

Biomarkers in respiratory diseases

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Biomarkers in respiratory diseases



Very difficult question



Chronic respiratory diseases:

- Asthma
- COPD
- Cancer
- Cystic fibrosis
- Obstructive sleep apnea

Infectious respiratory diseases:

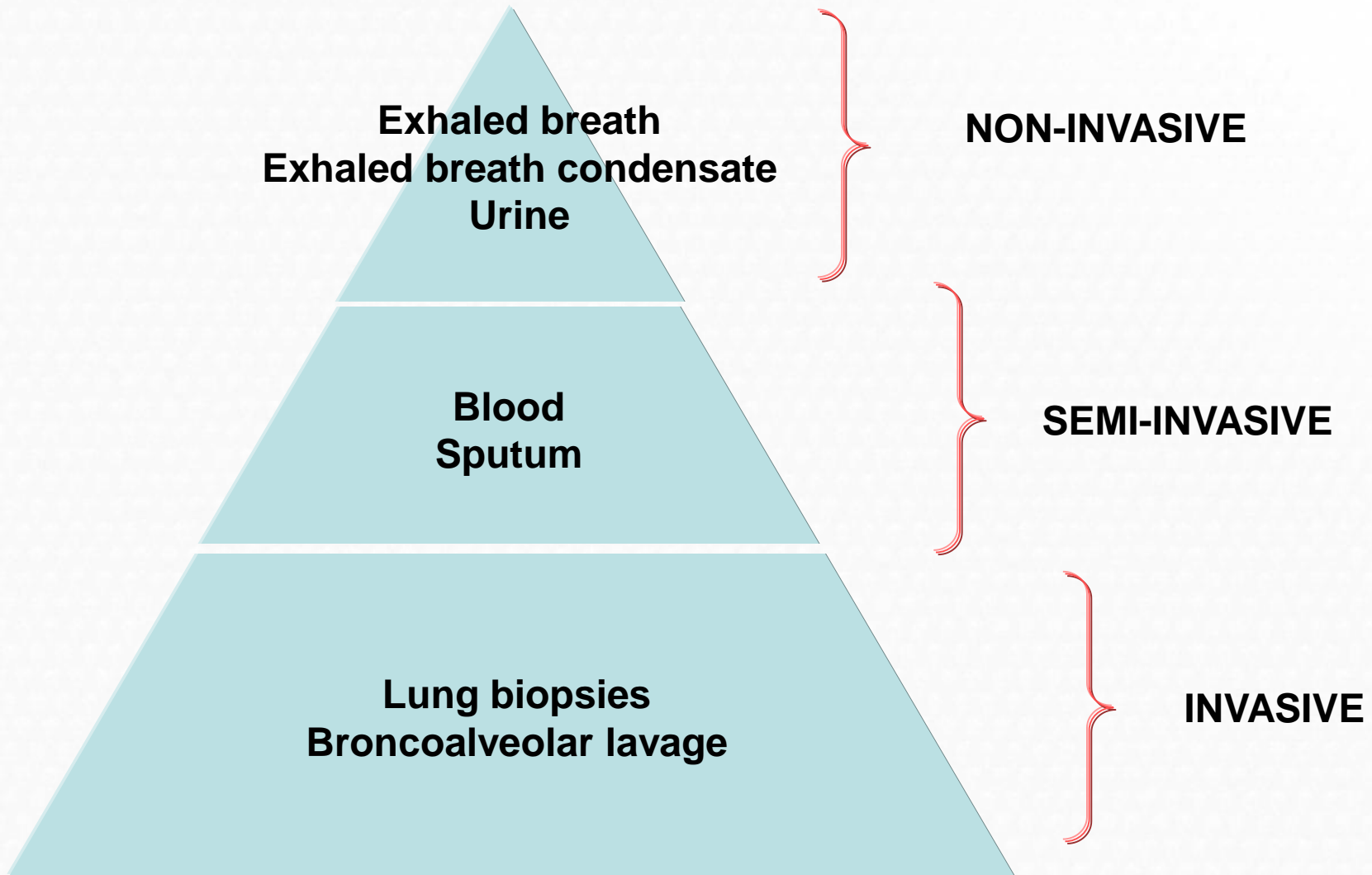
- Pneumonia
- Tuberculosis

Diffuse lung diseases:

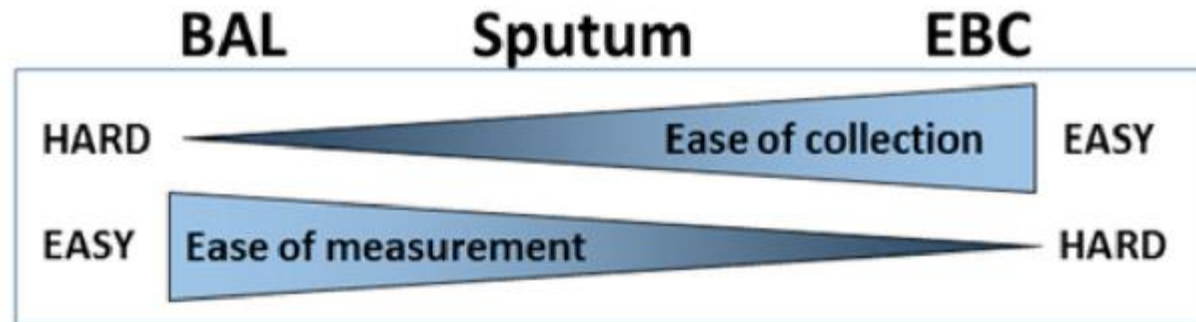
- Interstitial lung diseases
- Pulmonary hypertension
- Acute lung injury



TYPE OF BIOLOGICAL SAMPLES



TYPE OF BIOLOGICAL SAMPLES



Chronic respiratory diseases:

Asthma

COPD

Cancer

Cystic fibrosis

Obstructive sleep apnea

Infectious respiratory diseases:

Pneumonia

Tuberculosis

Diffuse lung diseases:

Interstitial lung diseases

Pulmonary hypertension

Acute lung injury

BIOMARKERS

Exhaled breath

Exhaled breath condensate

Urine

Blood

Sputum

Lung biopsies

Bronchoalveolar lavage

ASTHMA



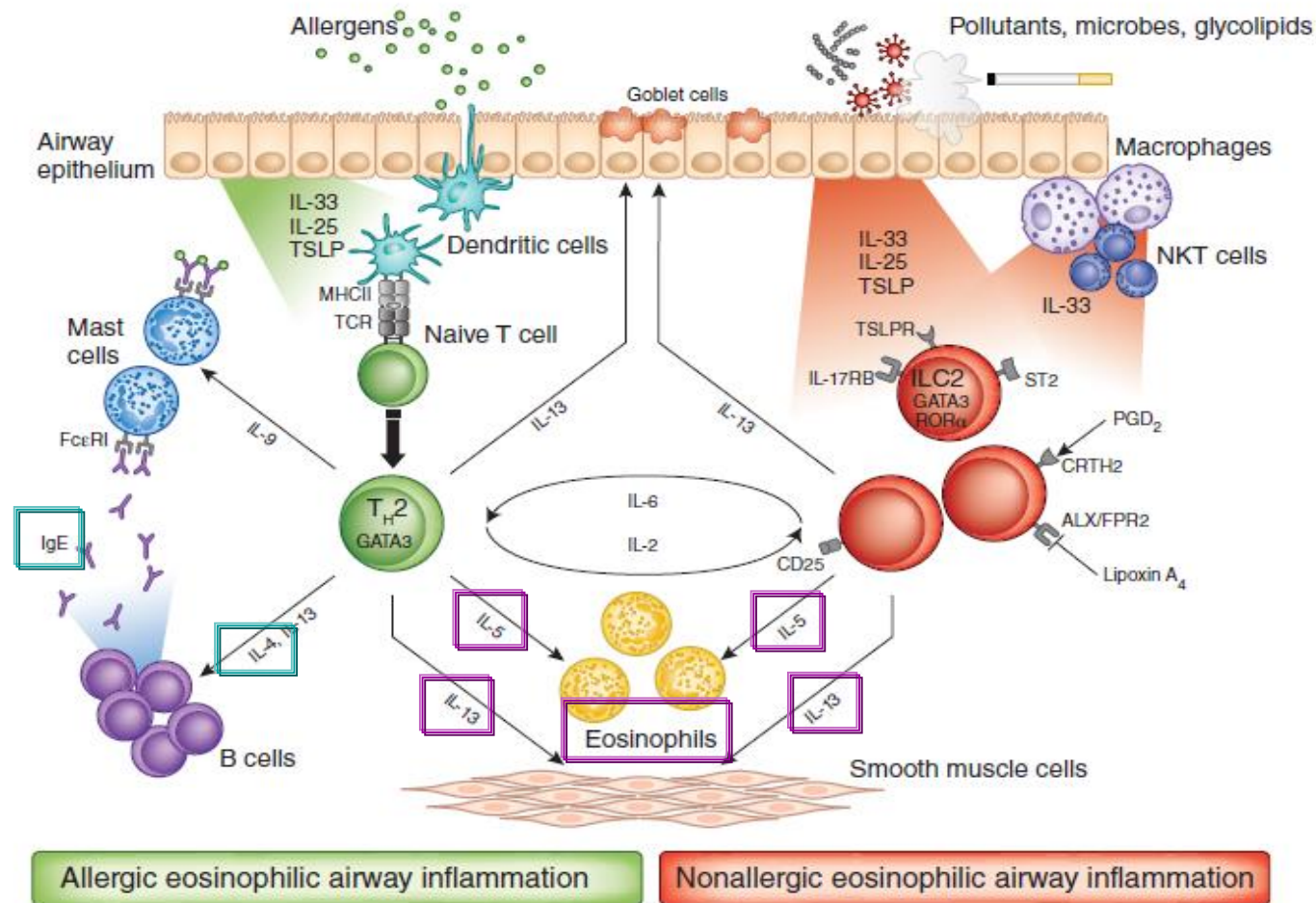
Asthma is a syndrome that **includes several clinical phenotypes** that share **similar clinical manifestations**, but probably of **different etiologies**.



