P. Fuzzy model to estimate the number of hospitalizations for asthma and pneumonia under the effects of air pollution. Revista de saude publica 2017 Jun 22;51:55.
2. César AC, Nascimento LF, Mantovani KC, Pompeo Vieira LC. Fine particulate matter estimated by mathematical model and hospitalizations for pneumonia and
Asthma in children. Revista paulista de pediatria: orgao oficial da Sociedade de Pediatria de Sao Paulo 2016 Jan-Mar;34(1):18-23.

3. Gold DR, Litonjua AA, Carey VJ, Manson JE, Buring JE, Lee IM et al. Lung VITAL: Rationale, design, and baseline characteristics of an ancillary study evaluating the effects of vitamin D and/or marine omega-3 fatty acid supplements on acute exacerbations of chronic respiratory disease, asthma control, pneumonia and lung function in adults. Contemporary clinical trials 2016 Mar;47:185-95.

4. Zhou H, Zhou B, Xiang B. Relationship between lifestyle and family care among asthma child. Journal of Modern Clinical Medicine 2007.

5. Sun GF. The effect of family intervention on compliance of asthma child who inhale cortex steroids. Chinese Journal of Behavioral Medical Science 2004.

6. Obata, Fumiya. Content of Asthma Child and Healthy Child's Internal Body Parts: Examination which Centers on Understanding of Function. Bulletin of Special Education 2000:115-22.

7. Hussein YM, Alzahrani SS, Alharthi AA, Ghonaim MM, Alhazmi AS, Eed EM et al. Association of serum cytokines levels, interleukin -10/1082G/A and interferon-γ +874T/A polymorphisms with atopic asthma children from Saudi Arabia. Cellular immunology 2014 May-Jun;289(1-2):21-6.

8. Gulen F, Zeyrek D, Can D, Altinoz S, Koksoy H, Demir E et al. Development of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. Asian Pacific journal of allergy and immunology 2007 Mar;25(1):7-11.

9. Pajno GB, Morabito L, Barberio G, Parmiani S. Clinical and immunologic effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, placebo-controlled study. Allergy 2000 Sep;55(9):842-9.

10. Klug B, Bisgaard H. Measurement of lung function in awake 2-4-year-old asthmatic children during methacholine challenge and acute asthma: a comparison of the impulse oscillation technique, the interrupter technique, and transcutaneous measurement of oxygen versus whole-body plethysmography. Pediatric pulmonology 1996 May;21(5):290-300.

11. Friend JA. Do smoking parents seek the best advice for their asthmatic children? Thorax 2001 Jan;56(1):1.

12. Li W, Ban C, Zhang J, Hu Y, Han B, Han B. Correlation study of cough variant asthma and mycoplasma pneumonia infection in children. Pakistan journal of pharmaceutical sciences 2017 May;30(3(Special)):1099-102.

13. Marchand E, Etienne-Mastroianni B, Chanez P, Lauque D, Leclerc P, Cordier JF. Idiopathic chronic eosinophilic pneumonia and asthma: how do they influence each other? The European respiratory journal 2003 Jul;22(1):8-13.

14. Juhn YJ, Kita H, Yawn BP, Boyce TG, Yoo KH, McGree ME et al. Increased risk of serious pneumococcal disease in patients with asthma. The Journal of allergy and clinical immunology 2008 Oct;122(4):719-23.

