Diagnostic test of Asthma

Presenter : Eung Gu Lee

Division of Pulmonary and Critical Care Medicine
Department of Internal Medicine
Bucheon St. Mary's Hospital
The Catholic University of Korea

Contents

- Definition and Pathophysiology
- Spirometry
- Bronchodilator response, Peak expiratory flow
- Bronchoprovocation challenge
- Sputum, blood eosinophil counts, Fraction of exhaled nitric oxide (FeNO), Periostin
- Serum specific IgE test, Skin prick test

Contents

- Definition and Pathophysiology
- Spirometry
- Bronchodilator response, Peak expiratory flow
- Bronchoprovocation challenge
- Sputum, blood eosinophil counts, Fraction of exhaled nitric oxide (FeNO), Periostin
- Serum specific IgE test, Skin prick test

Definition and Pathophysiology

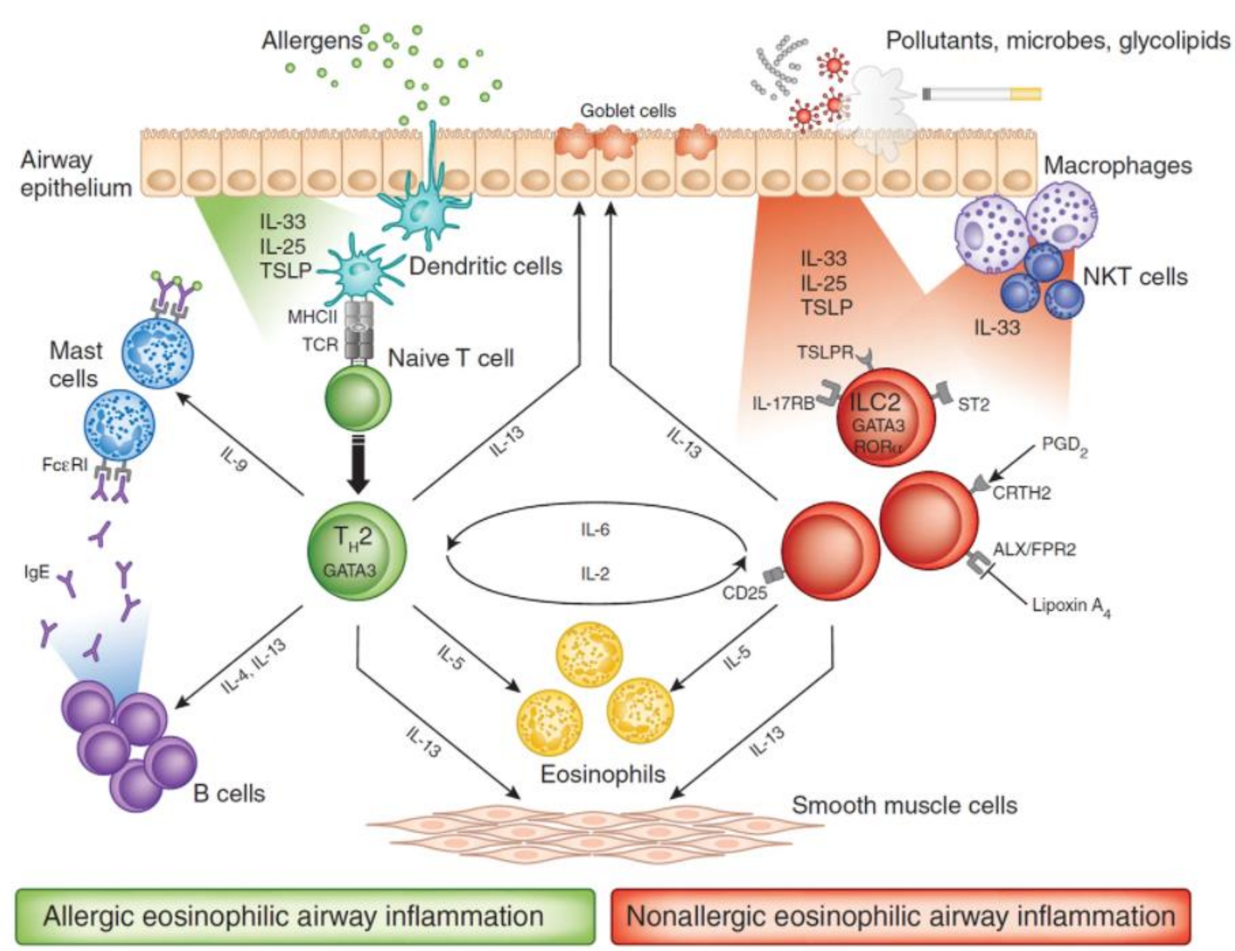
- Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation.

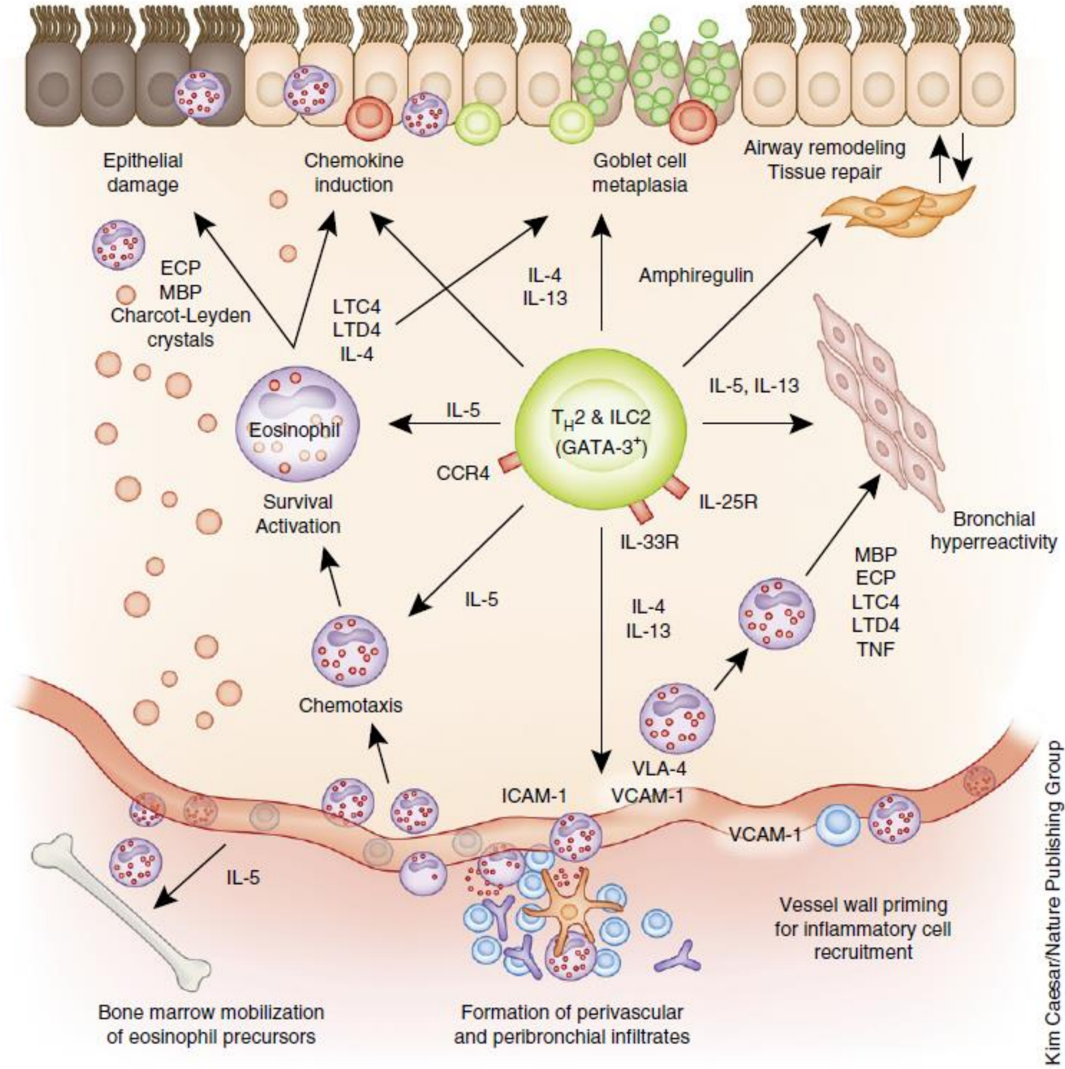
- History of **respiratory symptoms** such as
  - wheeze, shortness of breath, chest tightness, and cough that **vary over time and in intensity**, together with **variable airflow limitation**.

- Airway inflammation, Airway hyper-responsiveness, Variable airflow limitation, Airway remodeling
  → clinical symptoms

Global Strategy for Asthma Management and Prevention. 2021 GINA report

Definition and Pathophysiology



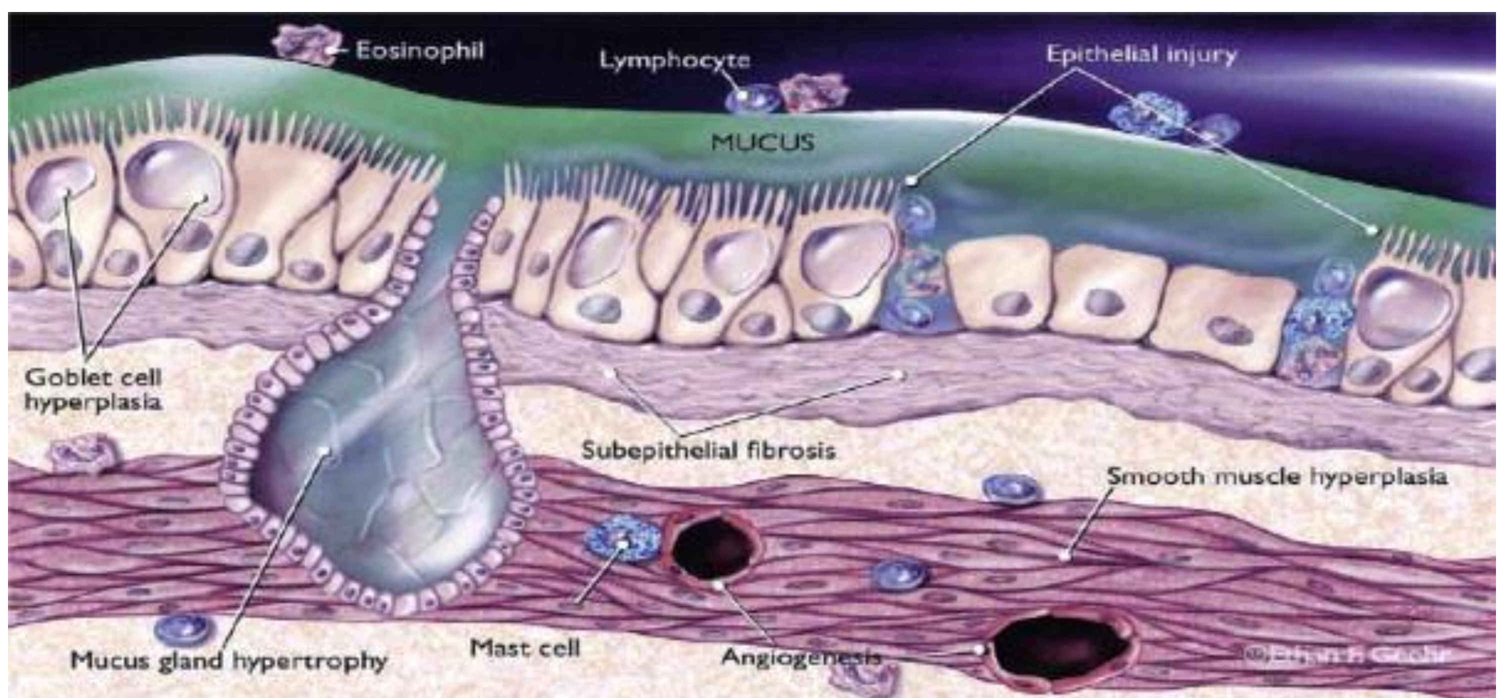


Nat Immunol. 2015 Jan;16(1):45-56.