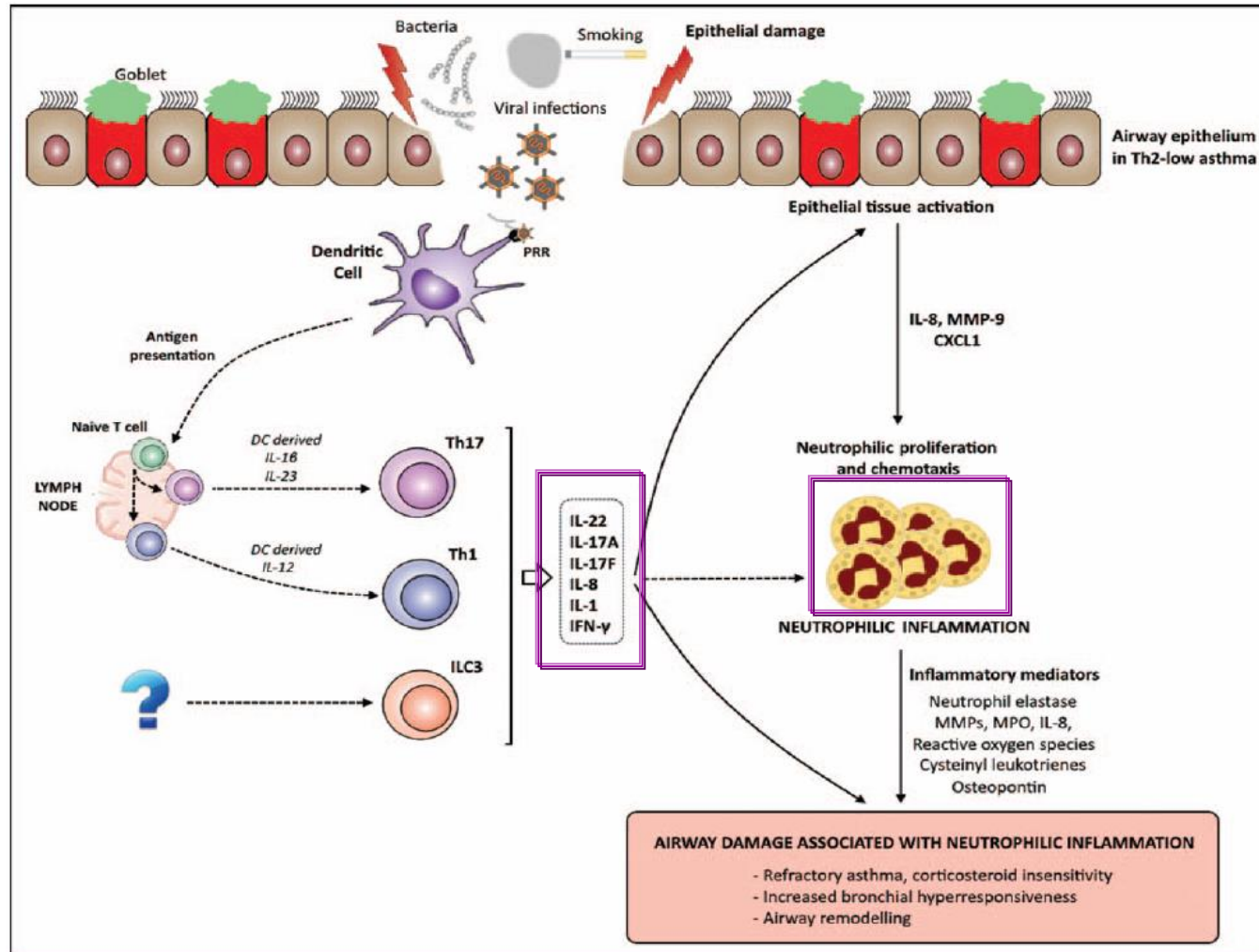
Type 2 response

Type 2 / Th2

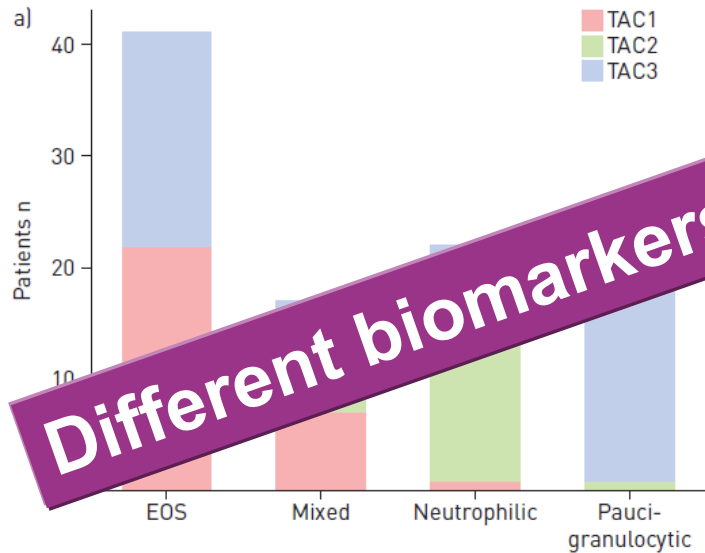
Type 2 / ILC2



Non-type 2 response



T-helper cell type 2 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-BIOPRED

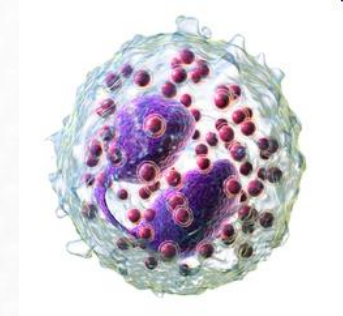


TAC 1: Highest sputum eosinophilia
TAC 2: Highest sputum neutrophilia
TAC 3: Paucigranulocytic

TAC: Transcriptome-associate clusters

Most of these biomarkers are useful for the diagnosis of allergic asthma.

Eosinophils (blood and sputum)
Exhaled Nitric Oxide (FeNO)
IgE
Eosinophil cationic protein (ECP)



There are few biomarkers for the diagnosis of non-allergic asthma

Neutrophils (sputum)
Th17 cytokines ???

Eosinophils

Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort

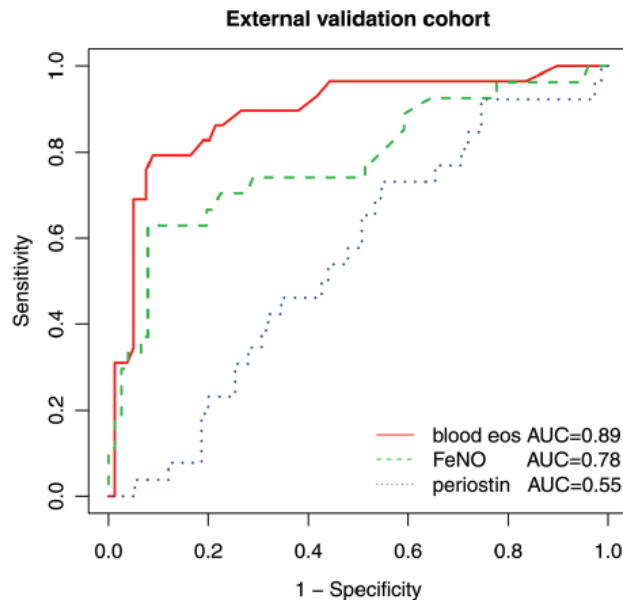


Figure 1 Receiver operating characteristics curve analyses of the sensitivity and the specificity of blood eosinophils (eos), FE_{NO} and serum periostin (in-house) for the diagnosis of eosinophilic inflammation. AUC, area under the curve.

TABLE 5 Biomarkers in blood, sputum and exhaled air

	Severe nonsmoking asthma	Smokers and ex-smokers with severe asthma	Mild/moderate nonsmoking asthma	Healthy nonsmoking controls	p-value	p-value [#]
Subjects n	311	110	88	101		
Exhaled NO ppb	26.5 [16–47] (n=290)	23.5 [12–42] (n=104)	25 [18–54] (n=87)	19.25 [13–29] (n=96)	<0.001	0.438
Sputum						
Sputum eosinophils %	2.75 [0–19] (n=128)	4.13 [1–14] (n=53)	1.05 [0–3] (n=43)	0 [0–0] (n=41)	<0.001	0.004
Sputum neutrophils %	53.69 [34–75] (n=128)	55.15 [35–65] (n=53)	44.5 [26–62] (n=43)	39.56 [21–56] (n=41)	0.002	0.042
Sputum differential eosinophil count >1.9%	74 (57.81%) (n=128)	32 (60.38%) (n=53)	17 (39.53%) (n=43)	1 (2.44%) (n=41)	<0.001	0.026
Blood						
Blood eosinophils %	2.94 [1–6] (n=302)	2.88 [1–5] (n=106)	3.00 [2–5] (n=88)	2.10 [1–3] (n=101)	0.001	0.295
Blood eosinophils absolute	0.2 [0.3] (n=302)	0.22 [0.29] (n=106)	0.23 [0.2] (n=88)	0.1 [0.11] (n=101)	0.001	0.295
Blood neutrophils %	62 [55–70] (n=302)	61.75 [55–69] (n=106)	56.83 [52–63] (n=88)	57.34 [51–64] (n=101)	<0.001	<0.001
Blood neutrophils absolute	4.73 [3.1] (n=302)	4.97 [2.87] (n=106)	3.64 [1.75] (n=88)	3.03 [1.6] (n=101)	<0.001	<0.001

Data are presented as median [interquartile range], unless otherwise stated. [#]: severe asthma (nonsmoking asthma group and smokers and ex-smokers with severe asthma group combined) versus mild/moderate nonsmoking asthma group.

