

BIOMARKERS

CCL11 as a potential diagnostic marker for asthma?

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Abstract

Objective: Asthma is an inflammatory airway disease characterized by airway eosinophilia, in which CCL11 (eotaxin) plays a crucial role. The aim of study is to determine the elevation of CCL11 levels in bronchoalveolar lavage fluid (BALF), blood, exhaled breath condensate (EBC) and sputum in asthma patients and to identify which medium yields the most significant change in CCL11 level. **Methods:** The databases of PubMed, Embase and Cochrane Centre Register of Controlled Trials were systematically searched from inception to September 2013. Controlled clinical trials that focused on CCL11 concentrations in asthma patients and controls, and their correlations with other asthma indicators were obtained. Data were analysed using Stata 12.0. **Results:** Thirty studies were included in this investigation. CCL11 levels in blood, EBC and sputum were significantly higher in asthma patients than in healthy subjects. Sputum CCL11 concentrations were significantly elevated in unstable asthma patients versus stable asthma patients and in uncontrolled asthma patients versus partially controlled asthma patients. CCL11 levels in sputum and blood were negatively correlated with the lung function as measured by FEV₁% predicted, and were positively correlated with BALF, EBC and sputum eosinophil counts. Similarly, CCL11 concentrations were positively correlated with eosinophil cationic protein in EBC, blood and sputum as well as with interleukin-5 in sputum and fractional exhaled nitric oxide in EBC. Steroid treatment had no significant effect on CCL11 levels. **Conclusions:** CCL11 is a potentially useful biomarker for the diagnosis and assessment of asthma severity and control, especially in sputum. CCL11 is crucial in eosinophil chemoattraction and activation in asthma pathogenesis. Further studies using anti-CCL11 approaches are needed to confirm a role for CCL11 in asthma pathogenesis particularly in patients with more severe disease.

Keywords

Asthma, blood, bronchoalveolar lavage fluid, CCL11, exhaled breath condensate, sputum

History

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Introduction

Asthma is a complex disease characterised by chronic airway inflammation involving eosinophils [1]. Airway eosinophilia is a characteristic feature of asthma and it persists in a certain population despite the use of steroids [2–7]. CCL11/eotaxin is the most potent and selective factor that modulates the function of eosinophils and has a key role in the infiltration of eosinophils into the airway via high-affinity binding to the CCR3 receptor on eosinophil cell surface [8]. CCL11 is mainly secreted from bronchial epithelial cells, eosinophils and endothelial cells [9–11], and promotes local eosinophil recruitment to the lungs and rapid mobilization of eosinophils and their progenitors of eosinophil from the bone marrow [1] via cooperation with interleukin 5 (IL-5) [12,13], resulting in peripheral and tissue eosinophilia [14]. CCL11 produced by

the airway epithelium also creates a gradient that attracts eosinophils from the blood to the airway after allergen challenge [15]. CCL11 expression in endobronchial biopsies is positively correlated with airway hyper-responsiveness [11,16] and the sensitivity of asthmatics to contractile agonists [15]. Previous studies reported increased CCL11 expression in bronchoalveolar lavage fluid (BALF) [10], blood [12], exhaled breath condensate (EBC) [17] and induced sputum [18] in asthma patients. These observations indicate that CCL11 levels are significantly correlated with asthma.

Only a few studies, including some small clinical trials, have focused on identifying the relationship between increased BALF, blood, EBC or sputum CCL11 level and asthma status. These studies have drawn inconsistent conclusions and no comprehensive meta-analysis is currently available. Thus, this study aims to evaluate whether CCL11 expression in BALF, blood, EBC and sputum in asthma patients is related to clinico-pathological features and asthma severity and to identify which medium yields the most significant changes in CCL11 level. This study may serve as a reference for the diagnosis and treatment of asthma.

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Methods

Data sources and searches

A comprehensive literature search was conducted in PubMed, EMBASE and the Cochrane Centre Register of Controlled Trials from inception to September 2013 with the following keywords: 'asthma', 'airway inflammation', 'bronchial constriction', 'bronchial spasm', 'bronchial hyperreactivity', 'wheeze' or 'wheezing' and 'Chemokine CCL11', 'CCL11' or 'eotaxin'. All available studies were searched without language restriction. The reference list of each primary study identified was also searched.

Inclusion and exclusion criteria

A priori inclusion criteria included the following: (1) observational studies; (2) studies that compared CCL11 levels between asthma patients and healthy controls, between asthmatics with different severities of the condition or among asthma patients in a population before and after steroid treatment; (3) studies that identified the relationship between CCL11 and indicators used in evaluating the condition of asthma patients; and (4) studies on non-smoking asthma patients and healthy subjects because smoking can affect CCL11 levels [1]. The exclusion criteria included the following: (1) adults or children without an established diagnosis of asthma; (2) subjects who contracted diseases, such as chronic obstructive pulmonary disease, pneumonia and other diseases, which may affect CCL11 levels; (3) asthma patients with allergic rhinitis [19]; (4) patients without recorded outcomes; (5) studies without healthy subjects as the control group; (6) studies lacking full texts even after e-mail communication with the authors; and (7) case reports/editorial.

Study selection

Systematic screening and review of the title, abstract and full-text article were conducted. Each study was evaluated independently by two investigators (D.W. and J.Z.). Any disagreement was resolved by discussion with the third party (X. Y.). Data representing the number of participants, age, gender, asthma severity, interventions (e.g. doses of inhaled corticosteroid (ICS) and β agonists), details of the CCL11 assays and study outcomes were extracted, recorded and verified (D.W. and J.Z.).

Statistical analysis

Data expressed as median (range) or mean $\pm$ standard errors of the mean were re-calculated as mean $\pm$ standard deviation (SD) and statistical data were combined using Stata 12.0. Heterogeneity was assessed using the I^2 statistics as the basis in the random or fixed effects model. If I^2 was significant ($>50\%$), the random effects model was selected. Otherwise, the fixed effects model was selected. The weighted mean difference (WMD) was used to describe outcome measurements across the studies. However, the standardised mean difference was used when a high-degree of variability was observed between units. Fisher Z and reverse Fisher Z transformation were used to combine correlation coefficients. Visual inspection of funnel plots along with

Begg's test and Egger's asymmetry test used to determine publication bias. Publication bias was assumed to exist at $p < 0.05$.

Results

Of the 4180 unique references identified, 3598 were excluded, including 2763 irrelevant studies and 842 animal experiments. Among the 575 remaining relevant studies, 312 were reviews, 7 were conferences, 200 were not about asthma, 2 were case reports and 4 were editorials. Further subsequent abstract review resulted in 50 candidate studies. After the final screening of the full text, 14 studies were found without available data, 1 focused on the asthma patients with allergic rhinitis, 1 had no control group and 4 controlled clinical studies lacked sufficient data. We attempted to obtain further information by sending e-mails to the authors. However, no additional essential information was gathered. Finally, 30 articles [1,3,8,12,14,15,17,18,20–41] involving 1214 asthma patients and 801 healthy controls were included in our meta-analysis. There may have been the publication bias in the blood group (Begg Test, $p = 0.064$; Egger's test, $p = 0.002$) (Supplementary material).

All obtained articles were divided into four subgroups according to their focus, namely, CCL11 concentrations in BALF, blood, EBC and induced sputum. ELISA was used for CCL11 measurement in 29 articles except one with Luminex [35].

CCL11 concentrations in asthmatics and healthy controls in different compartments

CCL11 concentrations were statistically different in the blood, EBC and sputum but not in the BALF of asthma patients compared with those of healthy subjects. The overall WMDs of CCL11 concentration between asthmatics and healthy controls were 58.33 pg/mL [95% confidence interval (CI), 35.27 to 81.40; $p < 0.001$; $I^2 = 93.4\%$; random effects model] in the blood, 3.93 pg/mL (95% CI, 1.16 to 6.71; $p = 0.006$; $I^2 = 94.7\%$; random effects model) in EBC, 176.04 pg/mL (95% CI, 105.70 to 246.38; $p < 0.001$; $I^2 = 90.2\%$; random effects model) in the sputum and 3.17 pg/mL (95% CI, -1.51 to 7.85; $p = 0.184$; $I^2 = 79.1\%$; random effects model) in BALF (Supplementary material).