Definition and Pathophysiology

- Airway remodeling

- **Goblet and mucous gland hyperplasia**: increased sputum, airway narrowing, airway wall thickness
- **Smooth muscle cell hypertrophy and hyperplasia**: inflammation/fibrosis products,
  airway narrowing, airway hyper-responsivessness
- **Angiogenesis**: airway wall edema, mediators delivery
- **Subepithelial fibrosis, Epithelial alteration**: Decreased protective barrier, AHR



J Allergy Clin Immunol. 2011 Sep;128(3):439-48

Definition and Pathophysiology

- Diagnosis of Asthma was based on

1) A history of characteristic symptom patterns
- Wheeze, shortness of breath, cough and/or chest tightness
- Experience more than one of these types of symptoms.
- Often worse at night or in the early morning.
- Vary over time and in intensity
- Triggered by viral infections, exercise, allergen exposure, changes in weather, laughter, or irritants such as car exhaust fumes, smoke or strong smells

Global Strategy for Asthma Management and Prevention. 2021 GINA report

Definition and Pathophysiology

- Diagnosis of Asthma was based on

2) Evidence of variable airflow limitation, Airway hyper-responsiveness (AHR)

→ **Spirometry, bronchodilator reversibility test, peak expiratory flow, Provocation test, Exercise challenge test**

3) Airway inflammation

→ **Sputum eosinophil count, Blood eosinophil count, Fractional Exhaled Nitric Oxide (FeNO), Periostin**

4) Allergy test

→ **Serum specific Immunoglobulin E, Skin prick test**

Global Strategy for Asthma Management and Prevention. 2021 GINA report

Contents

- Definition and Pathophysiology
- Spirometry
- Bronchodilator response, Peak expiratory flow
- Bronchoprovocation challenge
- Sputum, blood eosinophil counts, Fraction of exhaled nitric oxide (FeNO), Periostin
- Serum specific IgE test, Skin prick test

Diagnostic tests of asthma - Spirometry

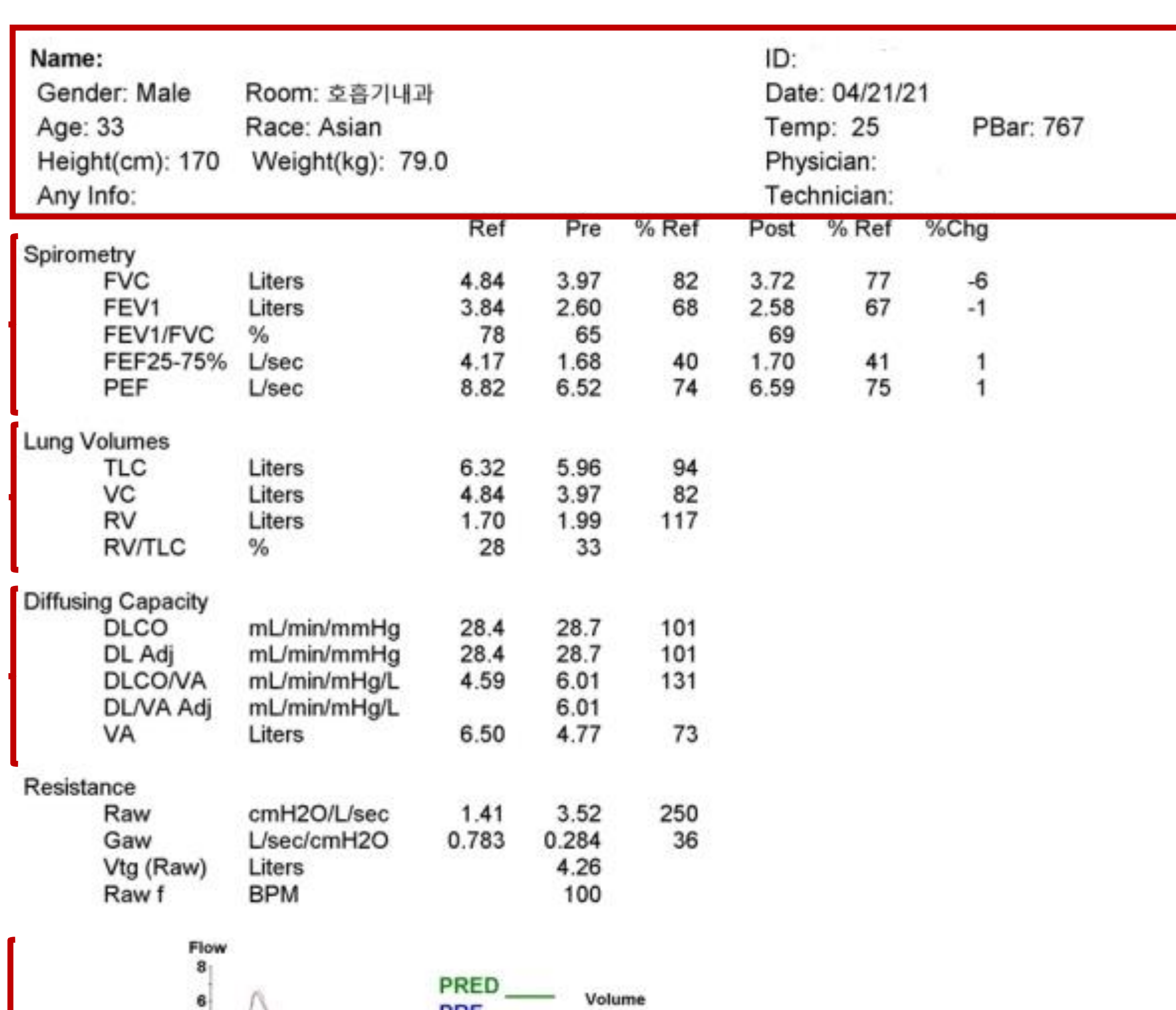
Patient information:
Name, ID, Date, Gender, Age, Race, Height, Weight, Temperature, Atmospheric pressure

Spirometry, Bronchodilator response

Plethysmography

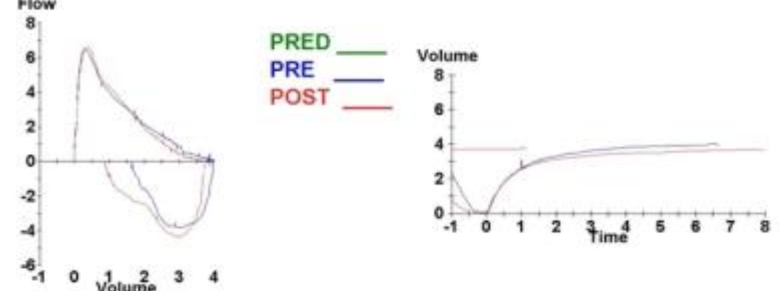
Diffusing capacity of lung for carbon monoxide

Flow-volume curve, Volume-time curve



Name: ID:
Gender: Male Room: 호흡기내과 Date: 04/21/21
Age: 33 Race: Asian Temp: 25 PBar: 767
Height(cm): 170 Weight(kg): 79.0 Physician:
Any Info: Technician:

		Ref	Pre	% Ref	Post	% Ref	%Chg
Spirometry							
FVC	Liters	4.84	3.97	82	3.72	77	-6
FEV1	Liters	3.84	2.60	68	2.58	67	-1
FEV1/FVC	%	78	65		69		
FEF25-75%	L/sec	4.17	1.68	40	1.70	41	1
PEF	L/sec	8.82	6.52	74	6.59	75	1
Lung Volumes							
TLC	Liters	6.32	5.96	94			
VC	Liters	4.84	3.97	82			
RV	Liters	1.70	1.99	117			
RV/TLC	%	28	33				
Diffusing Capacity							
DLCO	mL/min/mmHg	28.4	28.7	101			
DL Adj	mL/min/mmHg	28.4	28.7	101			
DLCO/VA	mL/min/mHg/L	4.59	6.01	131			
DL/VA Adj	mL/min/mHg/L		6.01				
VA	Liters	6.50	4.77	73			
Resistance							
Raw	cmH2O/L/sec	1.41	3.52	250			
Gaw	L/sec/cmH2O	0.783	0.284	36			
Vtg (Raw)	Liters		4.26				
Raw f	BPM		100				



Diagnostic tests of asthma - Spirometry

- Interpretation of PFT
  - Based on comparisons of data measured in an individual patient with reference (predicted) values based on healthy subjects.

  - Predicted values
    1) Obtained from studies of "NORMAL" or "HEALTHY" subjects
    2) With the same anthropometric (sex, age, height and weight) and ethnic characteristics of patient tested.
    3) Calculated with equations derived from large groups of representative sample of healthy subjects.
    4) FVC, FEV_1, FEV_1/FVC should come from same reference source

Eur Respir J 2005; 26: 948–968
DOI: 10.1183/09031936.05.00035205
Copyright©ERS Journals Ltd 2005

SERIES "ATS/ERS TASK FORCE: STANDARDISATION OF LUNG FUNCTION TESTING"
Edited by V. Brusasco, R. Crapo and G. Viegi
Number 5 in this Series

Interpretative strategies for lung function tests

R. Pellegrino, G. Viegi, V. Brusasco, R.O. Crapo, F. Burgos, R. Casaburi, A. Coates, C.P.M. van der Grinten, P. Gustafsson, J. Hankinson, R. Jensen, D.C. Johnson, N. MacIntyre, R. McKay, M.R. Miller, D. Navajas, O.F. Pedersen and J. Wanger

한국인의 정상 폐활량 예측치

[1]서울대학교 보건대학원, [2]산업안전보건연구원
최정근[1], 백도명[1], 이정오[2]

Normal Predictive Values of Spirometry in Korean Population

Jung Keun Choi[1], Domyung Paek[1], Jeoung Oh Lee[2]
[1]School of Public Health, Seoul National University, [3]Occupational Safety and Health Research Institute

Eur Respir J 2005; 26: 511–522.
Tuberculosis and Respiratory Diseases, 58(3), 230.

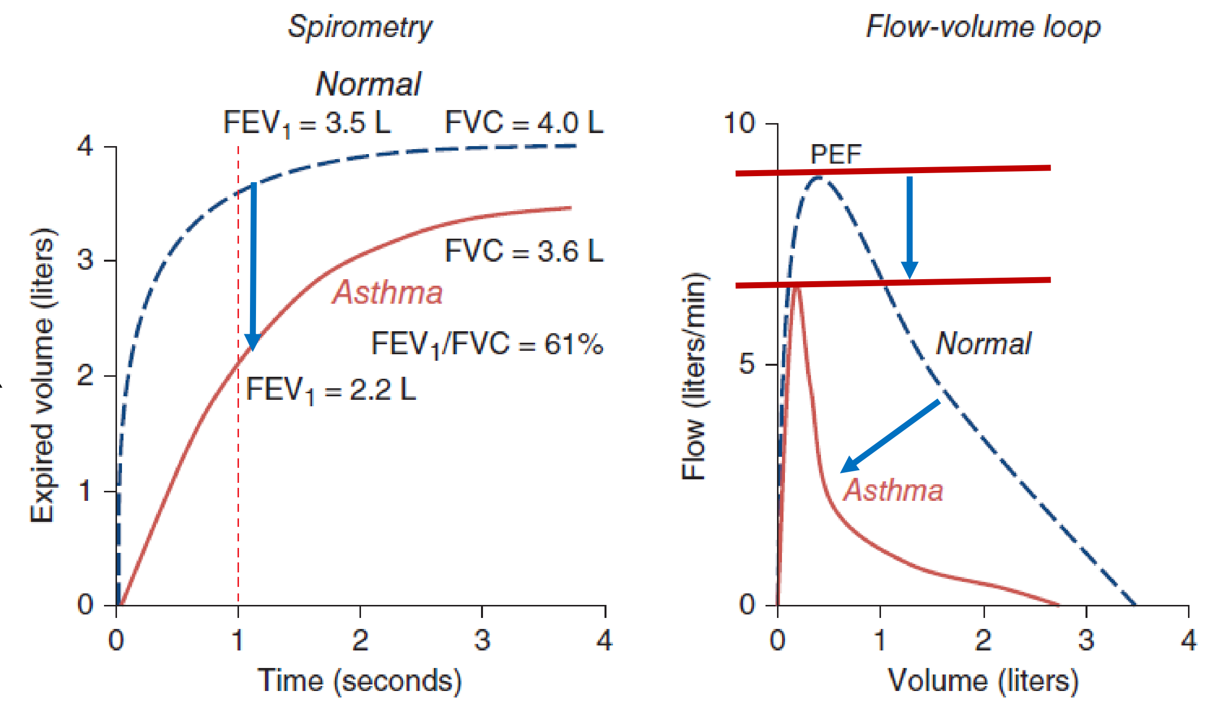
Diagnostic tests of asthma - Spirometry

- Spirometry confirms airflow limitation with a reduced FEV1, FEV1/FVC ratio, and PEF

- Documented expiratory airflow limitation
  - At a time when FEV1 is reduced, confirm that FEV1/FVC is reduced (< 0.75-0.80)

- Reversibility
  - >12% and 200 mL increase in FEV_1
  - 15 min after an inhaled SABA (albuterol 400μg) OR
  - 2~4 week trial of oral corticosteroids (OCS) (prednisone or prednisolone 30–40 mg daily)

  - Diurnal variations in airflow obstruction
  : Measurements of PEF twice daily



- Flow-volume loops: Reduced peak expiratory flow (PEF), reduced maximum expiratory flow (MEF)

Eur Respir J 2005; 26: 511–522.

