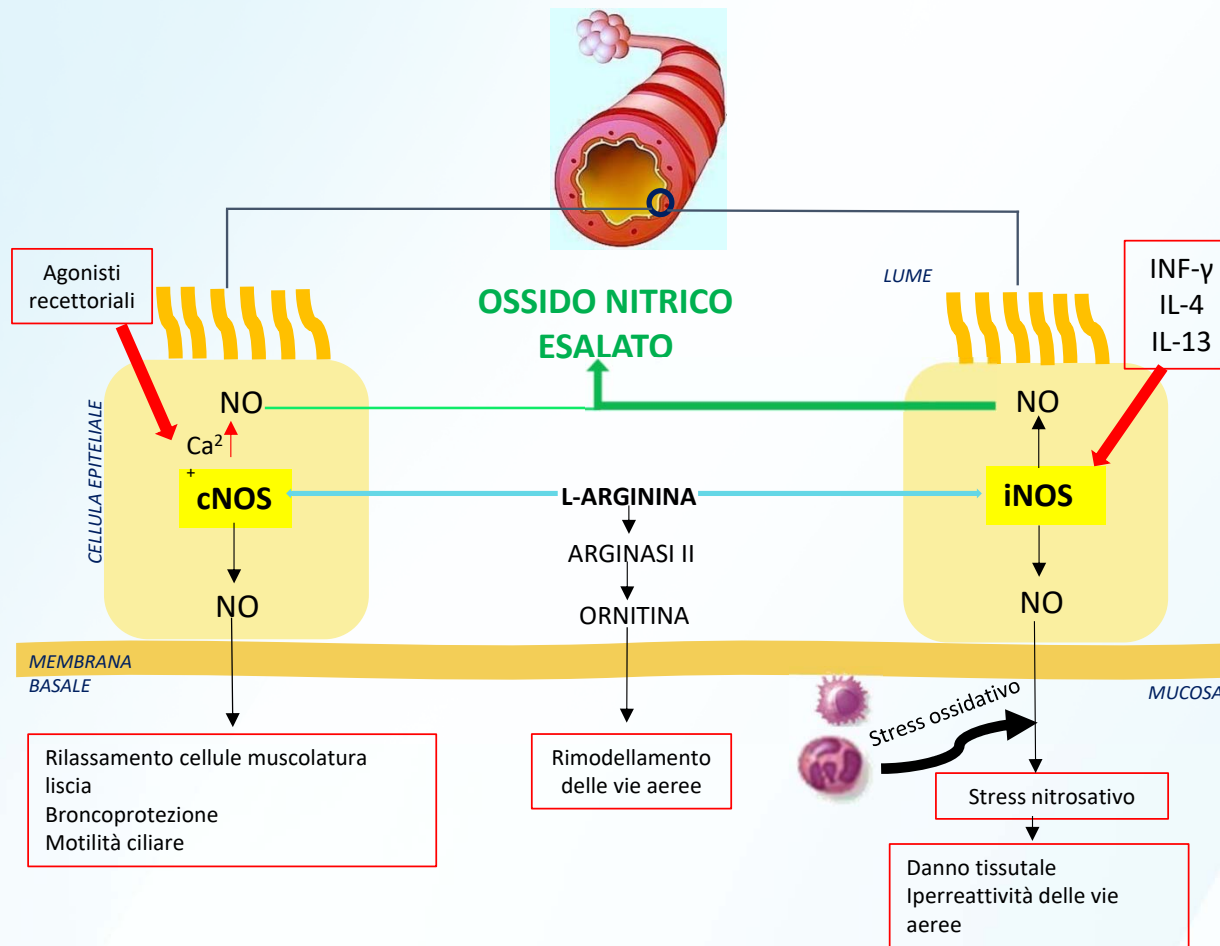


Focus sul FeNO nel paziente con asma grave

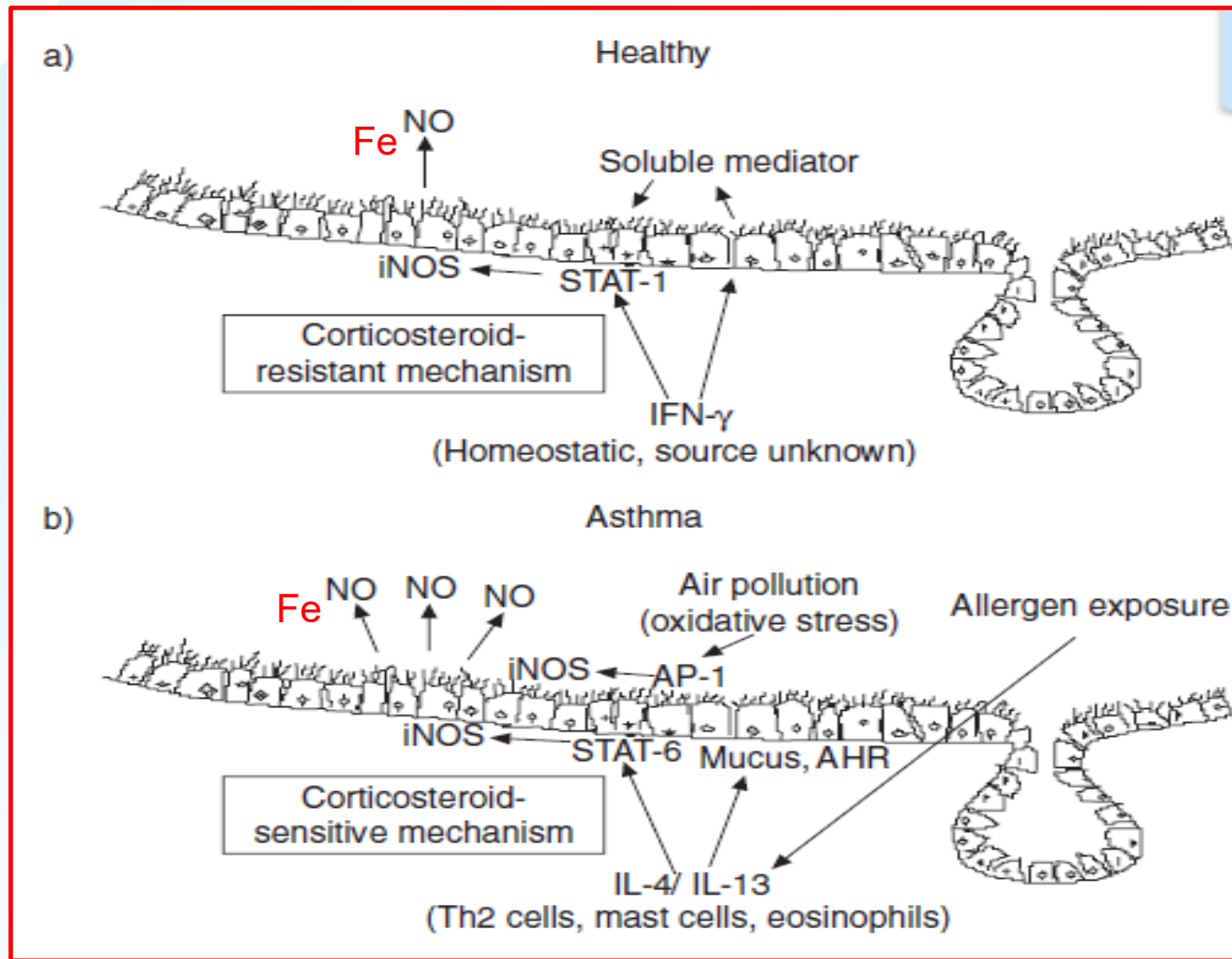
Fabio LM Ricciardolo, MD, PhD, FERS
Dipartimento di Scienze Cliniche e Biologiche
Università degli Studi di Torino



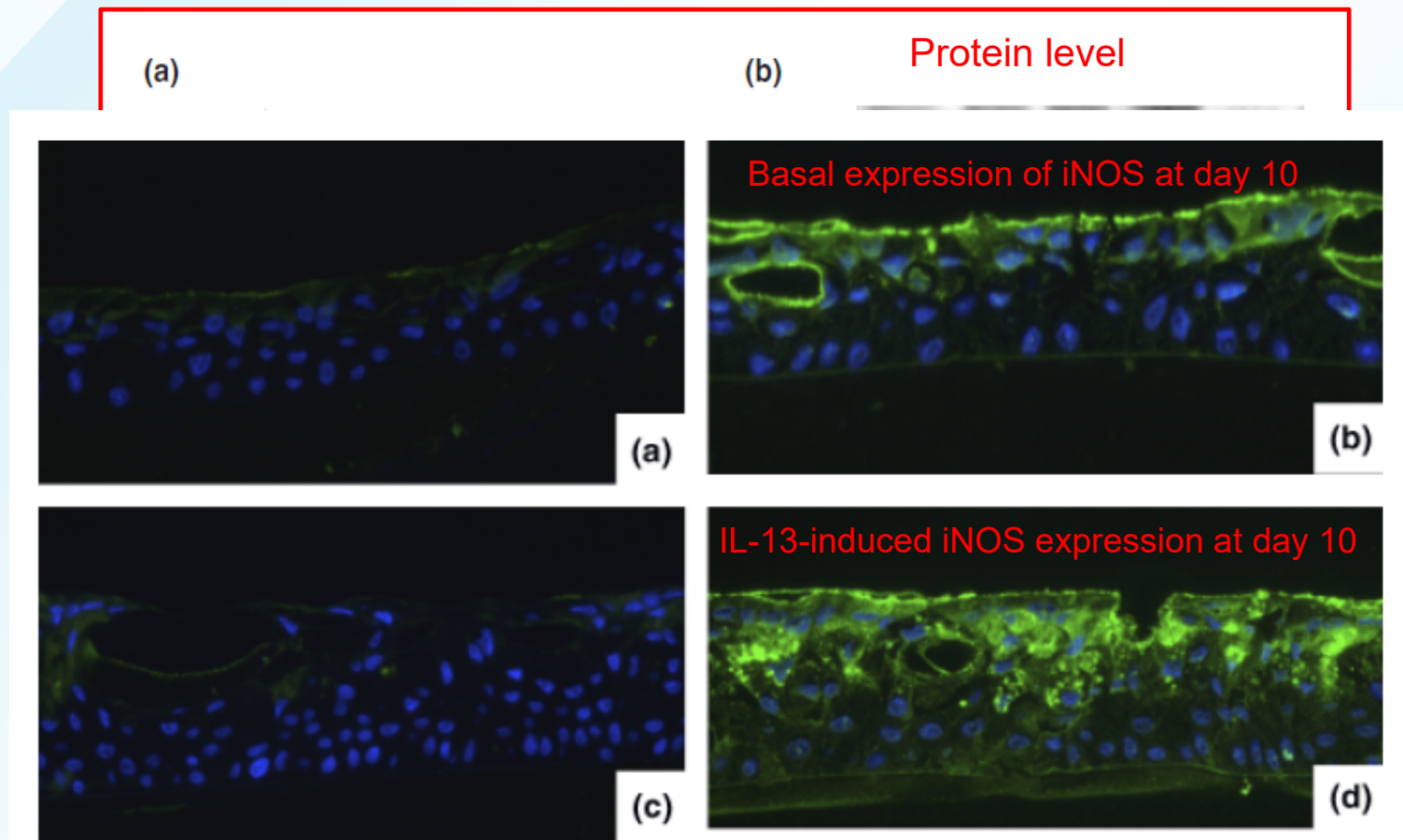
SINTESI DELL'OSSIDO NITRICO



Regulation of iNOS in bronchial epithelium



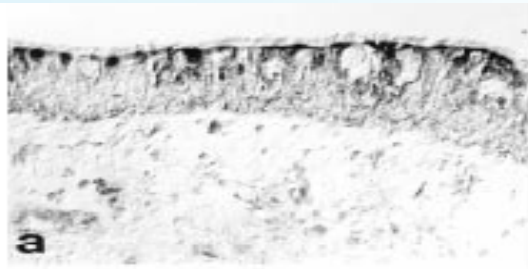
IL-13 induced iNOS expression in bronchial epithelial cells



iNOS and Asthma

Epithelial iNOS

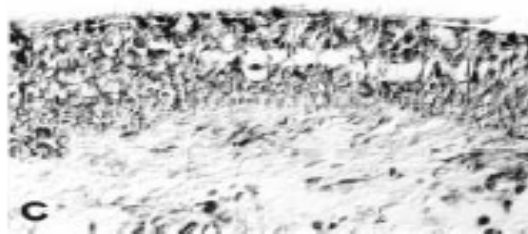
CTRL



Naive
Asthma

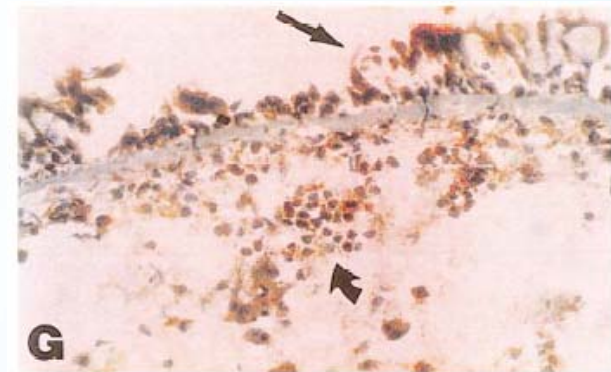
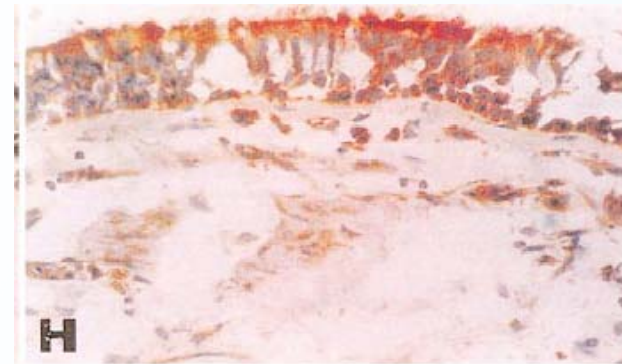


