

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Berry MA, Hargadon B, Shelley M, et al. Evidence of a role of tumor necrosis factor α in refractory asthma. *N Engl J Med* 2006;354:697-708.

Appendix

Measurement of sputum mediators

Sputum IL-8, Cysteinyl-leukotriene (LTC₄, D₄ & E₄) and Histamine were measured using standard ELISA kits (BD Pharmagen, Immunotech and Cayman chemicals respectively) and eosinophilic cationic protein (ECP) was measured using a fluorescence immunoassay (UniCAP test, Pharmacia, Uppsala, Sweden). These assays have been previously validated for use in sputum supernatants (S1). The sensitivity levels of the assay were 2, 13x10⁻³, 0.8x10⁻³, 50x10⁻³ ng/ml for ECP, cysteinyl leukotrienes, IL-8 and histamine. The intra-assay coefficient of variability was 5-10% and the interassay coefficient of variability 3-15% across a range of concentrations of mediators measured.

References

S1 Birring SS, Parker D, Brightling CE, Bradding P, Wardlaw AJ, Pavord ID. Induced sputum inflammatory mediator concentrations in chronic cough. Am J Respir Crit Care Med 2004; 169:15-19.

Supplementary table

Week	Placebo				Etanercept				Between treatment difference (95% C.I.)
	0	5	10	Within treatment difference (95% C.I.)	0	5	10	Within treatment difference (95% C.I.)	
Visual analogue scores									
-Cough	54 (25)	49 (24)	44 (24)	-10 (-24, 4)	43 (18)	33 (22)	27 (22)	-16 (-32, 1)	-5 (-28, 17)
-Wheeze	39 (37)	38 (31)	35 (29)	-3 (-17, 12)	26 (21)	25 (25)	17 (16)	-9 (-21, 4)	-6 (-29, 16)
-Breathlessness	53 (29)	49 (26)	57 (24)	4 (-15, 23)	56 (27)	35 (25)	32 (26)	-24 (-41, -7)*	-28 (-62, 6)
<i>Juniper Asthma Quality of Life Score</i>									
-Symptoms	3.8 (1.4)	4.1 (1.3)	3.8 (1.3)	0.01 (-0.30, 0.31)	3.7 (1.0)	4.5 (1.3)	4.8 (1.3)	1.13 (0.50, 1.77)**	1.15 (0.19, 2.1)*
-Activities	4.2 (1.2)	4.2 (1.3)	4.2 (1.2)	0.01 (-0.21, 0.21)	4.4 (0.9)	4.8 (0.9)	5.1 (1.1)	0.78 (0.18, 1.38)*	0.79 (-0.01, 1.59)
-Emotions	4.4 (1.4)	4.6 (1.5)	4.2 (1.7)	-0.2 (-0.88, 0.68)	4.2 (1.4)	4.6 (1.3)	5.0 (1.4)	0.72 (0.12, 1.30)*	0.88 (0.01, 1.75)*
-Environment	4.5 (1.4)	4.5 (1.4)	4.6 (1.4)	0.02 (-0.07, 0.1)	4.3 (1.0)	4.5 (1.2)	4.8 (1.3)	0.4 (0.02, 0.78)*	0.28 (-0.46, 1.0)
Alveolar NO concentration (ppb)	10.8 (17)	6.6 (3.8)	9.2 (9.8)	-1.6 (-9.2, 6.1)	9.8 (10)	4.7 (2.5)	6.1 (3.5)	-3.8 (-11, 3.1)	1.6 (-6.1, 9.3)
Sputum IL-8 (ngml ⁻¹)	164.8 (22)	-	143.9 (40)	-20.9 (-69, 27)	171.5 (23)	-	153.0 (23)	-18 (-48, 13)	2.5 (-2.3, 7.3)
Sputum Cys-Lt (ngml ⁻¹)	9.5 (2.9)	-	8.6 (2.5)	-0.8 (-5, 3.4)	10.0 (2.8)	-	10.2 (1.5)	0.2 (-4.7, 5.1)	1.0 (-3.0, 4.1)
Sputum ECP (µgml ⁻¹)	1.1 (0.2)	-	1.3 (0.2)	0.2 (-0.1, 0.4)	1.0 (0.2)	-	0.9 (0.2)	-0.07 (-0.2, 0.06)	-0.3 (-0.6, 0.1)

Supplementary table

Mean (S.D.) measurements from patients before and after placebo and etanercept treatment with within treatment and between treatment differences and 95% confidence intervals. IL-8 – interleukin 8, Cys-Lt – Cysteinyl leukotriene, ECP – eosinophilic cationic protein. Data given as mean (standard deviation) except where marked † geometric mean (log standard deviation). Between group results are given as mean difference (95% confidence intervals) except where marked † where fold difference (95% confidence intervals) are given. * denote $p < 0.05$ and ** $p < 0.01$.