Exhaled & serum biomarkers in pulmonary diseases

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What are biomarkers ?

Why resort to biomarkers ?

- Early diagnosis
- Differentiating diseases
  - Classic
    - Cardiogenic vs. non cardiogenic PE
    - Sepsis vs. no sepsis
- Monitoring disease activity
- Prognostication
- Monitoring response to therapy

Source

- Exhaled breath
  - FENO
  - Other Exhaled breath condensate (EBC)
- Serum
  - CRP
  - Procalcitonin
  - S-TREM 1
- Sputum / BAL
- Tissue

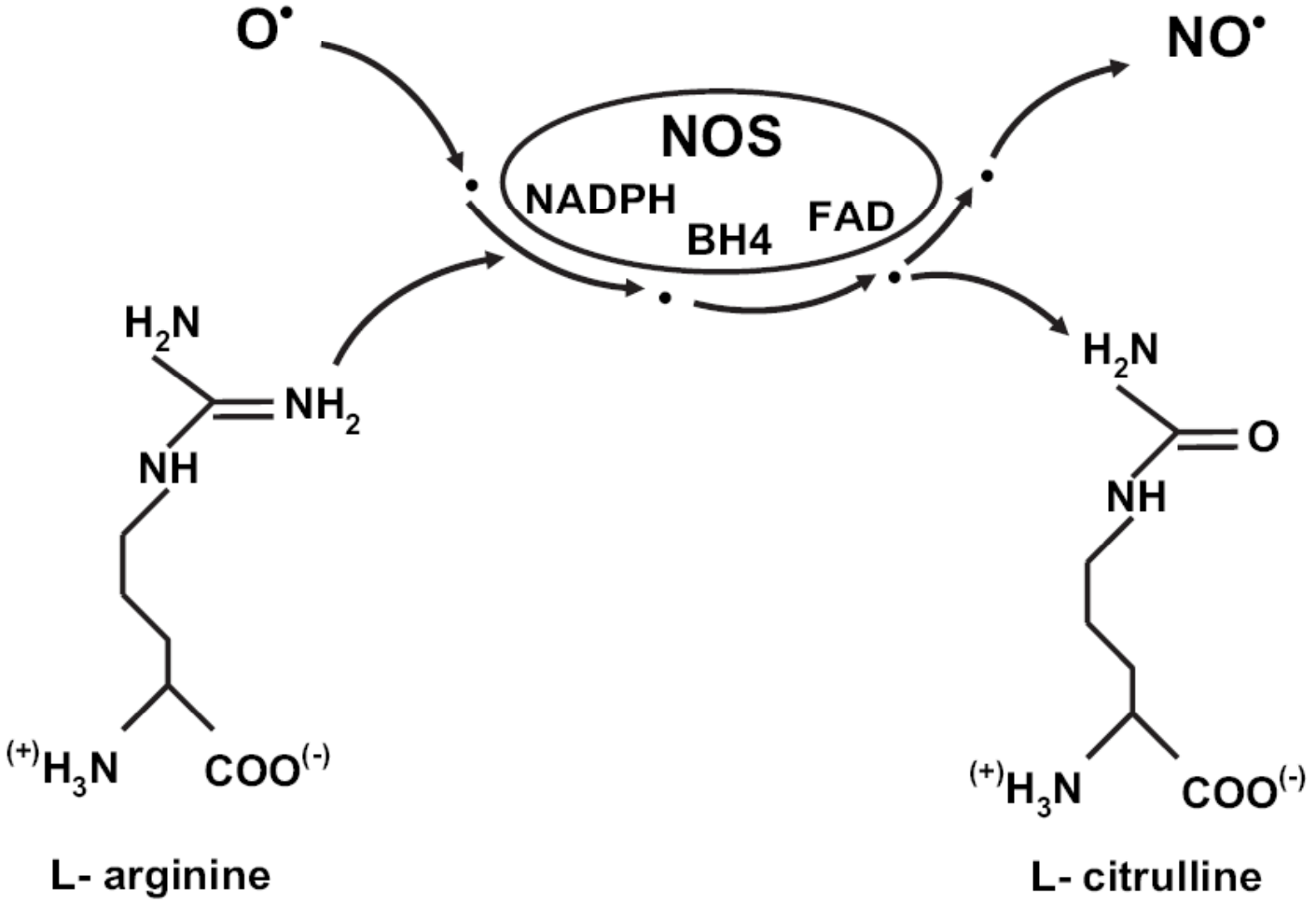
- Micron/ sub-micron particles emanating from mouth / ET have been identified