CS
Asthma



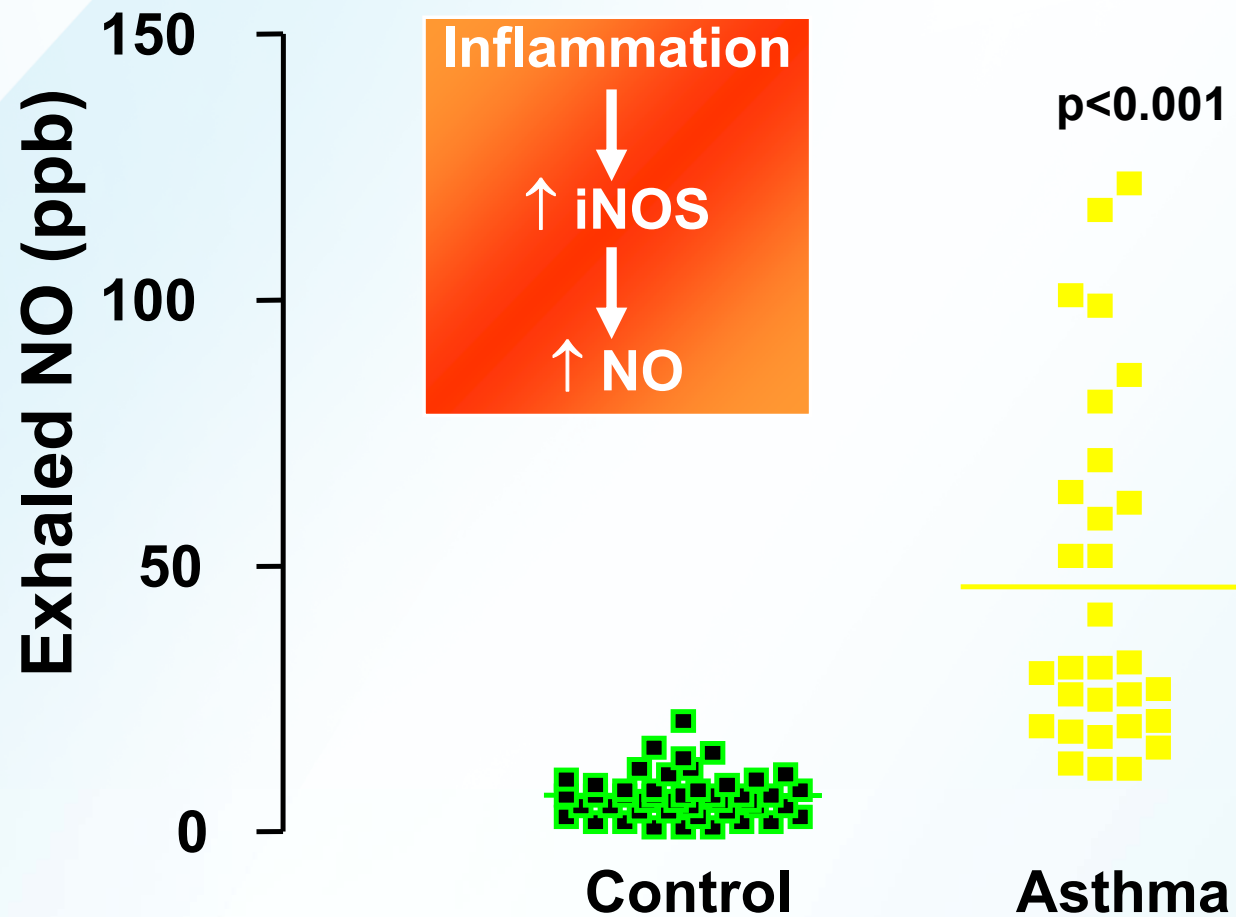
Redington et al. Thorax 2001

iNOS in inflammatory cells



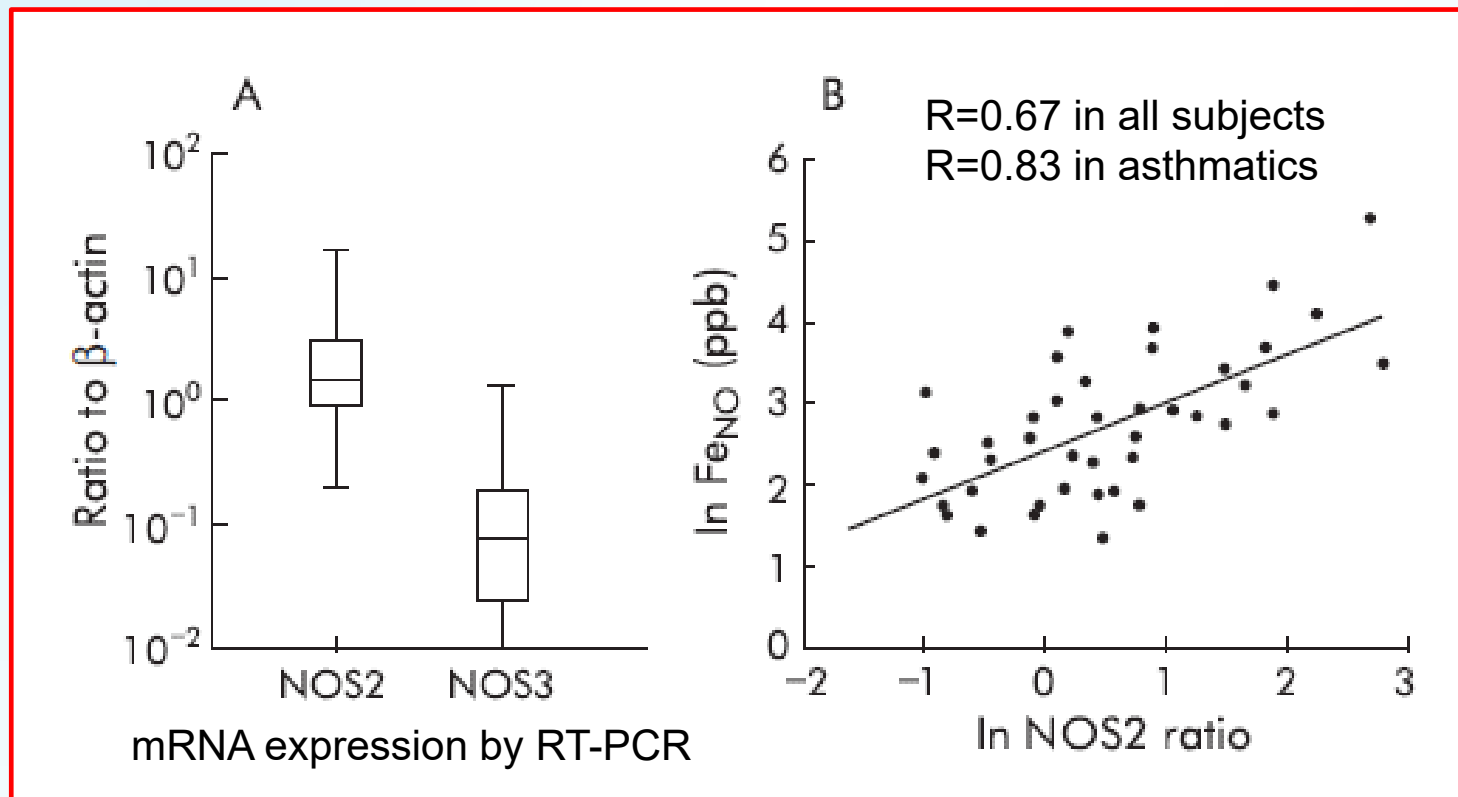
Saleh et al. FASEB J 1998

INCREASED EXHALED NO IN ASTHMA



iNOS expression and FeNO

24 Healthy; 10 atopic healthy; 9 atopic asthmatics: children
Brushing in distal trachea

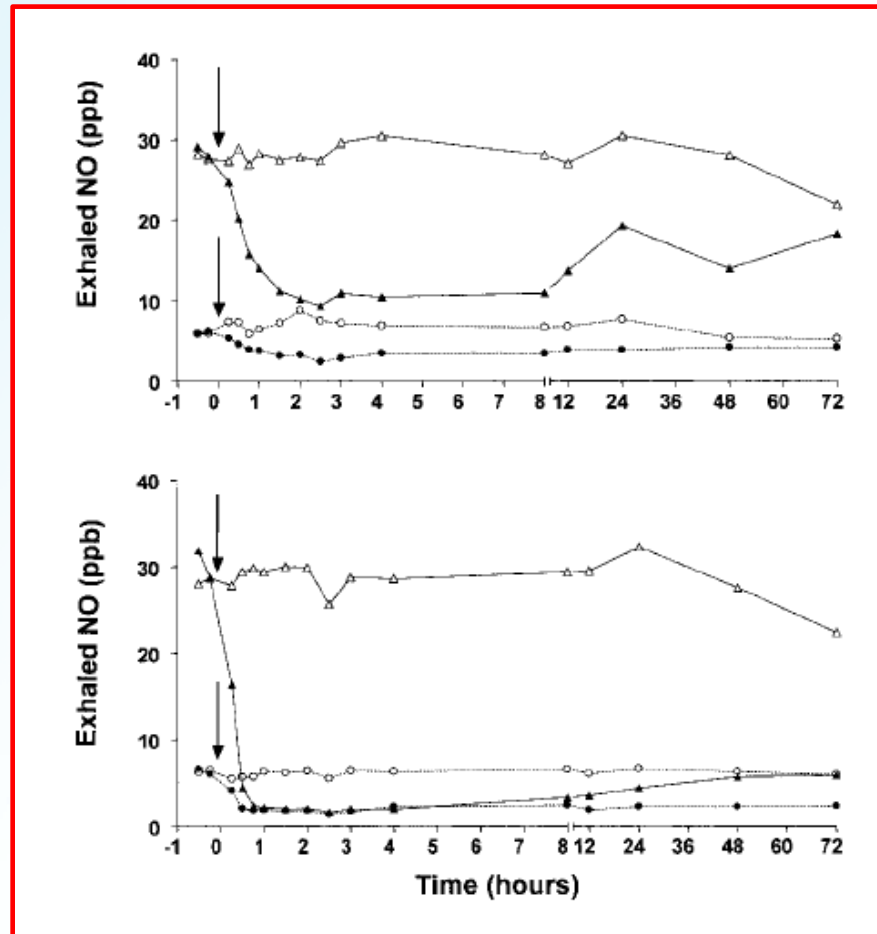


Exhaled NO and selective iNOS inhibitor (SC-51)

N=12 Asthma
vs
N=12 Controls

Triangle: Asthmatics
Circles: Controls
Closed: SC-51
Open: Placebo

Inhibitor effect in
15 minutes



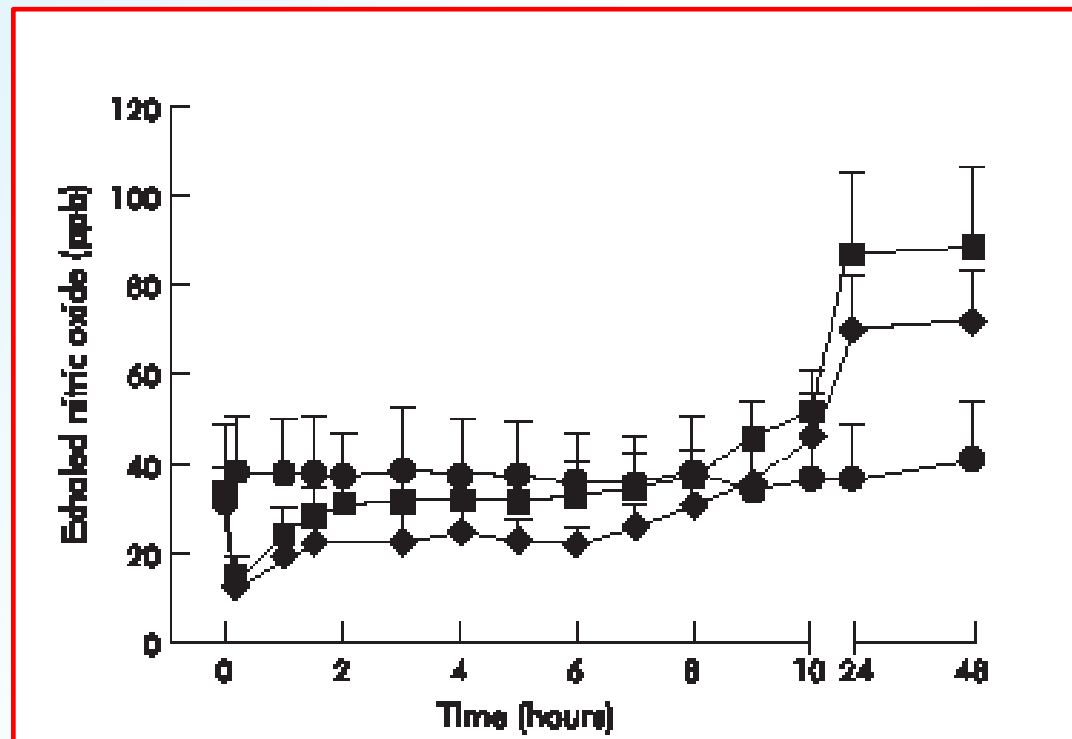
FeNO 250 ml/s

Oral dose of
SC-51: 20 mg

Oral dose of
SC-51: 200 mg

No effects on blood pressure and pulmonary function.

Exhaled NO after allergen in asthma

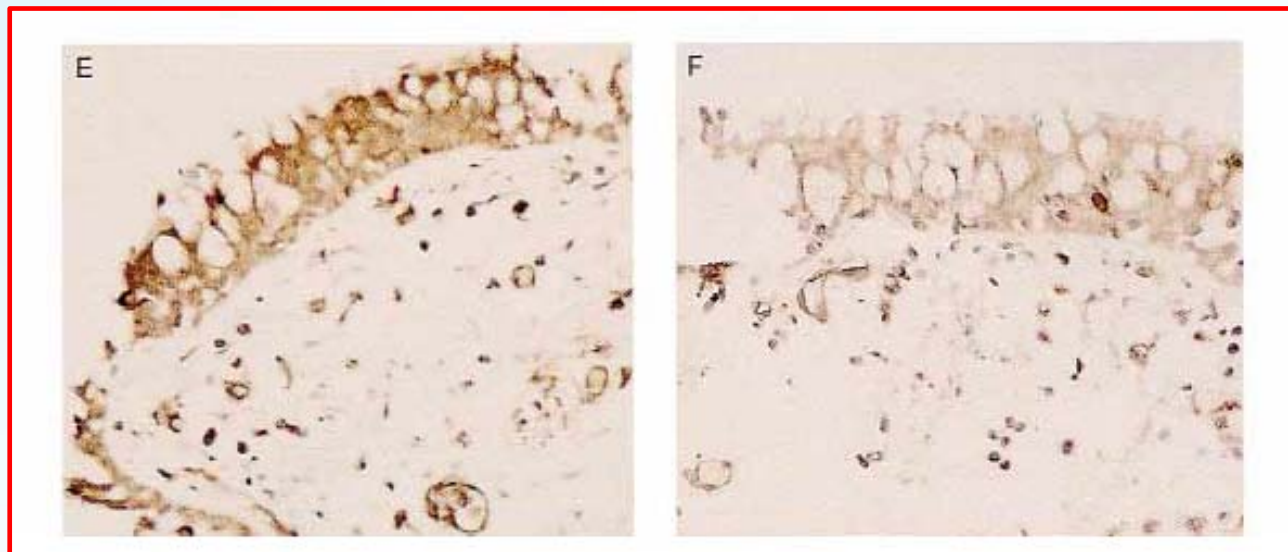


iNOS expression after allergen exposure in allergic asthma

iNOS EPITHELIAL EXPRESSION

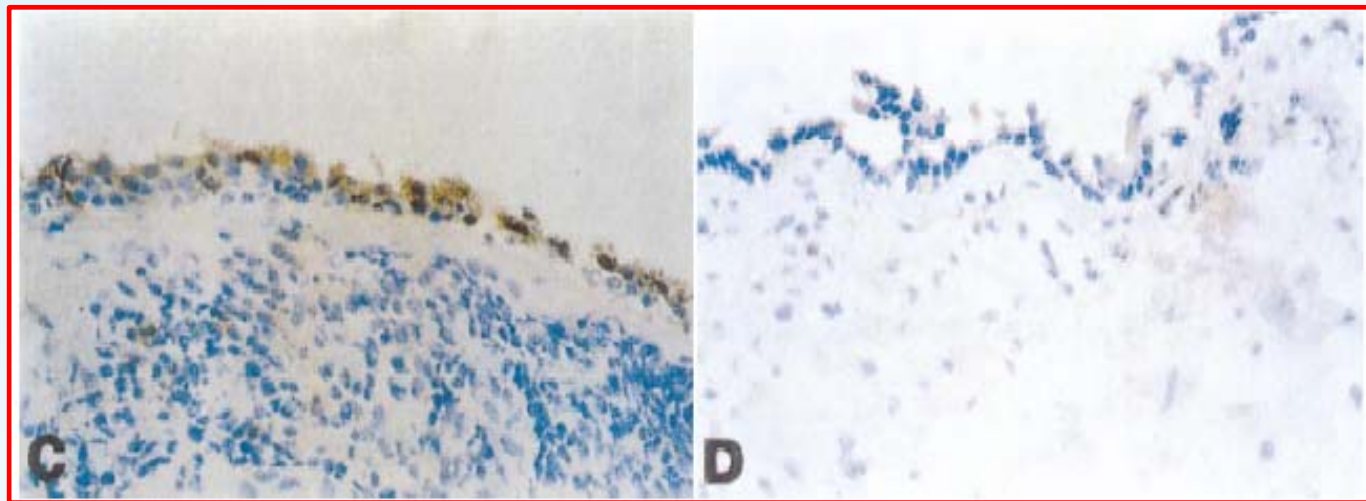
48 h after allergen exposure

48 h after diluent exposure



iNOS and Inhaled CS

Bronchial biopsies of an asthmatic patient



Inhalation of Placebo or Budesonide
(800 μg twice/day per 4 weeks)