CCL11 concentrations in adult and childhood asthma

There were 8 articles on adult asthma and 4 articles on childhood asthma that described blood CCL11 concentrations. The overall WMDs of CCL11 levels were 76.29 pg/mL (95% CI, 36.43 to 116.16; $p < 0.001$; $I^2 = 92.1\%$; random effects model) between asthmatic adults and control subjects and 28.22 pg/mL (95% CI, -3.94 to 60.38; $p = 0.086$; $I^2 = 93.7\%$; random effects model) between asthmatic children and healthy controls (Supplementary Material). The differences between asthmatic adults and control subjects were more pronounced than those in children where CCL11 levels did not vary between healthy controls and asthmatic subjects [76.29 pg/mL (95% CI, 36.43 to 116.16; $p < 0.001$; $I^2 = 92.1\%$; random effects model) versus 28.22 pg/mL (95% CI, -3.94 to 60.38; $p = 0.086$; $I^2 = 93.7\%$; random effects model)].

CCL11 concentration in patients with different asthma severities

Asthma severity was classified into the following four categories: intermittent, mild persistent, moderately persistent and severe persistent asthma according to the Global Initiative for Asthma (GINA) Guidelines. The subjects were separated into two groups because of the limited information provided by the articles namely mild/moderate persistent asthma and severe persistent asthma. The WMDs of blood CCL11 concentrations were 69.50 pg/mL (95% CI, 29.63 to 109.37; $p=0.001$; $I^2=88.1\%$; random effects model) between mild/moderate persistent asthmatics and healthy controls, 135.27 pg/mL (95% CI, -8.63 to 279.17; $p=0.065$; $I^2=95.7\%$, random effects model) between severe persistent asthmatics and healthy controls and 49.27 pg/mL (95% CI, -32.32 to 130.86; $p=0.237$; $I^2=90.1\%$, random effects model) between severe persistent asthmatics and mild/moderate persistent asthmatics (Supplementary material). Overall, severe persistent asthmatics had higher blood CCL11 concentrations than mild/moderate persistent asthmatics and healthy controls.

Higher blood CCL11 levels were also observed in unstable asthmatics compared with subjects with stable disease. Blood CCL11 levels were also analysed according to whether the patients were stable (defined as no exacerbations or changes in symptoms/medication use for at least 6 weeks and basal FEV₁ greater than 80%, requiring intermittent use of short-acting β_2 receptor agonists or ICS) or unstable (defined as who fulfilled at least one of the following criteria: (1) daytime symptoms were daily, Night-time symptoms were more than 2 times per month and basal FEV₁ was less than 80% predicted or PEFR variability was more than 30%; (2) step-up or additional treatment was necessary based on their clinical symptoms and pulmonary function; (3) exacerbation was diagnosed when the preexisting symptoms were aggravated within 7 days) [3,8,21,24,27]. The WMDs of CCL11 concentrations were 265.24 pg/mL (95% CI, 97.89 to 432.59; $p=0.002$; $I^2=84.2\%$, random effects model) between unstable asthmatics and healthy controls, 81.40 pg/mL (95% CI, 39.28 to 123.52; $p<0.001$; $I^2=85.1\%$, random effects model) between stable asthmatics and control group and 129.62 pg/mL (95% CI, -56.47 to 315.71; $p=0.172$; $I^2=81.7\%$, random effects model) between unstable and stable asthma patients (Supplementary material).

Sputum CCL11 levels in both unstable and stable asthmatics were statistically higher than those in healthy subjects. The WMDs for unstable and stable asthma patients were 340.99 pg/mL (95% CI, 102.81 to 579.18; $p=0.005$; $I^2=91.2\%$, random effects model) and 142.67 pg/mL (95% CI, 84.49 to 200.85; $p<0.001$; $I^2=82.4\%$, random effects model), respectively. CCL11 concentrations in unstable asthmatics were statistically higher than those in stable asthmatics, with a WMD of 167.47 pg/mL (95% CI, 96.97 to 237.98; $p<0.001$; $I^2=4.7\%$, fixed effects model) (Supplementary material).

CCL11 concentrations and asthma control

Asthma control is defined as controlled, partially controlled and uncontrolled according to GINA Guidelines.

Controlled asthma is defined as subjects presented over the last 4 weeks with twice or less per week of daytime symptoms, twice or less per week the need for reliever treatment, no limitations of daily activities, no nocturnal symptoms and normal or near normal lung function and uncontrolled asthma is defined as nocturnal wheezing, daily asthma symptoms, and use of β_2 agonist as required [34,42]. The WMDs of blood CCL11 levels were 142.77 pg/mL (95% CI, 38.60 to 246.95; $p=0.007$; $I^2=94.2\%$; random effects model) between uncontrolled asthmatics and healthy subjects, 42.38 pg/mL (95% CI, -27.32 to 112.08; $p=0.233$; $I^2=89.9\%$; random effects model) between partially controlled asthma patients and healthy controls and -1.67 pg/mL (95% CI, -26.70 to 23.36; $p=0.896$; $I^2=53.3\%$; random effects model) between uncontrolled and partially controlled asthmatics (Supplementary Material). No significant differences in blood CCL11 concentrations were observed between uncontrolled and partially controlled asthmatics.

In contrast, there was a significant difference in the WMDs of sputum CCL11 concentrations between the controlled and uncontrolled groups. The WMDs were 340.99 pg/mL (95% CI, 102.81 to 579.18; $p=0.005$; $I^2=91.2\%$; random effects model) between uncontrolled asthmatics and healthy controls, 39.80 pg/mL (95% CI, -59.40 to 139.00; $p=0.432$; $I^2=0.0\%$; fixed effects model) between partially controlled asthmatics and healthy subjects and 114.20 pg/mL (95% CI, 13.37 to 215.03; $p=0.026$; $I^2=0.0\%$; fixed effects model) between uncontrolled and partially controlled asthmatics (Supplementary material).

Correlation coefficient between CCL11 levels and other indicators

The negative correlation coefficients between CCL11 levels and FEV₁%pred were statistically significant in the EBC, blood and sputum groups. Fisher Z transformation was used to transform r to z , which is considered as the normal distribution. The Z values were -0.22 (95% CI, -0.50 to 0.07; $p=0.140$; $I^2=36.3\%$; fixed effects model), -0.23 (95% CI, -0.31 to -0.15; $p<0.001$; $I^2=0.0\%$; fixed effects model) and -0.48 (95% CI, -0.69 to -0.27; $p<0.001$; $I^2=0.0\%$; fixed effects model), respectively (Supplementary Material). A positive correlation was observed between the CCL11 level and eosinophil count in BALF, and EBC, but not in blood, with z values of 0.98 (95% CI, 0.64 to 1.32; $p<0.001$; $I^2=0.0\%$; fixed effects model), 0.82 (95% CI, 0.43 to 1.22; $p<0.001$; $I^2=0.0\%$; fixed effects model) and 0.02 (95% CI, -0.29 to 0.32; $p=0.907$; $I^2=75.0\%$; random effects model) (Supplementary Material), respectively. Similarly, the z values were 0.83 (95% CI, -0.24 to 1.91; $p=0.127$; $I^2=92.4\%$; random effects model) and 0.96 (95% CI, 0.65 to 1.27; $p<0.001$; $I^2=4.3\%$; fixed effects model) (Supplementary Material) in the sputum of unstable and stable asthma patients, respectively. No significant differences were observed between the CCL11 concentration and eosinophil percentage in the sputum, with a z value of 0.07 (95% CI, -0.47 to 0.62; $p=0.789$; $I^2=89.9\%$; random effects model) (Supplementary Material).

The CCL11 levels in the sputum, blood and EBC were also positively correlated with the ECP level, with z values

of 0.58 (95% CI, 0.39 to 0.77; $p < 0.001$; $I^2 = 0.0\%$; fixed effects model), 0.55 (95% CI, 0.21 to 0.89; $p = 0.002$; $I^2 = 0.0\%$; fixed effects model) and 0.91 (95% CI, 0.52 to 1.30; $p < 0.001$; $I^2 = 0.0\%$; fixed effects model), respectively (Supplementary Material). The positive correlation coefficients between CCL11 and IL-5 levels in the sputum of unstable and stable asthma patients were 0.94 (95% CI, -0.49 to 2.36; $p = 0.198$; $I^2 = 95.6\%$; random effects model) and 1.05 (95% CI, 0.74 to 1.36; $p < 0.001$; $I^2 = 0.0\%$; fixed effects model) (Supplementary Material), respectively. The CCL11 level in EBC was positively correlated with the baseline fractional exhaled nitric oxide (FENO), with a Z value of 1.44 (95% CI, 1.05 to 1.83; $p < 0.001$; $I^2 = 0.0\%$; fixed effects model) (Supplementary Material). Finally, we summarized the statistically significant findings of this study in Table 1.