15. Jung JA, Kita H, Yawn BP, Boyce TG, Yoo KH, McGree ME et al. Increased risk of serious pneumococcal disease in patients with atopic conditions other than asthma. The Journal of allergy and clinical immunology 2010 Jan;125(1):217-21.
16. Chan JY, Stern DA, Guerra S, Wright AL, Morgan WJ, Martinez FD. Pneumonia in childhood and impaired lung function in adults: a longitudinal study. Pediatrics 2015 Apr;135(4):607-16.
17. Nantanda R, Tumwine JK, Ndeezi G, Ostergaard MS. Asthma and pneumonia among children less than five years with acute respiratory symptoms in Mulago Hospital, Uganda: evidence of under-diagnosis of asthma. PloS one 2013;8(11):e81562.
18. Romero-Espinoza JA, Moreno-Valencia Y, Coronel-Tellez RH, Castillejos-Lopez M, Hernandez A, Dominguez A et al. Virome and bacteriome characterization of children with pneumonia and asthma in Mexico City during winter seasons 2014 and 2015. 2018;13(2):e0192878.
19. Nantanda R, Ostergaard MS, Ndeezi G, Tumwine JK. Clinical outcomes of children with acute asthma and pneumonia in Mulago hospital, Uganda: a prospective study. BMC pediatrics 2014 Nov 28;14:285.
20. Gui Y. Correlation between cough variant asthma in children and repeated mycoplasma pneumonia infection. Journal of clinical pulmonary 2017(2):276-79.
21. Van Bever HP, Desager KN, Lijssens N, Weyler JJ, Du Caju MV. Does treatment of asthmatic children with inhaled corticosteroids affect their adult height? Pediatric pulmonology 1999 Jun;27(6):369-75.
22. Saha MT, Laippala P, Lenko HL. Growth of asthmatic children is slower during than before treatment with inhaled glucocorticoids. Acta paediatrica (Oslo, Norway : 1992)1997 Feb;86(2):138-42.
23. Heaton T, Rowe J, Turner S, Aalberse RC, de Klerk N, Suriyaarachchi D et al. An immunoepidemiological approach to asthma: identification of in-vitro T-cell response patterns associated with different wheezing phenotypes in children. Lancet 2005 Jan 8-14;365(9454):142-9.
24. Munir AK, Björkstén B, Einarsson R, Schou C, Ekstrand-Tobin A, Warner A et al. Cat (Fel d I), dog (Can f I), and cockroach allergens in homes of asthmatic children from three climatic zones in Sweden. Allergy 1994 Aug;49(7):508-16.
25. Carswell F, Birmingham K, Oliver J, Crewes A, Weeks J. The respiratory effects of reduction of mite allergen in the bedrooms of asthmatic children--a double-blind controlled trial. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology 1996 Apr;26(4):386-96.
26. Leaderer BP, Belanger K, Triche E, Holford T, Gold DR, Kim Y et al. Dust mite, cockroach, cat, and dog allergen concentrations in homes of asthmatic children in the northeastern United States: impact of socioeconomic factors and population density. Environmental health perspectives 2002 Apr;110(4):419-25.
27. Bisgaard H, Gillies J, Groenewald M, Maden C. The effect of inhaled fluticasone propionate in the treatment of young asthmatic children: a dose comparison study. American journal of respiratory and critical care medicine Med 1999 Jul;160(1):126-31.
28. Koh YY, Chae SA, Min KU. Cough variant asthma is associated with a higher wheezing threshold than classic asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology 1993 Aug;23(8):696-701.
29. Gui Q, Qian GS, Han J, Zhao ZQ. Genetic analysis of serum total IgE of pedigree member in asthma. Immunological Journal 2004;20.
**Figure Legends**

**Figure 1.** The flow diagram of the study selection

**Figure 2.** Assessment of the quality of the included studies: low risk of bias (green hexagons), unclear risk of bias (blank hexagons), and high risk of bias (red hexagons).