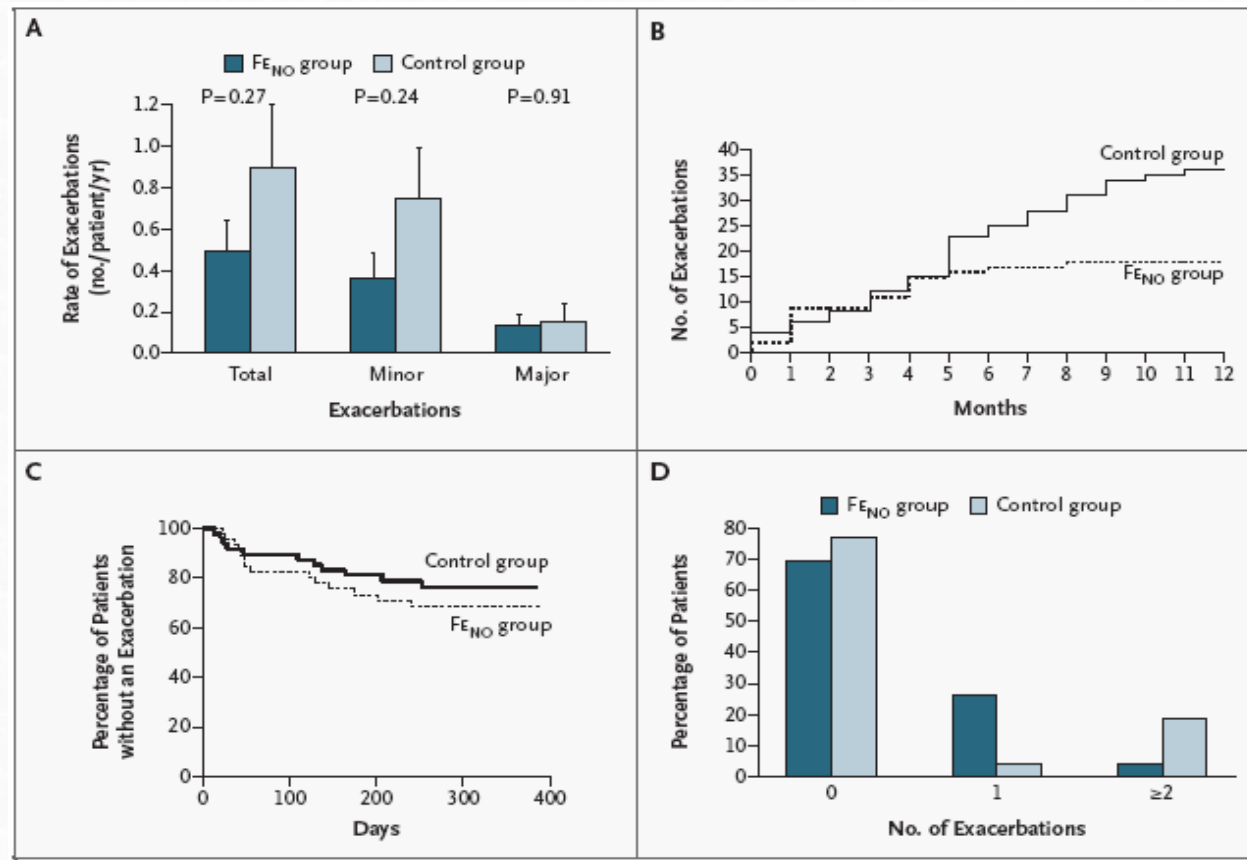
Shaw et al. Eur Respir J. 2015;46:1308-21.

Wagener et al. Thorax 2015; 70: 115-20.

Exhaled Nitric Oxide (FeNO)

46 asthmatics treated according to FeNO
48 asthmatics treated according to guidelines

Main variable: Exacerbations
Secondary variable: Corticosteroid dose



Exhaled Nitric Oxide (FeNO)

EDITORIALS

Am J Respir Crit Care Med 2009; 179: 87-92.

Against

NO more dogma
16 references

In favor

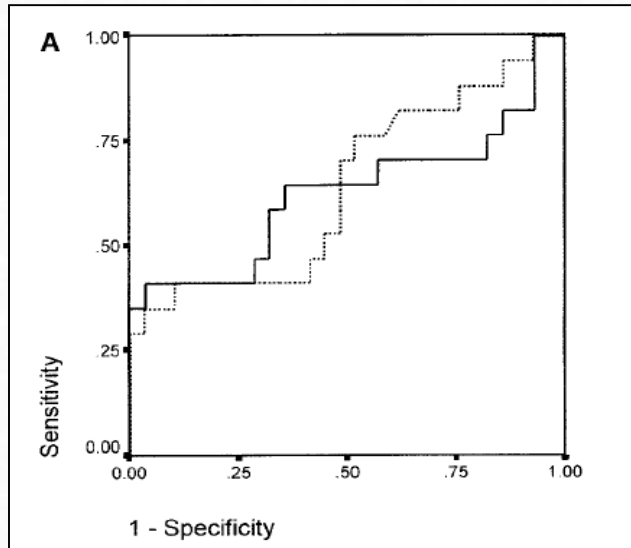
Exhaled NO:
Still alive, not laid to rest
15 references

Stick S et al. Am J Respir Crit Care Med 2009; 15;179(2):87-8.
Taylor DR et al. Am J Respir Crit Care Med 2009; 373(9661):382 .

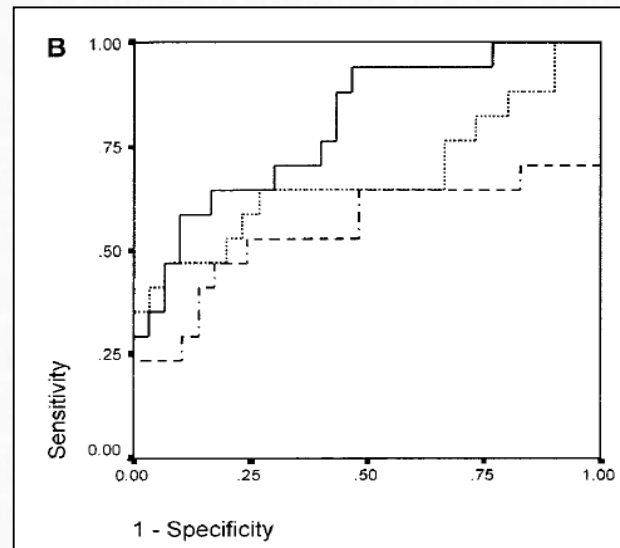
Exhaled Nitric Oxide (FeNO)

N = 47 asthmatic patients (8 – 75 years)

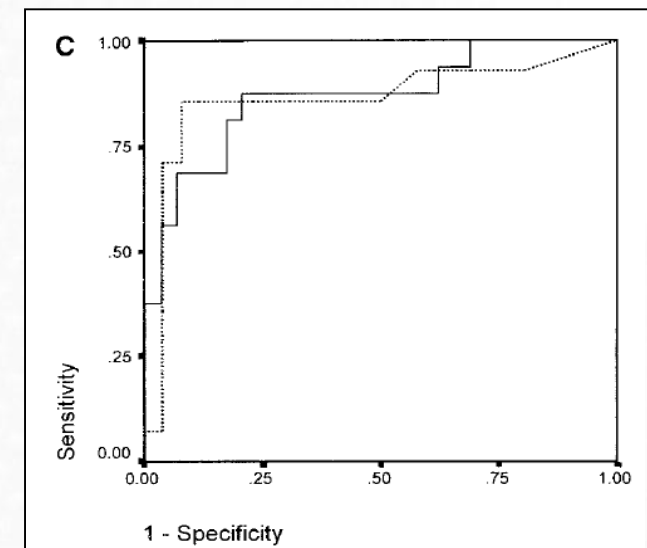
PEF



FEV1 - FVC



FENO

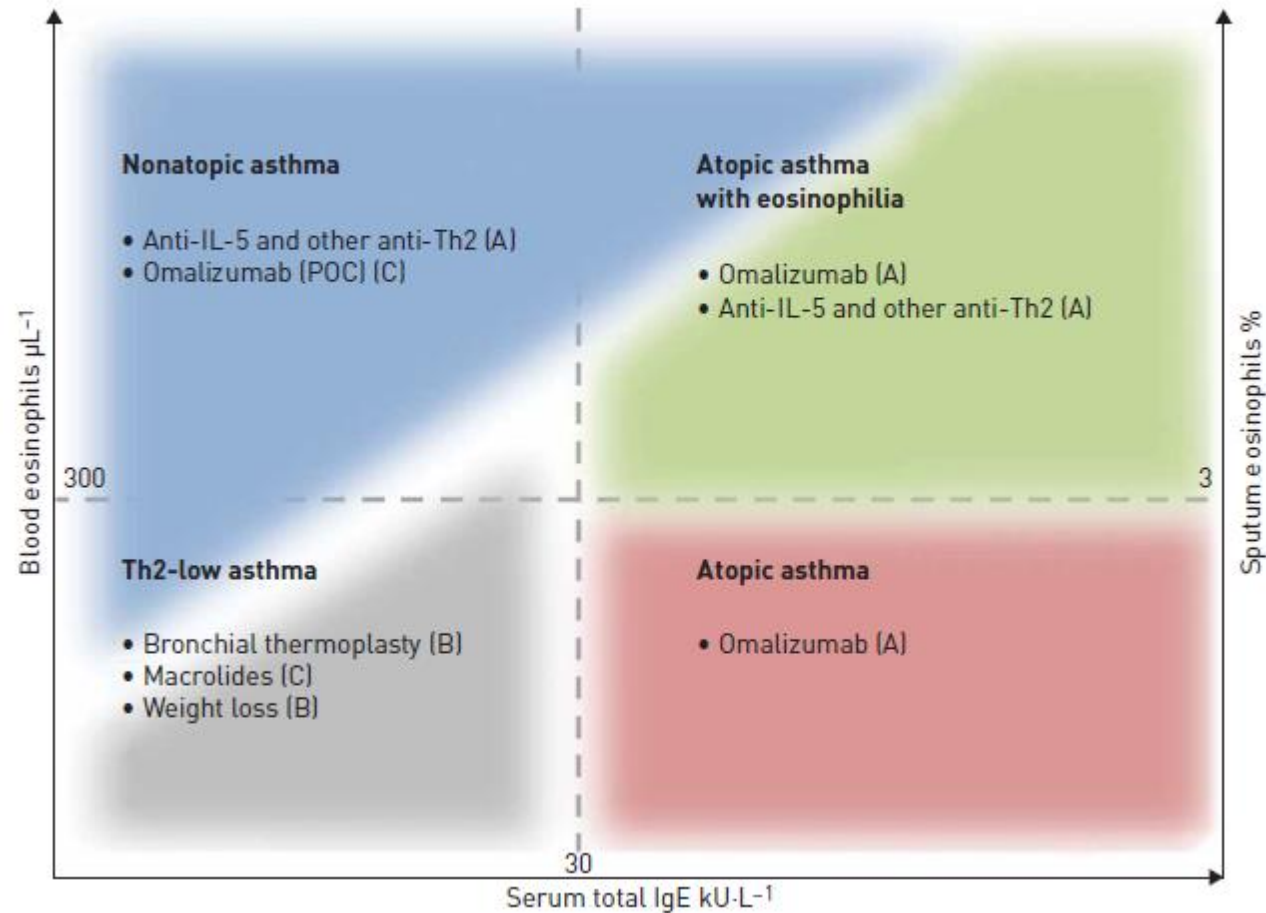


NO > 20 ppb

Eosinophil correlation: $r = 0.67$, $p < 0.001$

Hyperresponse: $r = -0.56$, $p < 0.001$

IgE



Eosinophil cationic protein (ECP)

Airway inflammation

- ECP correlates with activated eosinophils in bronchial mucosa of asthmatics²⁶
- ECP is more sensitive than blood and sputum blood eosinophils in assessing asthma severity^{27,28}
- Sputum ECP is more sensitive than serum ECP in assessing asthma severity¹⁵
- ECP levels appears to reflect virus-induced airway inflammation in asthma but is not affected by type of virus²⁹⁻³¹