Discussion

In the present study, CCL11 concentrations in the blood, EBC and sputum but not in BALF were markedly elevated in asthma patients as compared with those in healthy subjects. The unchanged CCL11 level in BALF may be attributed to the limited studies available in the literature. A positive correlation was found between the CCL11 concentration and eosinophil count in BALF, sputum and EBC; between CCL11 and ECP in EBC, blood and sputum; and between CCL11 and IL-5 in induced sputum. But there was no significant correlation shown between the CCL11 concentration and eosinophil percentage in the sputum, which may be due to the increased neutrophils in the acute asthmatics [6,43,44]. In addition, the CCL11 levels were inversely correlated with the lung function measured by FEV₁%pred in sputum and blood, and with FENO in EBC. This result further suggests that CCL11 has an important function in asthma diagnosis and may be used in the primary screening of asthma patients.

Infiltration of eosinophils into the bronchial wall and respiratory epithelial damage are distinctive features of asthma [31]. Inhaled allergen activates mast cells and Th2 lymphocytes in the lung to generate the cytokines IL-4, IL-13 and TNF- α , which stimulates the release of CCL11 by epithelial cells, fibroblasts, endothelial cells [24,31,45,46]. As the most potent and selective chemoattractant for eosinophils [8,17], CCL11 can stimulate migration [32], adhesion and recruitment of eosinophils into the airways [11,47,48], in cooperation with other mediators, such as RANTES and IL-5 [49,50]. This subsequently induces eosinophils to release more ECP, oxidising agents and CCL11 [8]. This may explain why CCL11 levels correlate well with IL-5 and ECP levels and with eosinophil counts.

CCL11 levels, particularly sputum CCL11, can also be used to distinguish between asthma of different severities and degrees of control. Sputum and blood CCL11 levels were inversely correlated with lung function as measured by FEV₁% pred and sputum CCL11 levels are higher in unstable compared with stable asthmatics. Uncontrolled asthma patients also had elevated sputum CCL11 levels compared with partially controlled asthma patients relating to the excessive influx of eosinophils seen in more severe asthma and may account for the increased bronchial mucosa injury seen in these patients [12].

Table 1. Summary of the statistically significant findings of the study.

	BAL (95% CI)	Blood (95% CI)	EBC (95% CI)	Sputum (95% CI)
Asthmatics versus healthy subjects (pg/ml)		58.33 (35.27 to 81.40) ^c	3.93 (1.16 to 6.71) ^b	176.04 (105.70 to 246.38) ^c
Adults with asthma versus healthy subjects (pg/ml)	3.17 (-1.51 to 7.85)	76.29 (36.43 to 116.16) ^c		142.67 (84.49 to 200.85) ^c
Mild to moderate persistent asthmatics versus healthy subjects (pg/ml)		69.50 (29.63 to 109.37) ^b		340.99 (102.81 to 579.18) ^b
Stable asthmatics versus healthy subjects (pg/ml)		81.40 (39.28 to 123.52) ^c		167.47 (96.97 to 237.98) ^c
Unstable asthmatics versus healthy subjects (pg/ml)		265.24 (97.89 to 432.59) ^b		340.99 (102.81 to 579.18) ^b
Unstable asthmatics versus stable asthmatics (pg/ml)		129.62 (-56.47 to 315.71)		114.20 (13.37 to 215.03) ^a
Uncontrolled asthmatics versus healthy subjects (pg/ml)		142.77 (38.60 to 246.95) ^b		-0.48 (-0.69 to -0.27) ^c
Uncontrolled asthmatics versus partially controlled asthmatics (pg/ml)		-1.67 (-26.70 to 23.36)	-0.22 (-0.50 to 0.07)	0.96 (0.65 to 1.27) ^{#c}
r between CCL11 levels and FEV ₁ %pred	0.98 (0.64 to 1.32) ^c	-0.23 (-0.31 to -0.15) ^c	0.82 (0.43 to 1.22) ^c	0.83 (-0.24 to 1.91) [*]
r between CCL11 levels and eosinophil counts		0.02 (-0.29 to 0.32)		0.58 (0.39 to 0.77) ^c
r between CCL11 levels and ECP levels		0.55 (0.21 to 0.89) ^b	0.91 (0.52 to 1.30) ^c	1.05 (0.74 to 1.36) ^{#c}
r between CCL11 levels and IL-5 levels				0.94 (-0.49 to 2.36) [*]
r between CCL11 levels and baseline FENO			1.44 (1.05 to 1.83) ^c	

r = Correlation of coefficient; ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$; #stable asthmatics; *unstable asthmatics.

No significant correlation between CCL11 levels and asthma severity was observed in the blood, probably because blood CCL11 levels reflect circulatory eosinophil inflammation and could therefore be influenced by other systemic factors. In addition, differences in CCL11 levels between asthma patients and control subjects in the sputum were more obvious than those in the blood and EBC. In the present meta-analysis we demonstrated that sputum CCL11 concentrations are more closely correlated with eosinophil count and lung function than blood and EBC CCL11 levels. These results suggest that sputum CCL11 levels are more reliable indicators of the severity and clinical control status of asthma than those in the blood and EBC.

Airway eosinophilia has been shown to be correlated with the degree of airway hyper-responsiveness and disease severity [3], and it was reported to be related to asthma exacerbation [4,51,52]; treatment targeted at eosinophil reduction in sputum reduces the frequency of asthma exacerbations [3,53–58] compared with traditional methods based on primarily clinical symptoms and lung functions as well as FENO [56]. Further studies are needed to compare the CCL11 levels to eosinophil counts as an indicator of control or severity, although the positive correlation between CCL11 and eosinophils has been observed in some studies [1,14,15,17,20,24,31].

Corticosteroids are effective in reducing eosinophilia and expression of eosinophil-associated chemoattractants including CCL11 [27,59], MCP-3 and MCP-4 *in vitro* [60,61]. However, four studies [12,17,24,29] concluded that CCL11 levels are relatively resistant to corticosteroid treatment. Yamada et al. [21] and Tateno et al. [27] also reported that CCL11 levels are not affected by steroids in unstable asthmatics (Table 2). One [24] of them was a self-controlled trial, whereas the others were controlled clinical trials. Discrepancies in the results among various studies may be attributed to differences in the *in vitro* and *in vivo* environmental conditions. CCL11 levels were detected after a week of systemic steroid treatment based on the acute exacerbation of asthma in the self-controlled trial [24], the unchanged CCL11 concentrations may therefore be attributed to the ongoing process of sub-clinical allergic inflammation in the lungs of asthma patients during clinical remission or due to a delay in the rate of CCL11 decrease in the airways compared with clinical remission. Alternatively, viral infection may reduce steroid sensitivity in these patients [62].

The increase in CCL11 levels revealed in two articles [27,38] may be attributed to more severe airway inflammation in asthma patients who required ICS maintenance therapy as compared with steroid-free asthma patients, the dose of steroids is usually dependent on the severity of asthma. Thus, the results suggest that a more severe eosinophilic inflammation is present in the airway of asthmatics that need to be treated with ICS and that this may, in part, hide any decremental effect of steroids on sputum CCL11 or eosinophils. Furthermore, some steroid-free asthma patients still use β_2 -agonists and β_2 -agonists can inhibit tumour necrosis factor- α -induced CCL11 promoter activity in transiently transfected cells [61,63]. The combination of these effects may also partially explain the unchanged CCL11 levels observed between steroid-treated and steroid-free asthma patients in the five controlled clinical trials [12,17,18,27,29]

Table 2. Studies showing comparison of CCL11 levels before and after steroid treatment.

Study	Study type	Group	Sample (Trial/control)	Duration of Steroid treatment	Results: CCL11 level ^c
Khor et al. [29]	Controlled clinical trial	High dose ICS treatment (FP: 1000 mg/day)/ICS-free	20/42	4–8 weeks	No significant difference
Hossny et al. [12]	Controlled clinical trial	Reduced dose ICS treatment (FP: 500 mg/day)/ICS-free	20/42		
Tateno et al. [27]	Controlled clinical trial ^a	ICS treatment/ICS-free	9/5	Not mentioned	No significant difference
		ICS + oral (BDP > 500 µg/day + PSL) treatment/steroid-free	8/25	≥3 Months	The combined value of three steroid treated group ↑ ($p < 0.05$)
		Medium or high-dose ICS treatment (BDP > 500 µg/day)/steroid-free	19/25		
		Low-dose ICS (BDP < 500 µg/day) treatment/steroid-free	27/25		
Ko et al. [38]	Controlled clinical trial ^b	ICS treatment (FP: 200–800 mg/day)/steroid-free ^d	7/15	≥3 Months	No significant difference
Zietkowski et al. [17]	Controlled clinical trial	ICS treatment/ICS-free	29/25	Not mentioned	ICS treated Group ↑ ($p = 0.02$)
Yamada et al. [21]	Controlled clinical trial ^a	ICS treatment/ICS-free	16/14	Not mentioned	No significant difference
		Steroid treatment/Steroid-free	8/15	Not mentioned	Steroid free group ↑ ($p < 0.05$)
Park et al. [24]	Controlled clinical trial ^b	Steroid treatment/Steroid-free	11/19	Not mentioned	No significant difference
	Self-controlled trial	Systemic steroid treatment (2 mg/kg/day)/steroid-free	7	1 week	No significant difference

BDP = beclomethasone dipropionate; FP = fluticasone propionate; PSL = prednisolone.