- Origin is of speculation
  - Sheer force of turbulence aerosolizing airway lining fluid
  - Alveolar origin – due to force applied to open alveoli- potential to kinetic conversion

Points to consider

- How to collect ?
- What to collect ?
- How to isolate ?
- Contribution with respect to particle size
- Standardization in disease & health
- Dilution factor
- Contamination factor

NO synthesis

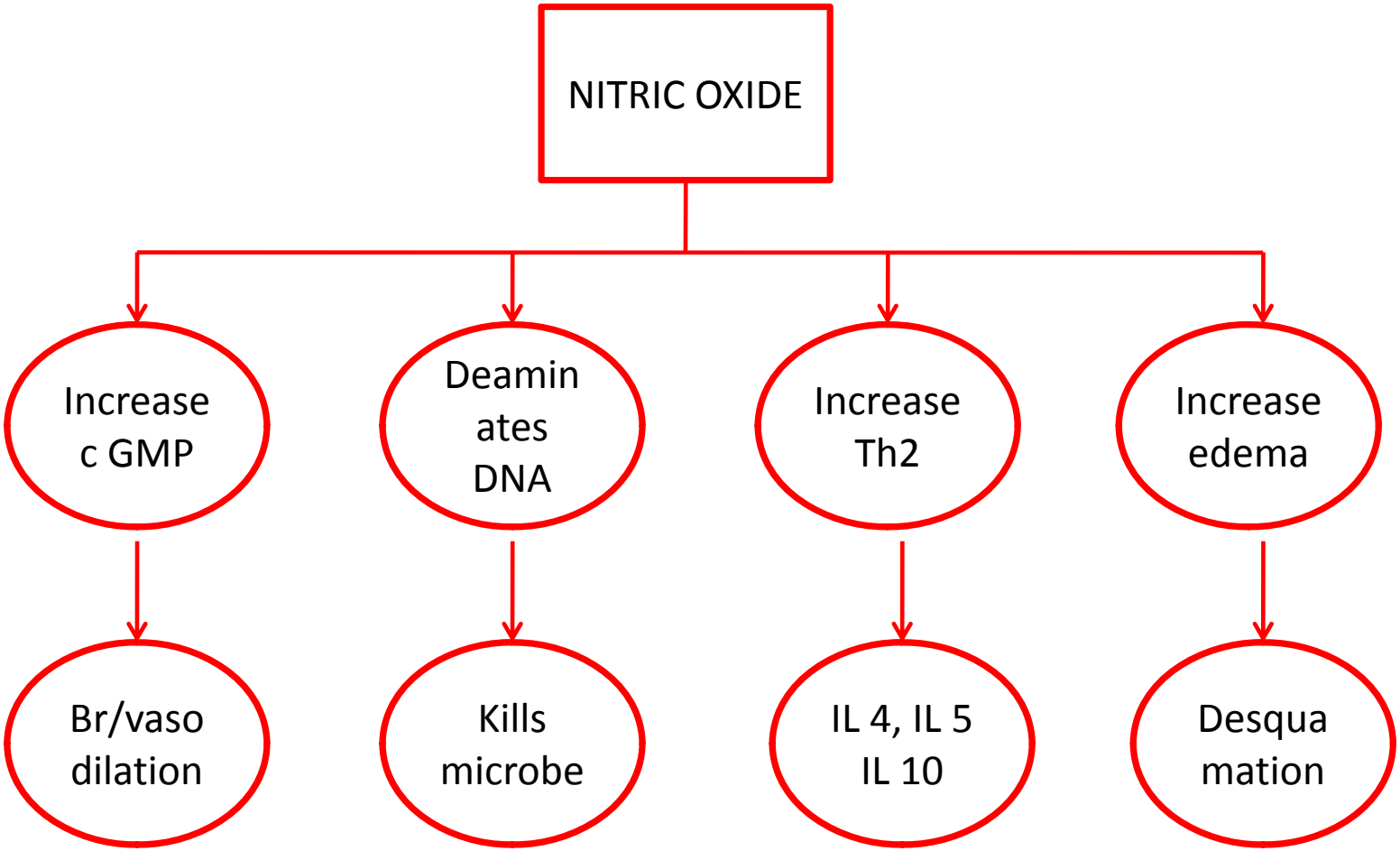


Clin Chest Med 28 (2007) 143–167

Fractional Exhaled Nitric Oxide (FENO)

