

Tobacco smoke as a risk factor for allergic sensitization in adults: Conclusions of a systematic review and meta-analysis



To the Editor:

Allergic sensitization to aeroallergens is a common phenomenon and a crucial step in the development of allergic diseases. Nonetheless, the influence of tobacco smoke exposure on the development of allergic sensitization in adults is quite complex and not totally understood.

Therefore, our objective was to systematically review the existing evidence regarding this topic and to perform a meta-analysis of the data collected from the included studies.

The search for studies that potentially matched the inclusion criteria (for inclusion and exclusion criteria, see this article's Online Repository at www.jacionline.org) was performed independently by 2 authors, in several databases (Fig 1). Discrepancies were resolved through discussion with the involvement of a third author when necessary. All data were independently extracted by 2 review authors, who also independently assessed the risk of bias in the included studies considering the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement for observational studies. For description of statistical methods, see this article's Online Repository at www.jacionline.org.

Fig 1 represents the flowchart of the inclusion and exclusion criteria used in the selection process. Of the 1387 retrieved studies, 16 were included in the meta-analysis.

Regarding the random-effects' meta-analysis for specific IgE, 6 studies with a total of 25,218 participants were included. The random-effects estimate showed that tobacco smoke exerted a statistically significant protective effect in the development of allergic sensitization among smokers, when compared with never smokers (odds ratio [OR], 0.692; 95% CI, 0.596-0.804; $P < .001$) (Fig 2, A). For summary of the meta-analysis, sensitivity analysis, and evaluation of publication bias, see this article's Online Repository at www.jacionline.org.

Concerning the fixed-effect meta-analysis for house dust mite (HDM)-specific IgE, 5 studies with a total of 31,782 participants were included. The fixed-effect estimate revealed that tobacco smoke exposure increases significantly the risk of allergic sensitization to HDM in smokers, when compared with never smokers (OR, 1.184; 95% CI, 1.113-1.259; $P < .001$) (Fig 2, B). For summary of the meta-analysis, sensitivity analysis, and evaluation of publication bias, see this article's Online Repository.

Relatively to the random-effects' meta-analysis for skin prick tests, 7 studies with a total of 11,041 participants were included. The random-effects estimate revealed that tobacco smoke exposure exerted a significant protective effect in the development of allergic sensitization among smokers when compared with never smokers (OR, 0.714; 95% CI, 0.596-0.856; $P < .001$) (Fig 2, C). For summary of the meta-analysis, sensitivity analysis,

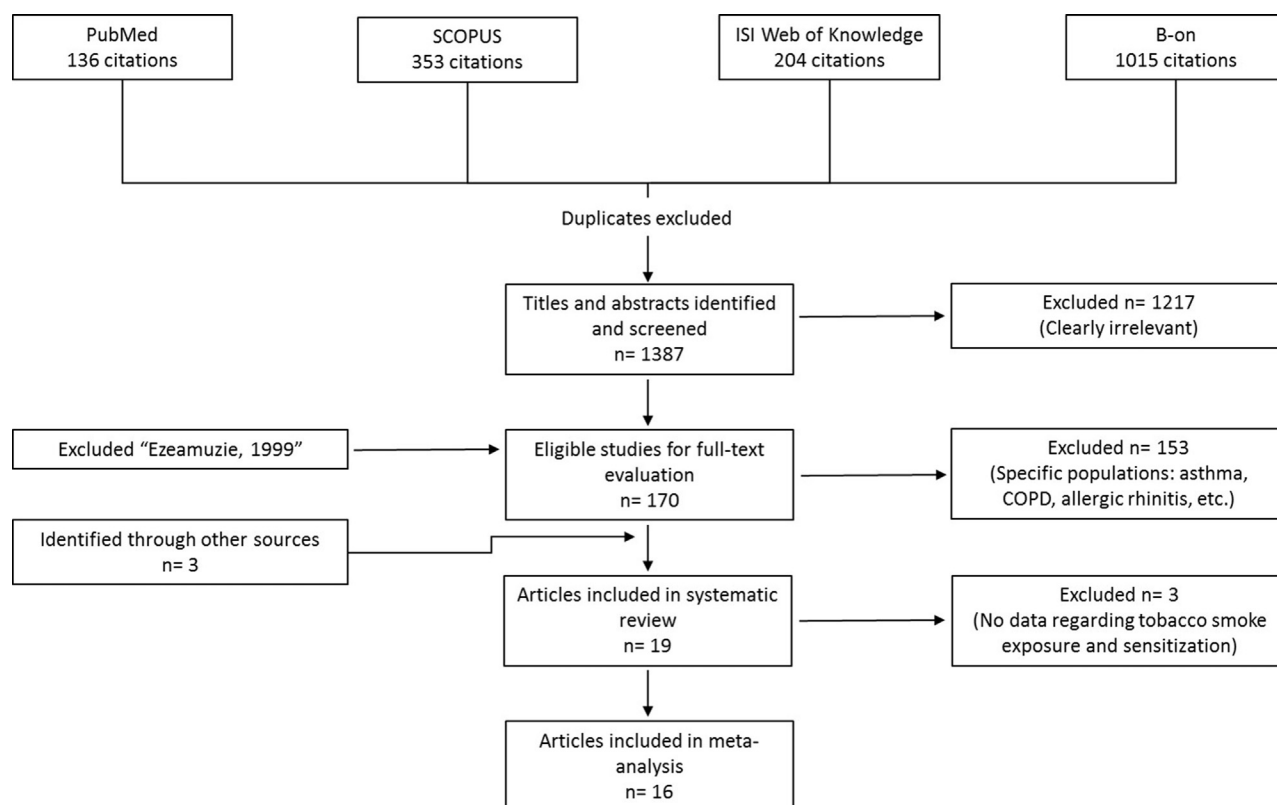


FIG 1. Flowchart of the inclusion and exclusion criteria used in this systematic review and meta-analysis. COPD, Chronic obstructive pulmonary disease.