Exhaled NO and Inhaled CS in Asthma

TABLE 2. Characteristics of study patients in the randomized trial^a

Patient no.	Budesonide					Placebo				
	NO (ppb)		FEV ₁ (% Predicted)		PC ₂₀	NO (ppb)		FEV ₁ (% predicted)		PC ₂₀
	Before	After	Before	After	After	Before	After	Before	After	After
1	9	4	79	84	0.25	5	10	93	88	12.02
2	76	35	74	93	15.99	30	25	91	99	1.99
3	65	19	100	120	3.28	55	53	116	116	0.25
4	18	10	97	102	7.94	6	—	100	97	1
5	30	8	97	100	7.94	15	33	98	97	0.25
6	32	40	101	100	3.98	28	25	91	91	0.25
7	65	17	84	89	0.5	90	65	85	86	0.125
8	45	34	86	87	0.07	33	20	84	88	1
9	67	25	97	113	1.5	67	60	110	110	0.75
10	18	12	100	105	2.93	16	11	98	100	1.3
Mean	42.5	20.4	91.5	99.3	4.44	34.5	33.6	96.8	97.2	1.96
(± SE)	7.7	3.9	3.1	3.6	1.57	8.8	6.9	3.3	3.1	1.27

^a Data for nitric oxide and FEV₁ are shown before and after Budesonide or placebo treatment.

Diagnosing Asthma

Comparisons between Exhaled Nitric Oxide Measurements and Conventional Tests

Andrew D. Smith, Jan O. Cowan, Sue Filsell, Chris McLachlan, Gabrielle Monti-Sheehan, Pamela Jackson, and D. Robin Taylor

N = 47 patients with symptoms suggestive asthma

TABLE 1. STUDY PLAN

	Visit 1		Visit 2		Visit 3
	Initial	2 wk	Initial	2 wk	
Clinical asthma assessment	X				
F _{ENO} measurement	X		X		X
Skin allergy test	X				
Spirometry	X		X		X
Bronchodilator reversibility	X				
Hypertonic saline challenge			X		X
Sputum induction			X		
Peak flow measurements		X		X	
Trial of oral prednisone				X	

Definition of abbreviation: F_{ENO} = exhaled nitric oxide.

Diagnosing Asthma

Comparisons between Exhaled Nitric Oxide Measurements and Conventional Tests

Andrew D. Smith, Jan O. Cowan, Sue Filsell, Chris McLachlan, Gabrielle Monti-Sheehan, Pamela Jackson, and D. Robin Taylor

TABLE 2. CHARACTERISTICS OF THE STUDY PARTICIPANTS BY ASTHMA DIAGNOSIS

	Patients with Asthma (n = 17)	Subjects without Asthma (n = 30)
Mean age, yr	41.6 (range, 9–72)	31.8 (range, 9–64)
Smoking history (mean pack-years)	14 nonsmokers, 3 ex-smokers (11.3)	28 nonsmokers, 2 ex-smokers (12.5)
Sex	8 female (47%), 9 male	19 female (63%), 11 male
FEV ₁ , L	2.71 (1.16)	3.18 (0.82)
FEV ₁ , % predicted	90.5 (18.4)	110.0 (13.5)*
FEV ₁ /FVC ratio, %	77.3 (11.9)	84.9 (6.0) [†]
Bronchodilator reversibility, %	11.6 (9.6)	4.2 (2.5)*
Peak flow variation, %	8.3 (5.4)	5.5 (2.5)
F _{ENO} , ppb (50 ml/second)	52.0 (34.0)	15.7 (12.9) [‡]
Sputum eosinophils, %	13.8 (10.0)	1.8 (5.0) [‡]
Sputum neutrophils, %	20.5 (16.7)	35.5 (21.6) [‡]

For definition of abbreviation see Table 1.

All values are reported as means (SD) unless otherwise stated. Bronchodilator reversibility is the percent increase in FEV₁ 15 minutes after inhalation of albuterol. Peak flow variability is expressed as the amplitude percent mean calculated over 7 days.

* p < 0.01, for between-group comparisons.

[†] p < 0.05, for between-group comparisons.

[‡] p < 0.001, for between-group comparisons.

Diagnosing Asthma

Comparisons between Exhaled Nitric Oxide Measurements and Conventional Tests

Andrew D. Smith, Jan O. Cowan, Sue Filsell, Chris McLachlan, Gabrielle Monti-Sheehan, Pamela Jackson, and D. Robin Taylor

TABLE 3. SENSITIVITY, SPECIFICITY, AND POSITIVE AND NEGATIVE PREDICTIVE VALUES FOR EACH OF THE DIAGNOSTIC TESTS FOR ASTHMA

	Asthma (n = 17)		Nonasthma (n = 30)		Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
	Yes	No	Yes	No				
Bronchodilator reversibility > 12%	7	10	0	30	—	—	—	—
Bronchial hyperresponsiveness < 20 ml	15	2	0	30	—	—	—	—
Peak flow variation > 20%	0	17	0	29*	0	100	NA	70
Peak flow improvement with steroid > 15%	4	13	0	29*	24	100	100	69
FEV ₁ < 80% predicted	5	12	0	30	29	100	100	71
FEV ₁ < 90% predicted	6	11	2	28	35	93	75	72
FEV ₁ /FVC ratio < 70%	6	11	0	30	35	100	100	73
FEV ₁ /FVC ratio < 80%	8	9	6	24	47	80	57	73
FEV ₁ improvement with steroid > 15%	2	15	0	29*	12	100	100	66
Sputum eosinophils > 3%	12	2*	3	23*	86	88	80	92
F _{ENO} > 20 ppb	14	2†	6	22†	88	79	70	92

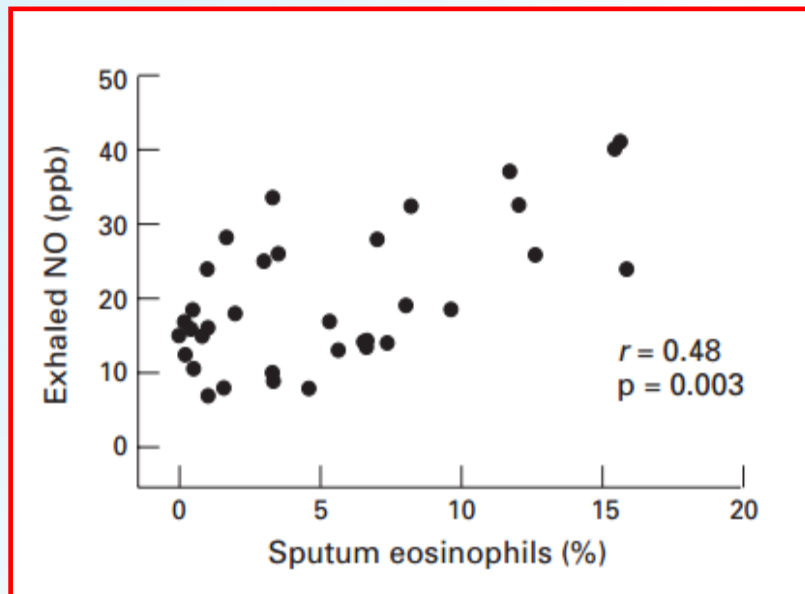
Figures for bronchodilator reversibility and bronchial hyperresponsiveness to hypertonic saline are not given because both these parameters were used to diagnose asthma.

* Patient unable or unwilling to complete procedure.

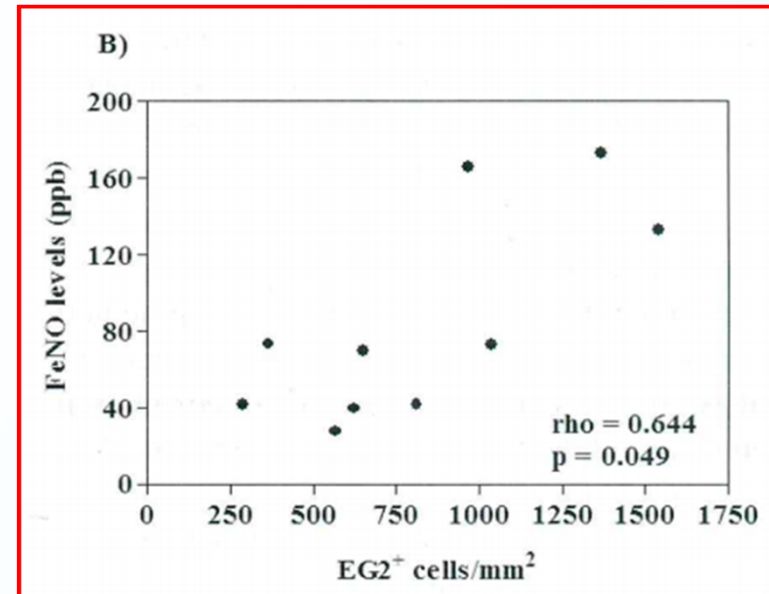
† Technical difficulties prevented completion of exhaled nitric oxide (F_{ENO}) measurements at 50 ml/second.