Airway hyper-responsiveness

- ECP does not correlate with airway hyper-responsiveness^{15,33-37}

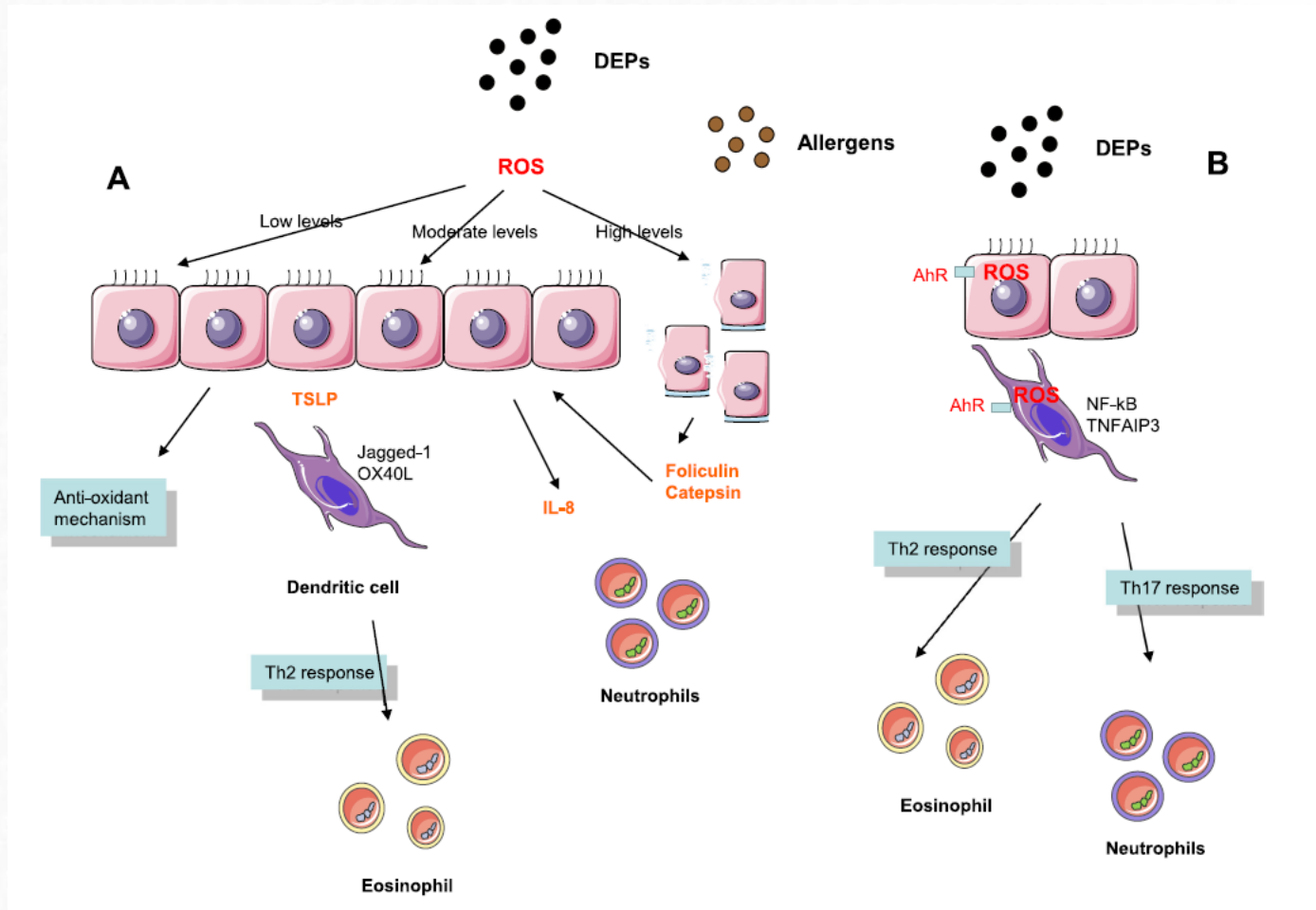
Diagnosis of asthma

- ECP is raised in other atopic diseases (e.g. allergic rhinitis, recurrent wheezing)⁴¹⁻⁴³ and infections (e.g. rhinovirus infections^{22,44} and bacterial sinusitis²²)
- ECP is not useful as a diagnostic tool for asthma because of its lack of specificity

Body fluid	Range of ECP levels ($\mu\text{g/L}$)		Advantages	Disadvantages
	Normal	Asthmatics		
Serum ^{11,13,14}	2–20	> 20	Reflects propensity of activated eosinophils to release granule proteins	Blood must be collected under tightly controlled and standardized conditions
Plasma ^{11,14}	5–10 times lower than in serum		Reflects true levels of ECP in blood	Low levels are found because EDTA inactivates <i>ex-vivo</i> release of ECP
Sputum ^{15–17}	20–1280	76.8–32,000	High levels of ECP found	<ul style="list-style-type: none"> • Requires induction of sputum with nebulized saline in non-productive asthmatics • There is wide overlap in range of normal and asthmatics
Saliva ¹⁷	250–450	450–850	<ul style="list-style-type: none"> • High levels of ECP found • Easy and painless collection process 	Insufficient research to support its repeatability and clinical utility
Nasal lavage fluid ^{20–23}	0–60	90–120*	Painless but uncomfortable collection process	<ul style="list-style-type: none"> • Difficult to obtain samples • Does not correlate well with asthma
Broncho-alveolar lavage fluid ^{24,25}	1–3	5–100	Moderate levels of ECP found	Difficult to obtain samples
Urine ^{3,13,20}	—	—	—	Not useful. Urine eosinophil protein X (EPX)/urine creatinine ratio is more useful. However, urinary EPX is not widely used in asthma research

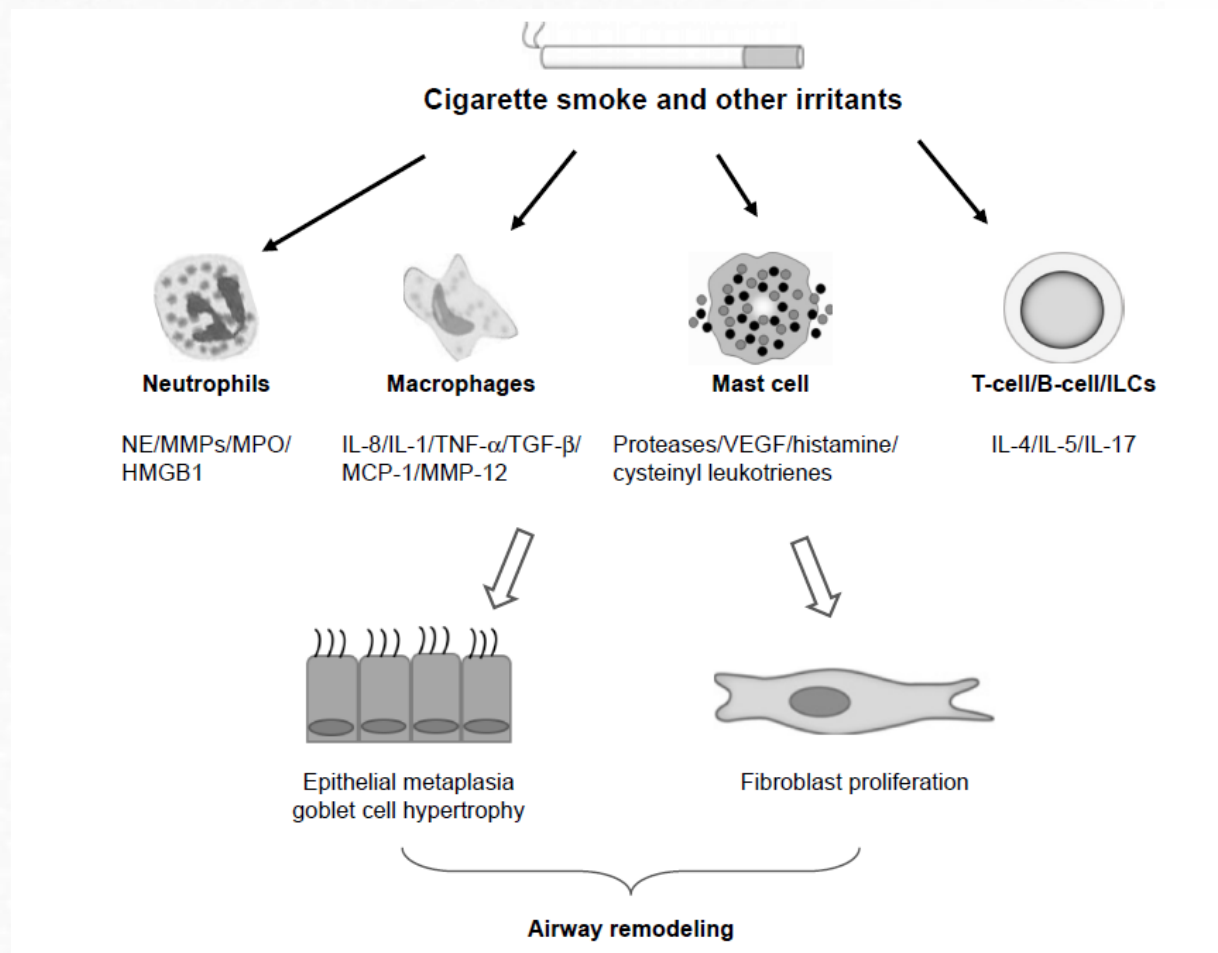
*Expressed as μg of ECP per g of protein.²¹

Effect of pollution



COPD





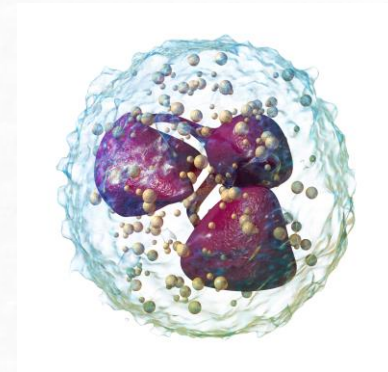
Neutrophils (Sputum)