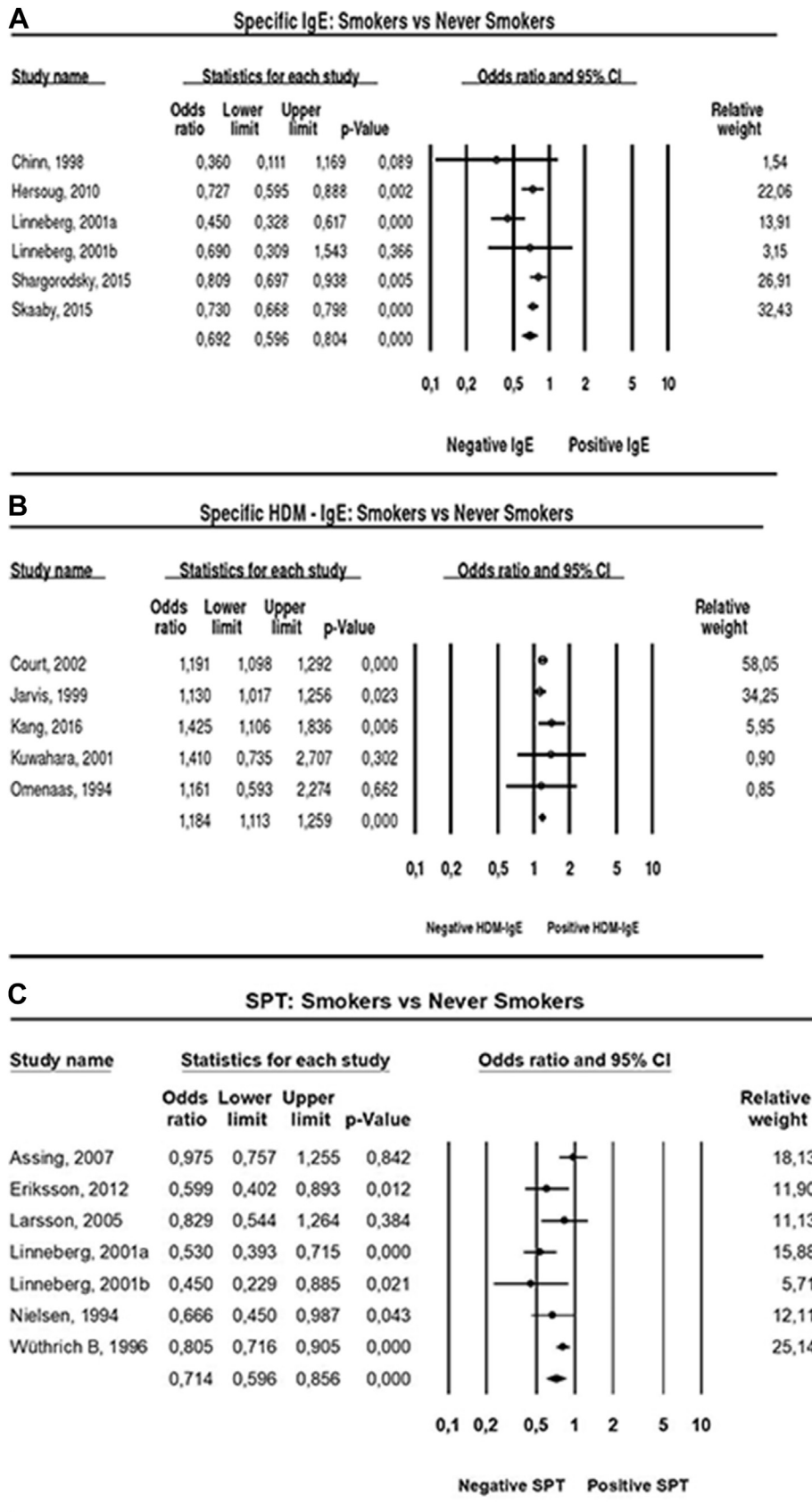


FIG 2. A, Forest plot representing the comparison of tobacco smoke exposure on specific IgE in smokers vs never smokers. B, Forest plot representing the comparison of tobacco smoke exposure on HDM-specific IgE in smokers vs never smokers. C, Forest plot representing the comparison of tobacco smoke exposure on SPTs in smokers vs never smokers. SPT, Skin prick test.

and evaluation of publication bias, see this article's Online Repository.

This study revealed that, in general, smokers have a lower risk of developing allergic sensitization to common aeroallergens, when compared with never smokers (for IgE: OR, 0.692; 95% CI, 0.596-0.804; $P < .001$; for skin prick tests: OR, 0.714; 95% CI, 0.596-0.856; $P < .001$). These results support the hypothesis that tobacco smoke exerts a protective effect in the development of allergic sensitization to some aeroallergens, most likely through its immunosuppressant effects. Chronic exposure to tobacco smoke affects both innate and adaptive immune responses.¹ Various components of tobacco smoke were found to induce an increase in suppressor T cells, a decrease in T_H cells,² a significant reduction in natural killer cells, and a decrease in serum levels of immunoglobulins.¹ Furthermore, high-dose cigarette smoke has also been shown to directly suppress T-cell function, including T_H2-cell response, which plays an important role in IgE production and in the development of immediate hypersensitivity.² This suppression in T_H2-cell function might result in a lower risk of developing allergic sensitization to certain aeroallergens. Nevertheless, the higher risk for HDM sensitization that was observed among smokers still remains to be explained.

Regarding HDM sensitization, the observed results show that smokers have a higher risk of developing allergic sensitization to HDM allergens, when compared with never smokers (OR, 1.184; 95% CI, 1.113-1.259; $P < .001$). The differences observed may be due to a difference in biochemical function. Almost all inhalant allergens are proteases.³ HDM proteases other than the group 1 allergens, to which belongs the major HDM allergen *Dermatophagoides pteronyssinus* 1 (a cysteine protease), induce only low levels of sensitization, and very few allergens from other sources are cysteine proteases.^{3,4} This may be a possible explanation to why tobacco smoke exposure is able to exert a protective effect on most aeroallergens, but not on *Der p* 1.