- 3 isotypes
  - Calcium dependent
    - Endothelial
    - Neurogenic
  - Calcium independent
    - Inducible (main constituent)
- Volatile EBC
  - Measured by its reaction with Ozone by chemiluminescence
- Measured
  - Offline
  - Online

Functions of NO



Factors affecting FENO

- Pulmonary
  - Flow – measured @ constant rate of 50 ml/s
  - Nasal contamination- breathing against closed palate
- Age
  - Increases with age esp children
- Sex
  - Male > females (recent studies contradictory)
- Anthropometric factors
    - Height -strong +ve corelation
    - BMI &Race- not enough evidence

- Smoking & alcohol
  - Decreases FENO
- Dietary habits
  - Radish , lettuce , water, caffeine & fats – increase FENO
- Medication
  - Steroids & montelukast decreases
  - L-arginine & B agonist increases
- Others
  - Decreases after exercise , bronchoprovocation , spirometry & sputum induction

Reference values

- Difficult to establish due to numerous confounding factors
- Largest study in normal subjects involved > 3,300 pts
- Defined normal value between 24-54 ppb depending on age & height

(CHEST 2007; 131:1852–1856)

FENO in Br asthma

- Over 400 papers looking at various aspects of asthma management

Diagnosis

- Small studies have shown FENO may be an useful screening tool in high risk individuals

- Results from BASALT study evaluating three different strategies in asthma control is to be published later this year

- Atopy
  - Elevated in atopic individuals as a marker of eosinophillic inflammation
  - Atopic asthmatics have even higher levels
  - FENO is also increased in allergic rhinitis
  - Factors need consideration while interpreting FENO values
  - Reduce sensitivity of FENO as a screening tool for asthma in community

- COPD
  - Conflicting data from studies
  - Inversely proportional to FEV1, DLCO, SaO2
  - Normal or only slightly elevated in COPD
    - Smoking decreases NO
    - Possibly due to conversion to peroxynitrite & nitrate
  - Usually elevated during exacerbations
  - Need to evaluate role in certain subsets like Ex-smokers
  - Data emerging for use of CalvNO as marker of early peripheral inflammation

- PAH
  - Etiology is due to reduced vasodilator activity
  - NO is a potent vasodilator in pulmonary circulation
  - Serial FENO levels to monitor disease activity
  - It is inversely proportional to pulmonary artery pressures
  - Increase with successful lowering of pressures with therapy
- ILD
  - Increased due to CalvNO secondary to reduced DLCO

- Lung transplantation
  - For detecting
    - Infection – low sensitivity (57%)- not a good tool
    - BOS – high FENO has a good NPV but low specificity & PPV
    - Acute rejection
- Cystic fibrosis & ciliary dyskinesia
  - Decreased levels