**Figure 3.** Quality assessment of the included studies

**Figure 4.** A Forest plot for serum IgE level between CVA and SC

**Figure 5.** A Forest plot for comparison of eosinophil count between CVA and SC

**Figure 6.** A Forest plot for the number of positive IgM between CVA and SC.

**Figure 7.** A Forest plot for the sensitivity analysis of serum IgE level.

**Figure 8.** Begg’s funnel plot of publication bias.
| Study          | Year | Language | Country | No. of patients (female/male) | Age range (mean) | Groups | n  | Years of onset                  |
|---------------|------|----------|---------|-------------------------------|------------------|--------|----|---------------------------------|
| Zhang[12]     | 2009 | Chinese  | China   | 86/110                        | 6.1±1.3          | CVA    | 106| October 2009 to March 2012      |
| Marchand[13]  | 2003 | English  | France  | 34/19                         | 6.8±2.3          | CVA    | 26 | April 1995 to June 2002         |
| Juhn[14]      | 2008 | English  | Korea   | 258/264                       | 5.7±2.4          | CVA    | 174 | June 2001 to March 2006         |
| Jung[15]      | 2010 | English  | Korea   | 258/260                       | 6.5±3.1          | CVA    | 170 | May 2003 to June 2008           |
| Chan[16]      | 2015 | English  | America | 312/288                       | 5.9±1.9          | CVA    | 242 | June 2003 to March 2011         |
| Nantanda[17]  | 2013 | English  | Denmark | 200/166                       | 5.5±2.7          | CVA    | 203 | August 2011 to June 2012        |
| Romero-Espinoza[18] | 2018 | English  | Germany | 55/65                         | 5.2±2.9          | CVA    | 42  | January 2013 to January 2015    |
| Nantanda[19]  | 2014 | English  | Uganda  | 180/186                       | 5.6±2.7          | CVA    | 200 | August 2011 to June 2012        |
| Gui[20]       | 2017 | Chinese  | China   | 54/66                         | 6.9±2.3          | CVA    | 60  | January 2014 to March 2016      |
Pubmed database (n=510)

Embase database (n=230)

Cochrane Library (n=121)

China journal full-text database (n=170)

Exclude duplications remaining (n=1031)

After reading title, abstract, Exclude irrelevant studies (n=941)

81 articles selected for full-text, 9 articles added after reference search. (n=90)

Reason for exclusion
Designing article (n=7)
Insufficient data to analysis (n=72)
Reviews (n=2)

Articles included (n=9)
|                  | Random sequence generation (selection bias) | Allocation concealment (selection bias) | Blinding of participants and personnel (performance bias) | Blinding of outcome assessment (detection bias) | Incomplete outcome data (attrition bias) | Selective reporting (reporting bias) | Other bias |
|------------------|---------------------------------------------|-----------------------------------------|----------------------------------------------------------|------------------------------------------------|----------------------------------------|--------------------------------------|-----------|
| Chan 2015        | +                                           | +                                       | +                                                        | +                                              | +                                      | +                                    | +         |
| Gui 2017         | +                                           | +                                       | +                                                        | +                                              | +                                      | +                                    | +         |
| Juhn 2008        | +                                           | +                                       | +                                                        | +                                              | +                                      | +                                    | +         |
| Jung 2010        | +                                           | +                                       | +                                                        | +                                              | +                                      | +                                    | +         |
| Marchand 2003    | +                                          | +                                       | +                                                        | +                                              | +                                      | +                                    | +         |
| Nantanda 2013    | +                                           | +                                       | +                                                        | +                                              | +                                      | +                                    | +         |
| Nantanda 2014    | +                                           | +                                       | +                                                        | +                                              | +                                      | +                                    | +         |
| Romero-Espinoza 2018 | +                              | +                                       | +                                                        | +                                              | +                                      | +                                    | +         |
| Zhang 2009       | +                                           | +                                       | +                                                        | +                                              | +                                      | +                                    | +         |
| Study or Subgroup     | CVA Mean | CVA SD | CVA Total | SC Mean | SC SD | SC Total | Weight | IV, Fixed, 95% CI       | IV, Fixed, 95% CI       |
|----------------------|----------|--------|-----------|---------|-------|----------|--------|-------------------------|-------------------------|
| Chan 2015            | 178.4    | 32.2   | 242       | 87.9    | 34.1  | 163      | 19.6%  | 90.50 [83.88, 97.12]     |                          |
| Gui 2017             | 178.3    | 31.4   | 60        | 79.3    | 41.2  | 60       | 5.0%   | 97.00 [83.89, 110.11]    |                          |
| Juhn 2008            | 169.3    | 33.3   | 174       | 87.2    | 34.2  | 348      | 23.0%  | 102.10 [95.99, 108.21]   |                          |
| Jung 2010            | 143.5    | 34.2   | 170       | 86.5    | 44.2  | 348      | 18.0%  | 58.00 [51.07, 64.93]     |                          |
| Marchand 2003        | 168.2    | 24.5   | 26        | 92.2    | 43.2  | 27       | 2.4%   | 76.00 [57.18, 94.82]     |                          |
| Nantanda 2013        | 157.2    | 45.2   | 203       | 99.4    | 45.3  | 163      | 9.9%   | 57.80 [48.47, 67.13]     |                          |
| Nantanda 2014        | 167.7    | 31.7   | 200       | 89.5    | 44.5  | 160      | 12.9%  | 78.20 [70.02, 86.38]     |                          |
| Romero-Espinoza 2018 | 146.6    | 35.2   | 42        | 100.2   | 42.5  | 78       | 4.3%   | 46.40 [32.18, 60.62]     |                          |
| Zhang 2009           | 183.5    | 42.4   | 106       | 91.4    | 51.2  | 90       | 4.9%   | 92.10 [78.79, 105.41]    |                          |