Der p 1 can act as an adjuvant in the sensitization process and elicit allergic responses both to itself and to other allergens. The 4 main mechanisms by which *Der p* 1 induces allergic sensitization are through (1) cleavage of CD23⁵ (a low-affinity IgE Fc receptor), which leads to IgE-mediated immune responses⁴; (2) degradation and inactivation of lung surfactant proteins A and D,⁶ which inhibit the binding of inhalant allergens to cell-sequestered IgE; (3) cleavage of α 1-antitrypsin,⁷ a pulmonary antiprotease, whose activity in smokers is already significantly reduced; and (4) disruption of the epithelial barrier function, which increases permeability to other allergens, facilitating their access to dendritic cells⁴ and promoting the development of allergic sensitization.

The mechanism by which tobacco smoke exerts a protective effect on allergic sensitization to some aeroallergens, but not others, has not yet been clarified. A study performed by Lanckacker et al⁸ showed that cigarette smoke short exposure was sufficient to promote dendritic cell-mediated transport of HDM allergens to lymph nodes and elicit a T_H2 immune response, thus facilitating the process of allergic sensitization to HDM allergens. Moreover, some components of tobacco smoke may be able to alter allergenic properties of some proteins, potentiating or abating their allergenic potential.⁹

These hypotheses may explain, at least partially, the differences observed between sensitization to HDM allergens and sensitization to other aeroallergens. Regardless, tobacco smoke has deeply detrimental effects in human health, and these results

suggest that a protective effect in the development of allergic sensitization should not be considered an argument in favor to initiate or perpetuate this harmful habit. In the future, more studies that illustrate the mechanism by which tobacco smokes induces allergic sensitization to HDM and prevents sensitization to other aeroallergens are needed, as well as enlightenment on other factors involved in the process.

Beatriz Mónico, MSc^{a,b}
Jorge M. R. Gama, PhD^c
M. Ramiro Pastorinho, PhD^{a,b}
Olga Lourenço, PhD^{a,b}

From ^athe Faculty of Health Sciences, ^bCICS – UBI Health Sciences Research Centre, ^cCMA – UBI Centre of Mathematics and Applications, University of Beira Interior, Covilhã, Portugal. E-mail: olga@fcsaude.ubi.pt.

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Lower perinatal exposure to Proteobacteria is an independent predictor of early childhood wheezing



To the Editor:

We postulated that perinatal risk factors for asthma would be associated with early exposure to bacteria or bacterial products. To evaluate this hypothesis, we assessed circulating-free bacterial DNA (cfbDNA) in umbilical cord blood from mother-child pairs (see Table E1 in this article's Online Repository at www.jacionline.org) from Project Viva, a longitudinal study from birth through childhood.¹ To assess cfbDNA, we used 16S rRNA sequencing of the V1-3 region from circulating DNA

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Supplemental Material

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16 ***Original Article to a Letter to the Editor."***

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METHODS

Inclusion Criteria

Studies were considered eligible if they met previously defined inclusion criteria:

- (1) Published between inception of database and December 2016;
- (2) Performed on a non-selected, random population;
- (3) Individuals over 18 years old;
- (4) Mention of participants' smoking habits;
- (5) Sensitization to aeroallergens determined at least by one of the following methods:
specific IgE, Phadiatop[®] or skin prick tests.
- (6) Written in English, French, Italian, Portuguese or Spanish.

An individual was considered sensitized when had a positive SPT or a positive specific IgE (IgE $\geq$ 0.35 kU_A/L) test to, at least, one of the allergens being tested. Regarding the definition of "smoker", we considered the definitions established by the authors of the different studies.

Exclusion Criteria

We excluded studies performed on children or adolescents under 18 years old; studies performed on laboratory animals (i.e., rats, mice, etc.); and studies focusing on a specific population (i.e., on individuals with asthma, rhinitis, COPD, specific occupation, etc.).

Statistical methods

The statistical analysis of the extracted data was performed using the software *Comprehensive Meta-Analysis 3.3*. A fixed-effect or, when necessary, a random-effects meta-analysis was performed for each outcome, considering, separately, the OR between non-smokers and smokers.

Statistical heterogeneity between studies was assessed by Cochran's Q test and by I² index (p<0.05 considered statistically significant). We also performed a sensitivity analysis for each meta-analysis, which consisted in removing one study at a time in order to evaluate the effect's strength. Publication bias was evaluated using three different methods: the funnel plot, Duval and Tweedie's trim and fill method, and Eger's regression test.

Table E-1: Summary of the characteristics of the studies included in the meta-analysis, including name, country or region, sample size, age range, smoking status and the range of allergen sensitizations studied.

Reference	Study	Country/ Region	Sample size	Age	Smoking status	Specific IgE allergens tested	Skin Prick Tests allergens tested
Assing, 2007¹	NA	Copenhagen, Denmark	1668	18 - 35	never smoker, former smoker, smoker		HDM, cat, dog, horse, <i>Cladosporium</i> , <i>Alternaria</i> , grass, birch, mugwort
Chinn, 1998²	ECRHS II	Cambridge, Ipswich and Norwich, England	1198	20 - 44	former smoker, smoker	HDM, cat, <i>Cladosporium</i> , Timothy grass, birch	HDM, cat, <i>Cladosporium</i> , <i>Alternaria</i> , Timothy grass, olive, birch, <i>Parietaria judaica</i> , common ragweed
Court, 2002³	Health Survey for England	England	22567	20 - 90+	never smoker, former smoker, smoker	HDM*	
Eriksson, 2012⁴	NA	West Gothia, Sweden	741	16 - 75	never smoker, former smoker, smoker		#HDM, cat, dog, horse, <i>Cladosporium</i> , <i>Alternaria</i> , Timothy grass, birch, mugwort, <i>Blatella</i>
Hersoug, 2010⁵	Health 2006 study	Copenhagen, Denmark	3471	18 - 69	never smoker, former smoker, smoker (current + occasional)	HDM, birch, cat, grass	
Jarvis, 1999⁶	ECRHS II	34 centers, 14 countries	13002	20 - 44	never smoker, former smoker, smoker	HDM*, grass*, cat*	
Kang, 2016⁷	KHANES	Korea	1969	19 - 60+	never smoker, smoker	HDM*	
Kuwahara, 2001⁸	NA	Osaka, Japan	426	30 - 74	never smoker, smoker	HDM*	
Larsson, 2005⁹	Swedish FinEsS	Sweden + Finland	692	22 - 74	never smoker, former smoker, smoker		HDM, <i>Lepidoglyphus destructor</i> , <i>Acarus siro</i> , cat, dog, cow, horse, <i>Cladosporium</i> , <i>Alternaria</i> , birch, Timothy grass, mugwort, <i>Blatella</i> , latex
Linneberg, 2001a¹⁰	The Copenhagen	Copenhagen, Denmark	734	15 - 69	former smoker, smoker	HDM, cat, dog, Timothy grass, birch, mugwort	HDM, cat, dog, horse, <i>Cladosporium</i> , <i>Alternaria</i> ,