Clinical application

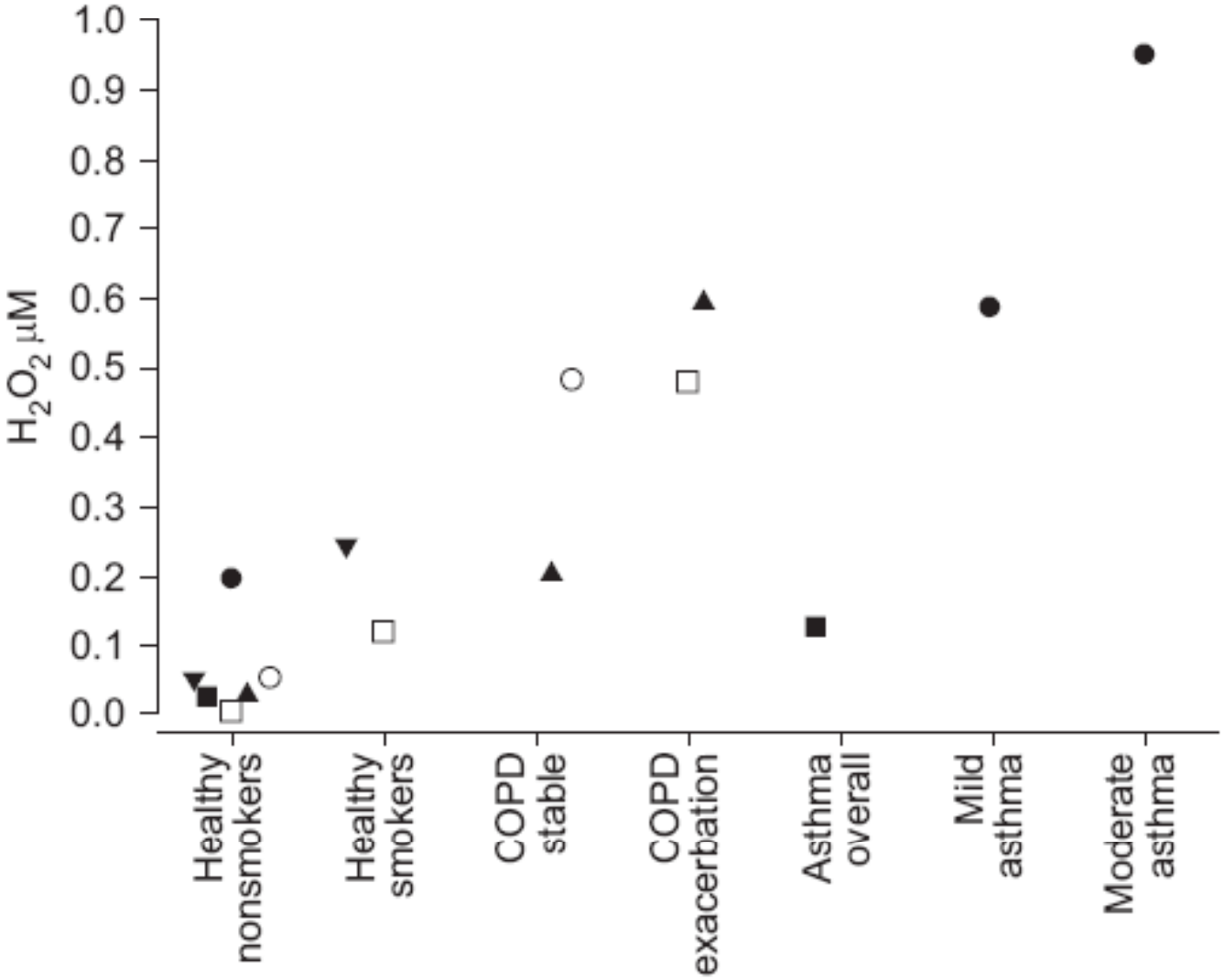
Table 1 Respiratory and non-respiratory conditions in which F_ENO measurements may have a role in diagnosis

Increased F_ENO	Variable changes in F_ENO reported	Decreased F_ENO
Asthma[1 79]	Bronchiectasis[91–93]	Cystic fibrosis[91 108–110]
Late asthmatic response[80 81]	COPD[17 75 78 94–102]	Primary ciliary dyskinesia[111 112]
Allergic rhinitis[19]	Fibrosing alveolitis[103]	Pulmonary hypertension[113]
Viral infections[43 44 82]	Sarcoidosis[104]	HIV infection[114]
Hepatopulmonary syndrome[83]	Systemic sclerosis[105–107]	ARDS[115]
Liver cirrhosis[84 85]		
Acute/chronic rejection of lung transplant including bronchiolitis obliterans[86–90]		

Thorax 2006;**61**:817–827.