FeNO and sputum/bronchial EOS in atopic asthma

35 stable atopic asthmatics
not on ICS

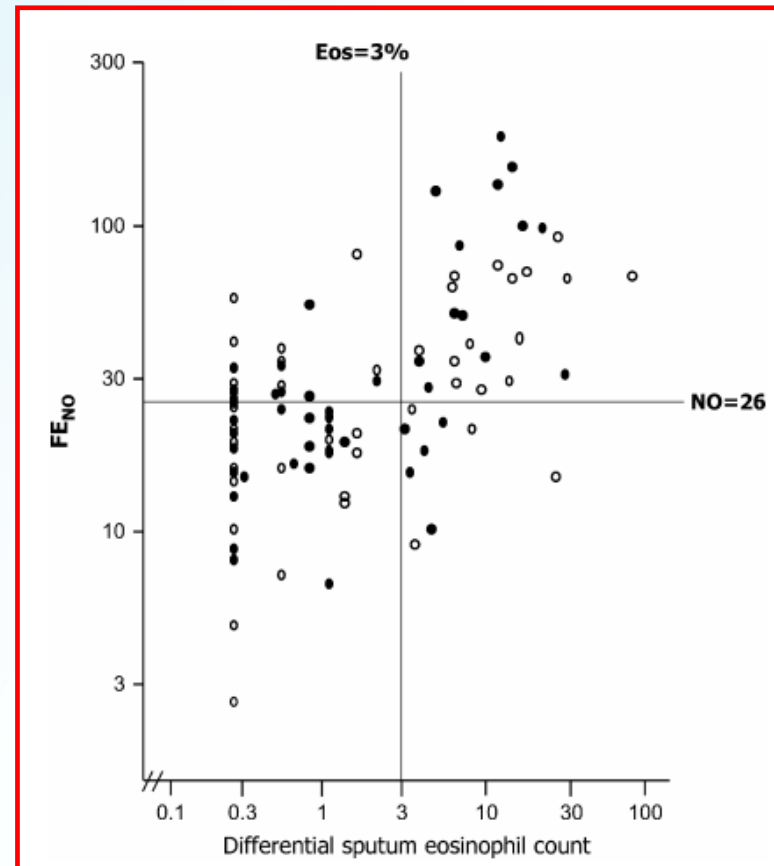


10 atopic mild asthmatics 48 hrs
after allergen challenge

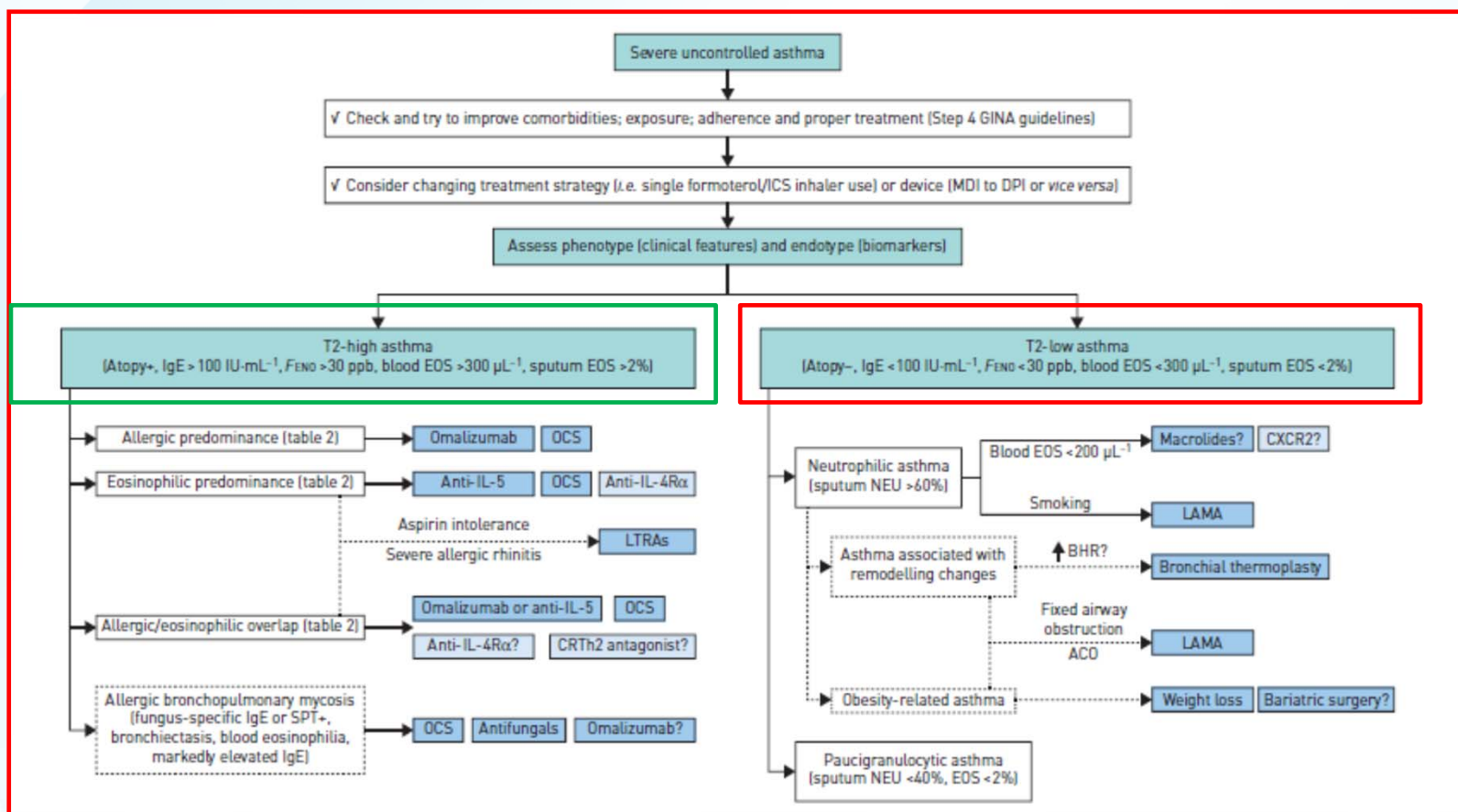


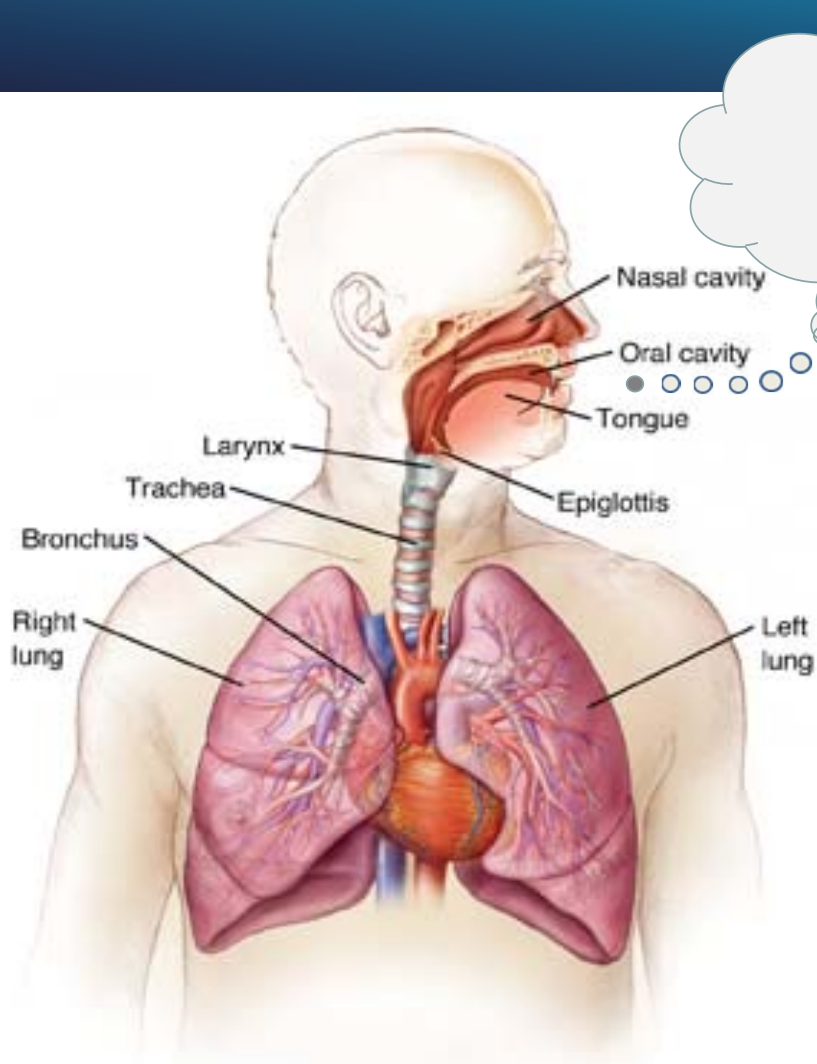
FeNO and asthma diagnosis: cut-off 26 ppb vs sputum eosinophils >3%

Negative Predictive
Value = 85%



Stratification in T2-high and T2-low Asthma





FeNO

↑ FeNO:

Children developing asthma

Allergic asthma

Eosinophilic refractory asthma

Non atopic severe asthma

Poorly controlled/exacerbated asthma

ASA-Intolerant asthma

↓ FeNO:

Non atopic asthma

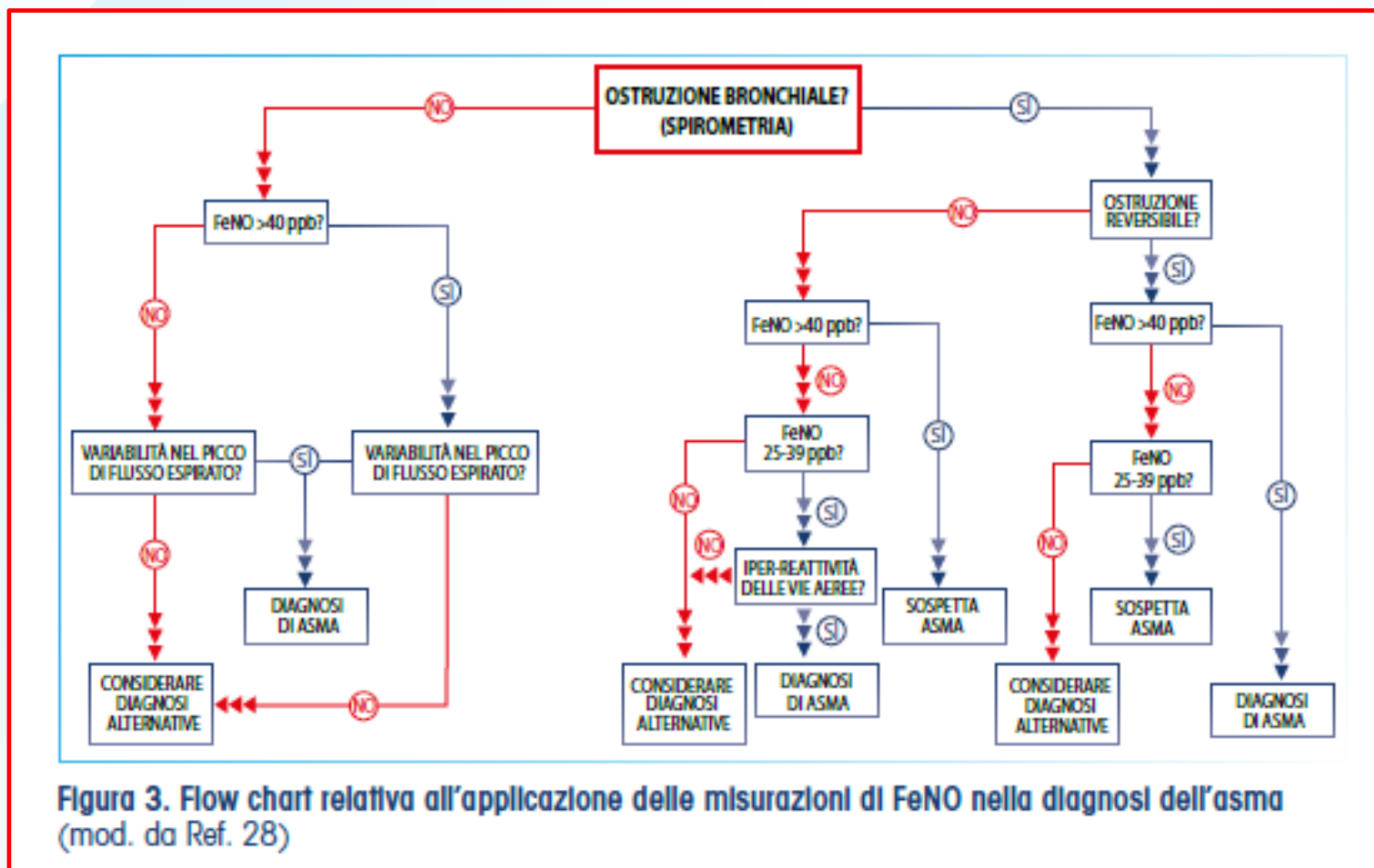
Smoking asthma

Obese asthma

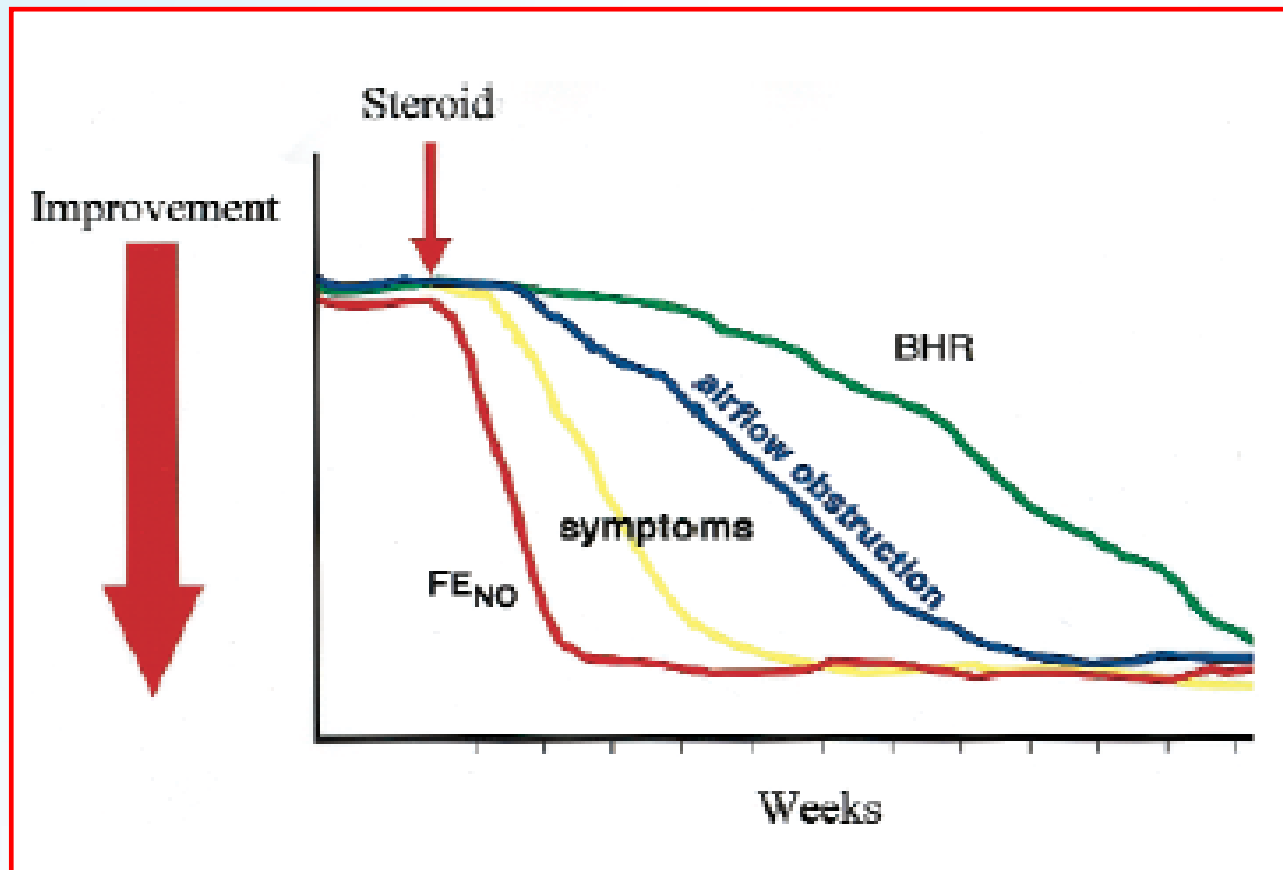
Neutrophilic severe asthma

Neonatal respiratory disorders with asthma

FeNO e diagnosi di asma



FeNO and ICS response



FeNO as predictor of steroid response

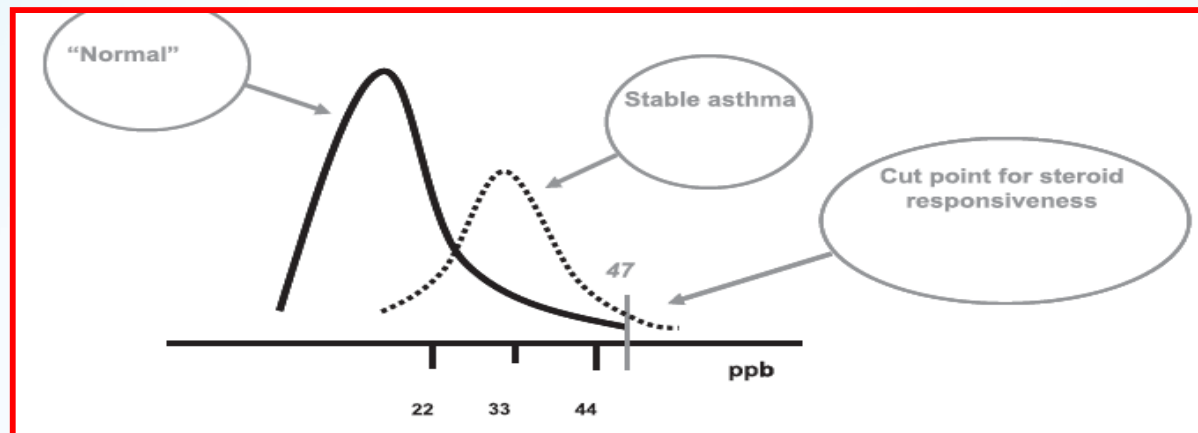
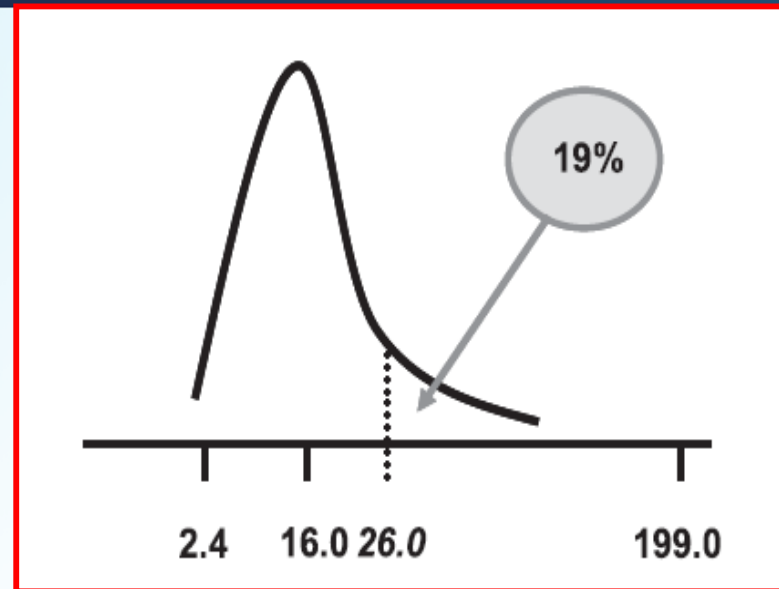
52 subjects with undiagnosed respiratory symptoms