	Allergy Study						Timothy grass, birch, mugwort
Linneberg, 2001b ¹¹	The Copenhagen Allergy Study	Copenhagen, Denmark	477	15 - 69	never smoker, former smoker, smoker	HDM, cat, dog, Timothy grass, birch, mugwort	HDM, cat, dog, horse, <i>Cladosporium</i> , <i>Alternaria</i> , Timothy grass, birch, mugwort
Nielsen, 1994 ¹²	Glostrup Population Studies	Copenhagen, Denmark	793	15 - 69	never smoker, former smoker, smoker		#HDM, cat, dog, horse, <i>Cladosporium</i> , <i>Alternaria</i> , Timothy grass, birch, mugwort
Omenaas, 1994 ¹³	NA	Hordaland, Norway	1512	18 - 73	never smoker, former smoker, smoker	HDM*, cat*, timothy grass*, birch*, <i>Cladosporium</i> *	
Shargorodsky, 2015 ¹⁴	NHANES 2005-2006	USA	4339	20 - 85	former smoker, smoker	HDM, cat, dog, cockroach, mouse, rat, <i>Alternaria</i> , <i>Aspergillus</i> , ragweed, rye grass, oak, birch, Russian thistle	
Skaaby, 2015 ¹⁵	Monica1, Inter99, Health2006, the 1936-cohort, Allergy98, Health2008	Denmark	15828	15 - 77	never smoker, former smoker, smoker		
Wüthrich B, 1996 ¹⁶	SAPALDIA	Switzerland	8344	18 - 60	never smoker, former smoker, smoker		HDM, cat, dog, <i>Cladosporium</i> , <i>Alternaria</i> , grass, birch, <i>Parietaria</i>

48 Unless indicated by * the results were presented in the studies as SPT positivity or Specific IgE positivity, defined as a positive SPT or Specific IgE to one or more allergens,
49 and not by single allergen. In the studies indicated by #, the results are presented by group of allergen (house dust mites, pollens, danders, molds, etc).

50 ECRHS - European Community Respiratory Health Survey, KHANES - Korean National Health and Nutrition Examination Survey; NHANES - National Health and Nutrition
51 Examination Survey; SAPALDIA - Swiss Study on Air Pollution and Lung Diseases in Adults

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META-ANALYSIS

1. Specific IgE: Smokers Vs. Never Smokers

Table E-2: Summary of random effects' meta-analysis and complementary tests for comparison of specific IgE between smokers and never smokers.

Specific IgE	
Random effects' meta-analysis: Smokers Vs. Never Smokers	
Number of studies	6
Number of participants	25218
OR (95% CI)	
Observed	0.692 (0.596 - 0.804)
Adjusted	0.699 (0.603 - 0.811) ^a
p-value	<0.001
Cochran's Q test	
Q statistics	12.278
df	5
p-value	0.031
I ² %	59.275

^a Duval and Tweedie's trim and fill

Six studies with a total of 25218 participants were included in this random effects' meta-analysis (Table E-2). The random effects estimate showed that tobacco smoke exerted a statistically significant protective effect in the development of allergic sensitization among smokers, when compared to never smokers (OR=0.692, 95%CI: 0.596 – 0.804, p<0.001) (Figure E-1).

The studies included in this meta-analysis revealed considerable heterogeneity (I²=59.275, Q(5)=12.278, p=0.031)¹⁷, which results from the study “Linneberg, 2001a”¹⁰ (Figure E-2). This heterogeneity is due to this study being the only case-control study in this meta-analysis, in which one of the groups is composed of individuals with rhinitis symptoms.

The funnel plot in Figure E-3 revealed a need for imputing one publication on the right side. The adjusted OR obtained by Duval and Tweedie's trim and fill method¹⁸ was OR=0.699, 95%CI: 0.603 - 0.811 which is not significantly different from the non-adjusted OR. Eger's regression test¹⁹ did not reveal a publication bias: IC 95%: -4.042 – 1.515, t=1.262, df=4, p=0.275.

2. HDM-Specific IgE: Smokers Vs. Never Smokers

Table E-3: Summary of fixed-effect meta-analysis and complementary tests for comparison of HDM-specific IgE between smokers and never smokers.

HDM-Specific IgE Fixed-effect meta-analysis: Smokers Vs. Never Smokers	
Number of studies	5
Number of participants	31782
OR (95% CI)	
Observed	1.184 (1.113 - 1.259)
Adjusted	1.168 (1.100 - 1.240) ^a
p-Value	<0.001
Cochran's Q test	
Q statistics	3.103
df	4
p-value	0.541
I ² %	0.000

^a Duval and Tweedie's trim and fill
HDM: House Dust Mite

Five studies with a total of 31782 participants were included in this fixed-effect meta-analysis (Table E-3). The fixed-effect estimate revealed that tobacco smoke exposure increases significantly the risk of allergic sensitization to HDM in smokers, when compared to never smokers (OR=1.184, 95%CI: 1.113 – 1.259, p<0.001) (Figure E-4).

Sensitivity analysis revealed no source of heterogeneity (Figure E-5). The funnel plot in Figure E-6 revealed the need of imputing two publications on the left side. The adjusted OR obtained by Duval and Tweedie's trim and fill method was only slightly overestimated: OR=1.168, 95%CI: 1.100 - 1.240. Additionally, Eger's regression test did not reveal publication bias: 95%CI: - 1.429 – 2.717, t=0.989, df=3, p=0.396.

3. SPTs: Smokers Vs. Never Smokers

Table E-4: Summary of the random effects' meta-analysis and complementary tests for comparison of SPTs between smokers and never smokers.