^astable asthmatics; ^bunstable asthmatics; ^csteroid treated group compared with the steroid free group; ^dsteroid used if asthma exacerbated.

above. Further studies are necessary to better understand the effect of ICS on CCL11 in the asthmatic airway.

No statistically significant differences in CCL11 concentrations were observed between asthmatic children and healthy subjects compared to adults. This finding may be the result of different underlying inflammatory mechanisms in adult and childhood asthma suggesting that children may not develop a complete immune system and have a weaker inflammatory response than adults.

This meta-analysis and systematic review has some limitations. Firstly, the effect of obesity on CCL11 was not considered. Choi et al. [64] found that blood CCL11 concentrations are higher in subjects with central obesity (waist circumference >80 cm) than in those without central obesity. Secondly, smoking status should be considered when using CCL11 to assess the severity of asthma because smoking increases sputum CCL11 concentrations both in asthma patients [1] and healthy subjects [18]. There may be a publication bias in the reporting of blood CCL11 and additional limitations reflecting differences between different geographical populations.

In summary, CCL11 levels were evaluated in the EBC, sputum, and blood of asthma patients. CCL11 concentrations in the sputum were closely correlated with asthma control and severity levels. In addition, CCL11 levels were inversely correlated with the lung function measured by FEV₁%pred and positively correlated with eosinophil count, IL-5 and ECP levels as well as the baseline FENO in EBC. The above correlations were more obvious in the sputum than in the blood, EBC and BALF. These observations suggest that measuring CCL11 concentration, especially in the sputum, is a good biomarker for the assessment of asthma severity and clinical control. Since increased CCL11 levels are involved in eosinophil chemo-attraction and activation in the pathogenesis of asthma, further studies are warranted to investigate whether CCL11 can be used as a target for the treatment of asthma by blocking eosinophilic chemo-attraction and activation as seen with anti-IL-5 therapy in subsets of patients with more severe eosinophilic asthma [65].

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Declaration of interest

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Authors' contributions

All authors read and met ICMJE criteria for authorship. DD.W and XY designed this study; DD.W and JZ extracted

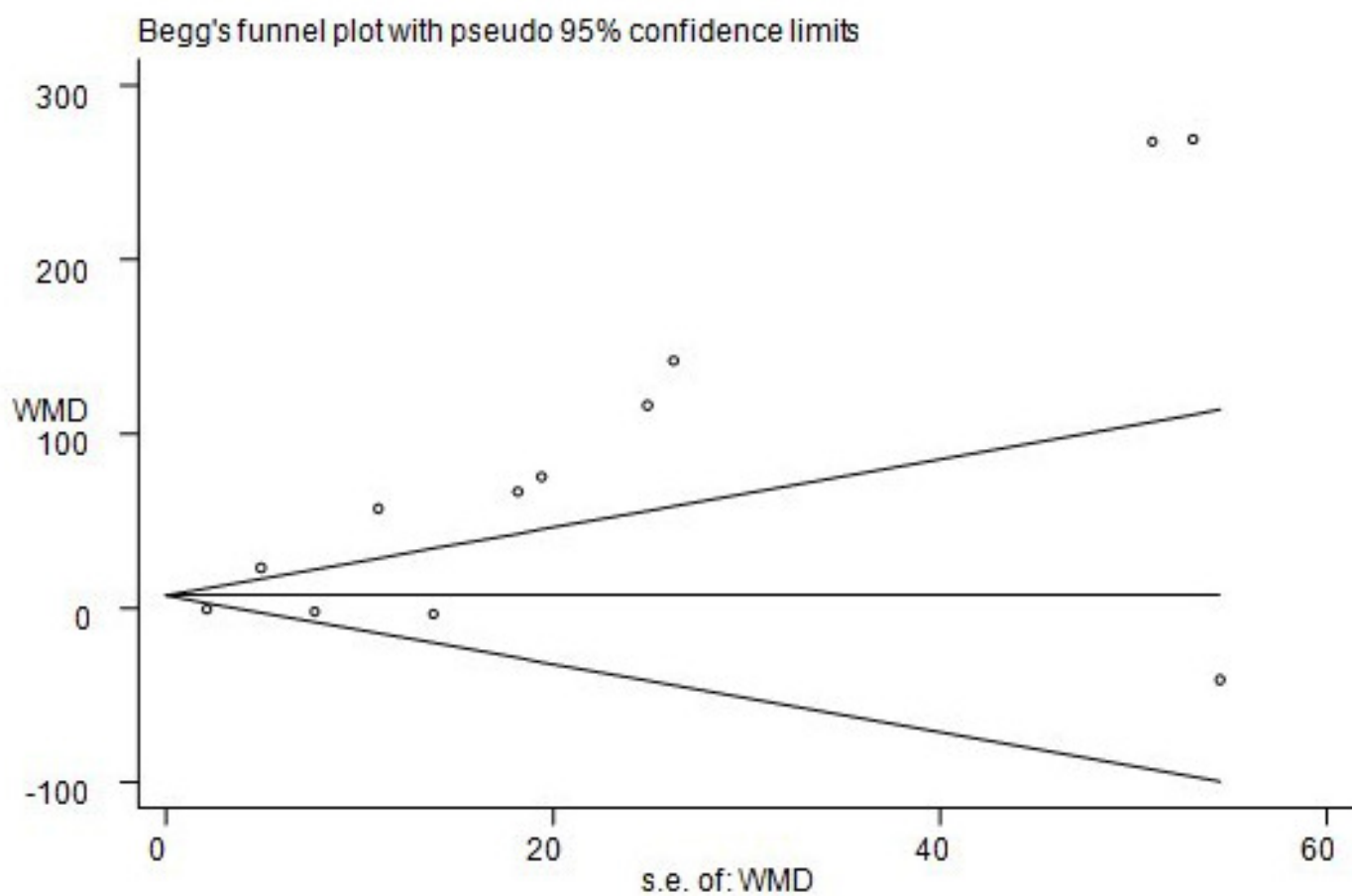
data; DD.W, HB, LL.L, WG performed the analysis; DD.W wrote the first draft of the manuscript and XY, MH, IMA and PJB critically revised the manuscript. All authors read and approved the final manuscript.