Total (95% CI) 1223 1437 100.0% 80.69 [77.75, 83.62]

Heterogeneity: Chi² = 151.55, df = 8 (P < 0.00001); I² = 95%
Test for overall effect: Z = 53.88 (P < 0.00001)
| Study or Subgroup    | Mean CVA | SD CVA | Total CVA | Mean SC | SD SC | Total SC | Weight | Mean Difference | IV, Fixed, 95% CI | Mean Difference | IV, Fixed, 95% CI |
|---------------------|----------|--------|-----------|---------|-------|----------|--------|-----------------|-----------------|-----------------|-----------------|
| Chan 2015           | 5.9      | 2.4    | 242       | 3.6     | 2.1   | 163      | 22.0%  | 2.30            | [1.16, 3.44]    |                 |                 |
| Gui 2017            | 6.1      | 2.5    | 60        | 3.8     | 2.3   | 60       | 5.8%   | 2.30            | [1.44, 3.16]    |                 |                 |
| Juhn 2008           | 6.6      | 3.2    | 174       | 4.1     | 1.9   | 348      | 16.1%  | 2.50            | [1.98, 3.02]    |                 |                 |
| Jung 2010           | 6.3      | 4.1    | 170       | 2.8     | 1.3   | 348      | 10.8%  | 3.50            | [2.87, 4.13]    |                 |                 |
| Marchand 2003       | 6.4      | 4.2    | 26        | 3.1     | 1.5   | 27       | 1.5%   | 3.30            | [1.59, 5.01]    |                 |                 |
| Nantanda 2013       | 7.2      | 3.4    | 203       | 3.4     | 2.4   | 163      | 12.1%  | 3.80            | [3.20, 4.40]    |                 |                 |
| Nantanda 2014       | 7.1      | 3.1    | 200       | 4.3     | 2.6   | 160      | 12.4%  | 2.80            | [2.21, 3.39]    |                 |                 |
| Romero-Espinoza 2018| 6.6      | 2.6    | 42        | 3.5     | 1.8   | 78       | 5.5%   | 3.30            | [2.42, 4.18]    |                 |                 |
| Zhang 2009          | 6.6      | 2.5    | 106       | 3.2     | 1.4   | 90       | 13.8%  | 3.40            | [2.64, 3.96]    |                 |                 |

Total (95% CI) 1223 1437 100.0% 2.93 [2.72, 3.13]