SPT Random effects' meta-analysis: Smokers Vs. Never Smokers	
Number of studies	7
Number of participants	11041
OR (95%CI)	
Observed	0.714 (0.596 – 0.856)
Adjusted	0.787 (0.650 – 0.953) ^a
p-value	<0.001
Cochran's Q test	
Q statistics	14.422
df	6
p-value	0.025
I ² %	58.398

^a Duval and Tweedie's trim and fill

Seven studies with a total of 11041 participants were included in this random effects' meta-analysis (Table E-4). The random effects estimate revealed that tobacco smoke exposure exerted a significant protective effect in the development of allergic sensitization among smokers when compared to never smokers (OR=0.714, 95%CI: 0.596 – 0.856, $p<0.001$) (Figure E-7).

The studies included in this meta-analysis revealed considerable heterogeneity ($I^2=58.398$, $Q(6)=14.422$, $p=0.025$)¹⁷, which results mainly from two studies (Figure E-8): the study “Assing, 2007”¹, due to being the only non-randomized study, and the study “Linneberg, 2001a”¹⁰, for the same reason mentioned before. If both of these studies were omitted, the heterogeneity index (I^2) decreases to 22.076 and the p-value of Cochran's Q test increases to 0.274 ($Q(4)=5.133$).

The funnel plot in Figure E-9 revealed the need for imputing two publications on the right side. The adjusted OR obtained by Duval and Tweedie's trim and fill method increased slightly in comparison with the observed OR (adjusted OR=0.787, 95% CI: 0.650 - 0.953). Additionally, Eger's regression test did not reveal a publication bias (95% CI: -4.130 – 1.177, $t=1.430$, $df=5$, $p=0.212$).

4. Combined analysis of IgE and SPT

Table E-5: Summary of the random effects' meta-analysis and complementary tests for the combined analysis of specific IgE and SPTs between smokers and never smokers.

Specific IgE and SPT	
Random effects' meta-analysis: Smokers Vs. Never Smokers	
Number of studies	11
Number of participants	35147
OR (95% CI)	
Observed	0.742 (0.674 - 0.815)
Adjusted	0.772 (0.693 - 0.860) ^a
p-value	<0.001
Cochran's Q test	
Q statistics	18.313
df	10
p-value	0.050
I^2 %	45.394

^a Duval and Tweedie's trim and fill

Eleven studies with a total of 35147 participants were included in this random-effects' meta-analysis combining the results from specific IgE and SPTs, between smokers and never smokers (Table E-5). The random effects' estimate revealed that when combined both results, tobacco smoke still exerts a protective effect in the development of allergic sensitization in adult smokers (OR=0.742; 95%CI: 0.674-0.815; $p<0.001$) (Figure E-10).

The studies included in this meta-analysis revealed considerable heterogeneity ($I^2=45.394$, $Q(10)=18.313$, $p=0.050$)¹⁷, which results mainly from two studies already mentioned (Figure E-11): the study “Assing, 2007”¹, and the study “Linneberg, 2001a”¹⁰, for the reasons previously indicated.

The funnel plot in Figure E-12 revealed the need for imputing three publications on the right side. The adjusted OR obtained by Duval and Tweedie’s trim and fill method increased slightly in comparison with the observed OR (adjusted OR=0.772, 95% CI: 0.693 – 0.860). Additionally, Eger’s regression test did not reveal a publication bias ($t=1.203$, $df=9$, $p=0.260$).

Strengths and weaknesses of the study

One of the strengths of this study was the inclusion of studies performed in randomized, non-selected populations. This allowed us to assess the influence of tobacco smoke in the development of allergic sensitization in adults with and without a genetic tendency towards atopy, which renders these results more reliable.

Furthermore, we only included studies which evaluated the influence of tobacco smoke exposure using objective markers of allergic sensitization (specific IgE determination and SPTs), which allowed us to objectively evaluate the influence of tobacco smoke exposure in the immune system, specifically in the initial step of an allergic response.

This systematic review and meta-analysis has some limitations. Firstly, the methods used to assess tobacco smoke exposure varied significantly between the included studies, especially regarding the different definitions of “smoker” presented. Some studies considered a smoker “someone who smokes daily”¹⁶, or “someone who had smoked more than five packs of cigarettes in their life”⁷, or even “someone who had smoked at least one cigarette a day for at least one year”¹². Furthermore, one of the studies¹⁴ used serum cotinine measurements to assess tobacco smoke exposure. Therefore, by grouping all of these different definitions into only one effect, we are ignoring the actual quantity of tobacco smoke that each individual was exposed to.

The fact that the majority of the included studies are cross-sectional studies can be another limitation, since the cause-effect relationship of the observed outcome may be debatable. Prospective cohort studies and randomized controlled studies are, undoubtedly, higher-evidence studies. Nevertheless, in the literature, there were few prospective cohort studies performed in adults, and it was not possible to include randomized controlled studies due to the absence of these studies in the literature, for obvious ethical reasons.

The inclusion criteria used in this systematic review also limited, in a way, the number of studies to be included (of the 1387 publications identified, only 18 were included in the systematic review and, of these, only 16 were included in the meta-analysis). Limiting the number of languages in the included studies in order to guarantee a full comprehension and analysis, may have also excluded potentially relevant publications.

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211

Figure Legends

Figure E-1: Forest plot representing the comparison of tobacco smoke exposure on specific IgE in smokers vs. never smokers. The dots represent the OR of individual studies and the error bars represent 95% CIs. The diamond represents the overall OR. The vertical line extending upwards from 1 represents the null value. CI: confidence interval.

Figure E-2: Sensitivity analysis of the random effects' meta-analysis comparing specific IgE between smokers and never smokers. The dots represent the resulting OR when the study is removed and the error bars represent 95% CIs. The vertical line extending upwards from 1 represents the null value. The diamond represents the observed OR. CI: confidence intervals.

Figure E-3: Funnel plot representing the analysis of publication bias for the random effects meta-analysis comparing specific IgE between smokers and never smokers. Open dots represent the studies included in the meta-analysis, and the observed OR is represented by the open diamond. Large studies are closer to the top and cluster around mean effect size. Smaller studies appear toward the bottom of the graph. Closed dot represents the imputed study according to the trim and fill method, and the adjusted OR is represented by the closed diamond.