TABLE 4. SENSITIVITIES, SPECIFICITIES, AND POSITIVE AND NEGATIVE PREDICTIVE VALUES FOR EACH OF THE BASELINE MEASUREMENTS ("PREDICTORS") USED TO PREDICT "STEROID RESPONSE"

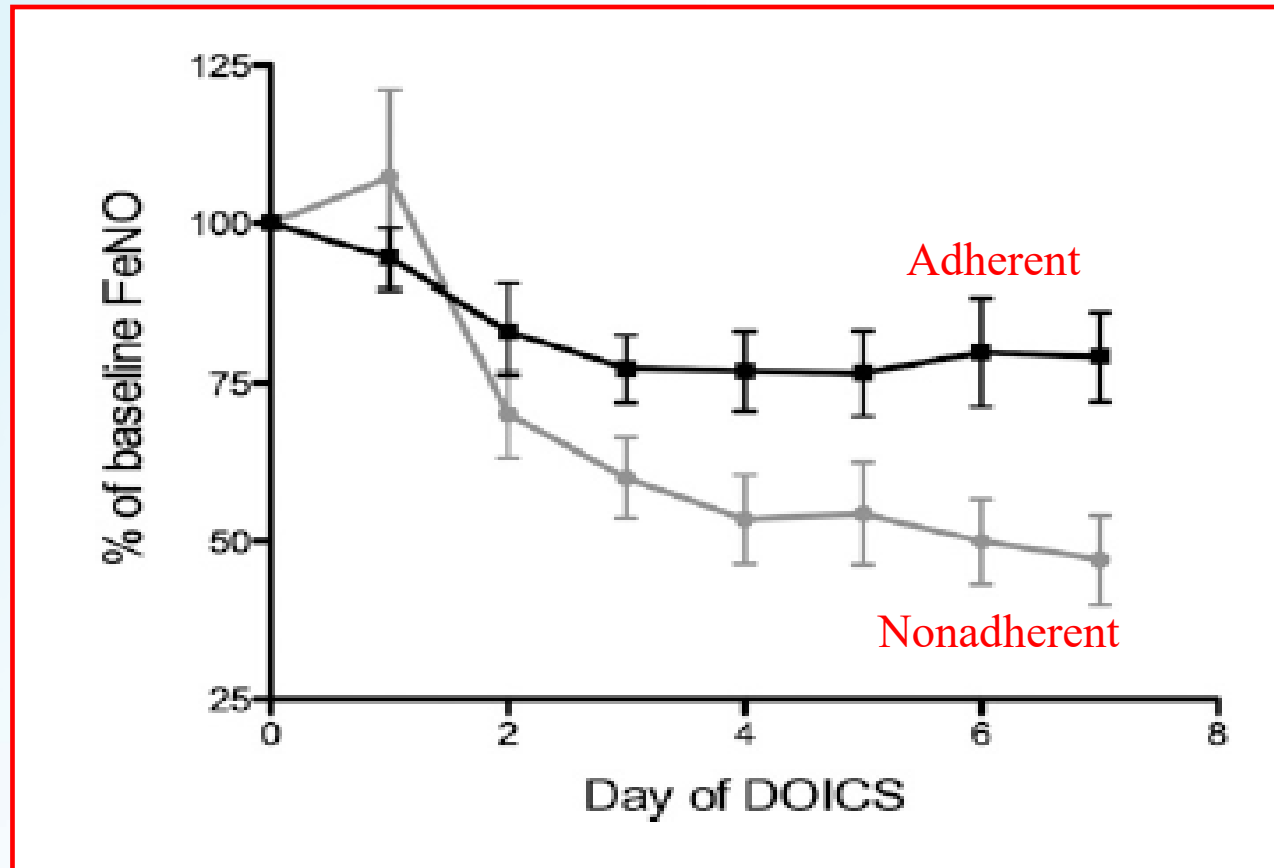
Steroid Response Endpoint	Predictors	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
FEV ₁ , increase of ≥ 12%	BD reversibility > 12%*	8	95	33	78
	FEV ₁ < 80% predicted [†]	17	88	29	78
	PD ₂₀ methacholine < 8 μmol [‡]	58	69	37	84
	PEFR variation > 20% [§]	0	97	NA	76
	FeNO > 47 ppb	67	78	47	89
Mean morning peak flow, increase of ≥ 15%	BD reversibility > 12%	18	98	67	82
	FEV ₁ < 80% predicted	36	93	57	84
	PD ₂₀ methacholine < 8 μmol	55	68	32	84
	PEFR variation > 20%	9	100	100	80
	FeNO > 47 ppb	82	81	53	94
Composite symptom score, reduction of ≥ 1 point	BD reversibility > 12%	7	95	33	74
	FEV ₁ < 80% predicted	7	84	14	71
	PD ₂₀ methacholine < 8 μmol	29	60	21	69
	PEFR variation > 20%	7	100	100	74
	FeNO > 47 ppb	43	71	35	77
PC ₂₀ AMP, increase of 2 doubling doses or more	BD reversibility > 12%	18	100	100	71
	FEV ₁ < 80% predicted	24	91	57	71
	PD ₂₀ methacholine < 8 μmol	82	85	74	90
	PEFR variation > 20%	6	100	100	69
	FeNO > 47 ppb	82	91	82	91

Placebo vs Fluticasone 500 mcg/day

F_ENO: cut points



FeNO suppression test in non-adherent difficult asthma

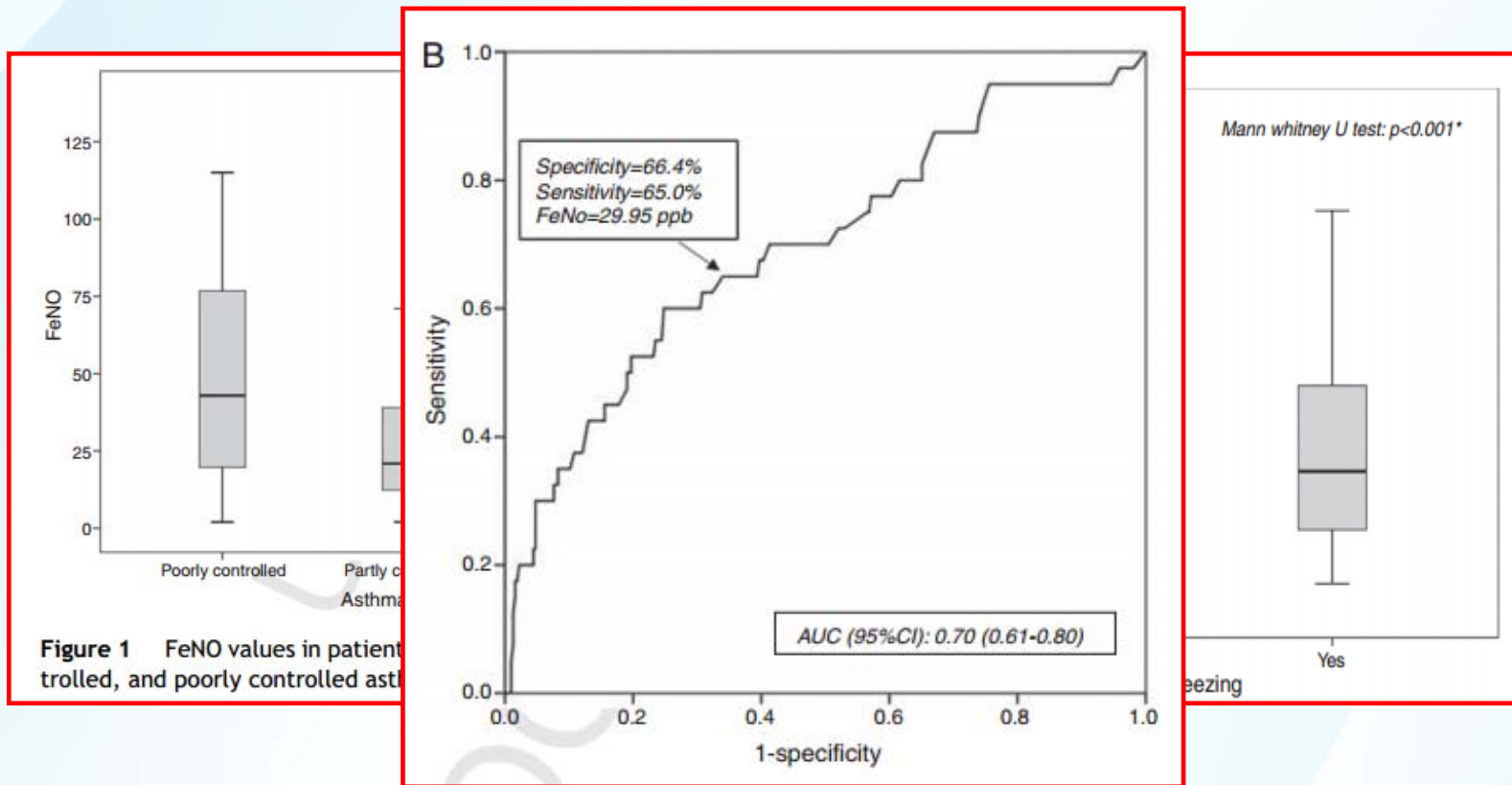


Directly observed inhaled steroid treatment for 7 days with daily FeNO measurement.

ICS: Budesonide 1.600 mcg/day

McNicholl et al. AJRCCM 2012;186:1102

FeNO and asthma control: a real life study



N = 363 patients

FeNO and future risk in asthma

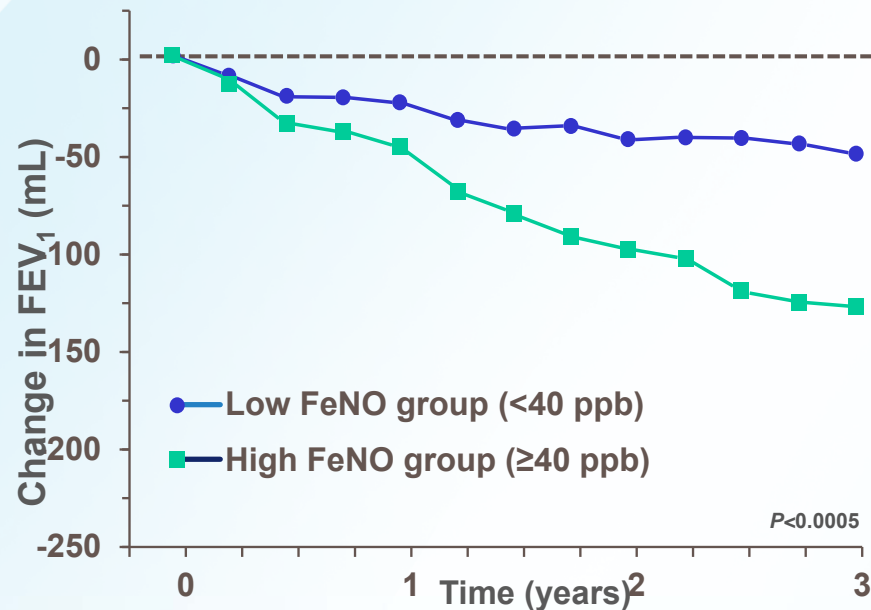
TABLE II. Unadjusted and adjusted* RR (95% CI) by Poisson regression with a robust error variance for the listed outcomes during the follow-up year

Outcomes during follow-up year	FeNO % predicted		P value
	≤300% (n = 199)	>300% (n = 105)	
Patients with ≥7 SABA canisters dispensed			
Unadjusted	1.00	2.46 (1.58-3.83)	<.001
Adjusted	1.00	2.26 (1.46-3.50)	<.001
Patients with ≥2 OCS courses with asthma exacerbations			
Unadjusted	1.00	3.41 (1.17-9.92)	.024
Adjusted	1.00	3.26 (1.17-9.10)	.024

Significant differences noted in bold.

*Variables adjusted in multivariate analyses were ACT scores, categorized by its 3 control categories: >19 (controlled), 16-19 (not well controlled), and <16 (very poorly controlled), and FEV₁ % predicted, categorized by >80% predicted (controlled) and ≤80% predicted (uncontrolled).

FeNO Assists in Identifying the Risk of Lung Function Decline



Persistently high FeNO (≥40 ppb) was associated with greater decline in lung function...

...along with greater decline in bronchodilator response over time

Figure from Matsunaga K, et al. *Allergol Int.* 2016;65(3):266-271. [https://www.allergologyinternational.com/article/S1323-8930\(15\)00236-1/fulltext](https://www.allergologyinternational.com/article/S1323-8930(15)00236-1/fulltext). Copyright © 2016 Japanese Society of Allergology. CC BY-NC-ND license: <http://creativecommons.org/licenses/by-nc-nd/4.0/>. FeNO=fractional exhaled nitric oxide; FEV₁=forced expiratory volume in 1 second. Matsunaga K, et al. *Allergol Int.* 2016;65(3):266-271.