Contents

- Definition and Pathophysiology
- Spirometry
- Bronchodilator response, Peak expiratory flow
- Bronchoprovocation challenge
- Sputum, blood eosinophil counts, Fraction of exhaled nitric oxide (FeNO), Periostin
- Serum specific IgE test, Skin prick test

Diagnostic tests of asthma – Bronchodilator response

- Increment of FEV_1 < 8% (or < 150mL): within measurement variability

→ "Significant" bronchodilation: FEV_1 >12% and 200mL compared with baseline

Population	Agent/mode of delivery	FVC	FEV_1	$MEF_{25-75\%}$ or $MEF_{50\%}$	Comments
Selected population studies					
1063 subjects 8–75 yrs of age; general population [108]	IP 2 puffs *via* MDI	10.7% (0.40 L)	7.7% (0.31 L)	20%	95th percentile for per cent change from baseline
2609 subjects; random sample of 3 areas in Alberta, Canada [109]	TB 500 µg *via* spacer		Males 9% (0.34 L); females 9% (0.22 L)		95th percentile for per cent change from baseline in asymptomatic never-smokers with FEV_1 >80% pred
75 selected normal subjects [110]	Two puffs *via* MDI	5.1% (0.23 L)	10.1% (0.36 L)	48.3%	Upper 95% CL (two-tailed) for per cent change from baseline
Selected patient studies					
40 patients referred to PFT laboratory [112]	Placebo	14.9% (0.34 L)	12.3% (0.18 L)	45.1%	Upper 95% CI change after placebo
985 COPD patients in the IPPB trial [111]	IP 250 µg *via* air nebuliser		15%		Per cent change from baseline
150 patients with airway obstruction [113]	SB 200 µg or TB 500 µg *via* MDI	15% (0.33 L)	10% (0.16 L)		95% CI for absolute change
78 patients with COPD/asthma [101]	SB 200 µg *via* MDI	14% (0.51 L)	15% (0.25 L)		95% CL per cent change of baseline

Thorax 1992;47:429-436
Eur Respir J 2005; 26: 511–522.

Diagnostic tests of asthma – Bronchodilator response

- Peak expiratory flow (PEF)
  - Absolute changes in PEF: simple technique to diagnose reversible airflow limitation
  - 정상 예측치 보다는 환자의 개인 최고값을 개인의 정상값으로 간주
  - After bronchodilator, improvement in PEF ≥ 60 L/min or ≥ 20%
  - 일중 최대호기유량 변동값 = [최대 호기유량-최소 호기유량]/[(최대 호기유량+최소 호기유량)/2] X 100
  - 2주 이상 평균 일중 최대호기유량 변동값 > 10%

Table 2 Sensitivity, specificity, and predictive values of absolute improvement in peak expiratory flow (PEF) after 400 μg salbutamol

	Improvement in FEV_1					
	% predicted values			Absolute (ml)		
Improvement in PEF (l/min)	≥9	<9	Total	≥190	<190	Total
≥60	21	3	24	22	2	24
<60	10	39	49	17	32	49
Total	31	42	73	39	34	73

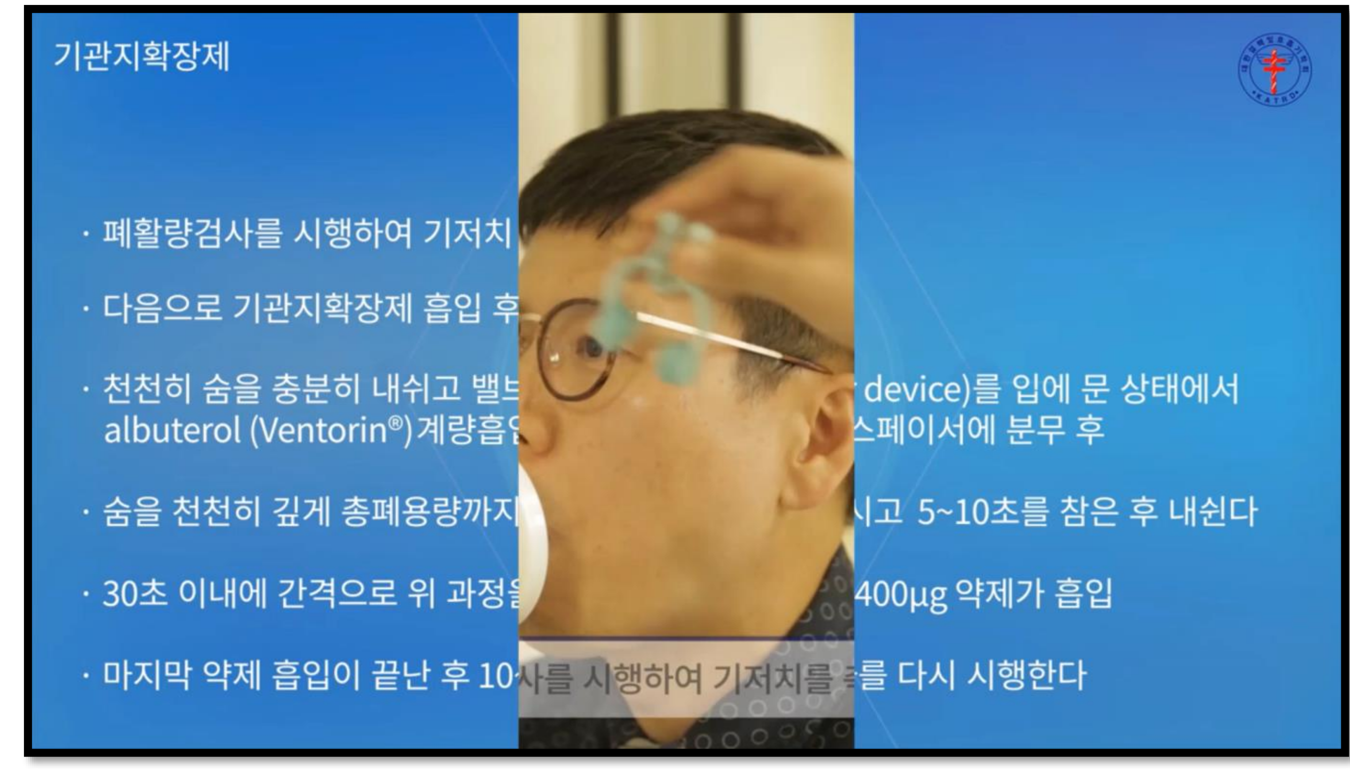
	% predicted values		Absolute (ml)	
	Sensitivity	67·7%	Sensitivity	56·4%
	Specificity	92·9%	Specificity	94·1%
	Positive predictive value	87·5%	Positive predictive value	91·7%
	Negative predictive value	79·6%	Negative predictive value	65·3%

Thorax 1992;47(3):162-166.
천식 진료지침 2020

Diagnostic tests of asthma – Bronchodilator response

- 검사 과정

1) 폐활량검사를 시행하여 기저치 측정
2) 기관지확장제 흡입 후 폐기능 검사과정을 진행
3) Valve spacer device 를 입에 문 상태에서
   Albuterol (Ventolin) 계량흡입기 100μg (1회) 을
   valve spacer 에 분무 후
4) 숨을 천천히 깊게 총폐용량까지 3~5초에 걸쳐서
   들이마시고 5~10초를 참은 후 내쉰다.
5) 30초 이내의 간격으로 위 과정을 4번 반복하여
   총 200~400μg 의 약제를 흡입.
6) 마지막 약제 흡입이 끝난 후 10~20분 사이에
   폐활량검사를 다시 시행



Spirometry (BTPS)

		PRED	PRE-RX BEST	PRE-RX %PRED	POST-RX BEST	POST-RX %PRED	% CHG
FVC	Liters	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
FEV1	Liters	3.24	2.60	80	2.96	92	14
FEV1/FVC	%	86	73		82		
FEF25-75%	L/sec	3.62	2.01	56	2.75	76	36
FET100%	Sec		4.94		3.45		-30
PEF	L/sec	7.00	6.89	98	6.89	98	0
FIVC	Liters	3.39	3.26	96	3.41	100	4
FVL ECode			_ 011		000011		
MVV	L/min	107					

Contents

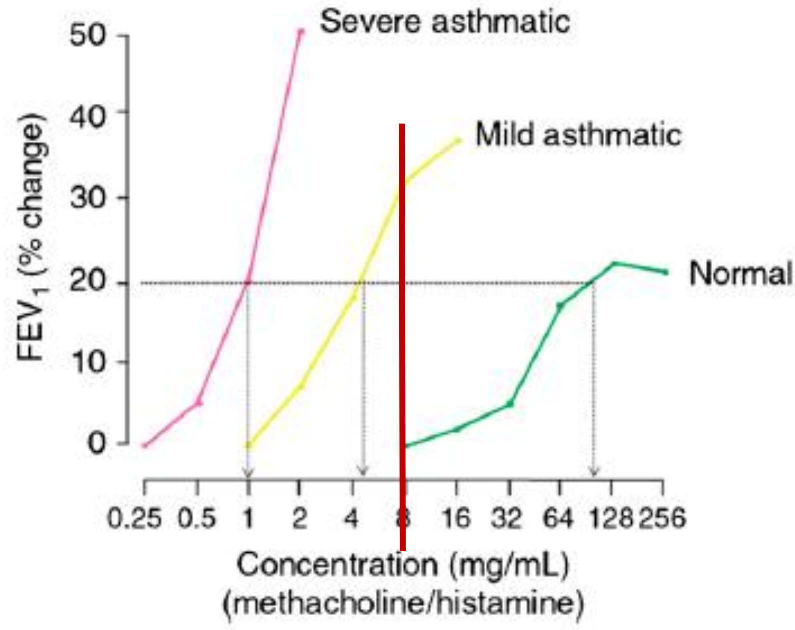
- Definition and Pathophysiology
- Spirometry
- Bronchodilator response, Peak expiratory flow
- Bronchoprovocation challenge
- Sputum, blood eosinophil counts, Fraction of exhaled nitric oxide (FeNO), Periostin
- Serum specific IgE test, Skin prick test

Bronchial provocation test (BPT)

- Useful in a clinical setting for normal spirometry and normal bronchodilator reversibility (BDR) test

- **Direct** Bronchial provocation test (BPT): **Methacholine** or **Histamine**
  → Act directly on receptors on the airway smooth muscle → Contraction

- 20% fall in FEV_1 of provocative concentration (**PC_{20}**): positive test



Dose-response curves to inhaled direct agonists

Front Physiol. 2012 Dec 10;3:460.
CHEST 2010; 138(2)(Suppl):4S–10S

Bronchial provocation test (BPT)

- **Indirect** Bronchial provocation test

: Dry air hyperpnea, stimuli – allergens, osmotic agents (**mannitol** or hypertonic saline)