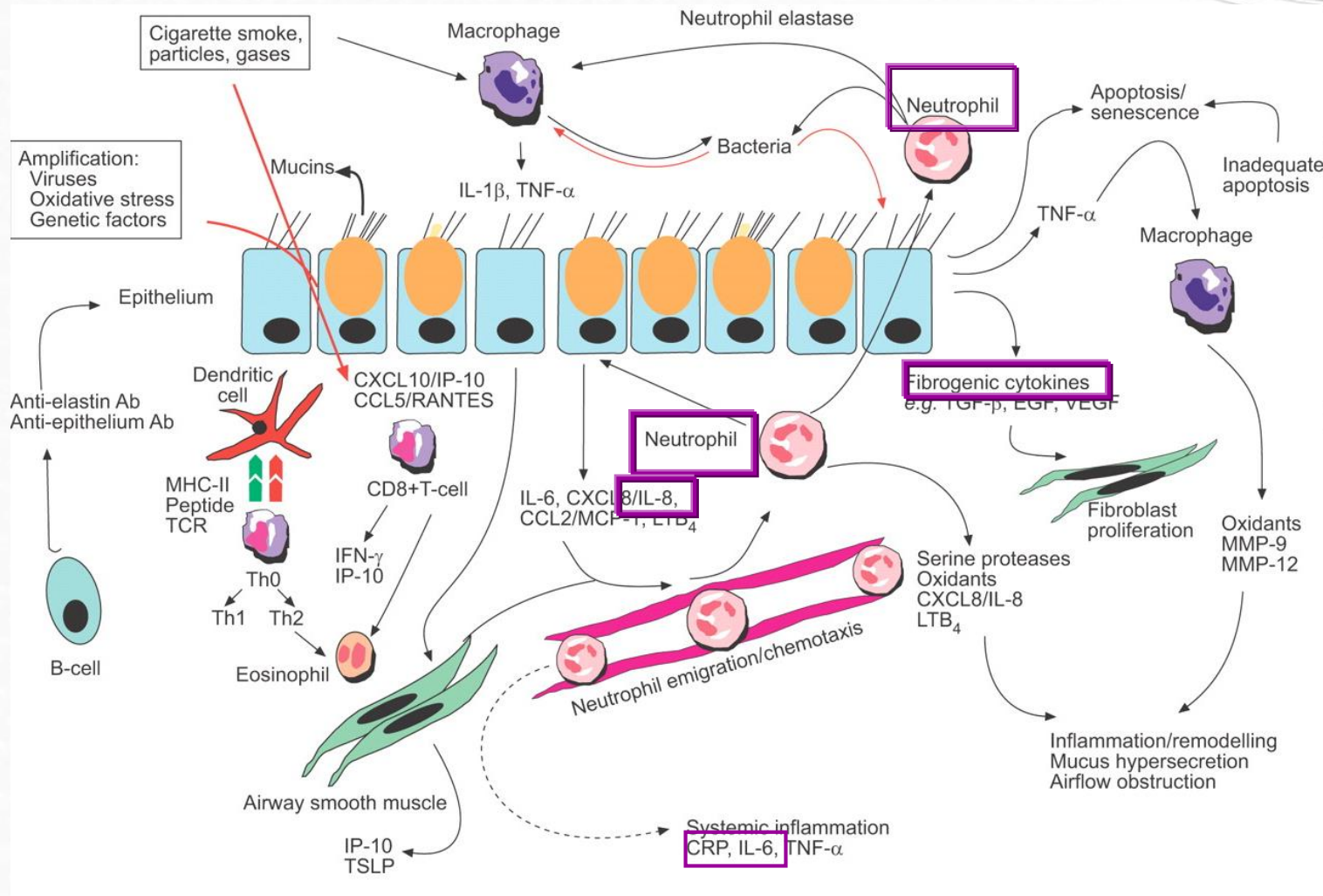
IL8

C-reactive protein

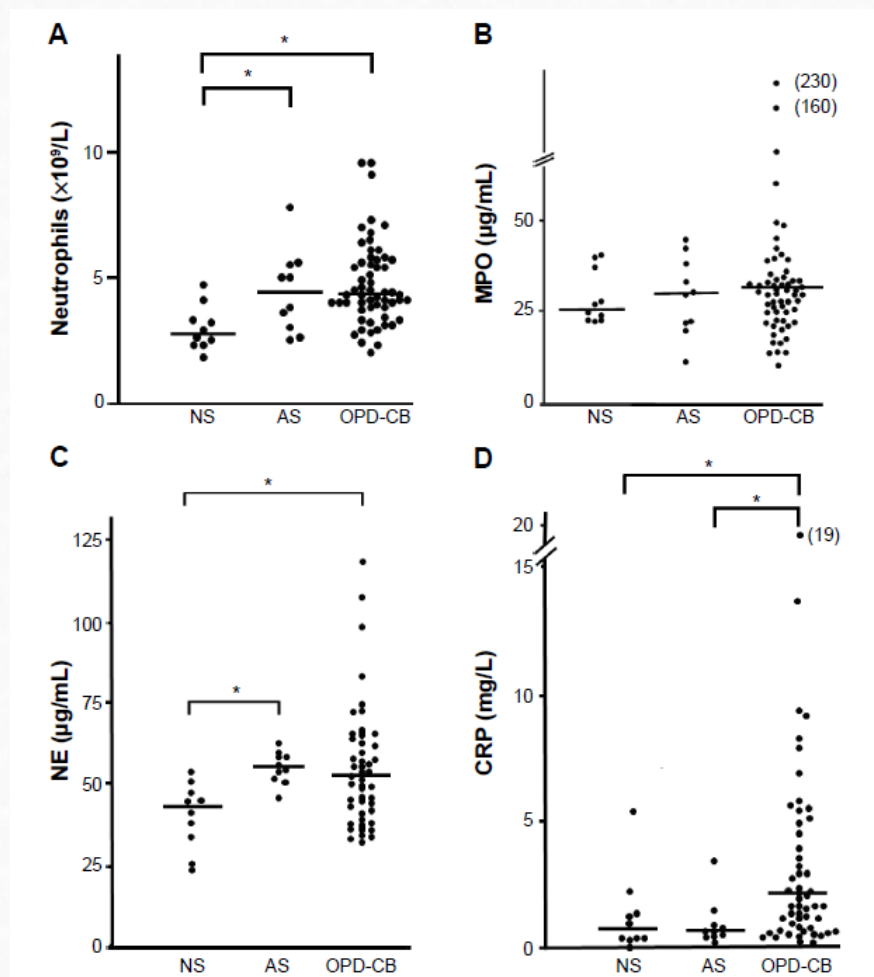
Fibrinogen

IL6

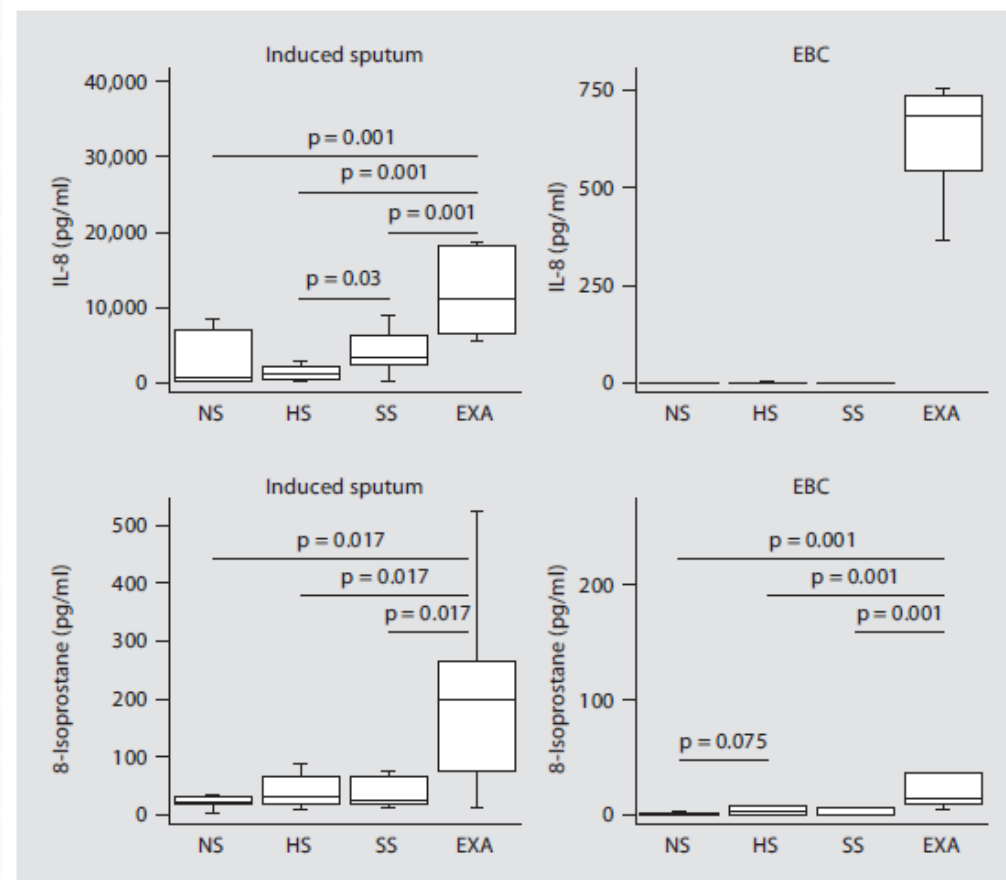




Neutrophils and IL8

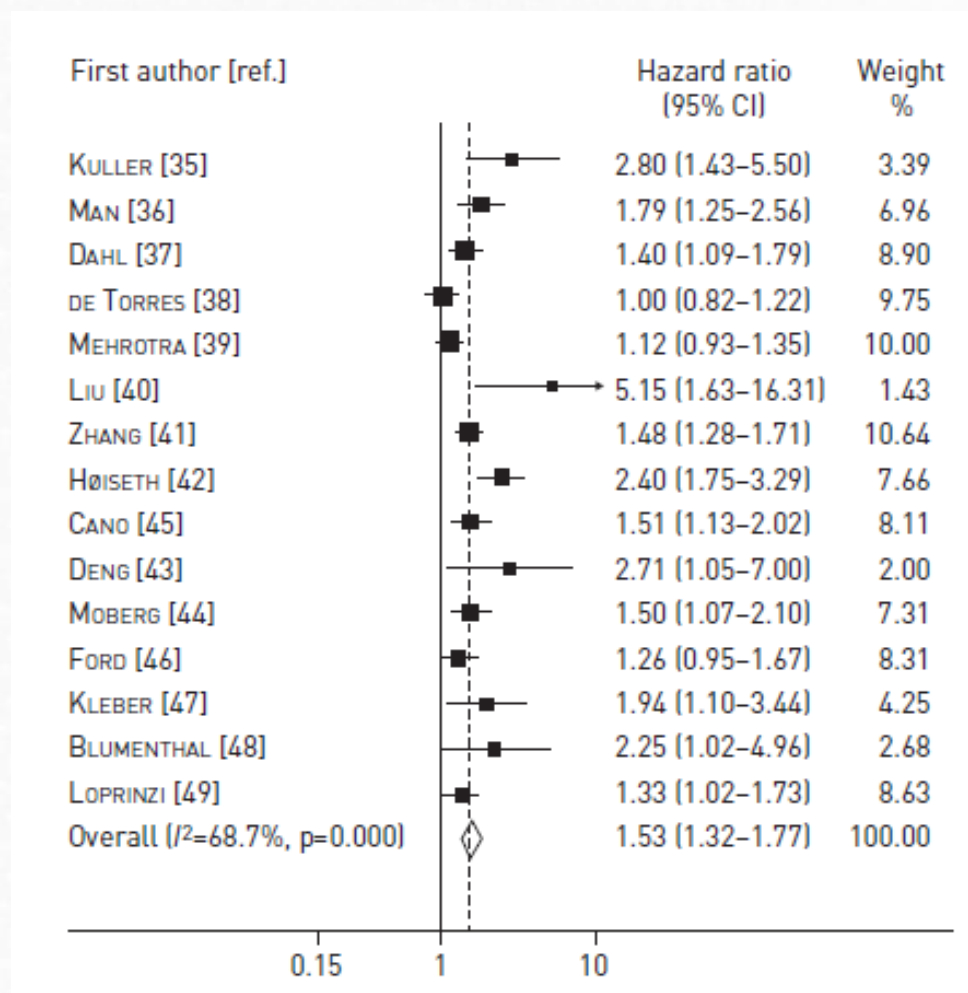


Andelid et al. Int J COPD 2015;10: 1253-63.