Figure E-4: Forest plot representing the comparison of tobacco smoke exposure on HDM-specific IgE in smokers vs. never smokers. The dots represent the OR of individual studies and the error bars represent 95% CIs. The diamond represents the overall OR. The vertical line extending upwards from 1 represents the null value. CI: confidence interval. HDM: house dust mite.

Figure E-5: Sensitivity analysis of the fixed-effect meta-analysis comparing HDM-specific IgE between smokers and never smokers. The dots represent the resulting OR when the study is removed and the error bars represent 95% CIs. The vertical line extending upwards from 1 represents the null value. The diamond represents the observed OR. CI: confidence intervals.

Figure E-6: Funnel plot representing the analysis of publication bias for the fixed-effect meta-analysis comparing HDM-specific IgE between smokers and never smokers. Open dots represent the studies included in the meta-analysis, and the observed OR is represented by the open diamond. Large studies are closer to the top and cluster around mean effect size. Smaller studies appear toward the bottom of the graph. Closed dots represent the imputed studies according to the trim and fill method, and the adjusted OR is represented by the closed diamond.

Figure E-7: Forest plot representing the comparison of tobacco smoke exposure on SPTs in smokers vs. never smokers. The dots represent the OR of individual studies and the error bars represent 95% CIs. The diamond represents the overall OR. The vertical line extending upwards from 1 represents the null value. CI: confidence interval.

Figure E-8: Sensitivity analysis of the random effects' meta-analysis comparing SPTs between smokers and never smokers. The dots represent the resulting OR when the study is removed and the error bars represent 95% CIs. The vertical line extending upwards from 1 represents the null value. The diamond represents the observed OR. CI: confidence intervals; OR: odds ratio; SPT: skin prick tests.

Figure E-9: Funnel plot representing the analysis of publication bias for the random effects' meta-analysis comparing SPTs between smokers and never smokers. Open dots represent the studies included in the meta-analysis, and the observed OR is represented by the open diamond. Large studies are closer to the top and cluster around mean effect size. Smaller

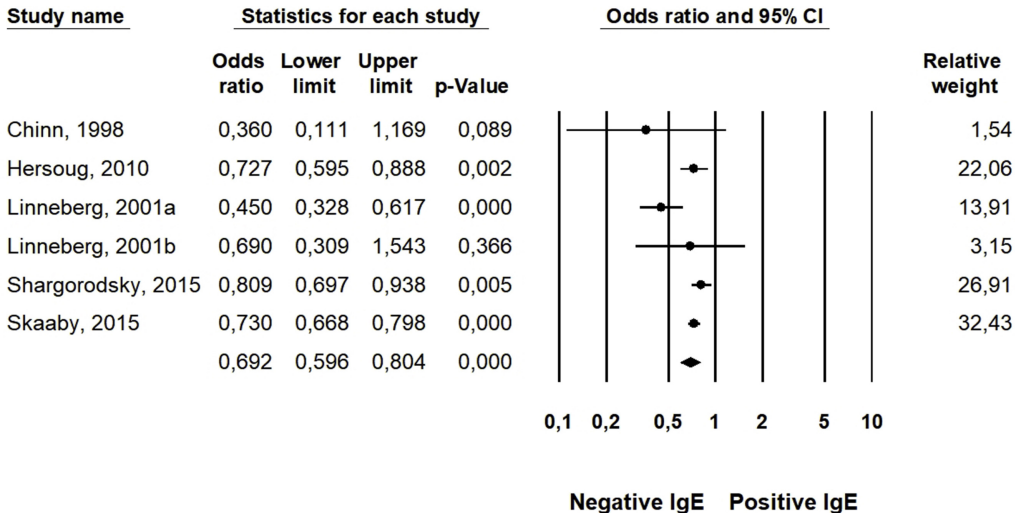
studies appear toward the bottom of the graph. Closed dots represent the imputed studies according to the trim and fill method, and the adjusted OR is represented by the closed diamond.

Figure E-10: Forest plot representing the combined analysis of specific IgE and SPTs comparison between smokers and never smokers. The dots represent the OR of individual studies and the error bars represent 95% CIs. The diamond represents the overall OR. The vertical line extending upwards from 1 represents the null value. CI: confidence interval; HDM: house dust mite; OR: odds ratio; SPTs: skin prick tests.

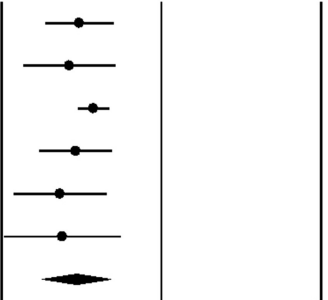
Figure E-11: Sensitivity analysis of the random-effect meta-analysis comparing specific IgE and SPTs between smokers and never smokers. The dots represent the resulting OR when the study is removed and the error bars represent 95% CIs. The vertical line extending upwards from 1 represents the null value. The diamond represents the observed OR. OR: odds ratio; CI: confidence interval; SPT: skin prick tests.

Figure E-12: Funnel plot representing the analysis of publication bias for the random effects' meta-analysis comparing specific IgE and SPTs between smokers and never smokers. Open dots represent the studies included in the meta-analysis, and the observed OR is represented by the open diamond. Large studies are closer to the top and cluster around mean effect size. Smaller studies appear toward the bottom of the graph. Closed dots represent the imputed studies according to the trim and fill method, and the adjusted OR is represented by the closed diamond.