→ Administered via an aerosol

→ Inflammatory cells

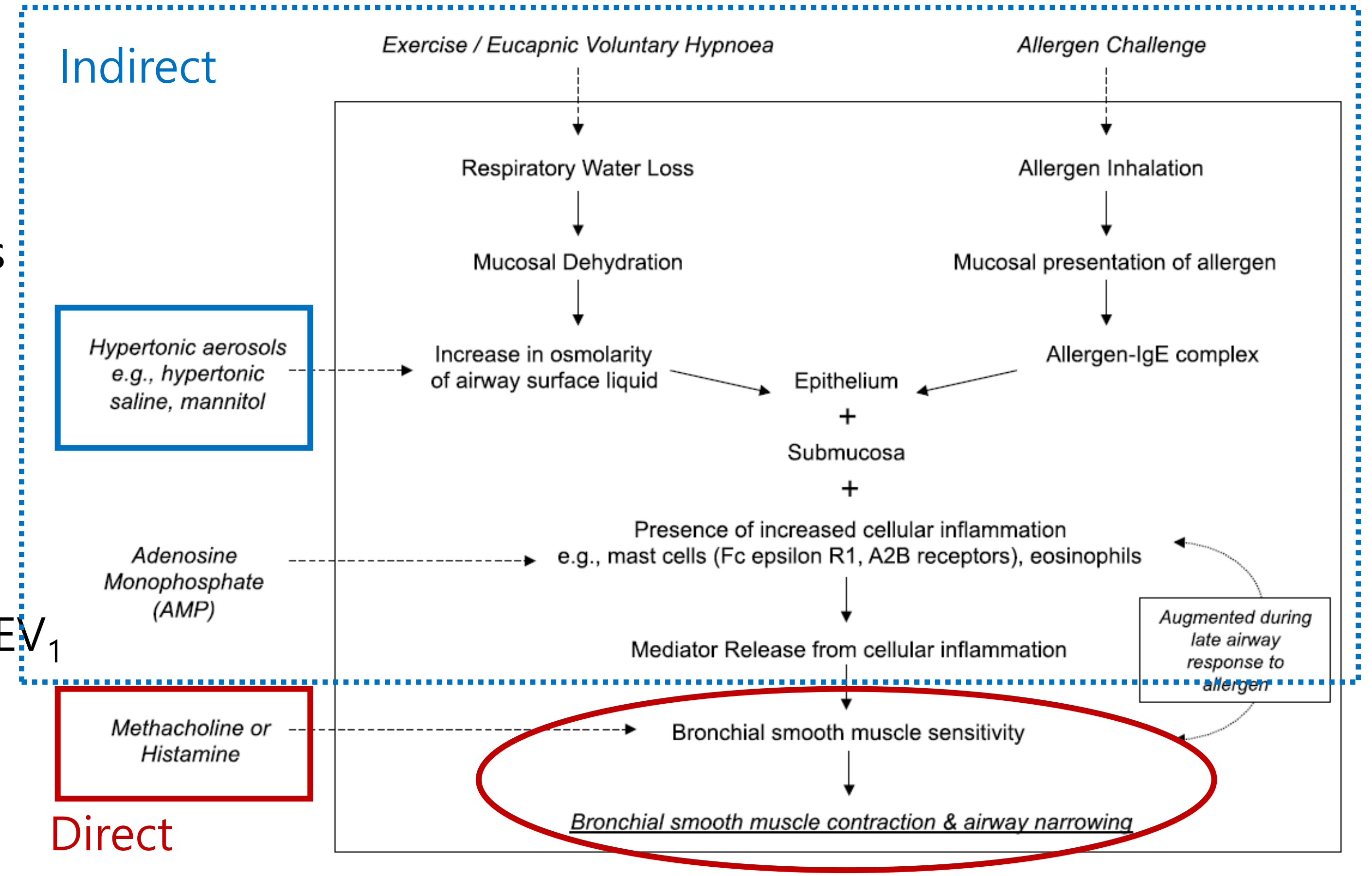
→ Release of bronchoconstriction mediators (histamine, prostaglandins, leukotrienes)

→ Airway smooth muscle contraction

- Airway sensitivity

: provoking dose of stimulus to 15% fall in FEV_1 of the dose-response curve (**PD_{15}**)

- Similar to most clinically relevant stimuli that cause AHR.



Front Physiol. 2012 Dec 10;3:460.

Bronchial provocation test (BPT)

- β_2-agonists, leukotriene antagonist, anticholinergics, ICS

  : protective effect against bronchial smooth muscle contraction (direct stimuli) and indirect stimuli

FACTORS THAT DECREASE BRONCHIAL RESPONSIVENESS

Factor	Minimum Time Interval from Last Dose to Study
Medications	
Short-acting inhaled bronchodilators, such as isoproterenol, isoetharine, metaproterenol, albuterol, or terbutaline	8 h
Medium-acting bronchodilators such as ipratropium	24 h
Long-acting inhaled bronchodilators, such as salmeterol, formoterol, tiotropium	48 h (perhaps 1 wk for tiotropium)
Oral bronchodilators	
Liquid theophylline	12 h
Intermediate-acting theophyllines	24 h
Long-acting theophyllines	48 h
Standard β_2-agonist tablets	12 h
Long-acting β_2-agonist tablets	24 h
Cromolyn sodium	8 h
Nedocromil	48 h
Hydroxazine, cetirizine	3 d
Leukotriene modifiers	24 h
Foods	
Coffee, tea, cola drinks, chocolate	Day of study

TABLE 1 Withholding times prior to indirect challenge testing

	Withholding time	Maximum duration of protection[#]
SABA (albuterol, terbutaline)	8 h	<6 h
LABA (salmeterol, eformoterol)	36 h	12 h
LABA in combination with an ICS (salmeterol/fluticasone, formoterol/budesonide)	36 h	NA
Ultra-LABAs (indacaterol, olodaterol, vilanterol)	48 h	NA
ICS (budesonide, fluticasone propionate, beclomethasone)	6 h	NA
Long-acting ICS (fluticasone furoate, ciclesonide)	24 h	NA
Leukotriene receptor antagonists (montelukast, zafirlukast)	4 days	24 h
Leukotriene synthesis inhibitors (zileuton/slow-release zileuton)	12 h/16 h	4 h
Antihistamines (loratadine, cetirzine, fexofenadine)	72 h	<2 h
Short-acting muscarinic acetylcholine antagonist (ipratropium bromide)	12 h	<0.5 h
Long-acting muscarinic acetylcholine antagonist (tiotropium bromide, aclidinium bromide, glycopyrronium)	72 h	NA
Cromones (sodium cromoglycate, nedocromil sodium)	4 h	2 h
Xanthines (theophylline)	24 h	NA
Caffeine	24 h	NA
Vigorous exercise	4 h	<4 h

Front Physiol. 2012 Dec 10;3:460.
Eur Respir J 2018; 52: 1801033

Bronchial provocation test (BPT): Methacholine

- Diluted methacholine solution

- FEV1 값이 20% 이상 감소하지 않는다면
 연무기를 비운 후 다음 농도의 메타콜린 용액으로 반복
- 0.0625, 0.25, 1, 4, 16, 25 mg/mL
- FEV1 값이 기저치보다 20% 이상 감소할 경우 검사를 중단
- 속효성 베타 항진제를 흡입하고 5분, 10분 후 폐기능을
 측정하여 기도폐쇄의 호전여부를 평가

Table 2. Contraindications for methacholine provocation testing

Absolute contraindications
Severe airflow limitation (FEV_1 < 50% predicted or < 1.0 L)
Heart attack or stroke in last 3 months
Uncontrolled hypertension
Known aortic aneurysm
Relative contraindications
Moderate airflow limitation (FEV_1 < 60% predicted or < 1.5 L)
Inability to perform acceptable-quality spirometry
Pregnancy or nursing mothers
Nursing mothers
Current use of cholinesterase inhibitor medication (for myasthenia gravis)

TABLE 4 Example of doses using the English Wright nebuliser for 2 min

Doubling increments		Quadrupling increments	
Concentration mg·mL^{-1}	Dose µg	Concentration mg·mL^{-1}	Dose µg
0.0625	1.425	0.0625	1.425
0.125	2.969		
0.25	5.938	0.25	5.938
0.5	11.875		
1	23.75	1	23.75
2	47.5		
4	95	4	95
8	190		
16	380	16	380

Shown are the incremental doses at specific concentrations for both doubling and quadrupling increments.

Eur Respir J 2017; 49: 1601526
Allergy Asthma Respir Dis 6(1):14-25

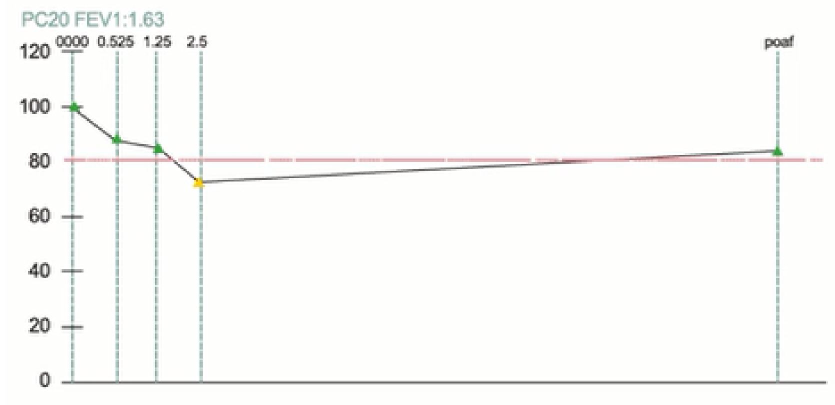
Bronchial provocation test (BPT): Methacholine

- PC_{20} < 8 mg/mL: positive test, PC_{20} 8~16 mg/mL: borderline
- A normal (negative) methacholine challenge (PC_{20} >16 mg/mL) effectively excludes current asthma
- A methacholine PC_{20} <1 mg/mL is highly specific for a diagnosis of asthma
- High diagnostic sensitivity: 77% in asthmatics taking regular ICS
- Sensitivity: Atopic (82%) vs. Non-atopic (52%)
- Exercise-induced bronchoconstriction (EIB)

: No AHR to direct stimuli → Low sensitivity to EIB

		Ref	pre Meas	Baseline Meas	Level 1 Meas	Level 2 Meas	Level 3 Meas	Level 4 Meas	Level 5 Meas	Level 6 Meas	Level 7 Meas	Level 8 Meas	Level 9 Meas	Post Meas
Dose					0.625	1.25	2.5							
FVC	Liters	3.88	3.83	3.79	3.58	3.52	3.16							3.67
%Ref			99	98	92	91	81							95
%Chg				-1	-6	-7	-17							-3
Dose					0.625	1.25	2.5							
FEV_1	Liters	3.31	3.41	3.01	2.65	2.55	2.18							2.55
%Ref			103	91	80	77	66							77
%Chg				-12	-12	-15	-28							-15
Dose					0.625	1.25	2.5							
$FEF_{25-75\%}$		3.40	4.38	2.93	2.11	1.85	1.64							1.72
%Ref			129	86	62	54	48							50
%Chg				-33	-28	-37	-44							-41
Dose					0.625	1.25	2.5							
PEF	Liters	6.62	7.17	6.93	5.67	5.73	3.54							5.54
%Ref			108	105	86	87	53							84
%Chg				-3	-18	-17	-49							-20

PD_{20} µmol (µg)	PC_{20} mg·mL^{-1}	Interpretation
>2 (>400)	>16	Normal
0.5–2.0 (100–400)	4–16	Borderline AHR
0.13–0.5 (25–100)	1–4	Mild AHR
0.03–0.13 (6–25)	0.25–1	Moderate AHR
<0.03 (<6)	<0.25	Marked AHR



Eur Respir J 2017; 49: 1601526
Allergy Asthma Respir Dis 2018; 6(1):14-25

Bronchial provocation test (BPT): Mannitol

- Absolute contraindication
  - Hypersensitivity to mannitol or gelatin
  - Heart attack or stroke in last 6 months
  - Uncontrolled HTN
  - Known cerebral aneurysm, aortic aneurysm

- Relative contraindication
  - Airflow limitation (FEV_1 < 70% or < 1.5L)
  - Pneumothorax, hemotysis
  - Inability to perform acceptable quality spirometry
  - Recent abdominal, thoracic surgery
  - Airway infection in last 2 weeks

Eur Respir J 2018; 52: 1801033
Allergy Asthma Respir Dis 6(1):14-25

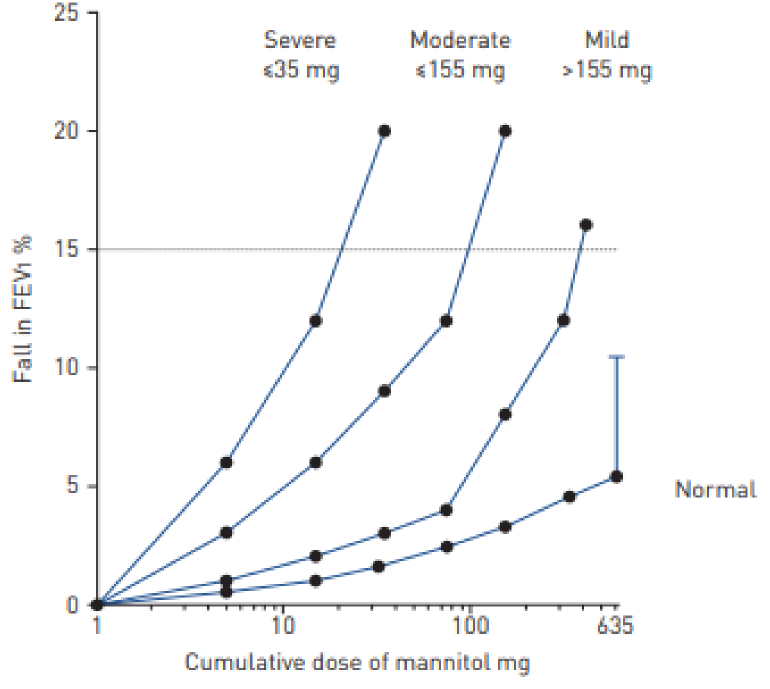
Bronchial provocation test (BPT): Mannitol