Heterogeneity: Chi² = 27.65, df = 8 (P = 0.0005); I² = 71%
Test for overall effect: Z = 27.69 (P < 0.00001)
| Study or Subgroup      | Events | Total | Events | Total | Weight | M-H, Fixed. 95% CI | M-H, Fixed. 95% CI |
|------------------------|--------|-------|--------|-------|--------|---------------------|---------------------|
| Chan 2015              | 131    | 242   | 40     | 163   | 18.1%  | 3.63 [2.34, 5.62]   |                     |
| Gui 2017               | 33     | 60    | 13     | 60    | 4.8%   | 4.42 [1.99, 9.81]   |                     |
| Juhn 2008              | 94     | 174   | 67     | 348   | 17.0%  | 4.93 [3.30, 7.35]   |                     |
| Jung 2010              | 90     | 170   | 69     | 348   | 17.6%  | 4.55 [3.05, 6.79]   |                     |
| Marchand 2003          | 12     | 26    | 8      | 27    | 3.5%   | 2.04 [0.66, 6.30]   |                     |
| Nantanda 2013          | 121    | 203   | 31     | 163   | 11.5%  | 6.28 [3.88, 10.17]  |                     |
| Nantanda 2014          | 99     | 200   | 35     | 160   | 16.3%  | 3.50 [2.20, 5.58]   |                     |
| Romero-Espinoza 2018   | 25     | 42    | 18     | 78    | 4.2%   | 4.90 [2.18, 11.03]  |                     |
| Zhang 2009             | 58     | 106   | 17     | 90    | 6.9%   | 5.19 [2.70, 9.96]   |                     |
| **Total (95% CI)**     | 1223   | 1437  | 100.0% |       | 4.44 [3.73, 5.29]   |                     |
| **Total events**       | 663    | 298   |        |       |        |                     |                     |

Heterogeneity: Chi² = 6.20, df = 8 (P = 0.63); I² = 0%

Test for overall effect: Z = 16.77 (P < 0.00001)
| Study or Subgroup | CVA Mean | SD | Total | SC Mean | SD | Total | Weight | Mean Difference | IV, Fixed, 95% CI | Fixed, 95% CI |
|------------------|----------|----|-------|----------|----|-------|--------|----------------|------------------|---------------|
| Chan 2015        | 178.4    | 32.2| 242   | 87.9     | 34.1| 163   | 25.5%  | 90.50          | [83.88, 97.12]  |
| Gui 2017         | 176.3    | 31.4| 60    | 79.3     | 41.2| 60    | 6.5%   | 97.00          | [83.89, 110.11] |
| Jung 2010        | 143.5    | 34.2| 170   | 85.5     | 44.2| 348   | 23.3%  | 58.00          | [51.07, 64.93]  |
| Marchand 2003    | 168.2    | 24.5| 26    | 92.2     | 43.2| 27    | 3.2%   | 76.00          | [57.18, 94.82]  |
| Nantanda 2013    | 157.2    | 45.2| 203   | 99.4     | 45.3| 163   | 12.9%  | 57.80          | [48.47, 67.13]  |
| Nantanda 2014    | 167.7    | 31.7| 200   | 89.5     | 44.5| 160   | 16.7%  | 78.20          | [70.02, 86.38]  |
| Romero-Espinoza 2018 | 146.6 | 35.2| 42    | 100.2    | 42.5| 78    | 5.5%   | 46.40          | [32.18, 60.62]  |
| Zhang 2009       | 183.5    | 42.4| 106   | 91.4     | 51.2| 90    | 6.3%   | 92.10          | [78.79, 105.41] |

Total (95% CI) 1049 1089 100.0% 74.28 [70.93, 77.62]

Heterogeneity: Chi² = 90.35, df = 7 (P < 0.00001); I² = 92%
Test for overall effect: Z = 43.51 (P < 0.00001)