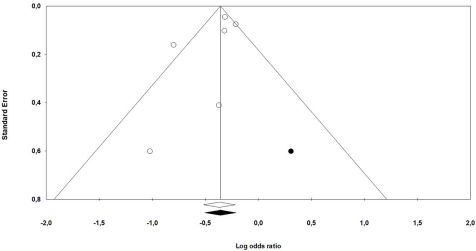
Specific IgE: Smokers vs Never Smokers



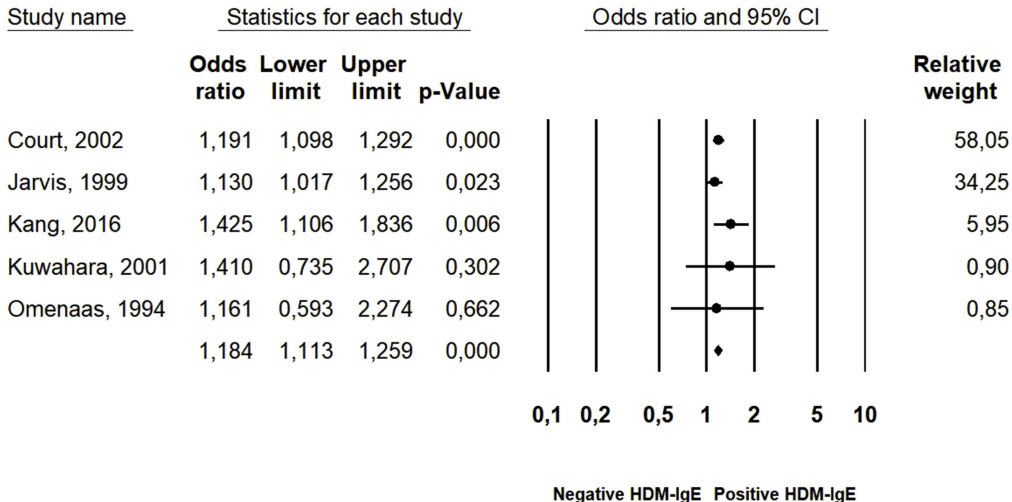
Specific IgE: Smokers vs Never Smokers

Study name	Statistics with study removed				Odds ratio (95% CI) with study removed
	Point	Lower limit	Upper limit	p-Value	
Chinn, 1998	0,700	0,603	0,812	0,000	
Hersoug, 2010	0,670	0,549	0,818	0,000	
Linneberg, 2001a	0,745	0,694	0,799	0,000	
Linneberg, 2001b	0,689	0,587	0,808	0,000	
Shargorodsky, 2015	0,644	0,526	0,787	0,000	
Skaaby, 2015	0,650	0,505	0,837	0,001	
	0,692	0,596	0,804	0,000	
					0,5 1 2
					Negative IgE Positive IgE

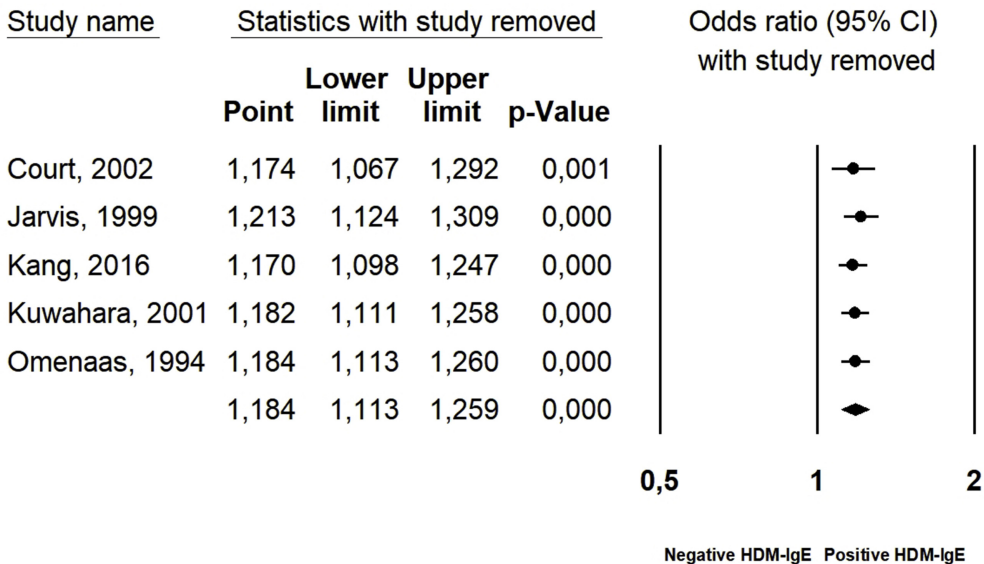
Funnel Plot of Standard Error by Log odds ratio



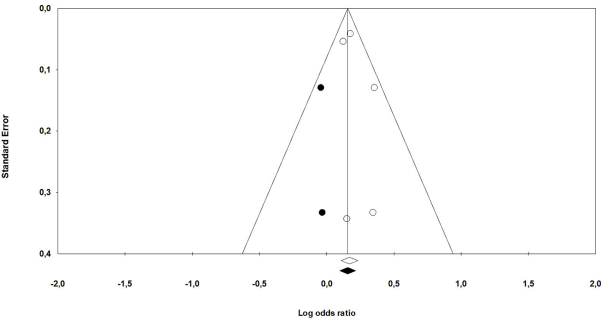
Specific HDM - IgE: Smokers vs Never Smokers



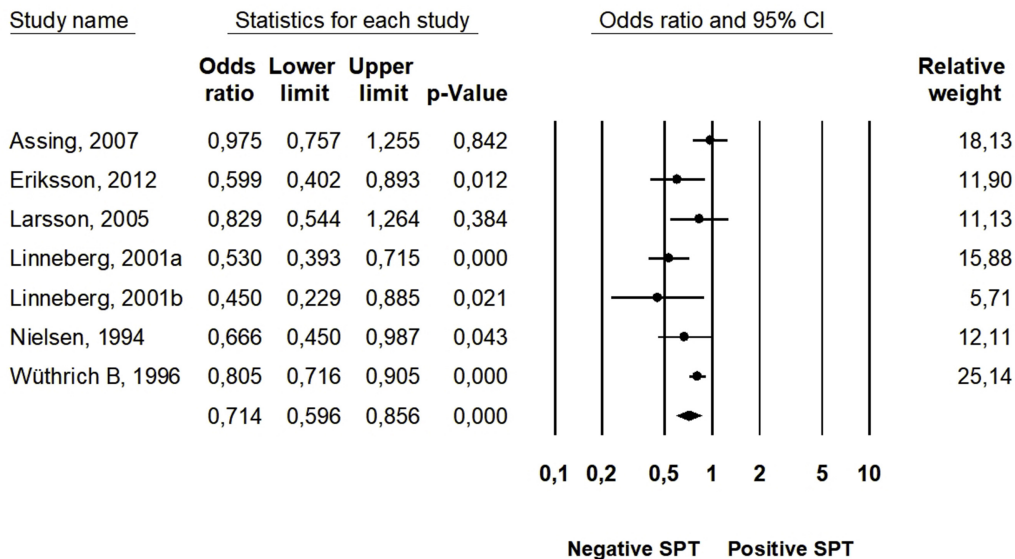
Specific HDM - IgE: Smokers vs Never Smokers