- 5, 10, 20, 40, 80, 160, 160, 160mg (total 635mg)
- Positive mannitol test: ≥15% fall within FEV_1 ≤ 635mg (cumulative dose)
- Cumulative dose 가 635 mg 에 이르거나, PD_{15} 가 정해지면 검사 종료
- Response dose ratio (RDR)

 - FEV_1 이 기저치의 15% 만큼 감소되는 시점의 FEV_1 (%) / mannitol dose

 - Mannitol 635mg 사용 후 FEV1 이 15% 감소시

 RDR = 15%/635mg = 0.024 %/mg



Eur Respir J 2018; 52: 1801033
Allergy Asthma Respir Dis 6(1):14-25

Bronchial provocation test (BPT): Mannitol

- High specificity: detecting asthma patients with significant disease activity
- Negative mannitol test
  - A low level of disease activity with no requirement for anti-inflammatory therapy
- Represent airway inflammation that will respond to steroid treatment
- Important role in detecting exercise induced bronchospasm (EIB) in subjects with exercise-related symptoms

Table 5. Interpretation of mannitol provocation test

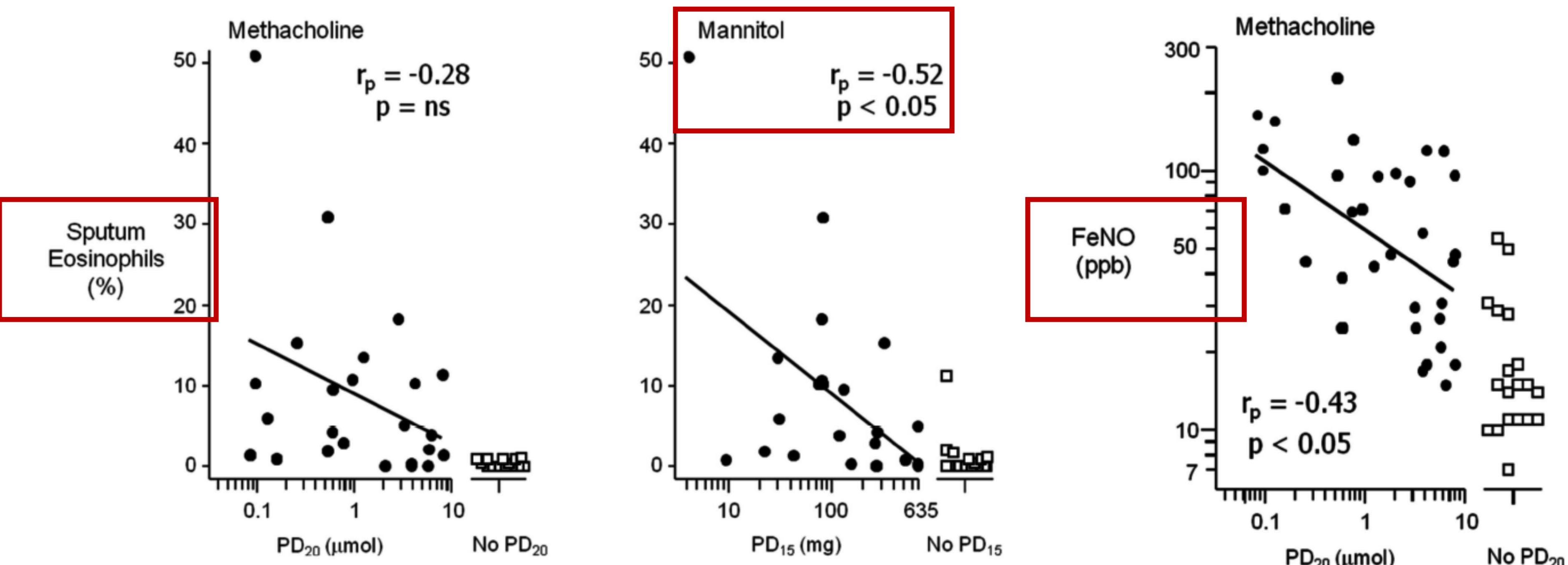
	Patients with clinical diagnosis of asthma			
	Positive for mannitol provocation		Negative for mannitol provocation	
	Not using ICS	Using ICS	Not using ICS	Using ICS
Interpretation	Patient with active airway inflammation	Patient has active airway inflammation despite current ICS therapy	Airway inflammation not detected	Patient's asthma is controlled on current ICS therapy
Response or planned action	Consider using ICS	Consider increasing ICS dose, ascertain adherence to therapy	Consider alternative diagnosis	Consider maintaining or reducing dose of ICS
	Patients with suspected asthma			
	Positive for mannitol provocation		Negative for mannitol provocation	
Interpretation	Asthma likely		Asthma cannot be ruled out	
Response or planned action	Consider using ICS		Further diagnostic work-up (e.g. peak flow monitoring) If symptoms are uncharacteristic, consider alternative diagnosis	

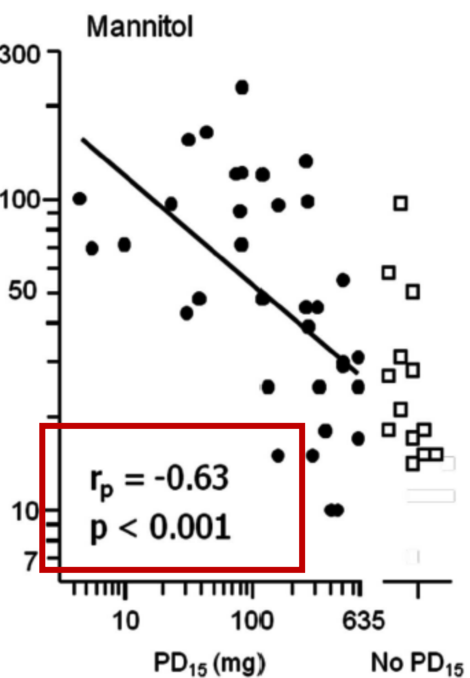
Eur Respir J 2018; 52: 1801033
Allergy Asthma Respir Dis 6(1):14-25

Bronchial provocation test (BPT)

- The relationship between the airway sensitivity to inhaled methacholine and mannitol compared to the sputum eosinophils and the FeNO in steroid naive asthmatic subjects

- Positive mannitol test: ongoing eosinophilic airway inflammation
- Negative mannitol test: very low likelihood of airway eosinophilia





Front Physiol. 2012 Dec 10;3:460.

Bronchial provocation test (BPT)

- Methacholine: 음성이면 천식이 아닐 가능성이 높음 (high negative predictive value), false-positive 가능
  → 천식을 배제하는 데 유용
- Mannitol: 양성이면 천식일 가능성이 높음 (high specificity), 음성이어도 천식을 배제할 수 없음
  → 천식을 진단하는 데 유용

Table 1 Specificity and sensitivity for mannitol and methacholine to identify a physician diagnosis of asthma in a variety of patient groups

	Entry	Number with Dx of asthma	Specificity for Dx of asthma	Sensitivity for Dx of asthma	Number taking ICS	Source
$N=592$	Asthmatics and healthy					Brannan *et al.* [46]
Mannitol		487	94.5	59.8	363	
excluding Mann −ve taking ICS		328	95.0	89.0	159	
$N=101$	Firefighters	?				Miedinger *et al.* [2]
Mannitol			97	92		
Methacholine			97	78		
$N=238$	Civil registration list	51			8	Sverrild *et al.* [47••]
Mannitol			98	59		
Mech 8 μmol			80	69		
$N=375$	Sx but no Dx of asthma	240			0	Anderson *et al.* [9••]
Mannitol			73	56		
Mech 16 mg/ml			75	51		
$N=115$ children	Sx but no Dx of asthma				0	Anderson *et al.* [9••]
Mannitol			81.4	63.2		
Mech 16 mg/ml			62.9	66.2		

Curr Opin Allergy Clin Immunol. 2011, Feb;11:46–52

Contents

- Definition and Pathophysiology
- Spirometry
- Bronchodilator response, Peak expiratory flow
- Bronchoprovocation challenge
- Sputum, blood eosinophil counts, Fraction of exhaled nitric oxide (FeNO), Periostin
- Serum specific IgE test, Skin prick test

Sputum eosinophil counts

- Sputum eosinophilia

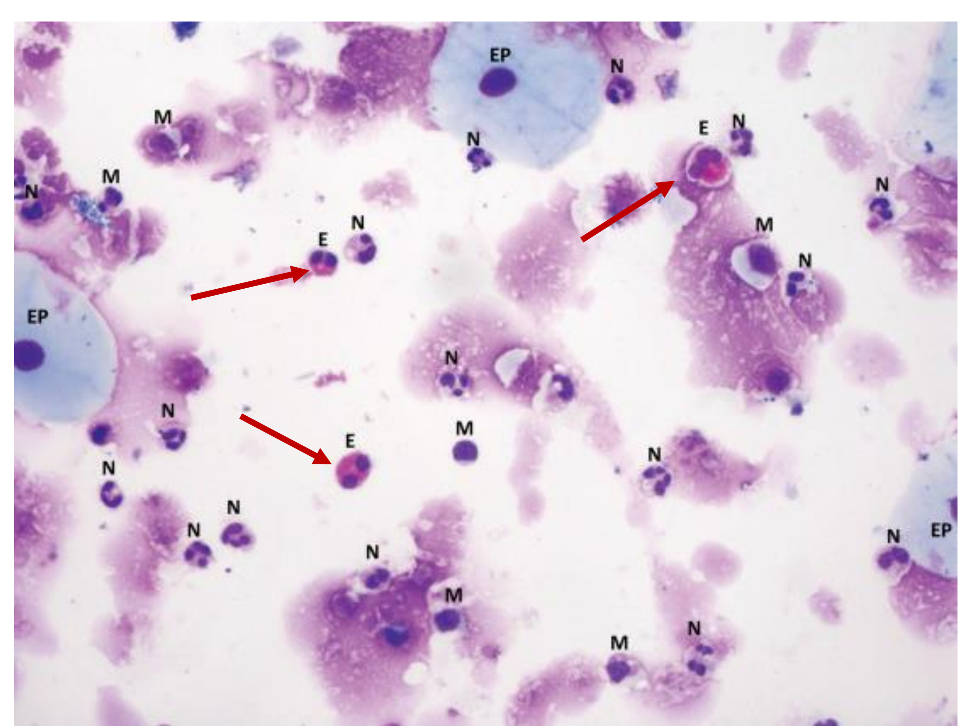
- Common feature of asthma with variable airflow limitation
- Lower respiratory secretions: eosinophilic bronchitis, chronic cough, COPD
- Sputum eosinophil (%) = Eosinophil counts / Total cell counts (Epithelial cells + inflammatory cells)

: **Excess proportion of eosinophils (≥3%)** in spontaneous or induced sputum

- Uncontrolled asthma, eosinophilic bronchitis
- Allergen or chemical sensitizer exposure
- Steroid-reactive chronic airway obstruction

Table 4. Report form of induced sputum processing

	Cell counts	%
Bronchial epithelial cells		
Macrophages		
Neutrophils		
Eosinophils		
Lymphocytes		
Total cell counts	400 (500)*	100



Am J Respir Crit Care Med 1999;160:S53–S57.
Allergy Asthma Respir Dis. 2017 Nov;5(6):307-311.

Sputum eosinophil counts

- Sputum induction

1) FEV_1, FVC: first measured to identify the presence and severity of any airflow limitation.
2) SABA (e.g. salbutamol, 200 μg): delivered to inhibit any possible bronchoconstriction from the saline inhalation
3) FEV_1 measurement after 10 minutes of inhalation of pre-treatment Salbutamol
4) Set the ultrasonic nebulizer output (≥ 1 mL/min) and sterile hypertonic saline (4.5% saline)
5) Start inhalation of saline with tidal breathing through a nebulizer.
   The sputum is collected every 5 minutes by coughing, during 15 to 20 minutes in total.
6) Measure FEV_1 (or PEF) every 5 minutes after the start of the test.
   Examination should be stopped when symptoms occur or FEV_1 is reduced by more than 20% of postbronchodilator FEV_1.

AM J RESPIR CRIT CARE MED 1999;160:S53–S57.
Allergy Asthma Respir Dis. 2017 Nov;5(6):307-311.

Sputum eosinophil counts

- Disadvantage
  - The requirement of lab facilities, the duration of the analyses
    → Difficult to use in actual clinical practice.