Mazur et al. Respiration 2009;78: 209-16.

C-reactive protein



Level > 3 mg/ml

Fibrinogen

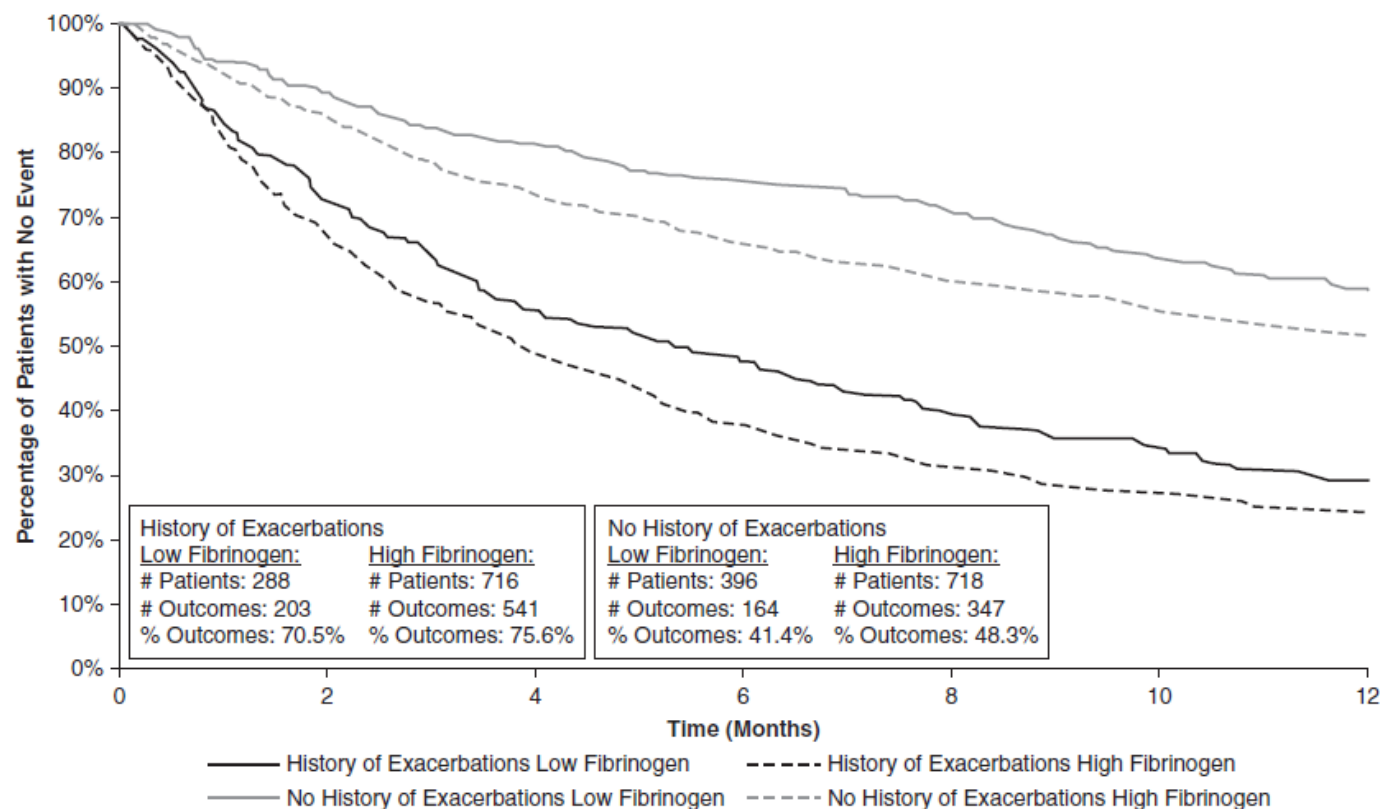
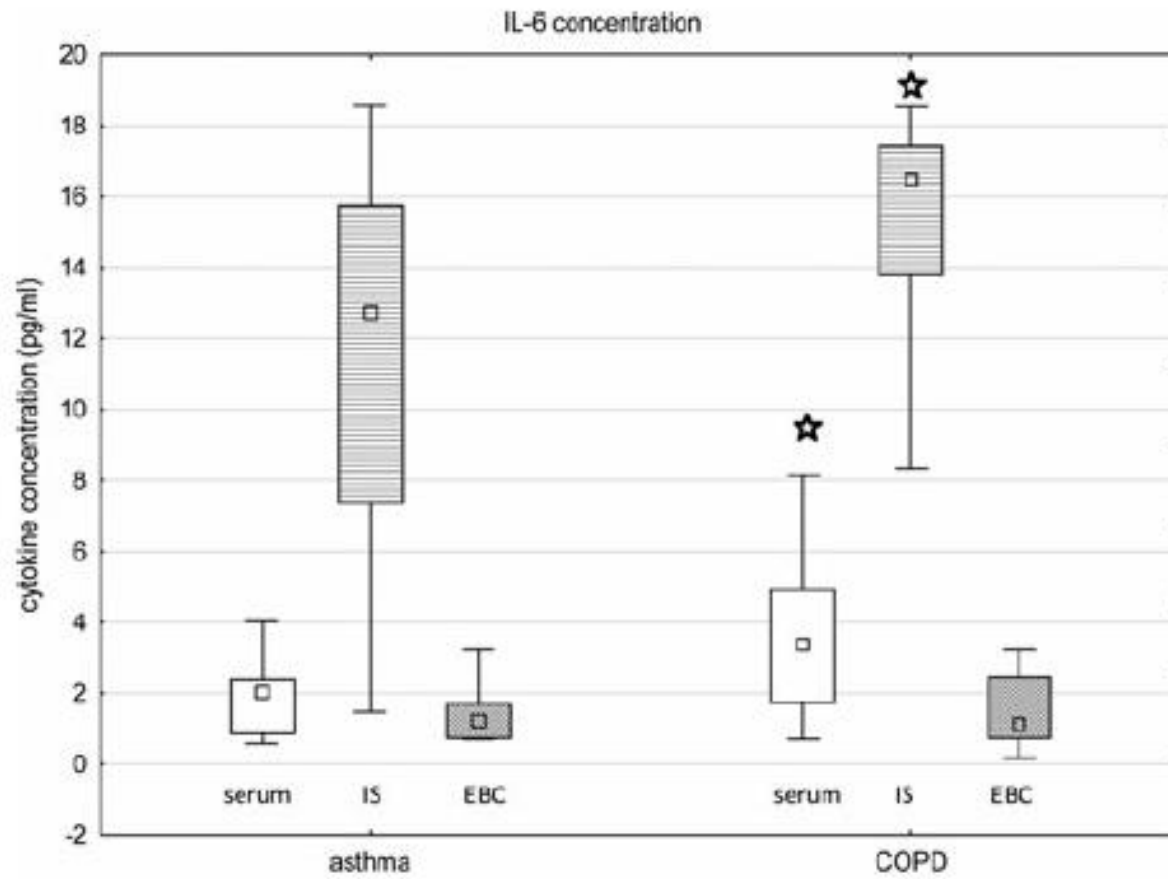


Figure 3. Kaplan-Meier plot of time to first chronic obstructive pulmonary disease exacerbations within 12 months in the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) study. High plasma fibrinogen was defined as a plasma fibrinogen concentration greater than or equal to 350 mg/dl at enrollment. Reprinted by permission from Reference 22.