H_2O_2

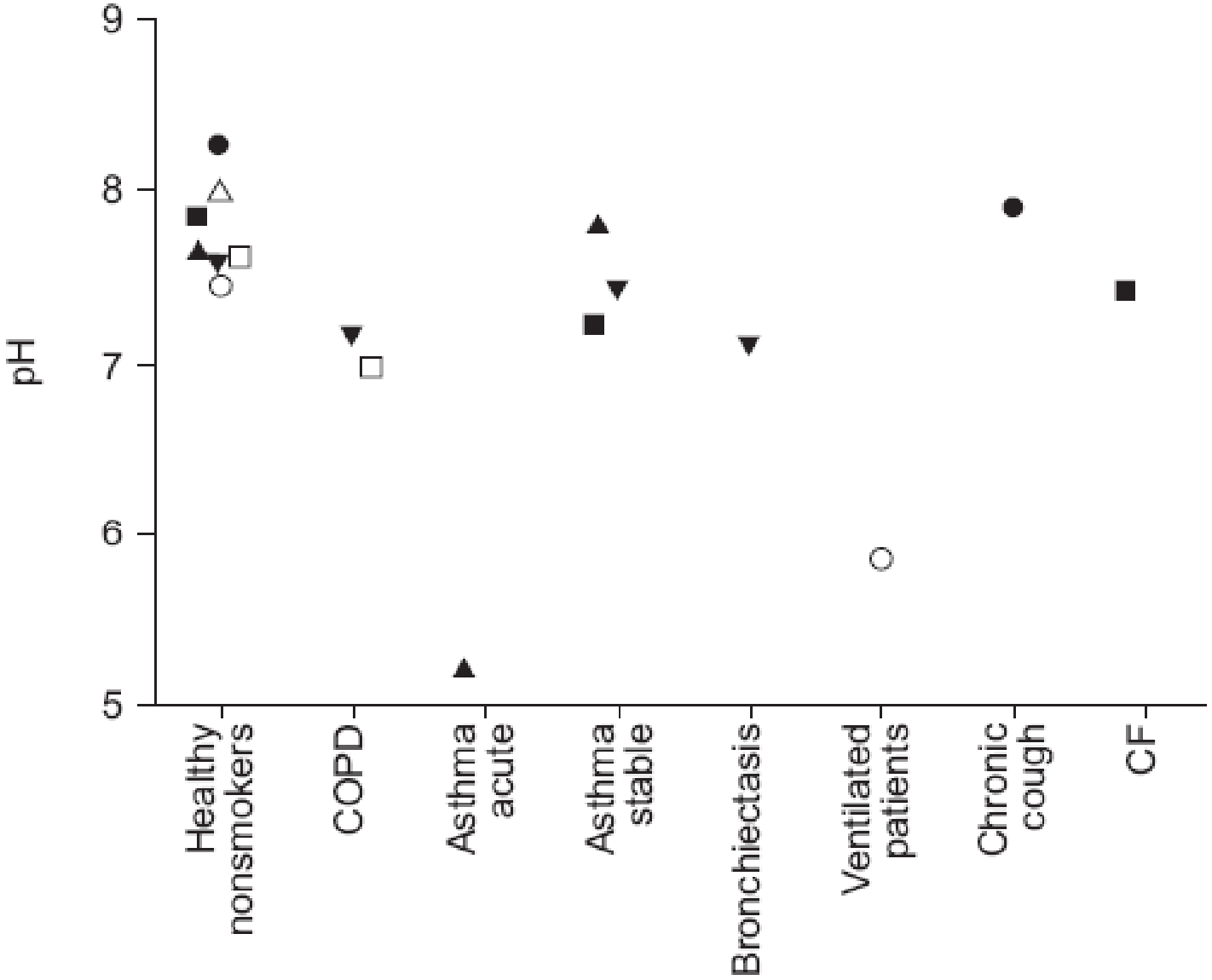
- Produced by
  - Superoxide dismutase mediated conversion of superoxide ions
- Detected by
  - Spectrophotometric method using horseradish peroxide
- Overlap in levels found in asthma & COPD hence may be non specific biomarker
- Levels proportional to dyspnoea , sputum neutrophils – s/o disease activity



Eur Respir J 2008; 32: 472–486

pH

- Airway acidification & regulation – implicated in pathogenesis of obstructive lung disease
- Unlike other EBCs pH in normal healthy volunteers from different studies similar
- Median pH ~ 8 (data from > 400 subjects)
- This suggest reproducibility across laboratories
- pH is decreased across spectrum of pulmonary diseases in the limited studies available
- Significant overlap across different diseases present