  - Induction of sputum can be problematic in patients with severe and uncontrolled asthma
    → hypertonic saline induced bronchoconstriction

- Adequate surrogate markers of eosinophilic inflammation in asthma
  : Blood eosinophil, FeNO, Periostin

Table 2. INDUCED SPUTUM: MARKERS OF INFLAMMATION (MEDIAN)*

	Healthy	Stable Asthma	Smokers' Bronchitis		
			No CAL	CAL − Eo	CAL + Eo
TCC, × 10^6/g	3.1	3.3	3.9	11.1	8.3
Eosinophils, %	0.5	5.2	0.3	1	5.4
Neutrophils, %	24.5	46.9	33	83.4	73.4
ECP, µg/L	288	1,040	352	2,560	5,240
Elastase, µg/L	297	534	241	2,512	2,996
Fibrinogen, µg/L	440	2,080	708	18,100	24,600
IL-5, pg/ml	UL	46.3	UL	UL	UL

Definition of abbreviations: CAL = chronic airflow limitation; ECP = eosinophil cationic protein; Eo = eosinophilia (> 3%) in sputum; TCC = total cell count; UL = under limit of detection.

* Data from References 5 and 7.

Thorax, 70 (2015), pp. 115-120
Am J Respir Crit Care Med 1999;160:S53–S57.

Blood eosinophil counts

- Blood eosinophil counts correlated with sputum eosinophilia
- Effector cells in Th2-mediated inflammation
- Cutoff between 250 cells/μL and 300 cells/μL (less than 5% of the leukocytes)

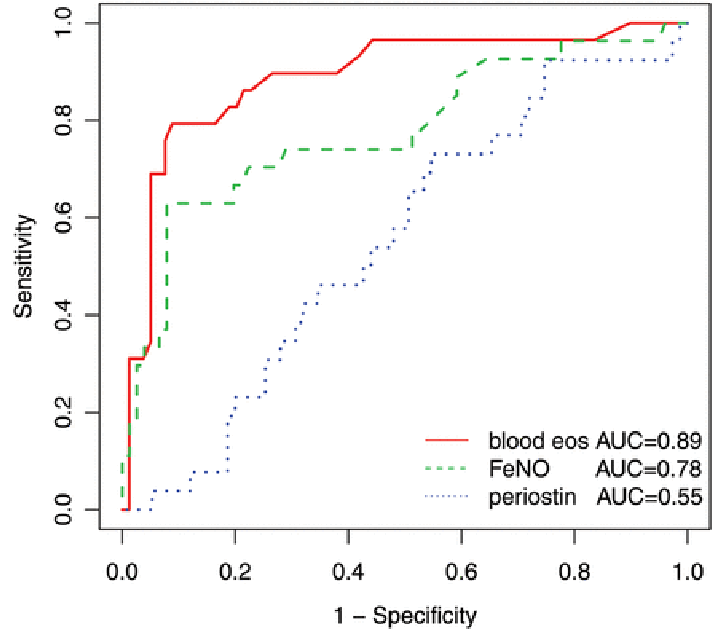


Table 2 Sensitivity, specificity, PPV and NPV of different surrogate markers using alternative cut-points to diagnose eosinophilic airway inflammation (less than, more than or equal to 3% sputum eosinophils)

	Threshold	Sensitivity	Specificity	PPV	NPV
Blood eosinophils	$>0.22\times10^9$/L	86	79	60	93
Blood eosinophils	$\geq0.25\times10^9$/L	79	84	64	91
Blood eosinophils	$\geq0.27\times10^9$/L	78	91	79	91
FE_{NO} level	>20 ppb	74	57	40	87
FE_{NO} level	≥24 ppb	74	63	42	87
FE_{NO} level	≥42 ppb	63	92	74	89
FE_{NO} level	>50 ppb	56	92	67	84

Thorax 2015;70:115–120.
J Allergy Clin Immunol Pract. 2020 Feb;8(2):442-450.

Blood eosinophil counts

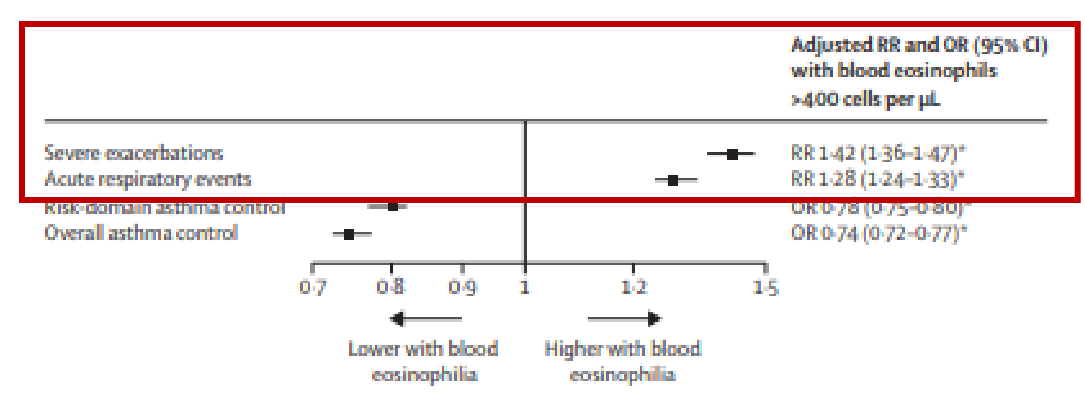
- Associated with airway eosinophilic inflammation and treatment response

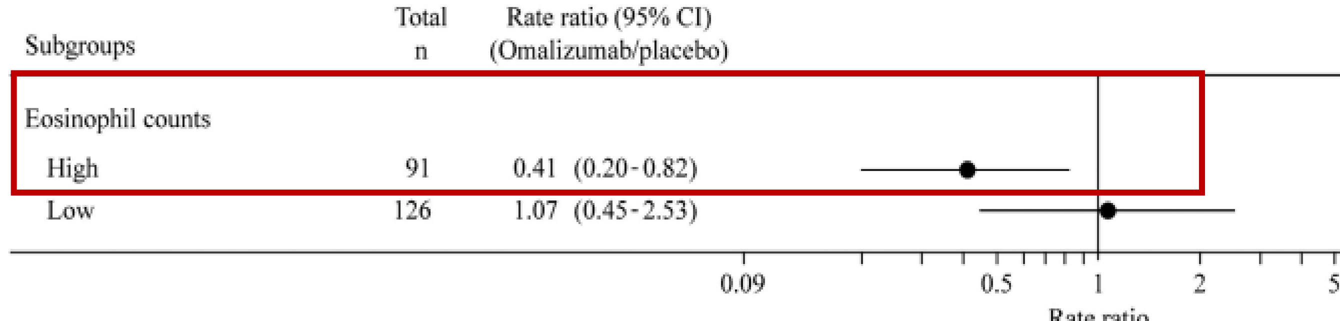
- Higher baseline blood eosinophil counts
  - Greater risk of exacerbation/predict future exacerbations
  - Predicts responsiveness to anti-IgE, anti-IL5 therapeutic targets

Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study

David B Price, Anna Rigazio, Jonathan D Campbell, Eugene R Bleecker, Christopher J Corrigan, Mike Thomas, Sally E Wenzel, Andrew M Wilson, Mary Buatti Small, Gokul Gopalan, Valerie L Ashton, Anne Burden, Elizabeth V Hillyer, Marjan Kerkhof, Ian D Pavord

Interpretation Patients with asthma and blood eosinophil counts greater than 400 cells per μL experience more severe exacerbations and have poorer asthma control. Furthermore, a count–response relation exists between blood eosinophil counts and asthma-related outcomes. Blood eosinophil counts could add predictive value to Global Initiative for Asthma control-based risk assessment.

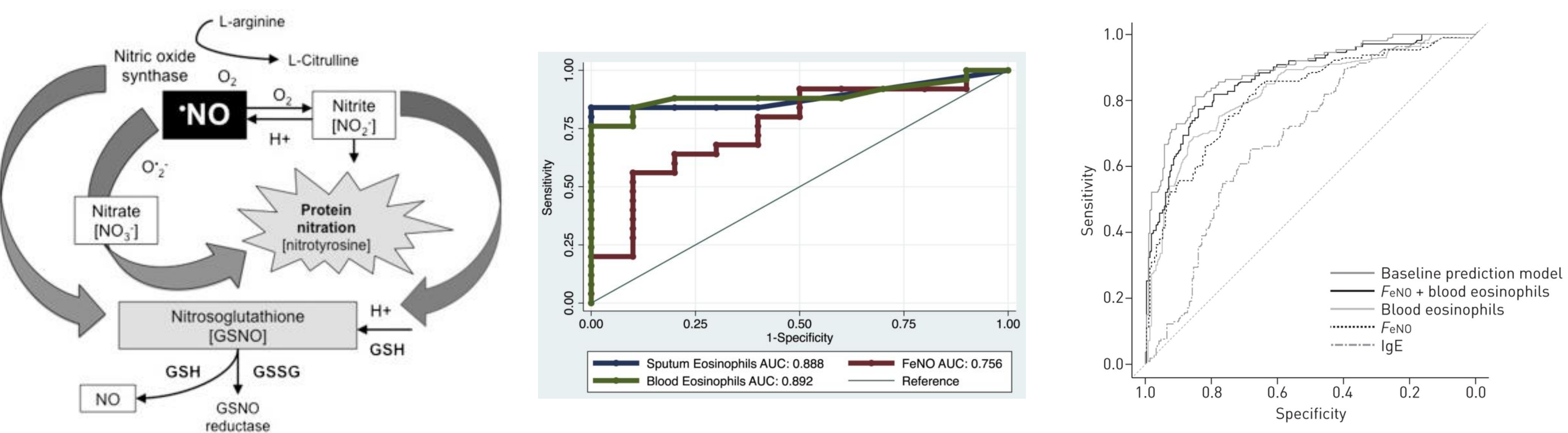




Lancet Respir Med 2015; 3: 849–58
Current Drug Targets, 2018, Vol. 19, No. 16

Fractional exhaled nitric oxide (FeNO)

- Noninvasive measure of airway Th2 high inflammation, biomarker of asthma severity
- Airway epithelial exposure to IL-13 → nitric oxide synthase upregulation → nitric oxide
- Modestly associated with levels of sputum and blood eosinophils
- Elevated in non-asthma conditions (e.g. eosinophilic bronchitis, atopy, allergic rhinitis)
- Useful in demonstrating insufficient anti-inflammatory therapy and may be useful in down-titrating ICS.



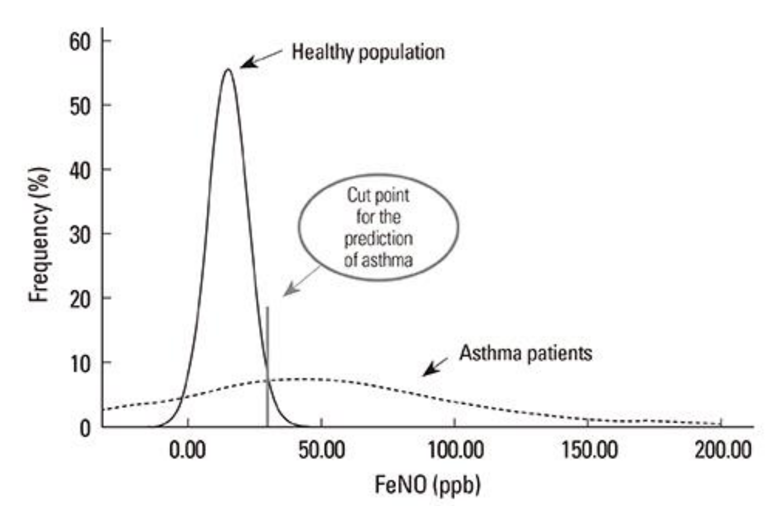
J Allergy Clin Immunol, 133 (2014), pp. 388-394

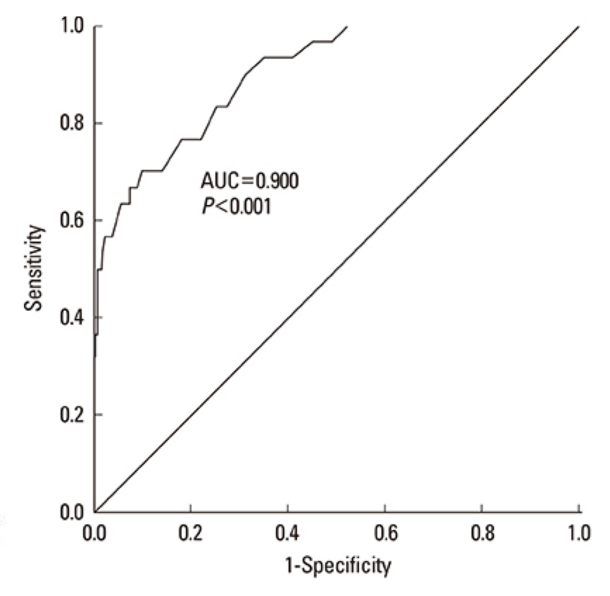
Thorax, 70 (2015), pp. 115-120 Eur Respir J, 46 (2015), pp. 688-696

Fractional exhaled nitric oxide (FeNO)

- In Korea, Ansung cohort
- Age ≥ 19 years
- N=570 (mean age, 59.9±12.3; male, 37.0%)
- Investigated FeNO reference ranges and determinant
- ROC curve: AUC = 0.900

	Sensitivity (%)	Specificity (%)
FeNO in males (n=241)		
>37.4 ppb*	60	95.3
>30.5 ppb†	70.0	90.0
FeNO in females (n=403)		
>27.0 ppb*	65.9	95.3
>20.5 ppb†	79.5	86.9





Study	Population	Demographics	Cutoff values	Sensitivity	Specificity
Oh et al. (2008)[53]	Patients with cough >3 wk (n=211)	Adults (mean, 46 yr)	31.7 ppb	86%	76%
Yune et al. (2015)[19]	Patients with symptoms suggestive of asthma (n=40)	Adults (mean, 53 yr)	37.5 ppb	90%	81%

Allergy Asthma Immunol Res. 2014 Nov;6(6):504-510.
Allergy Asthma Respir Dis 2017;5:185-192.