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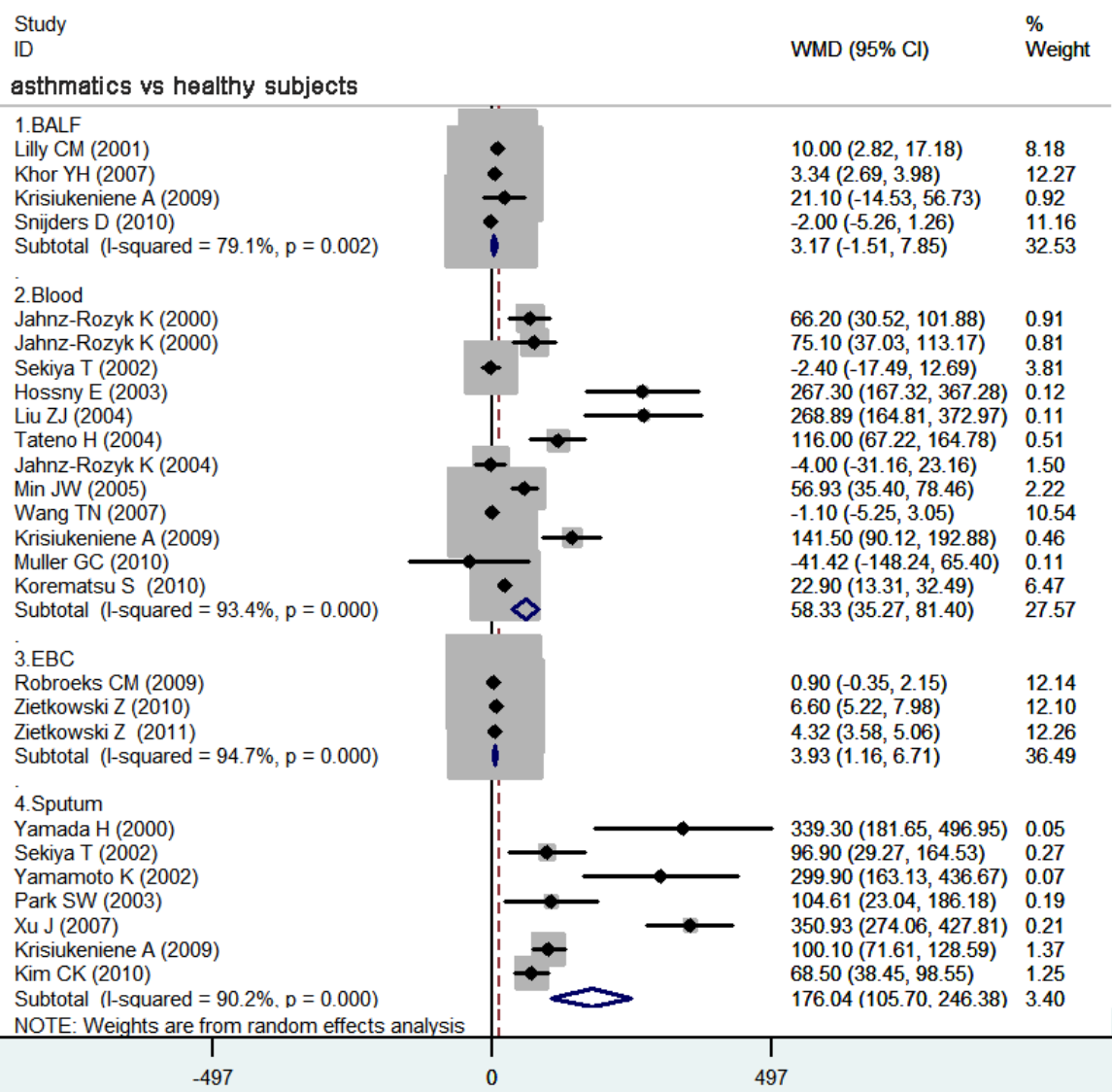
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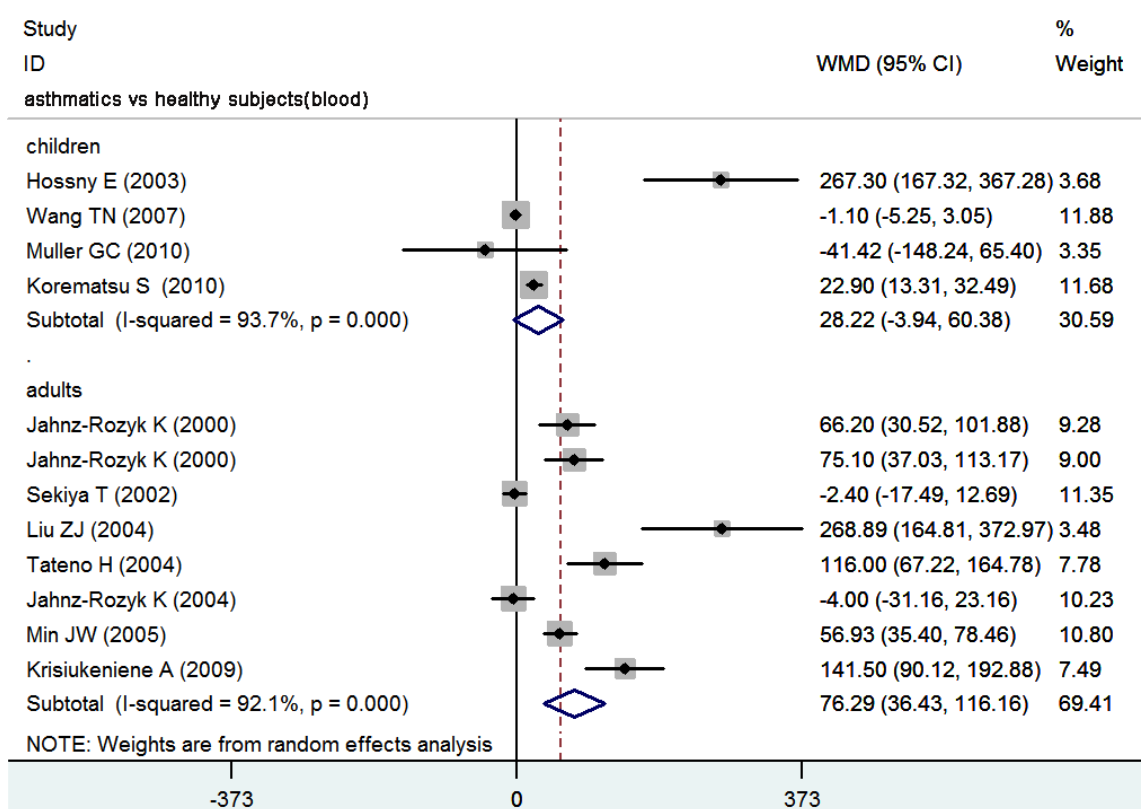
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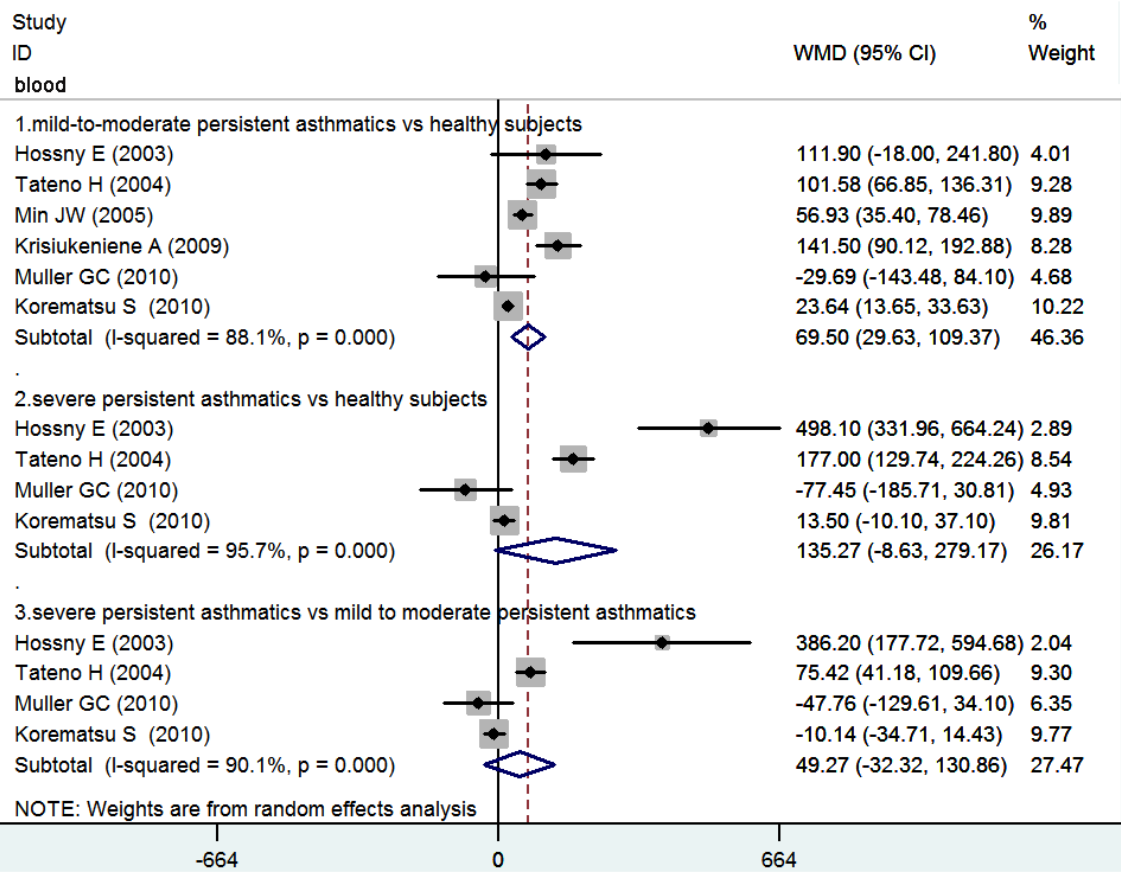
Additional file 1. The Begg funnel plot. WMD=weighted mean difference