IL6



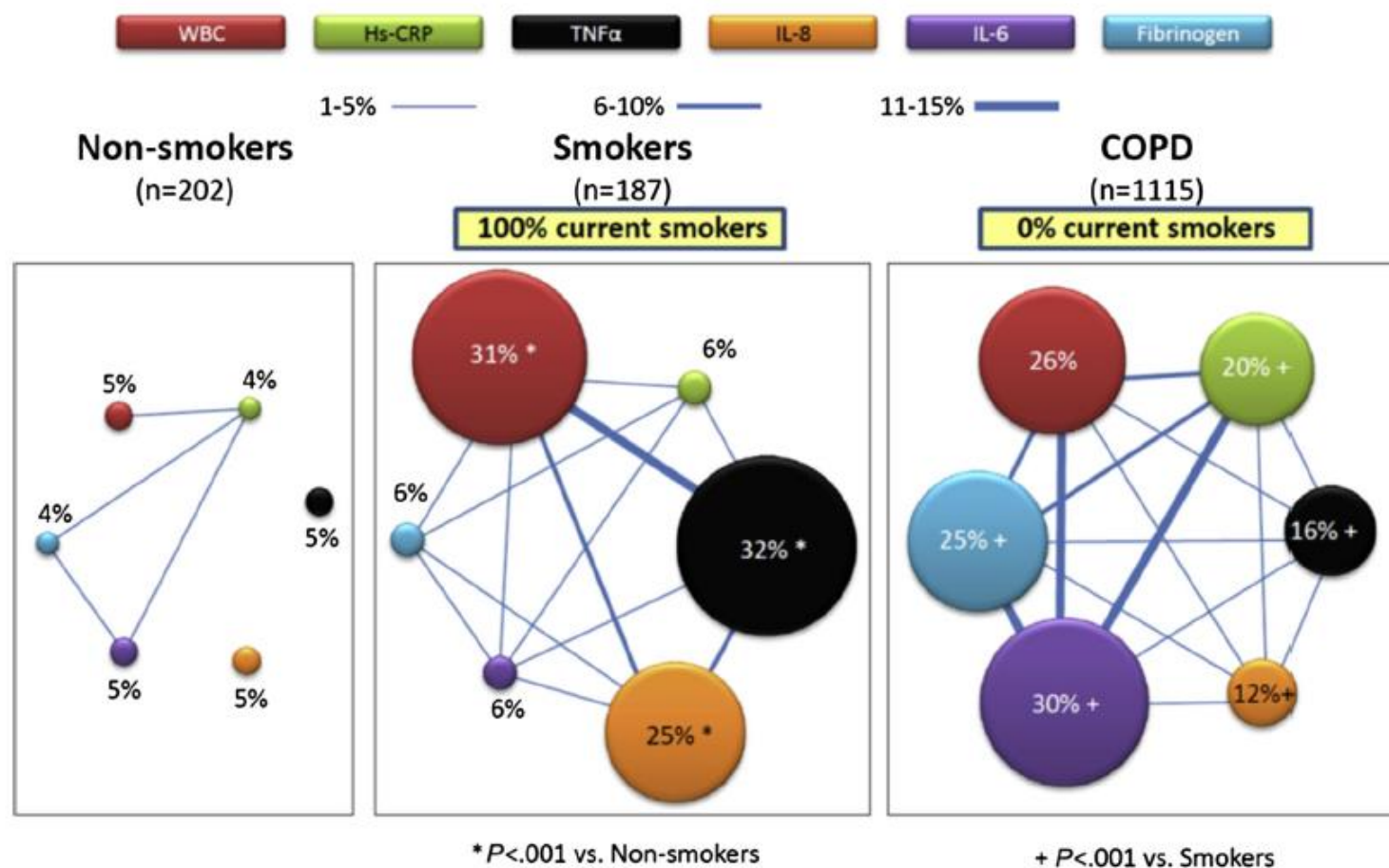
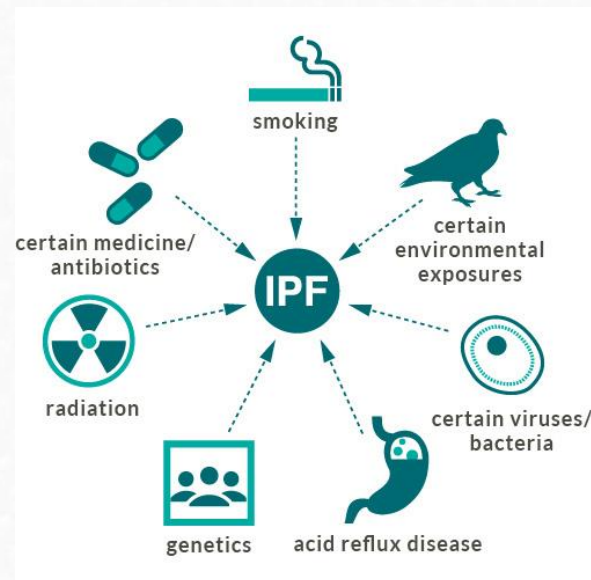
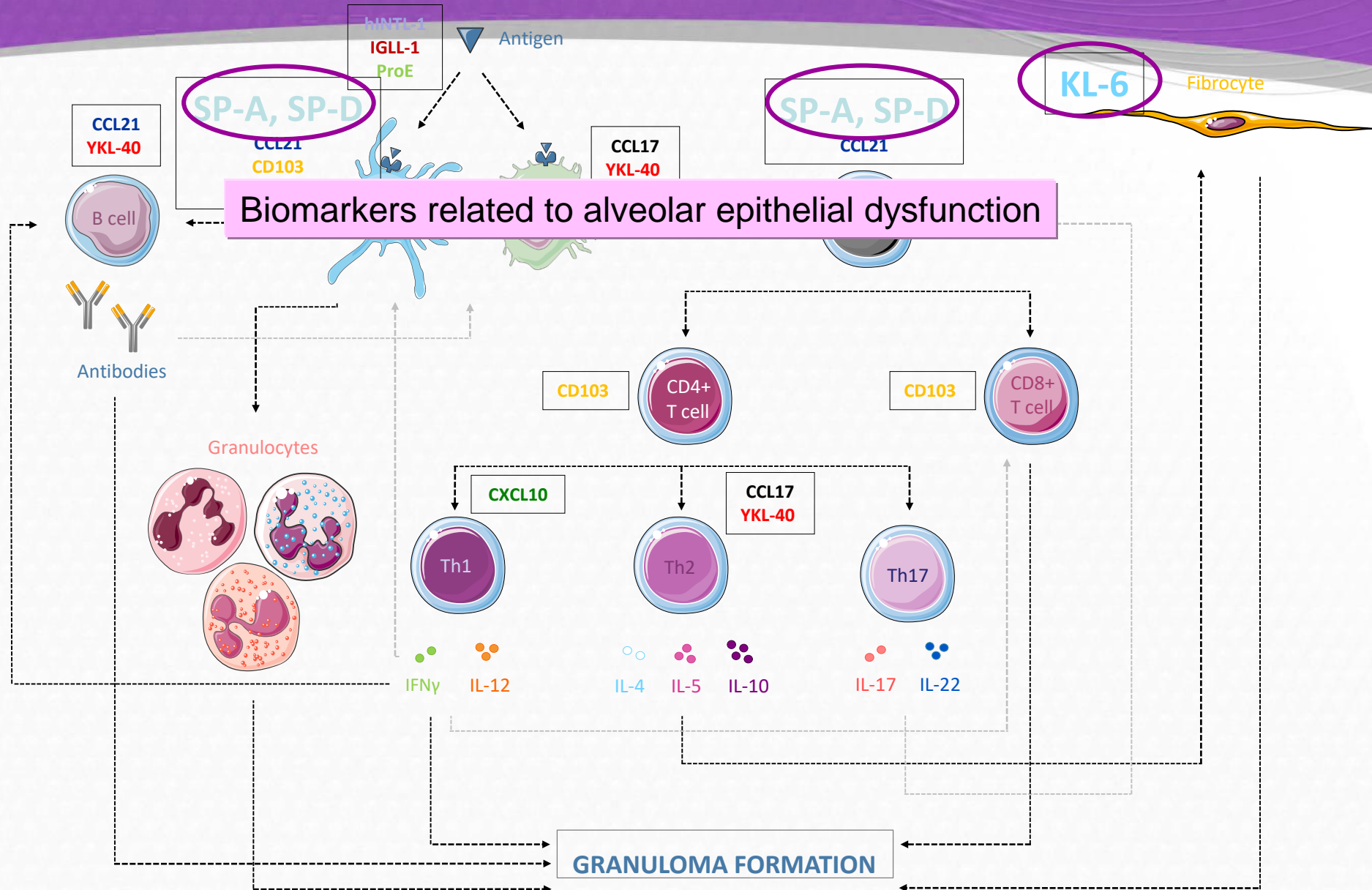


Fig. 3. Systemic inflammation as described in the ECLIPSE study. For further explanations see text. WBC, white blood cells. Hs-CRP, High-sensitivity-C reactive protein. (Adapted from Agustí A, Edwards LD, Rennard SI, et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. PLoS ONE 2012;7:e37483.)

INTERSTITIAL LUNG DISEASE



PULMONARY FIBROSIS



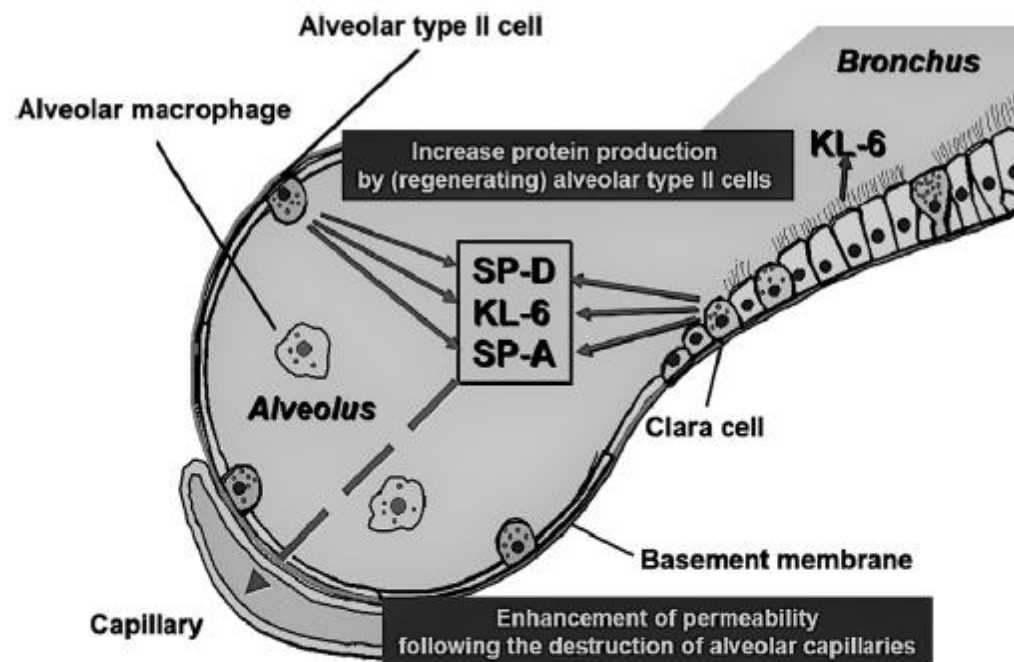


Fig. 3 – The mechanism for the efflux into the blood of KL-6, SP-A, and SP-D. The increment in serum levels of KL-6, SP-A, and SP-D could be attributed to an increase in the production of these proteins by regenerating alveolar type II cells and enhanced permeability following the destruction of the alveolar-capillary barrier.