Fractional exhaled nitric oxide (FeNO)

- 정상군과 환자군 간 overlap: 정상 참고치 보다는, 진단 목적의 cutoff value 로 활용
- High specificity

→ FeNO 가 높으면 천식 가능성을 높게 추정. FeNO 가 낮게 측정되어도 천식을 배제할 수 는 없음.

- Methacholine provocation test 와 상호 보완적.

→ FeNO 가 일정 cutoff value 이상의 양성인 경우 provocation 의 필요가 줄어든다.

- >50 ppb: 호산구성 기도염증 가능성이 상당히 높다.

  <25 ppb: 호산구성 기도염증 가능성이 상당히 낮다.

  25-50 ppb: 검사 조건과 임상 상황을 고려하여 주의 깊게 해석

Fractional exhaled nitric oxide values	Allergic airway inflammation	Consideration
>50 ppb (in adults) >35 ppb (in children)	Likely	Increase inhaled corticosteroids Check inhaler errors Check adherence Control allergen exposure (if patients have allergic asthma) Consider corticosteroid-resistant eosinophilic asthma
25–50 ppb (in adults) 20–35 ppb (in children)	Possible; cautious interpretation is recommended.	Increase inhaled corticosteroids Check inhaler errors Check adherence Control allergen exposure (if patients have allergic asthma)
<25 ppb (in adults) <20 ppb (in children)	Less likely	Review asthma diagnosis Consider noneosinophilic asthma Consider comorbid diseases like rhinosinusitis, gastroesophageal reflux diseases, or cardiac diseases

Allergy Asthma Immunol Res. 2014 Nov;6(6):504-510.
Allergy Asthma Respir Dis 2017;5:185-192.

Periostin

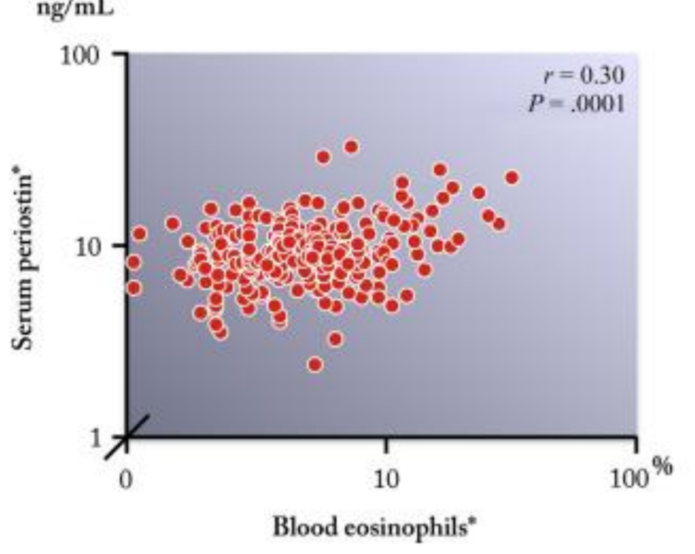
- Extracellular protein secreted from bronchial epithelial cells through stimulation by IL-13
- Serum periostin levels: associated with blood eosinophil counts and serum IgE
- Airway eosinophilia refractory to high dose ICS → higher serum periostin level

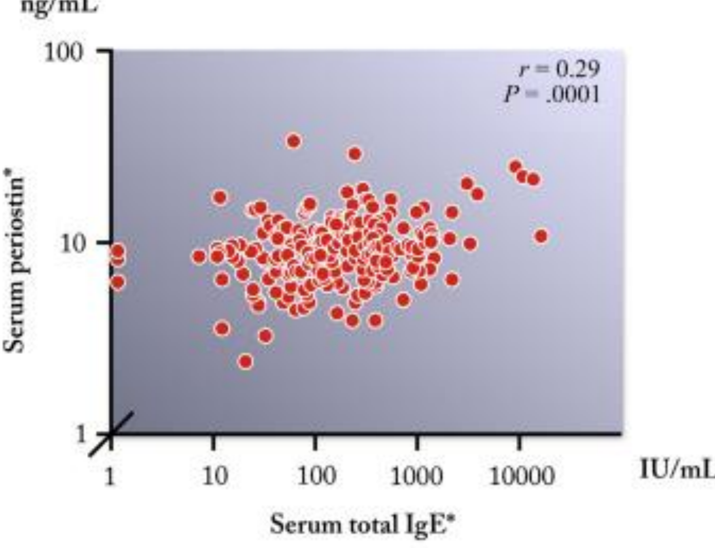
→ Useful biomarker for predicting airway eosinophilia and asthma exacerbation

Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids

Yoshihiro Kanemitsu, MD,[a,b]* Hisako Matsumoto, MD, PhD,[a,b]* Kenji Izuhara, MD, PhD,[c] Yuji Tohda, MD, PhD,[b,d] Hideo Kita, MD, PhD,[b,e] Takahiko Horiguchi, MD, PhD,[b,f] Kazunobu Kuwabara, MD, PhD,[b,f] Keisuke Tomii, MD, PhD,[b,g] Kojiro Otsuka, MD, PhD,[a,b,g] Masaki Fujimura, MD, PhD,[b,h] Noriyuki Ohkura, MD, PhD,[b,h] Katsuyuki Tomita, MD, PhD,[b,d] Akihito Yokoyama, MD, PhD,[b,i] Hiroshi Ohnishi, MD, PhD,[b,i] Yasutaka Nakano, MD, PhD,[b,j] Tetsuya Oguma, MD, PhD,[b,j] Soichiro Hozawa, MD, PhD,[b,k] Tadao Nagasaki, MD,[a] Isao Ito, MD, PhD,[a] Tsuyoshi Oguma, MD,[a] Hideki Inoue, MD,[a] Tomoko Tajiri, MD,[a] Toshiyuki Iwata, MD,[a] Yumi Izuhara, MD,[a] Junya Ono, BS,[l] Shoichiro Ohta, MD, PhD,[m] Mayumi Tamari, MD, PhD,[n] Tomomitsu Hirota, DDS, PhD,[n] Tetsuji Yokoyama, MD, PhD,[o] Akio Niimi, MD, PhD,[a,b,p] and Michiaki Mishima, MD, PhD[a,b] *Kyoto, Saga, Osaka, Aichi, Hyogo, Kanazawa, Kochi, Shiga, Hiroshima, Kanagawa, and Saitama, Japan*

Conclusions: Serum periostin appears to be a useful biomarker for the development of airflow limitation in asthmatic patients on ICS. (J Allergy Clin Immunol 2013;132:305-12.)





J Allergy Clin Immunol 2013;132;305-12.

Periostin

- Serum periostin levels are relatively stable and less variable than FeNO
  → Advantageous for long-term monitoring
- FeNO levels weakly associated with serum periostin levels, stronger in treatment step 4 or 5
- Measured with ELISA
  → available for research purposes only, no clinically marketed assay is available

- Reference range: 35.0 ~ 71.1 ng/mL (90% CI)

doi: 10.1111/cea.12763
Clinical & Experimental Allergy, 46, 1303–1314
© 2016 The Authors. Clinical & Experimental Allergy
Published by John Wiley & Sons Ltd.

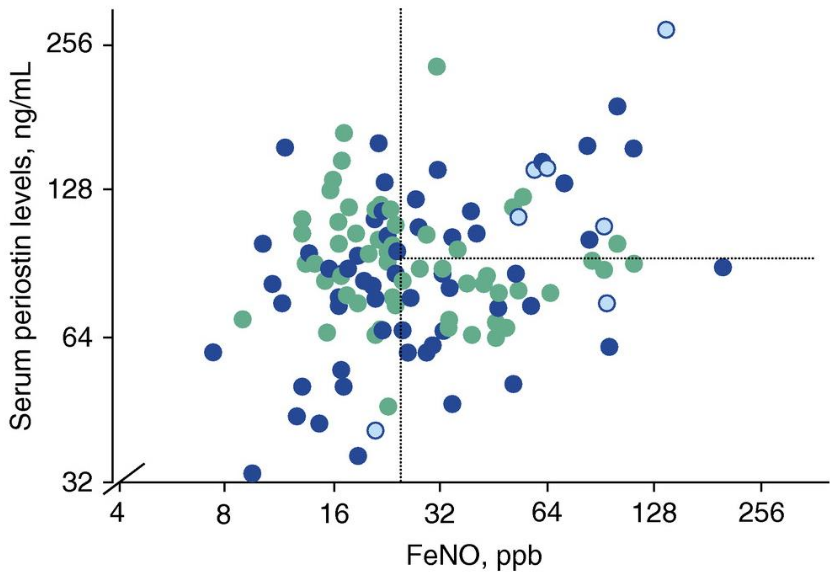
ORIGINAL ARTICLE Asthma and Rhinitis

Reference ranges for serum periostin in a population without asthma or chronic obstructive pulmonary disease

R. Caswell-Smith[1,2], A. Hosking[1,3], T. Cripps[1], C. Holweg[4], J. Matthews[4], M. Holli
I. Braithwaite[1,2,6] and R. Beasley[1,2,6], On behalf of the Periostin Study Team
[1]Medical Research Institute of New Zealand, Wellington, New Zealand, [2]Victoria University of
Auckland, Auckland, New Zealand, [4]Genentech Inc, San Francisco, CA, USA, [5]University of Ota
Health Board, Wellington, New Zealand

Table 2. Serum periostin levels by sex and age*

Variable	Mean (SD)	Median (IQR)	Min to Max
Periostin			
Male n = 239	50.6 (11.8)	49.0 (41.8 to 57.6)	29.8 to 105.7
Female n = 240	51.5 (10.8)	50.7 (44.0 to 56.5)	28.1 to 92.8



Am J Respir Crit Care Med, 190 (2014), pp. 1449-1452
J Allergy Clin Immunol Pract. 2020 Feb;8(2):442-450.

Biomarkers for detecting airway eosinophilic inflammation

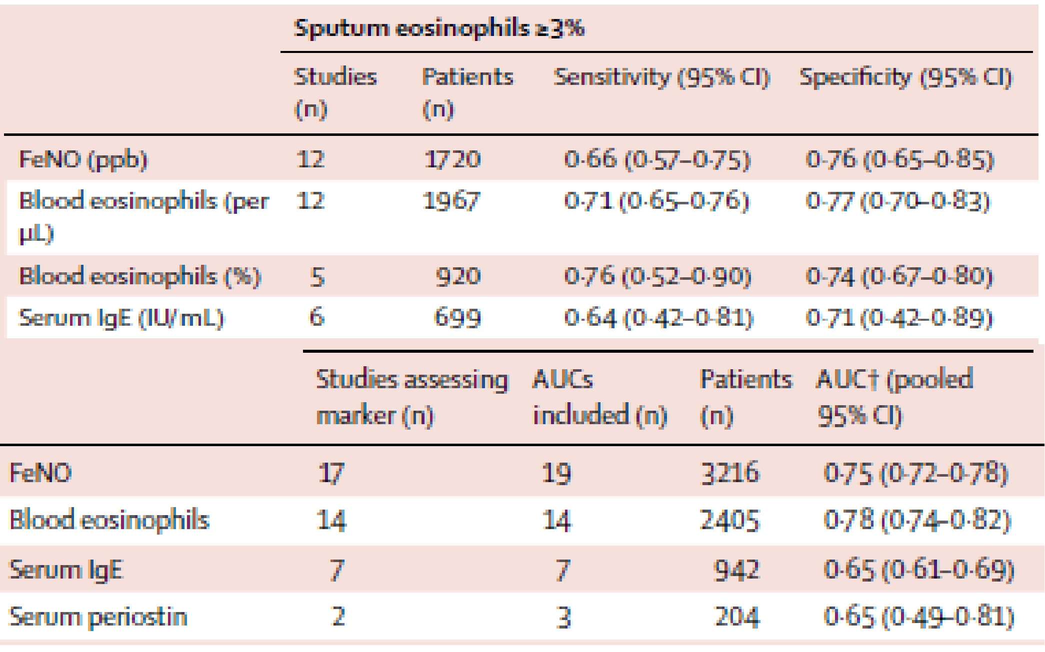
- Meta-analysis, 32 studies included

Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis

Daniël A Korevaar, Guus A Westerhof, Junfeng Wang, Jérémie F Cohen, René Spijker, Peter J Sterk, Elisabeth H Bel, Patrick M M Bossuyt

Lancet Respir Med 2015; 3: 290–300
Published Online

Summary
Background Eosinophilic airway inflammation is associated with increased corticosteroid responsiveness in asthma, but direct airway sampling methods are invasive or laborious. Minimally invasive markers for airway eosinophilia could present an alternative method, but estimates of their accuracy vary.