Data from a 3-year prospective cohort study in 128 patients

FeNO e gestione clinica di asma

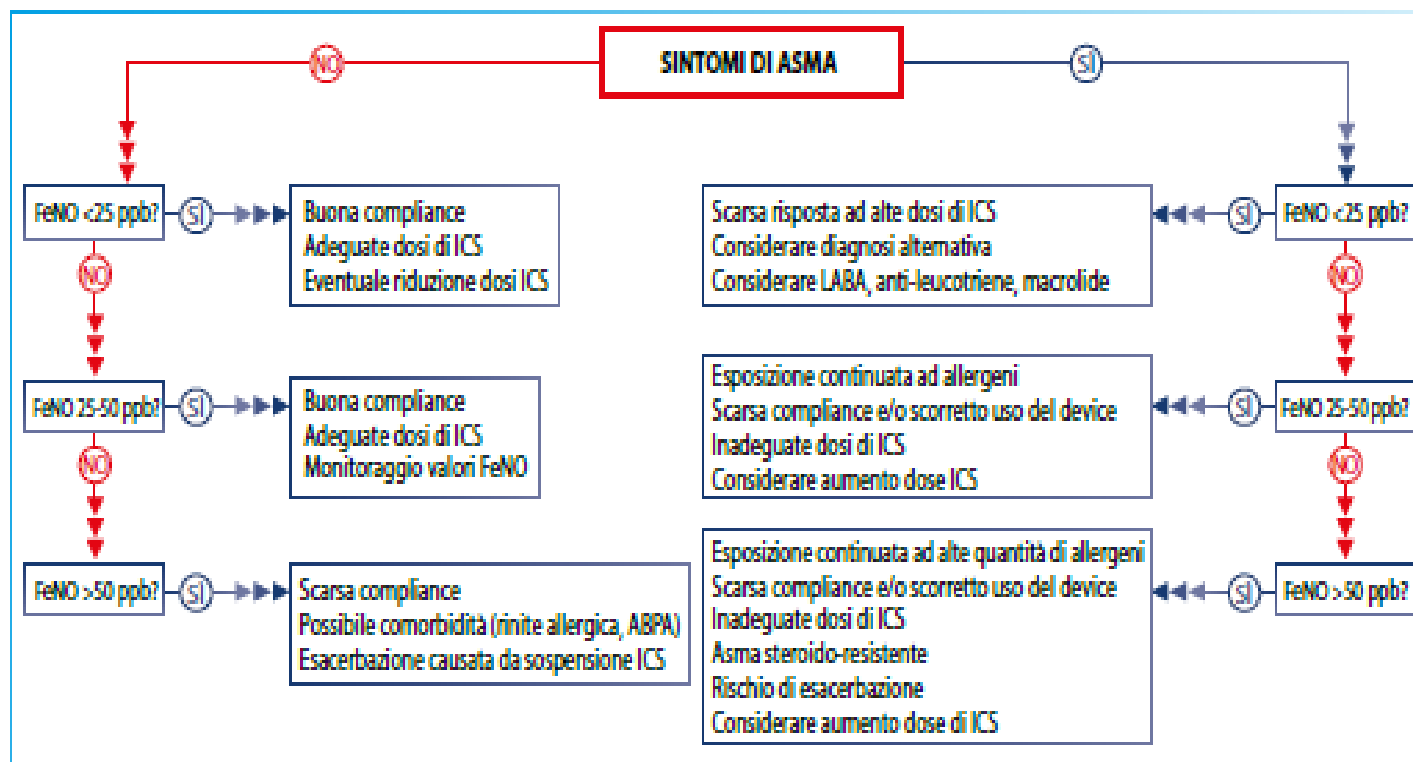
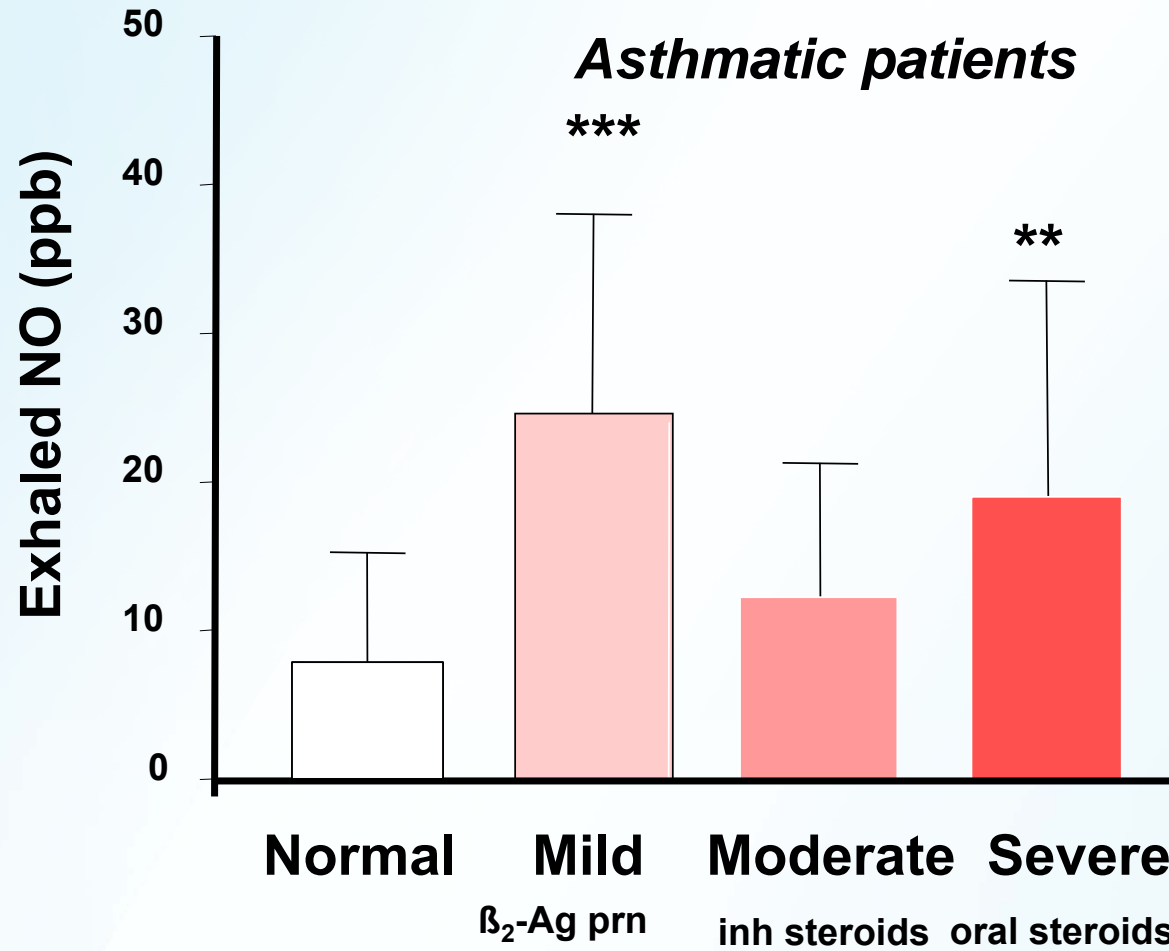
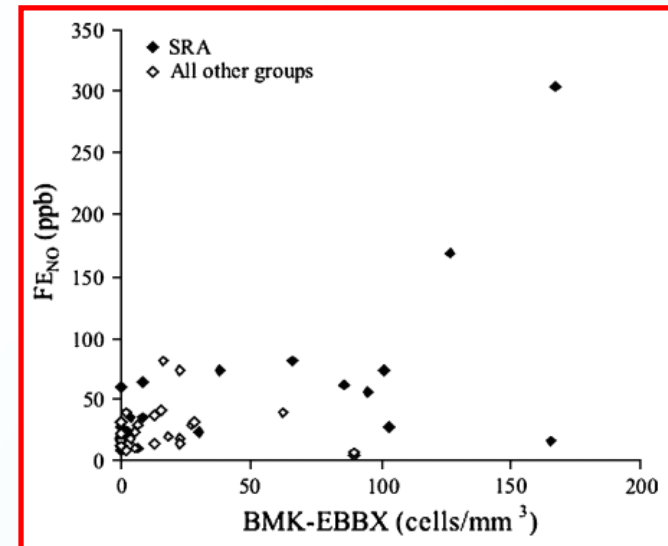
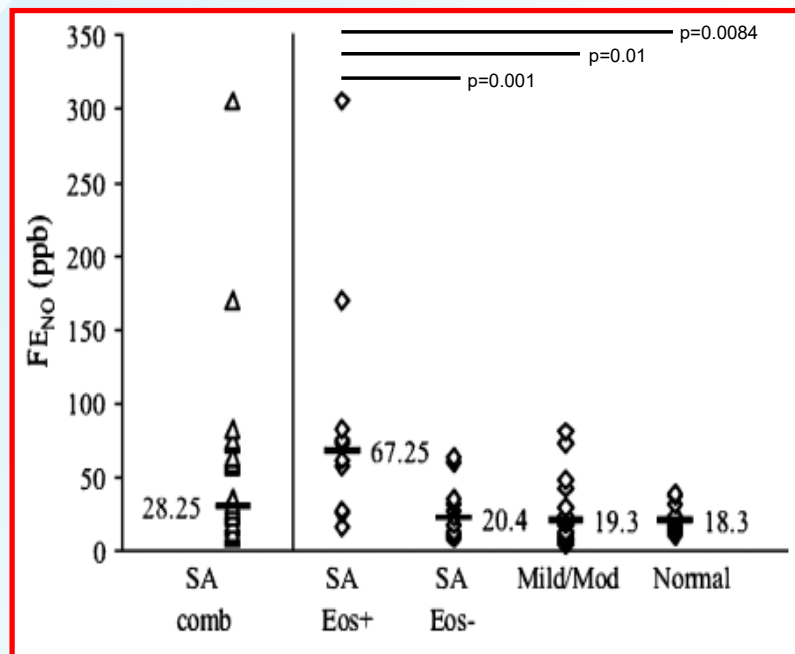


Figura 4. Flow chart relativa all'applicazione delle misurazioni di FeNO nella gestione clinica dell'asma (mod. da Ref. 15)

ASTHMA SEVERITY AND EXHALED NO



FeNO identifies the eosinophilic phenotype in severe refractory asthma

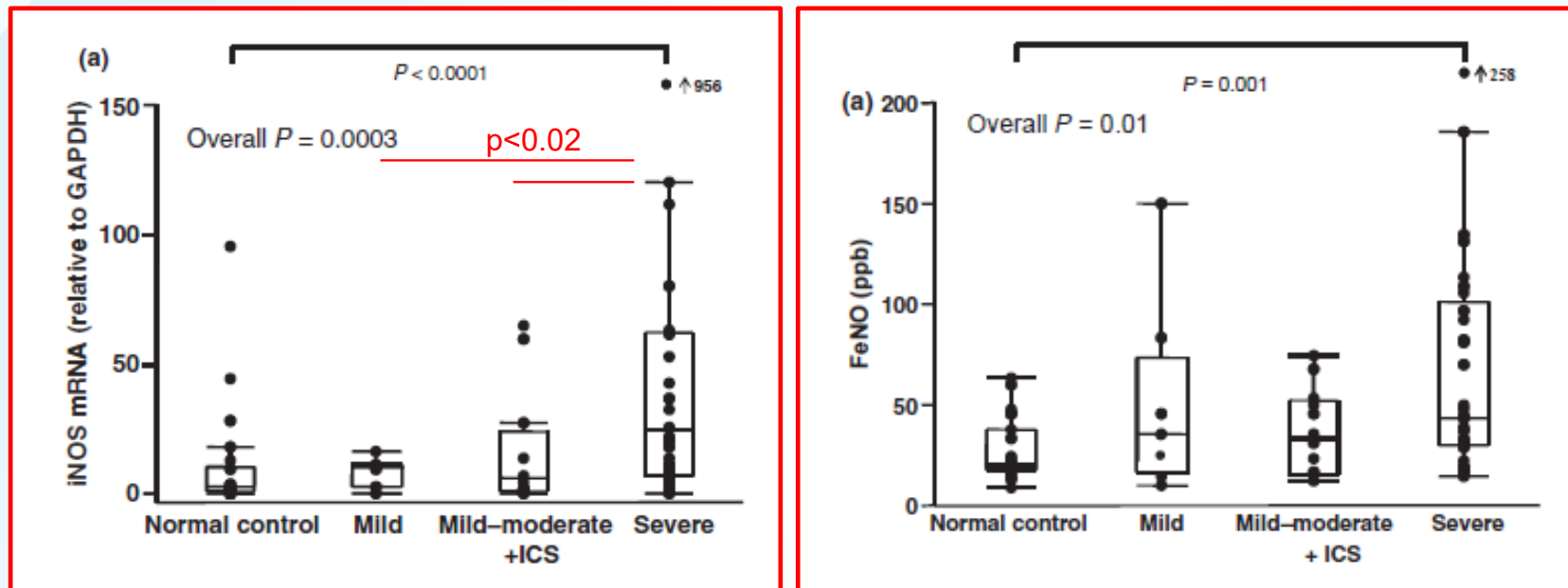


SRA: oral prednisone 10 mg/day or high ICS dose
+ FEV1<70%pred + 2 urgent care visit in previous year

BMK: eosinophil major basic protein
EBBX: endobronchial biopsy

iNOS and FeNO in Severe Asthma

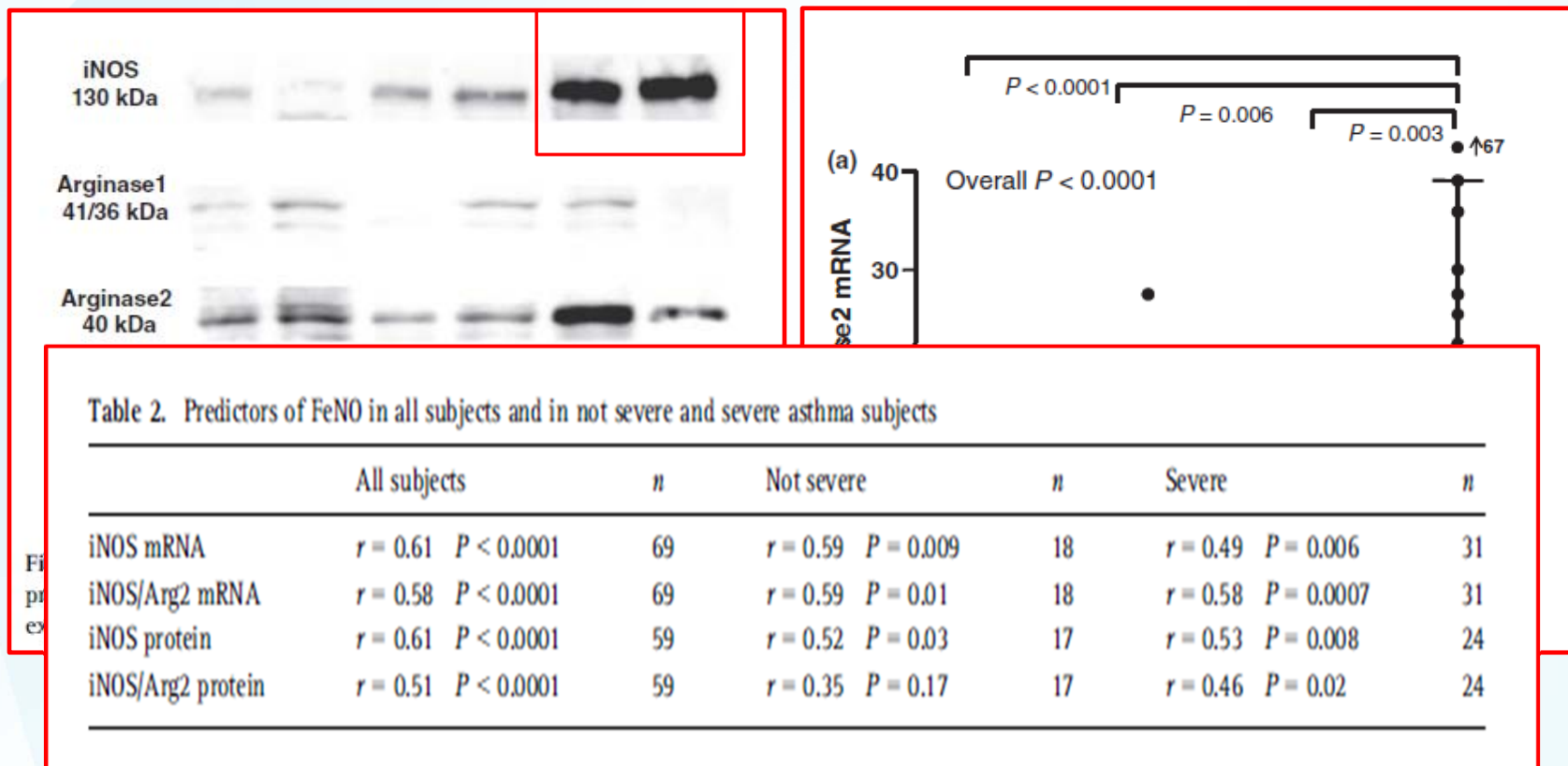
Bronchoscopy: bronchial epithelial brushings