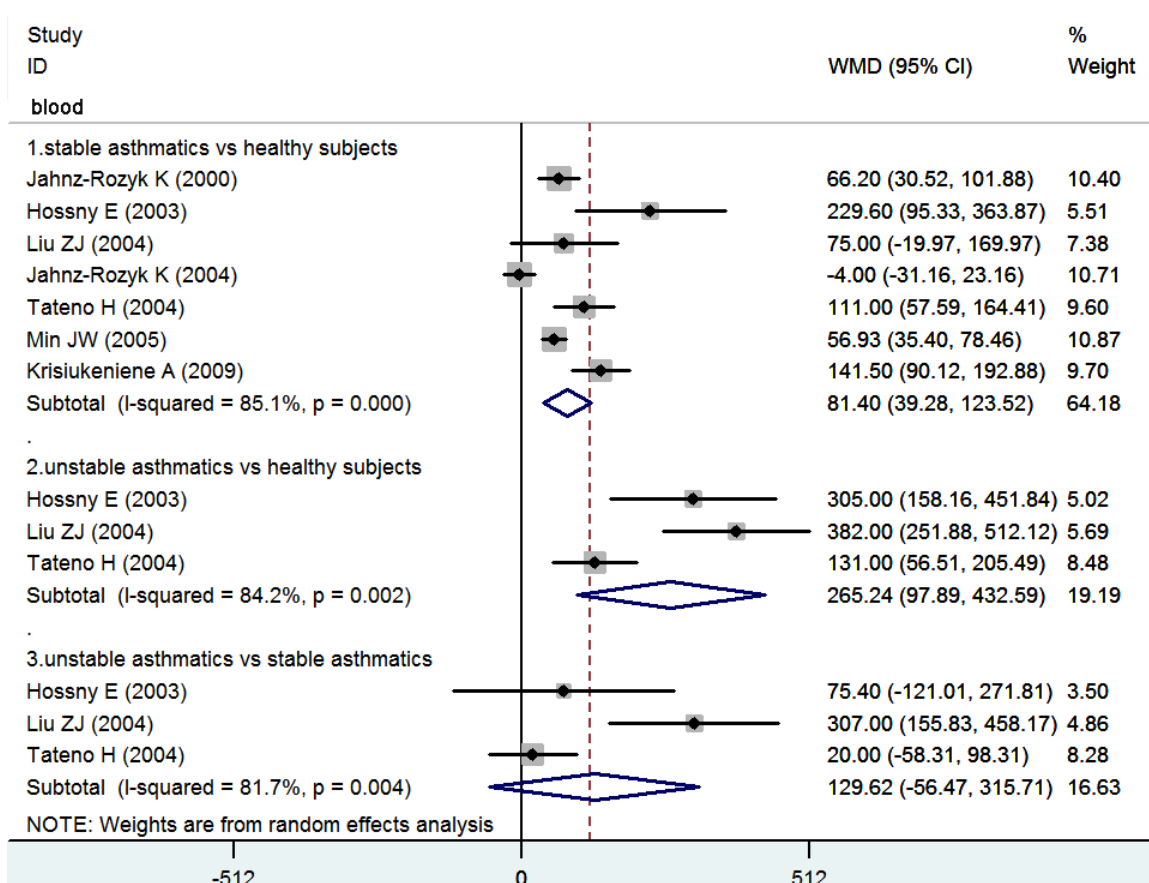
Additional file 2. Overall CCL11 levels in patients with asthma.



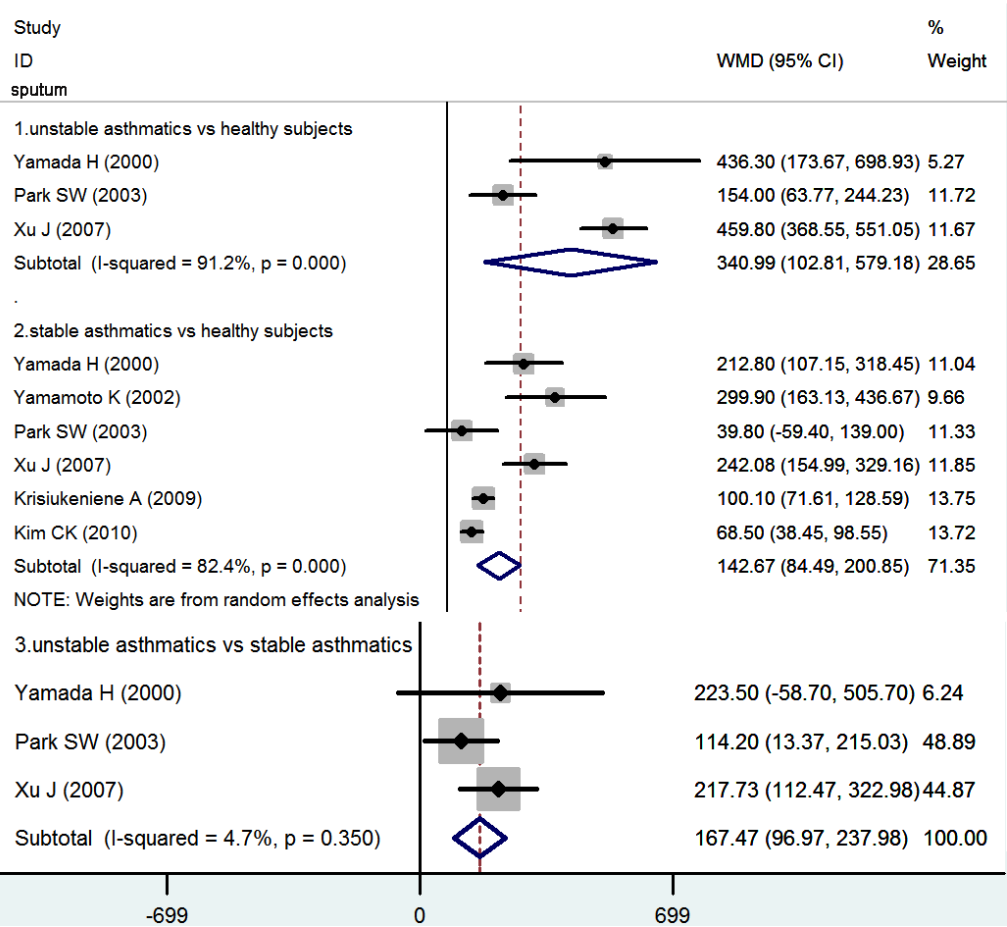
Additional file 3. Blood CCL11 levels in adults and children with asthma.



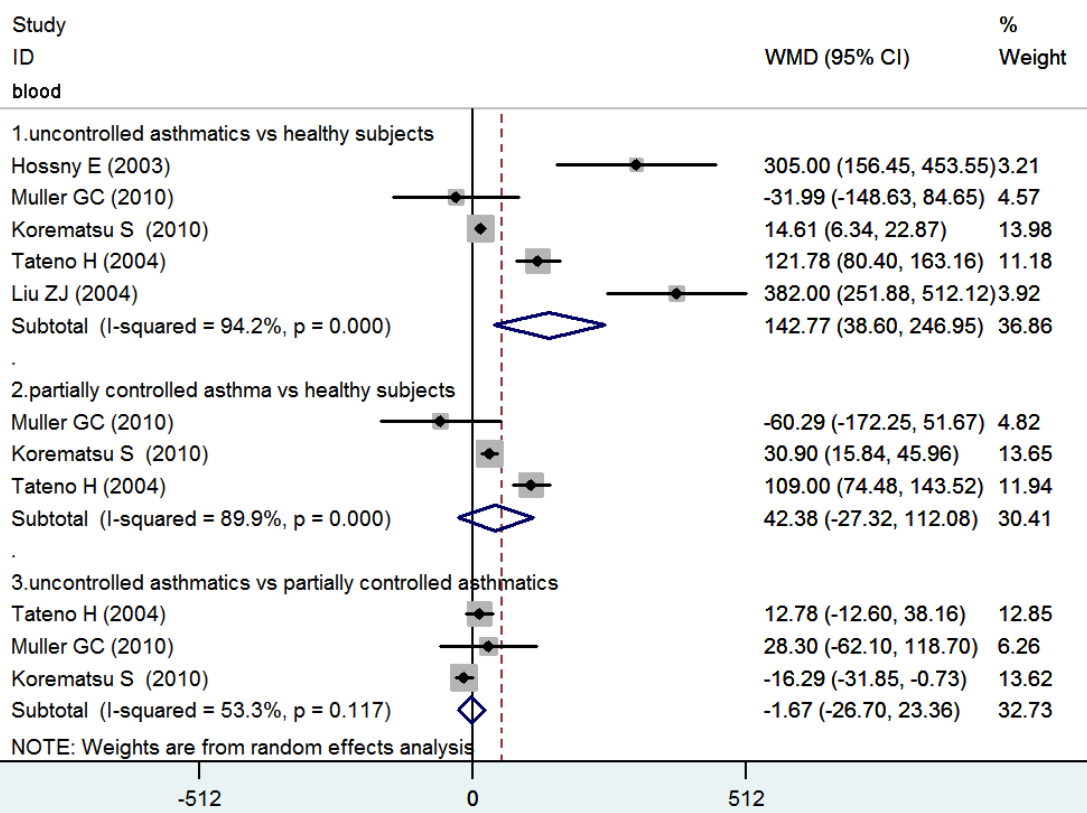
Additional file 4. Blood CCL11 levels in patients with different asthma severity.