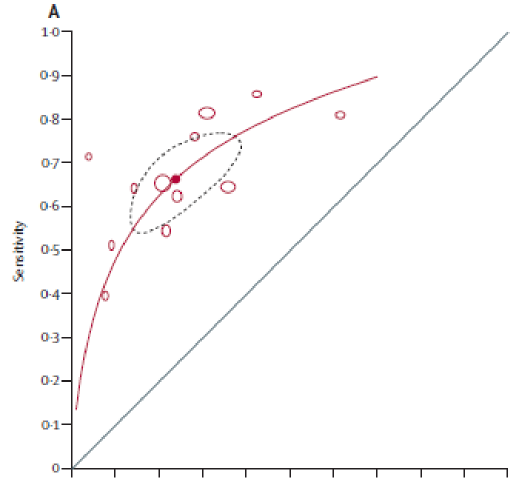


	Sputum eosinophils ≥3%			
	Studies (n)	Patients (n)	Sensitivity (95% CI)	Specificity (95% CI)
FeNO (ppb)	12	1720	0·66 (0·57–0·75)	0·76 (0·65–0·85)
Blood eosinophils (per μL)	12	1967	0·71 (0·65–0·76)	0·77 (0·70–0·83)
Blood eosinophils (%)	5	920	0·76 (0·52–0·90)	0·74 (0·67–0·80)
Serum IgE (IU/mL)	6	699	0·64 (0·42–0·81)	0·71 (0·42–0·89)

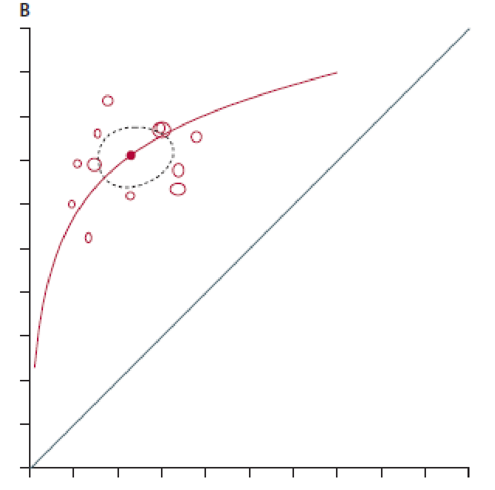
	Studies assessing marker (n)	AUCs included (n)	Patients (n)	AUC† (pooled 95% CI)
FeNO	17	19	3216	0·75 (0·72–0·78)
Blood eosinophils	14	14	2405	0·78 (0·74–0·82)
Serum IgE	7	7	942	0·65 (0·61–0·69)
Serum periostin	2	3	204	0·65 (0·49–0·81)

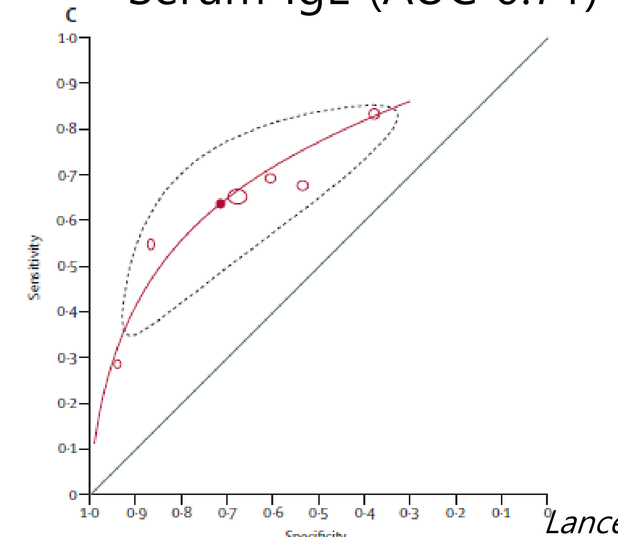
- ROC curve for detecting sputum eosinophils ≥ 3%

FeNO (AUC 0.75)



Blood eosinophil (AUC 0.78)





Lancet Respir Med 2015;3: 290–300

Biomarkers for detecting airway eosinophilic inflammation

- FeNO, blood eosinophils, Serum IgE

 - Sensitivities and specificities of these markers for detecting sputum eosinophilia are moderate
 - Lead to many false positives or false negatives
 → Use as a single surrogate marker for airway eosinophilia in patients with asthma requires caution.

- FeNO >50 ppb

 - Indicate that eosinophilic inflammation in symptomatic patients
 - Good short-term response to ICS
 - It is **NOT** meant to withhold ICS in a patients with low initial FeNO.

Global Strategy for Asthma Management and Prevention. 2021 GINA report
Lancet Respir Med 2015;3: 290–300

Contents

- Definition and Pathophysiology
- Spirometry
- Bronchodilator response, Peak expiratory flow
- Bronchoprovocation challenge
- Sputum, blood eosinophil counts, Fraction of exhaled nitric oxide (FeNO), Periostin
- Serum specific IgE test, Skin prick test

Serum specific IgE test, Skin prick test

- Allergic status
- Skin prick test, specific immunoglobulin E (sIgE) in serum
- Not specific for asthma
- Positive test results DOES NOT mean that the allergen is causing symptoms

→ The relevance of allergen exposure and symptoms MUST BE confirmed by the patient's history

- 증상을 일으키는 원인 물질을 규명

Comparison of characteristics of allegen-specific IgE tests

	Serum specific IgE test	Allergen skin prick test
Method	*In vitro*	*In vivo*
Risk of allergic reaction	No	Yes
Affected by antihistamine	No	Yes
Affected by systemic steroid	No	Sometimes
Affected by skin condition[a]	No	Yes
Convenient	Yes	Less
Skilled person	Not-needed	Needed
Sensitivity	Less sensitive	Sensitive
Selection of various allergens	Limited in singleplex test	Yes
Cost	Expensive	Less expensive
Time-required to result	Time-consuming	Immediate

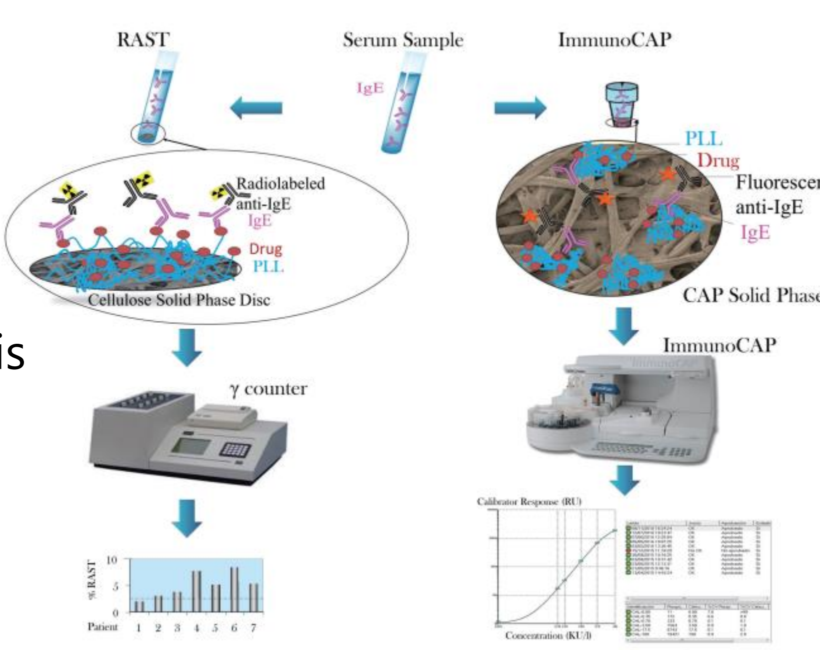
[a] Dermographism or extensive dermatitis.

Global Strategy for Asthma Management and Prevention. 2021 GINA report

Serum specific IgE test, Skin prick test

- Serum specific IgE test
  - RAST (Radioallergosorbent test: 방사성 동위원소),
    ImmunoCAP (형광효소면역측정법), MAST (Multiple allergen simultaneous test: 화학발광법)

  - Easy to collect and are frequently used to quantify the degree of atopy.
  - Biomarker of airway T2 inflammation → limited
  - Atopy alone is not essential for the generation of the T2 cytokines
  - Total serum IgE and specific IgE to inhaled allergens
  - More expensive, No more reliable than skin prick tests
    → Uncooperative patients, widespread skin disease, risk of anaphylaxis

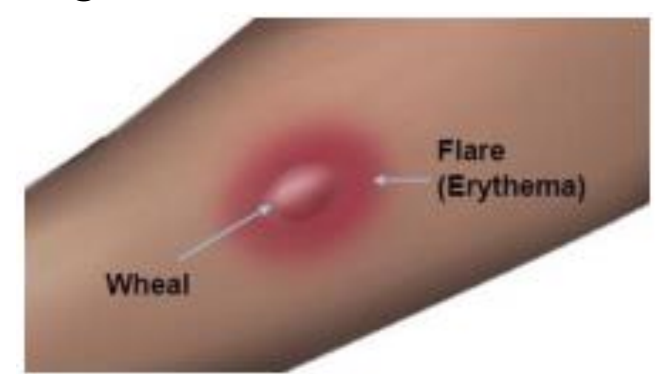


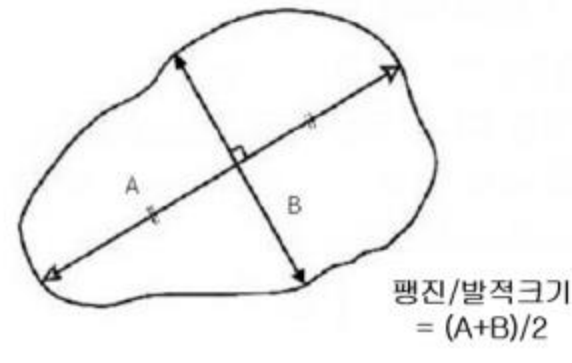
Korean J Med. 2018;93(6):497-500.
Allergy Asthma Immunol Res. 2017 July;9(4):288-298.

Serum specific IgE test, Skin prick test

- Skin prick test
  - Simple and rapid to perform: inexpensive, high sensitivity
  - Positive skin test: presence of allergic sensitization, but not the presence of allergic disease
    → 30–60% of sensitized-only individuals subsequently develop allergic symptoms

  - Positive control: histamine / Negative control: saline
  - 검사 시행 후 15~50분에 피부에 나타나는 팽진 (Wheal) 과 발적 (Flare) 의 크기로 판정
  - 팽진과 발적의 크기: (장경 + 단경)/2
  - 양성
    : 팽진 (Wheal) > 3mm and 발적 (Flare) > 10mm
    : Allergen/histamine ratio ≥ 1





Grade	Wheal	Flare
Negative	0	0
1+	R* < 1	<21 mm
2+	R < 1	≥21 mm
3+	1 ≤ R < 2	≥21 mm
4+	2 ≤ R < 3	≥21 mm
5+	3 ≤ R < 4	≥21 mm
6+	R ≥ 4	≥21 mm

Abbreviations : A, Allergen; H, Histamine
* R : ratio of wheal of allergen and histamine (1 mg/mL)

Korean J Med. 2018;93(6):497-500.

Summary

- Evidence of variable airflow limitation, Airway hyper-responsiveness (AHR)

  → **Spirometry, bronchodilator reversibility test, peak expiratory flow, Provocation test, Exercise challenge test**

- Airway inflammation

  → **Sputum eosinophil count, Blood eosinophil count, Fractional Exhaled Nitric Oxide (FeNO), Periostin**

- Allergy test

  → **Serum specific Immunoglobulin E, Skin prick test**

경청해 주셔서 감사합니다.