Reverse transcription RT-PCR

iNOS and FeNO in Severe Asthma

Bronchoscopy: bronchial epithelial brushings



Use of Exhaled Nitric Oxide Measurement to Identify a Reactive, at-Risk Phenotype among Patients with Asthma

Raed A. Dweik¹⁻³, Ronald L. Sorkness³, Sally Wenzel³, Jeffrey Hammel¹, Douglas Curran-Everett³, Suzy A. A. Comhair^{1,3}, Eugene Bleeker³, William Busse³, William J. Calhoun³, Mario Castro³, Kian Fan Chung³, Elliot Israel³, Nizar Jarjour³, Wendy Moore³, Stephen Peters³, Gerald Teague³, Benjamin Gaston³, and Serpil C. Erzurum¹⁻³; for the National Heart, Lung, and Blood Institute Severe Asthma Research Program*
Am J Respir Crit Care Med Vol 181. pp 1033–1041, 2010

TABLE 3. PULMONARY FUNCTION AND EXHALED NITRIC OXIDE LEVEL BY SEVERITY

Characteristic	Severe Asthma, Low F _{ENO}		Nonsevere Asthma, Low F _{ENO}		Severe Asthma, High F _{ENO}		Nonsevere Asthma, High F _{ENO}		Low F _{ENO} : Severe vs. Nonsevere Asthma	High F _{ENO} : Severe vs. Nonsevere Asthma	Severe Asthma: Low vs. High F _{ENO}	Nonsevere Asthma: Low vs. High F _{ENO}
	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n				
Baseline FVC, % predicted	75 ± 18	105	92 ± 15	162	75 ± 21	70	95 ± 14	109	<0.001	<0.001	0.97	0.13
Maximal FVC, % predicted	88 ± 17	101	97 ± 14	153	95 ± 18	67	103 ± 12	103	<0.001	<0.001	0.004	<0.001
Baseline FEV ₁ , % predicted	60 ± 19	105	83 ± 16	162	56 ± 22	70	83 ± 17	109	<0.001	<0.001	0.24	0.76
Maximal FEV ₁ , % predicted	74 ± 20	101	91 ± 15	153	80 ± 19	67	96 ± 15	103	<0.001	<0.001	0.009	0.04
FEV ₁ /FVC ratio, % predicted	79 ± 15	105	90 ± 11	162	74 ± 14	70	86 ± 12	109	<0.001	<0.001	0.011	0.03
Maximal FEV ₁ reversal, %	18 ± 23	101	11 ± 9	153	23 ± 19	67	17 ± 15	103				
Median (IQR)*	14 (6–22)	101	8 (5–14)	153	21 (9–29)	67	13 (7–22)	103	0.002	0.01	0.005	<0.001
PC ₂₀	3.9 ± 6	53	4.4 ± 6	149	1.5 ± 3	27	1.7 ± 3	96				
Median (IQR)*	1 (0.2–4.5)	53	2 (0.6–5)	149	0.6 (0.2–1.7)	27	0.7 (0.3–1.6)	96	0.10	0.40	0.01	<0.001
TLC, % predicted	107 ± 13	41	104 ± 12	31	117 ± 17	21	112 ± 10	22	0.55	0.28	0.006	0.05
FRC, % predicted	103 ± 27	38	96 ± 21	31	124 ± 34	18	115 ± 26	22	0.40	0.36	0.005	0.01
FRC/TLC, % predicted	96 ± 21	38	92 ± 14	31	104 ± 16	18	102 ± 17	22	0.43	0.84	0.10	0.03
RV, % predicted	143 ± 44	41	109 ± 34	31	176 ± 58	21	131 ± 47	22	0.004	0.001	0.005	0.11
RV/TLC, % predicted	128 ± 30	41	99 ± 21	31	141 ± 41	20	111 ± 33	22	<0.001	0.002	0.12	0.21
ER in past 12 mo	38%	105	12%	161	53%	70	18%	109	<0.001	<0.001	0.05	0.14
Ever had an ICU admission due to asthma	34%	105	5%	162	44%	70	12%	108	<0.001	<0.001	0.26	0.04
BMI	32 ± 8	103	30 ± 9	162	30 ± 8	66	28 ± 7	109	0.08	0.13	0.08	0.016

Definition of abbreviations: BMI = body mass index; ER = emergency room; ICU = intensive care unit; IQR = interquartile range; RV = reserve volume; TLC = total lung capacity.

High F_{ENO} defined as >35 ppb, and low F_{ENO} as ≤35 ppb.

* Wilcoxon rank sum *P* values reported rather than those based on contrasts from analysis of variance.

FeNO and Asthma Management: Meta-analysis (16 studies on FeNO-based management)

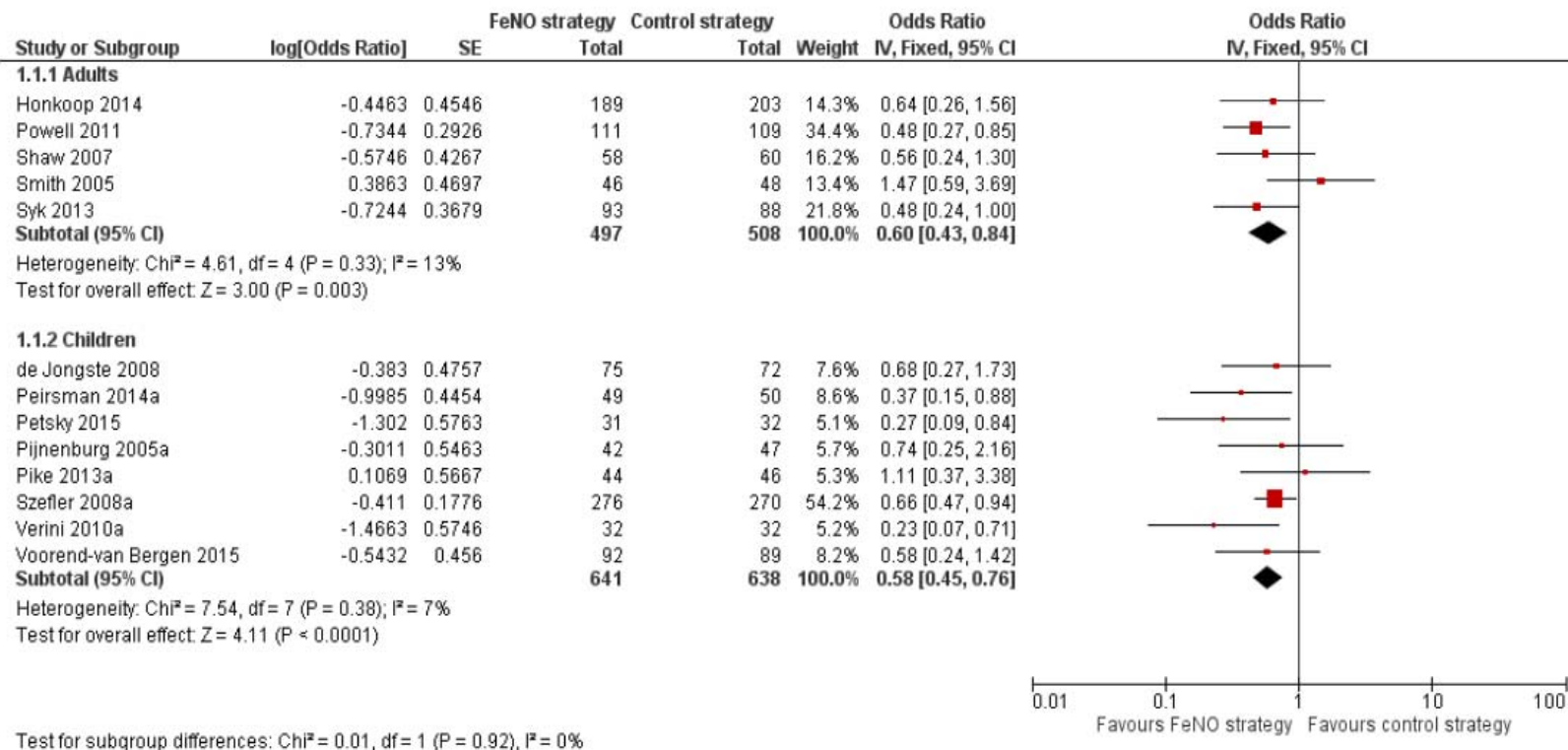


Figure 2 Number of subjects who had ≥ 1 exacerbation over the study period (FeNO).

Association between exhaled nitric oxide and nasal polyposis in severe asthma

Mauro Maniscalco^{a,*,1}, Cecilia Calabrese^{b,1}, Maria D'Amato^b, Pietro Guida^a, Antonio Molino^b, Maria Aliani^c, Renato De Tullio^c, Mariapia Foschino Barbaro^d, Fabio Luigi Massimo Ricciardolo^e, Giovanna Elisiana Carpagnano^d

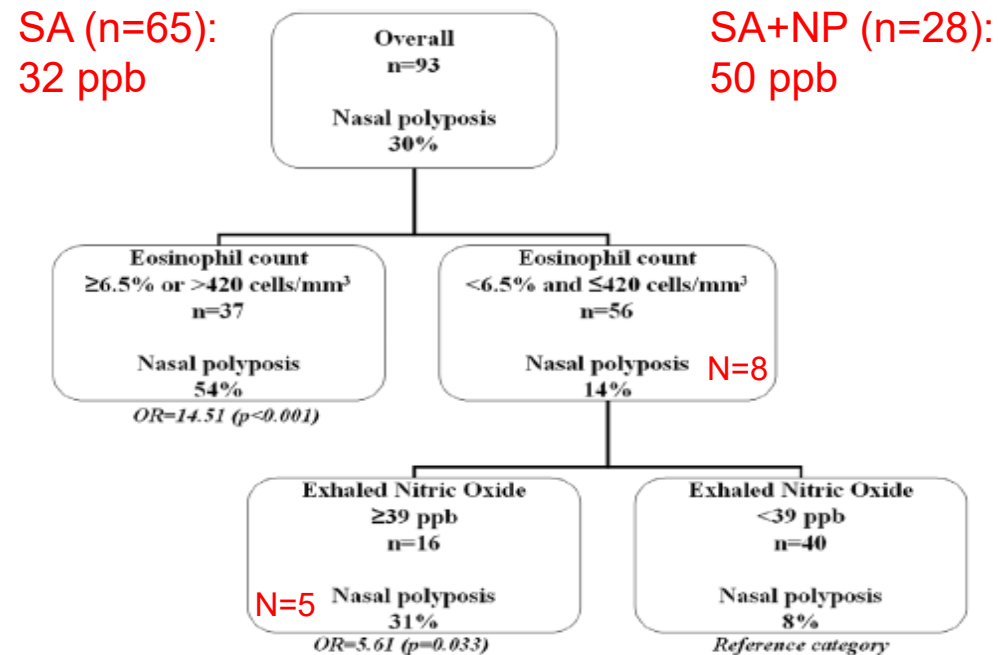
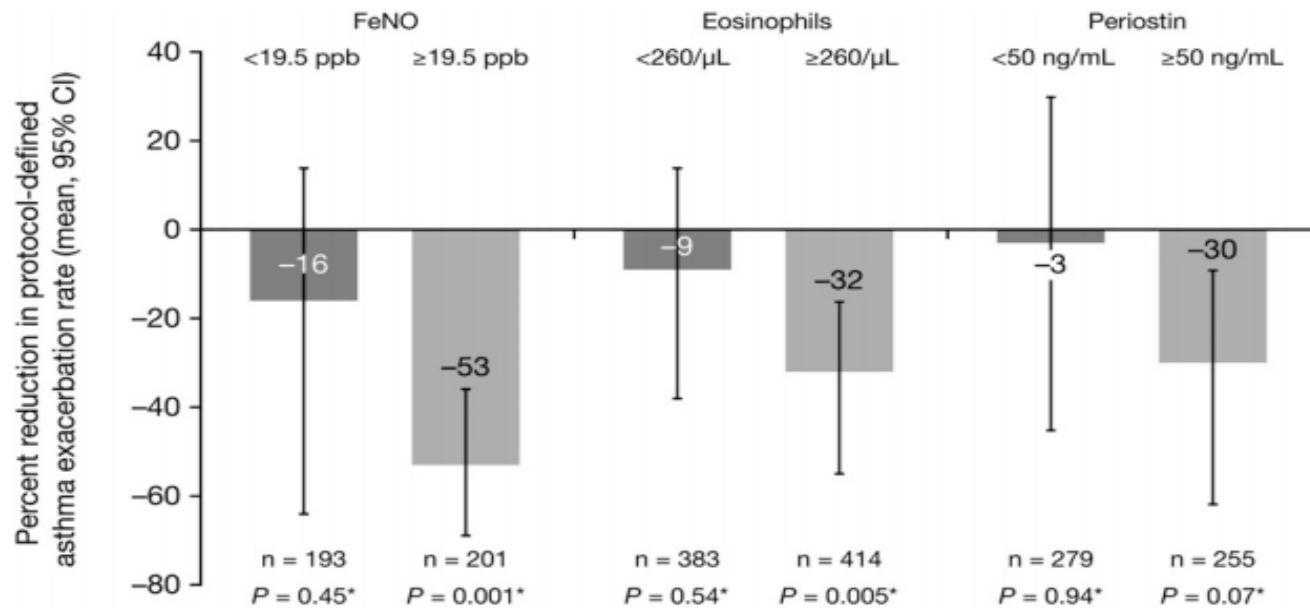