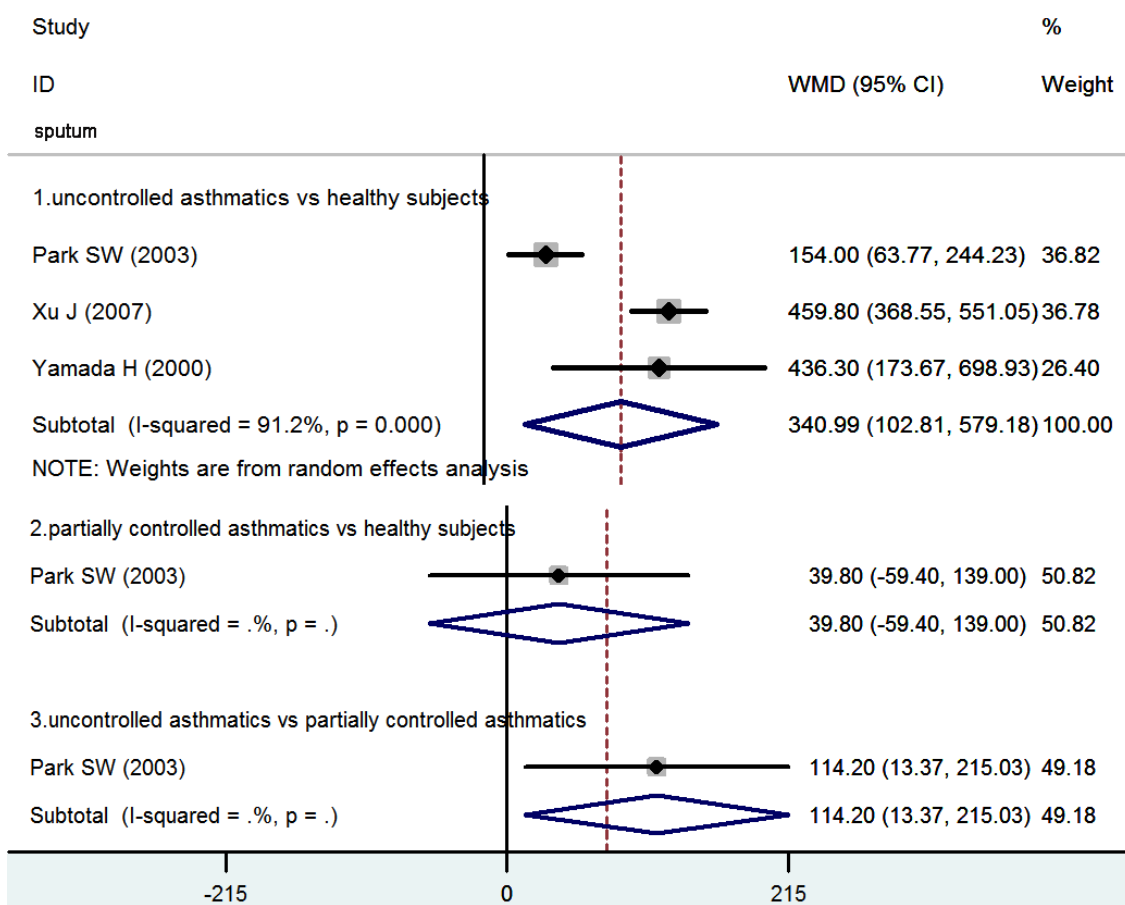
Additional file 5. Blood CCL11 concentrations in patients with stable or unstable asthma.



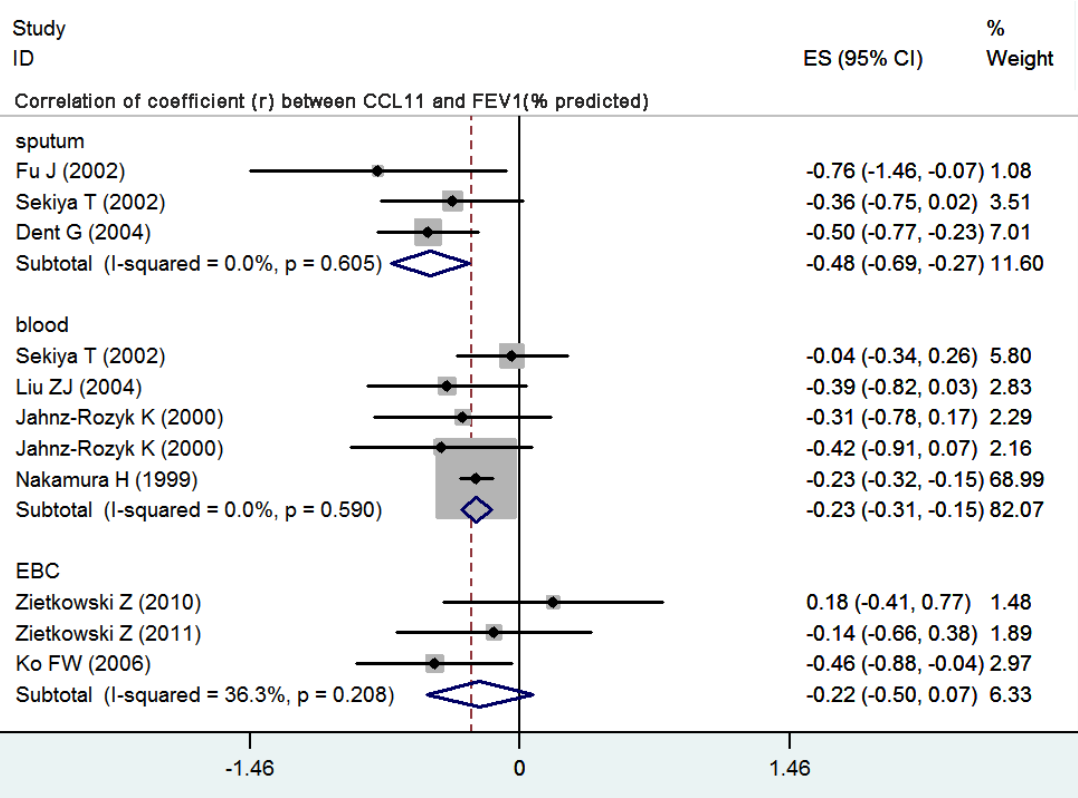
Additional file 6. Sputum CCL11 concentrations in patients with stable or unstable asthma.