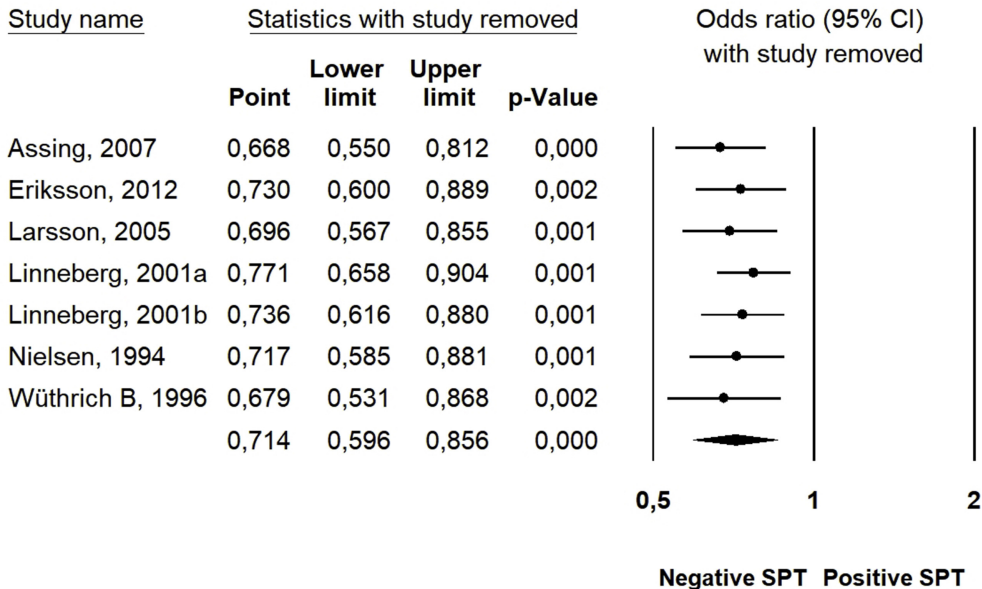
Funnel Plot of Standard Error by Log odds ratio



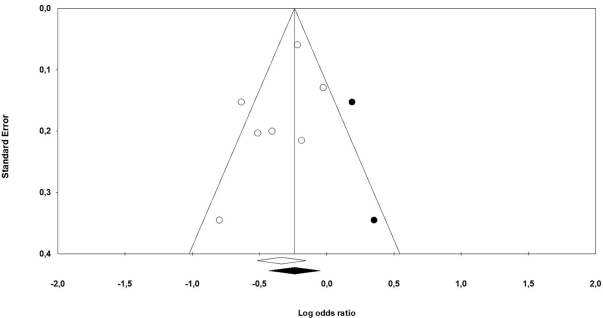
SPT: Smokers vs Never Smokers



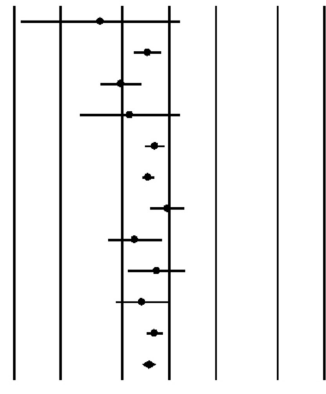
SPT: Smokers vs Never Smokers



Funnel Plot of Standard Error by Log odds ratio



Specific IgE and SPT: Smokers vs Never Smokers

Study name	Outcome	Statistics for each study				Odds ratio and 95% CI						Relative weight
		Odds ratio	Lower limit	Upper limit	p-Value							
Chinn, 1998	Sp. IgE	0,360	0,111	1,169	0,089							0,63
Hersoug, 2010	Sp. IgE	0,727	0,595	0,888	0,002							12,06
Linneberg, 2001a	Combined	0,488	0,359	0,664	0,000							6,99
Linneberg, 2001b	Combined	0,557	0,265	1,172	0,123							1,54
Shargorodsky, 2015	Sp. IgE	0,809	0,697	0,938	0,005							15,95
Skaaby, 2015	Sp. IgE	0,730	0,668	0,798	0,000							21,26
Assing, 2007	SPT	0,975	0,757	1,255	0,842							9,14
Eriksson, 2012	SPT	0,599	0,402	0,893	0,012							4,67
Larsson, 2005	SPT	0,829	0,544	1,264	0,384							4,25
Nielsen, 1994	SPT	0,666	0,450	0,987	0,043							4,78
Wüthrich B, 1996	SPT	0,805	0,716	0,905	0,000							18,72
		0,742	0,674	0,815	0,000							
						0,1	0,2	0,5	1	2	5	10
						Negative			Positive			

Specific IgE and SPT: Smokers vs Never Smokers

Study name	Outcome	Statistics with study removed				Odds ratio (95% CI) with study removed
		Point	Lower limit	Upper limit	p-Value	
Chinn, 1998	Sp. IgE	0,745	0,679	0,819	0,000	
Hersoug, 2010	Sp. IgE	0,741	0,665	0,825	0,000	
Linneberg, 2001a	Combined	0,767	0,716	0,821	0,000	
Linneberg, 2001b	Combined	0,744	0,675	0,820	0,000	
Shargorodsky, 2015	Sp. IgE	0,727	0,651	0,811	0,000	
Skaaby, 2015	Sp. IgE	0,736	0,652	0,832	0,000	
Assing, 2007	SPT	0,727	0,665	0,795	0,000	
Eriksson, 2012	SPT	0,749	0,680	0,826	0,000	
Larsson, 2005	SPT	0,736	0,666	0,814	0,000	
Nielsen, 1994	SPT	0,744	0,673	0,823	0,000	
Wüthrich B, 1996	SPT	0,725	0,647	0,812	0,000	
		0,742	0,674	0,815	0,000	
						0,5
						1
						2
						Negative
						Positive

Funnel Plot of Standard Error by Log odds ratio