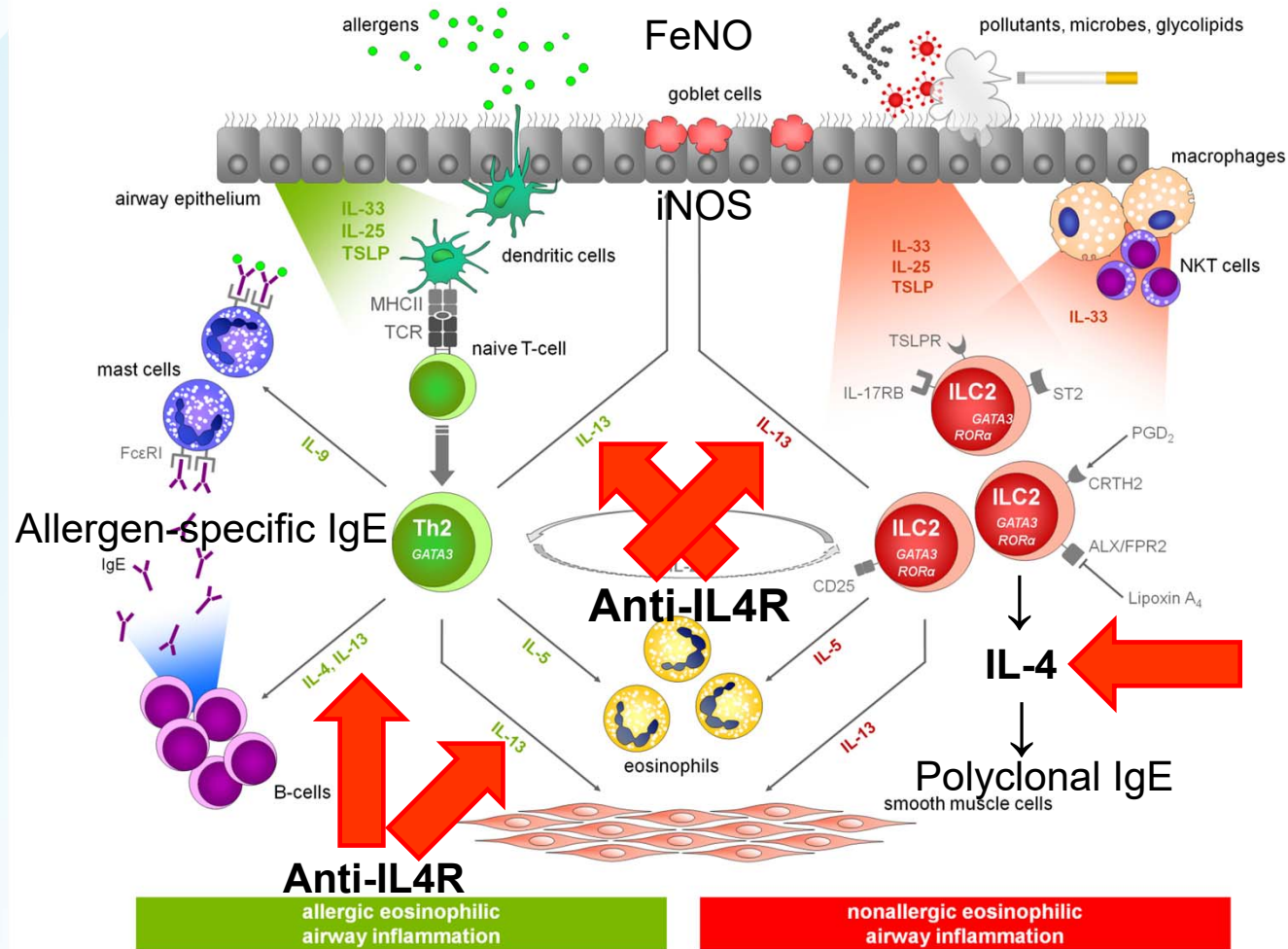
Fig. 1. Recursive partitioning analysis based on progressive binary splits to identify patients with nasal polyposis by eosinophil count and FeNO. Eosinophil count was the best predictor of nasal polyposis. In the subgroup with low eosinophil count, patients with high FeNO had a significant higher occurrence of nasal polyposis.

Biomarkers as predictors for omalizumab



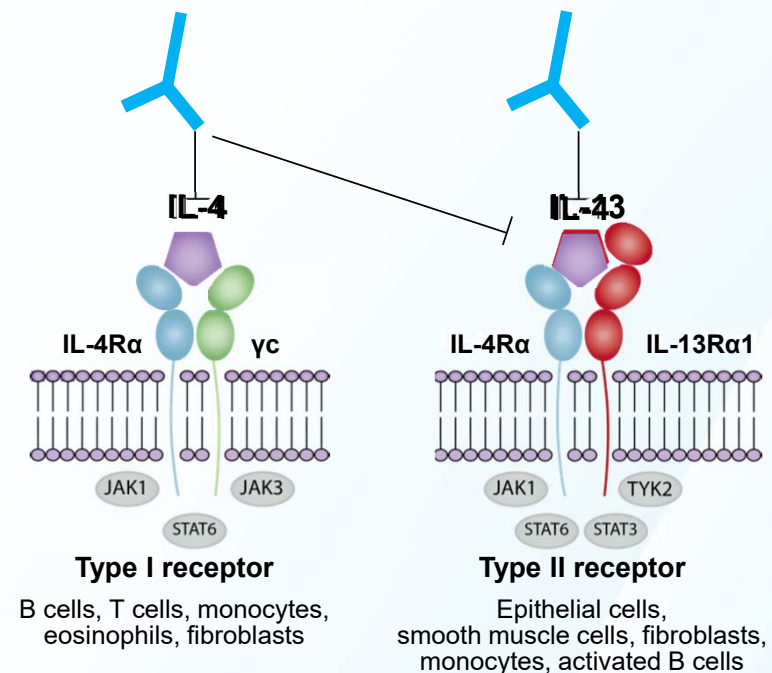
	Exacerbation rates					
	Low FeNO at baseline	High FeNO at baseline	Low eosinophils at baseline	High eosinophils at baseline	Low periostin at baseline	High periostin at baseline
Omalizumab	0.60	0.50	0.65	0.70	0.73	0.66
Placebo	0.71	1.07	0.72	1.03	0.72	0.93

Anti-IL4 receptor (IL4R) monoclonal antibody dupilumab

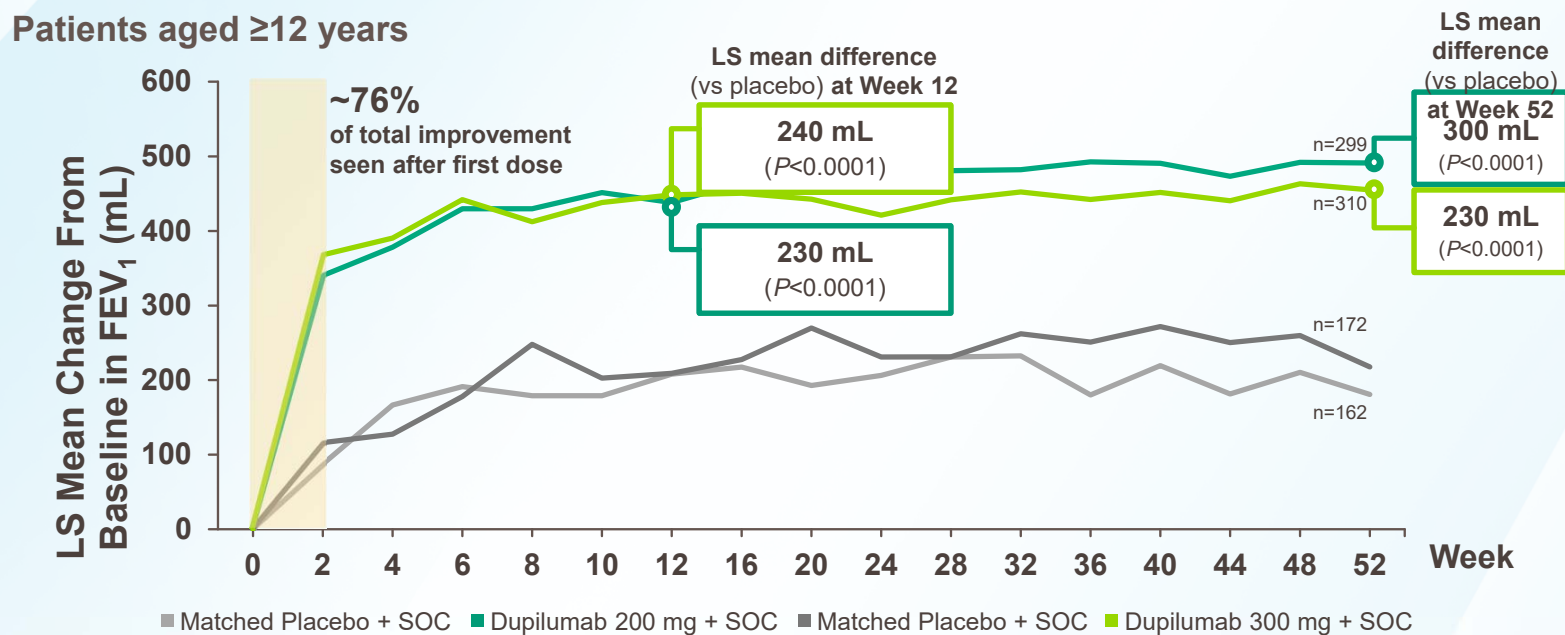


Anti-IL4R α monoclonal antibody dupilumab: mechanism of action

- **Dupilumab** is a fully human IL-4R α monoclonal antibody inhibiting **IL-4** and **IL-13** signaling pathways, key drivers of Type 2 inflammation.
- **Dupilumab** is approved for the treatment of adults with moderate-to-severe atopic dermatitis; and has also shown efficacy in patients with other Type 2 inflammatory diseases including nasal polyposis with chronic rhinosinusitis.



Dupilumab Led to Significant Improvement in Lung Function in Patients With FeNO Levels ≥ 25 ppb¹



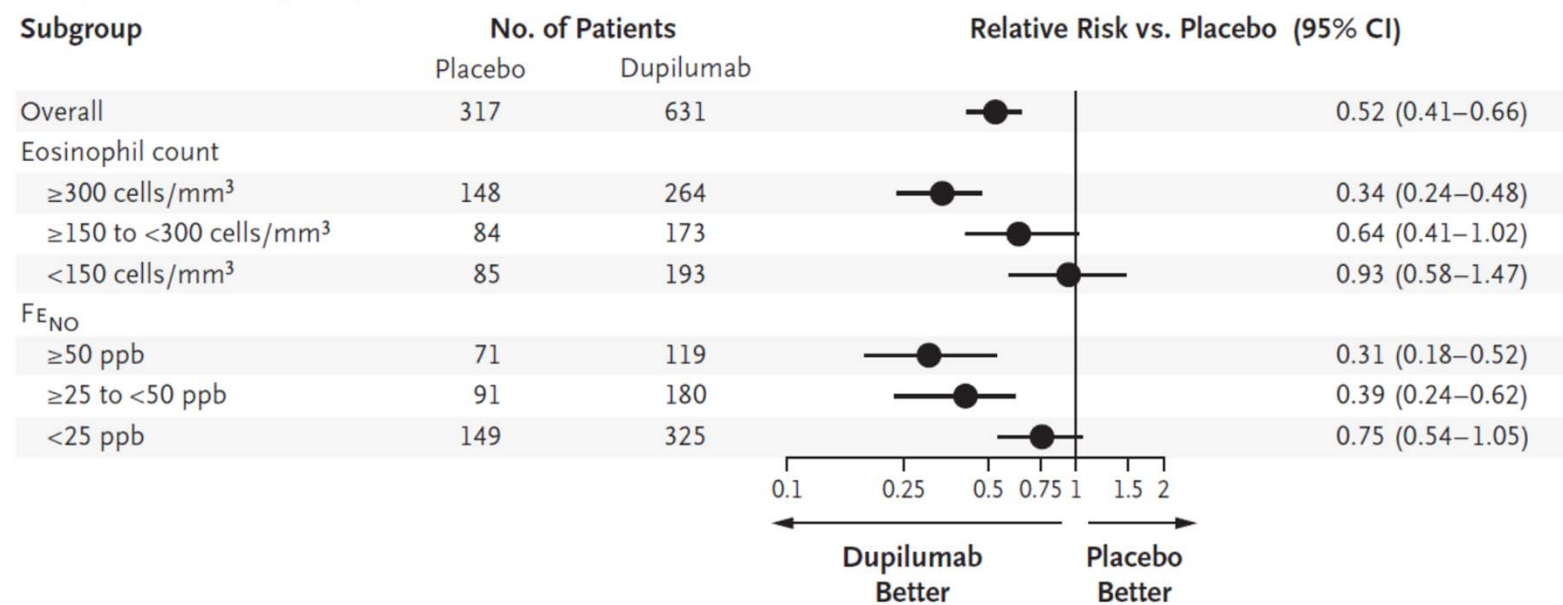
FEV₁=forced expiratory volume in 1 second; LABA=long-acting β -agonist; LAMA=long-acting muscarinic antagonist; LD=loading dose; LS=least squares; LTRA=leukotriene receptor antagonist; SABA=short-acting β -agonist; SOC=standard of care.

Study participants were randomised 2:2:1:1 to receive dupilumab 200 mg (LD=400 mg) or 300 mg (LD=600 mg) as add-on therapy every 2 weeks or a matched-volume placebo (1.14 mL or 2.00 mL, respectively) for each active dose. SOC therapy permitted throughout study included LABA, LAMA, LTRA, methylxanthines, and SABA (as needed).²

1. Dupixent (dupilumab) [summary of product characteristics]. Paris, France: sanofi-aventis groupe; 2019. 2. Castro M, et al. *N Engl J Med*. 2018;378(26):2486-2496.

Dupilumab prevents exacerbations in uncontrolled moderate-to-severe asthma

A Dupilumab, 200 mg Every 2 Wk, vs. Matched Placebo



NO minimum requirement for blood eosinophil count or FENO.

Livelli di FeNO associati a diversi fenotipi di asma



FeNO

↑FeNO = T2 high: 1) Asma allergica
2) Asma eosinofila refrattaria
3) Asma eosinofila non atopica
4) Asma in riacutizzazione

↓FeNO = T2 low: 1) Asma neutrofilico
2) Asma paucigranulocitico
3) Asma associato ad obesità
4) Asma da fumo

Effetto dei farmaci biologici

dupilumab → ↓ FeNO

omalizumab → ↓ FeNO

mepolizumab e benralizumab → ∅ FeNO

Conclusions

- FeNO is an easy-to-obtain and non-invasive biomarker for allergic/eosinophilic airway inflammation in T2-high asthma;
- FeNO is useful for diagnosis, severe asthma phenotyping, prediction of the loss of control and exacerbation, monitoring ICS and biologic therapy efficacy in selected severe asthmatics.