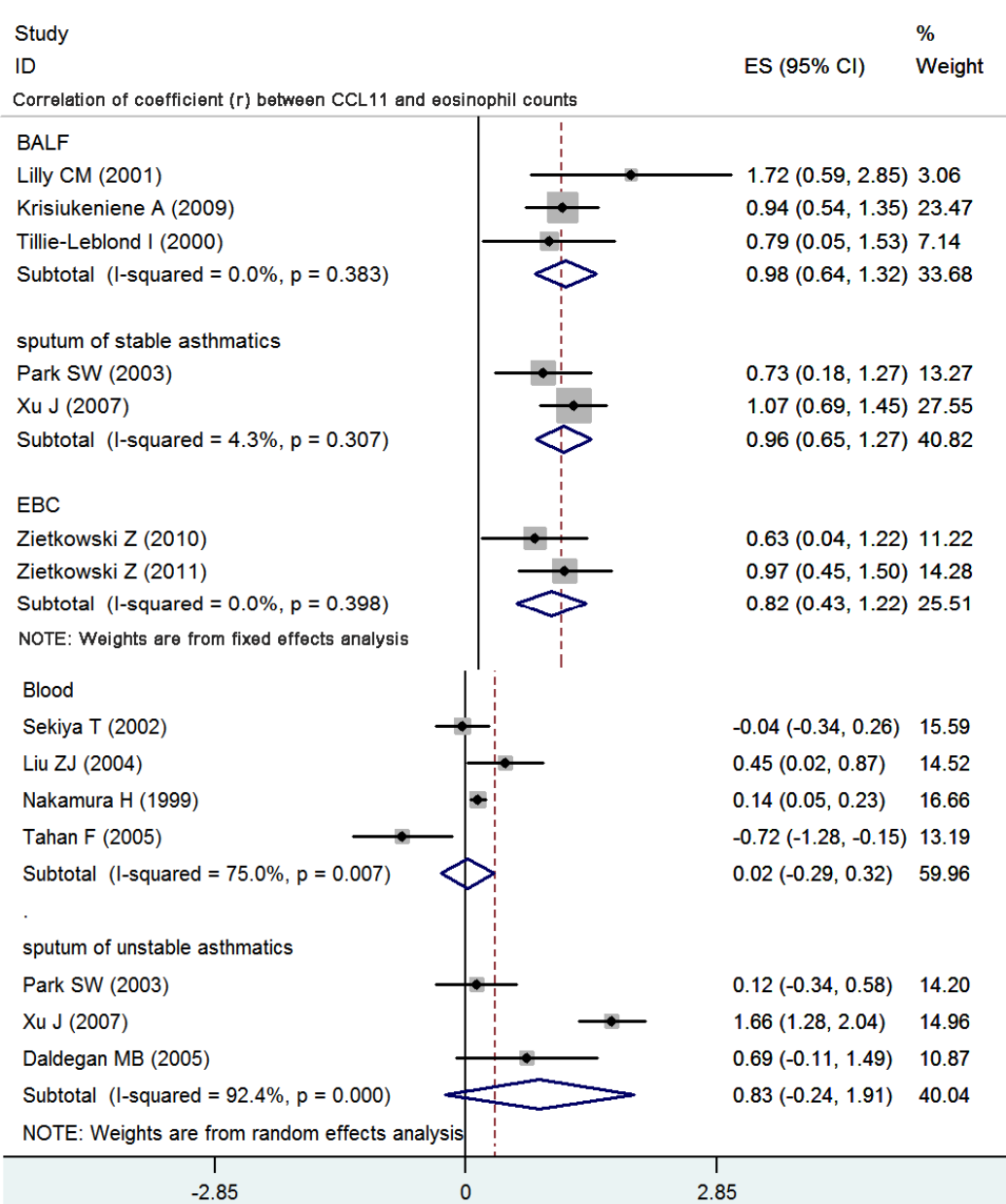
Additional file 7. Blood CCL11 concentrations and levels of asthma control.



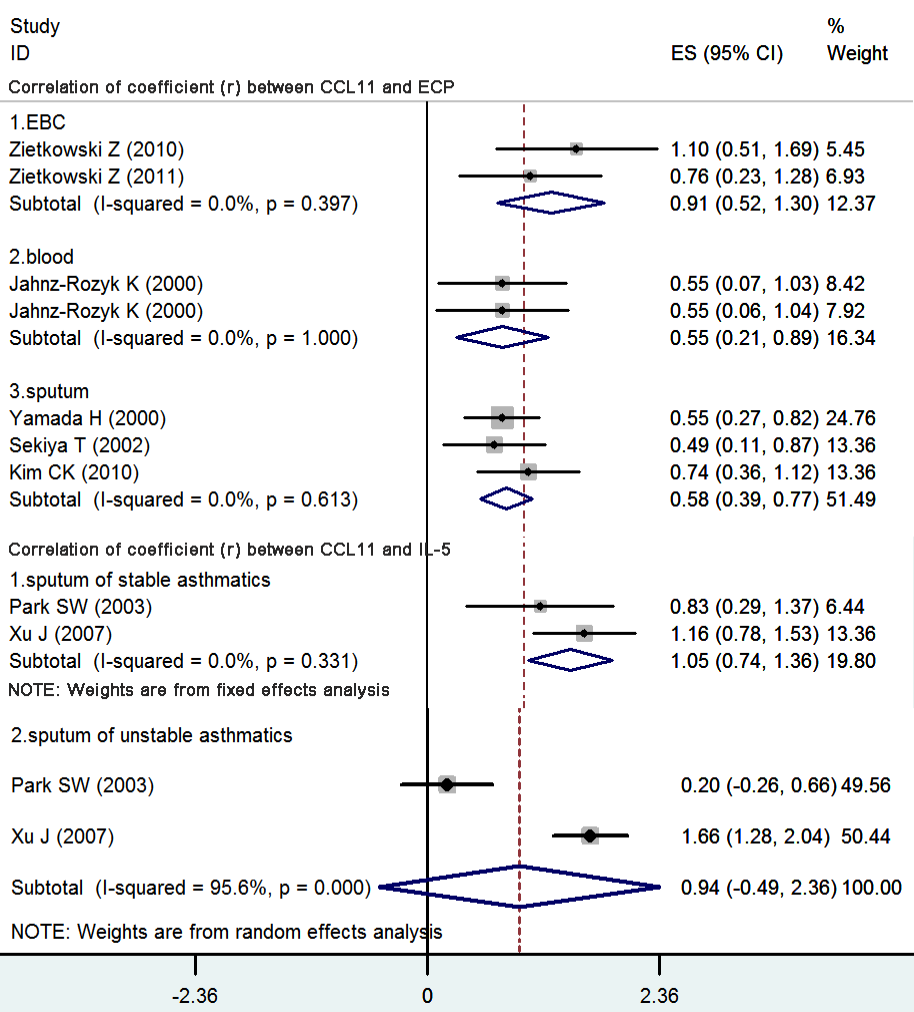
Additional file 8. Sputum CCL11 concentrations and levels of asthma control.



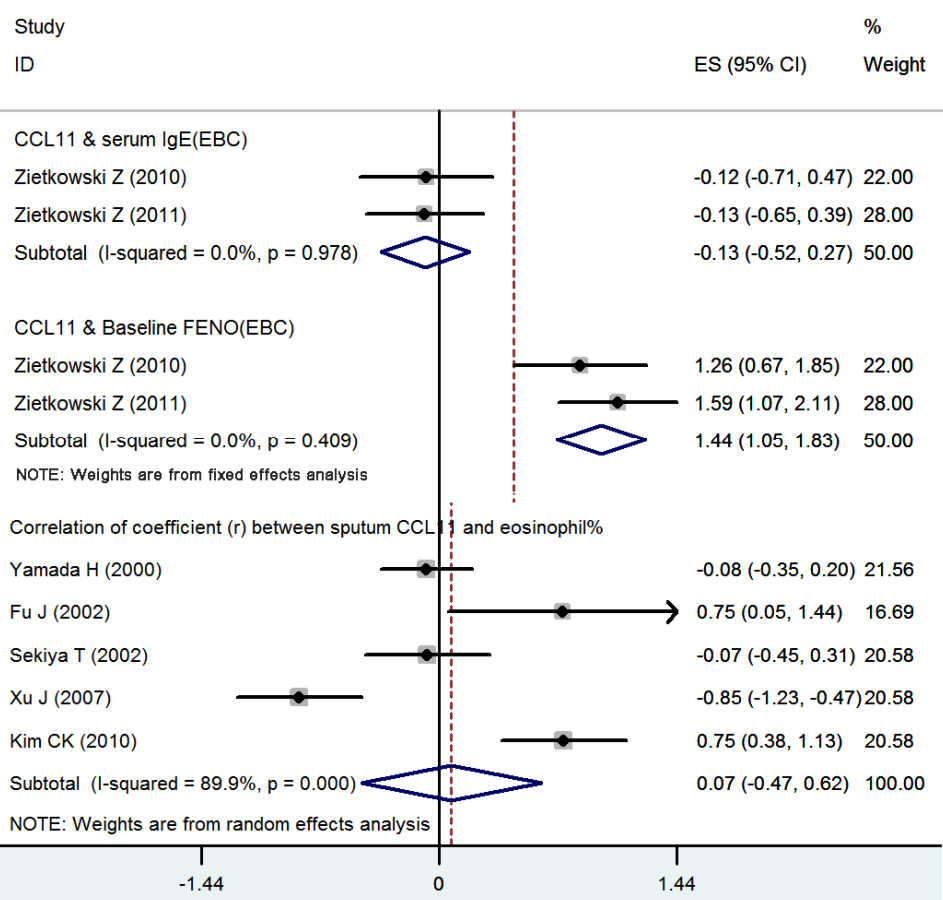
Additional file 9. Correlation coefficient (r) between CCL11 and the percentage predicted values of FEV₁.



Additional file 10. Correlation coefficient (r) between CCL11 and the eosinophil counts.



Additional file 11. Correlation coefficient (r) between CCL11 and ECP as well as between CCL11 and IL-5.



Additional file 12. Correlation coefficient (r) between CCL11 and IgE as well as baseline FENO in EBC and between CCL11 and the percentage of eosinophils in the blood.