Surfactant proteins A and D

1) Characteristics

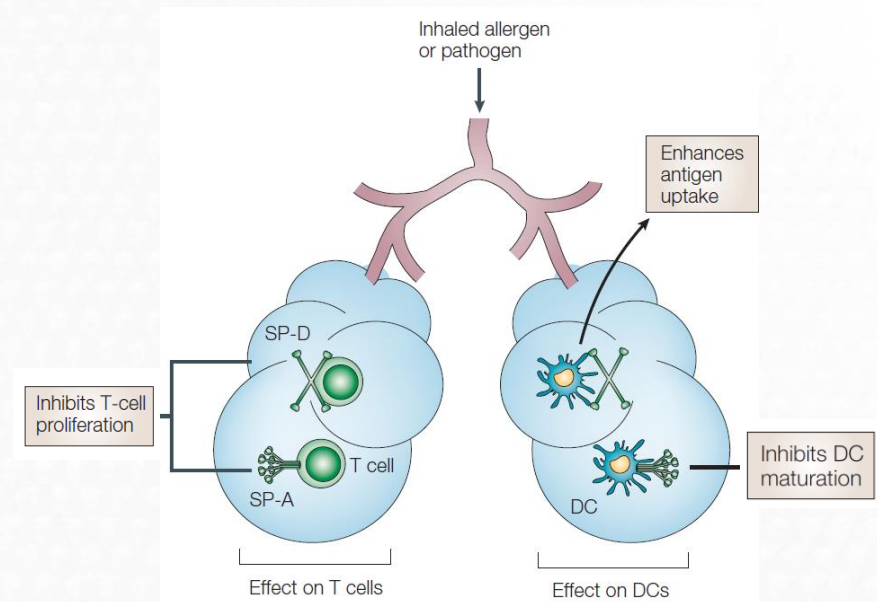
- Tetrameric structure (520 KDa) VS hexameric (630 KDa).
- Type C lectins superfamily.

2) Origin

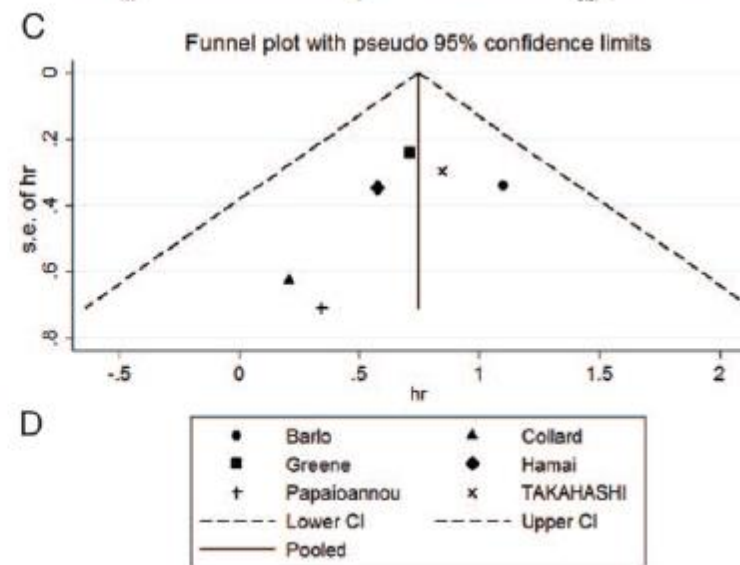
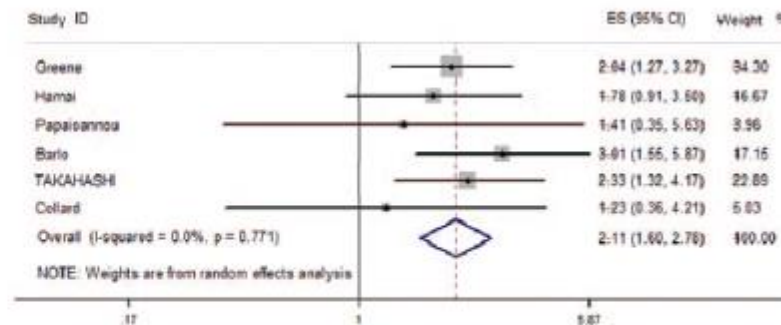
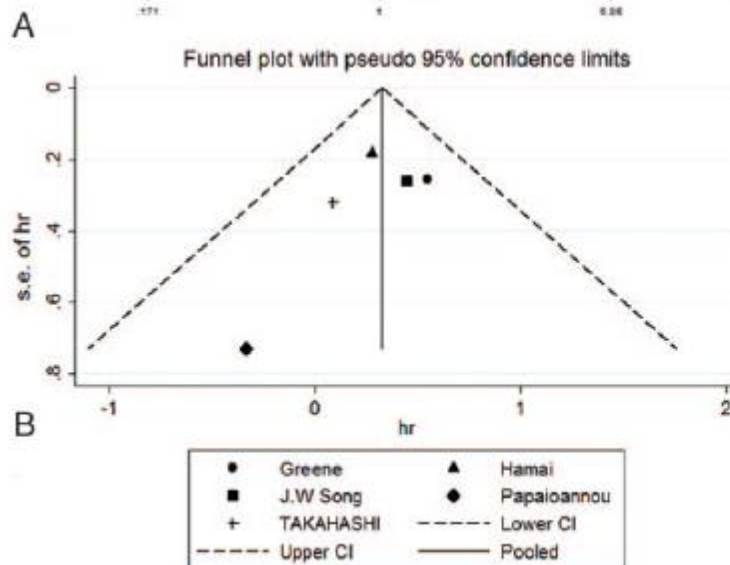
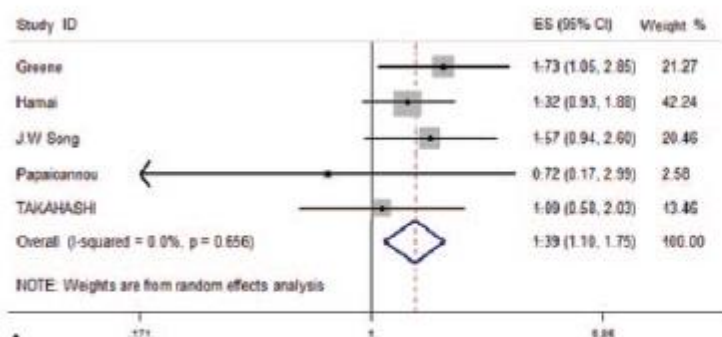
- Produced mainly by type II pneumocytes.

3) Function

- Homeostasis of the surfactant
- Pulmonary immunity
 - opsonins
 - inflammatory mediators
 - T cells and DC



PULMONARY FIBROSIS



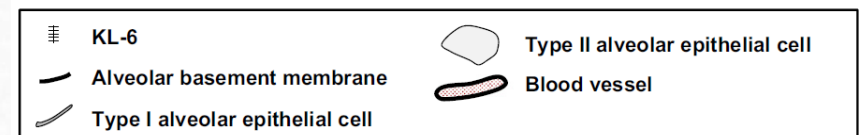
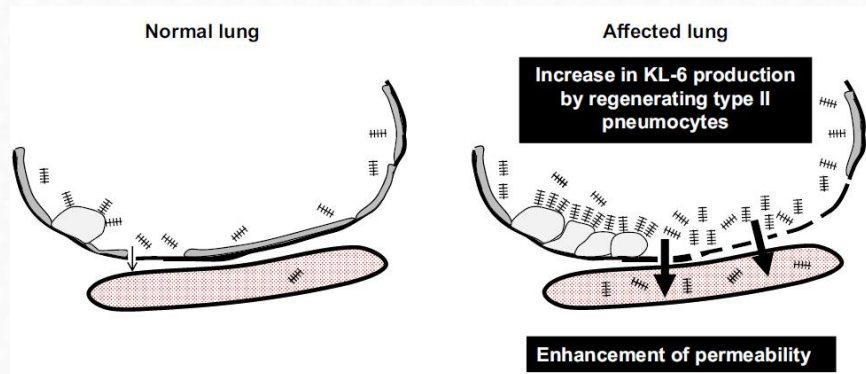
KL-6

1) Characteristics

- Glicoprotein of 200 KDa.

2) Origin

- Expressed in type II pneumocytes and bronchial epithelium in situations of tissue remodeling.
- It increases in serum after the destruction of the alveolar-capillary barrier and an increase in permeability.



3) Function

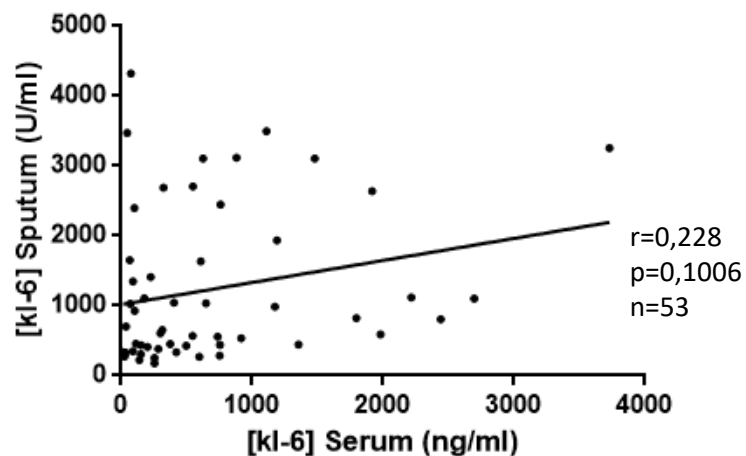
- Chemotactic and antiapoptotic effect in fibroblasts.

Ohshimo S, Yokoyama A, et al. *Biochem Biophys Res Commun*. 2005;338(4):1845-1852.

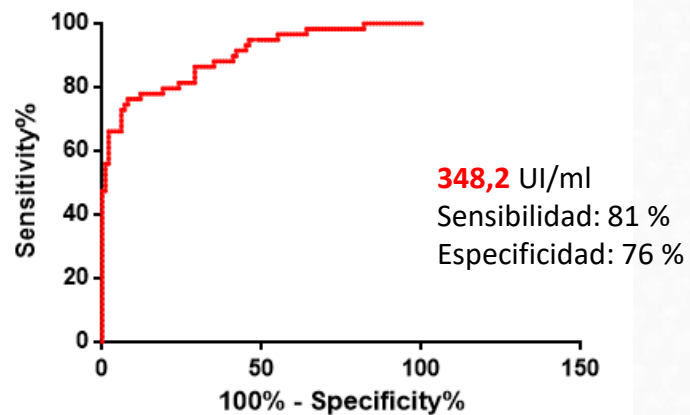
Ishikawa N, Hattori N, et al. *Respir Investig*. 2012 Mar;50(1):3-13.

KL-6

HP Patients



ROC curve KL-6



Type 2 response

Eosinophils (blood and sputum)
Exhaled Nitric Oxide (FeNO)
IgE
Eosinophil cationic protein (ECP)

Non-type 2 response

Neutrophils (sputum)
Th17 cytokines ???

ASTHMA

BLOOD AND SPUTUM

Neutrophils (Sputum)
IL8
C-reactive protein
Fibrinogen
IL6

COPD

Surfactant proteins A and D
KL-6

**PULMONARY
FIBROSIS**

BLOOD

THANKS YOU FOR YOUR ATTENTION