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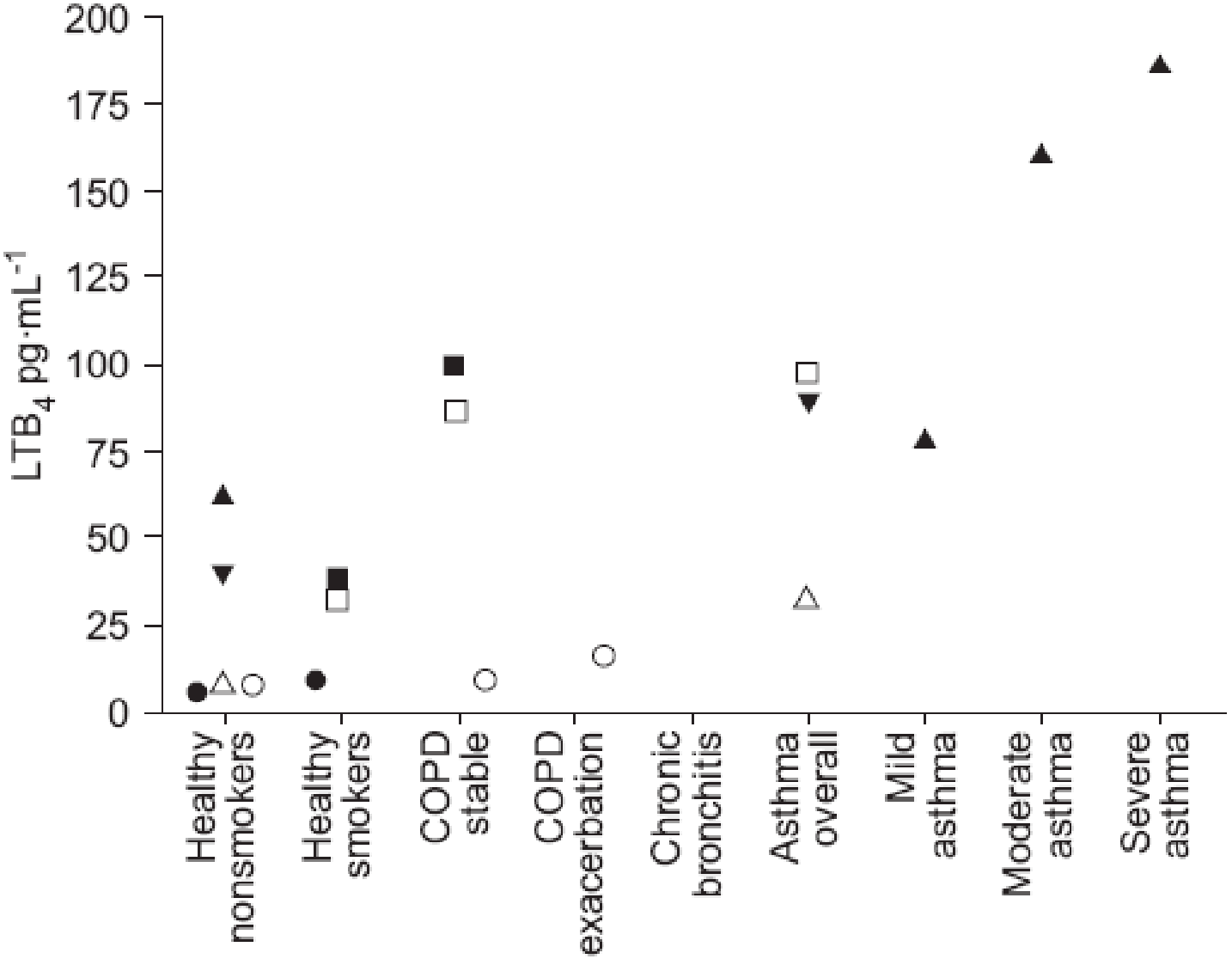
Leukotriene B4

- Produced from arachidonic acid by 5-lipooxygenase
- Estimated using ELISA
- Potent neutrophil chemoattractant – role in airway inflammation
- Mainly been evaluated in COPD & asthma
- Significant variability seen among patients with similar profile across different study groups
- Overlap between pts & healthy controls



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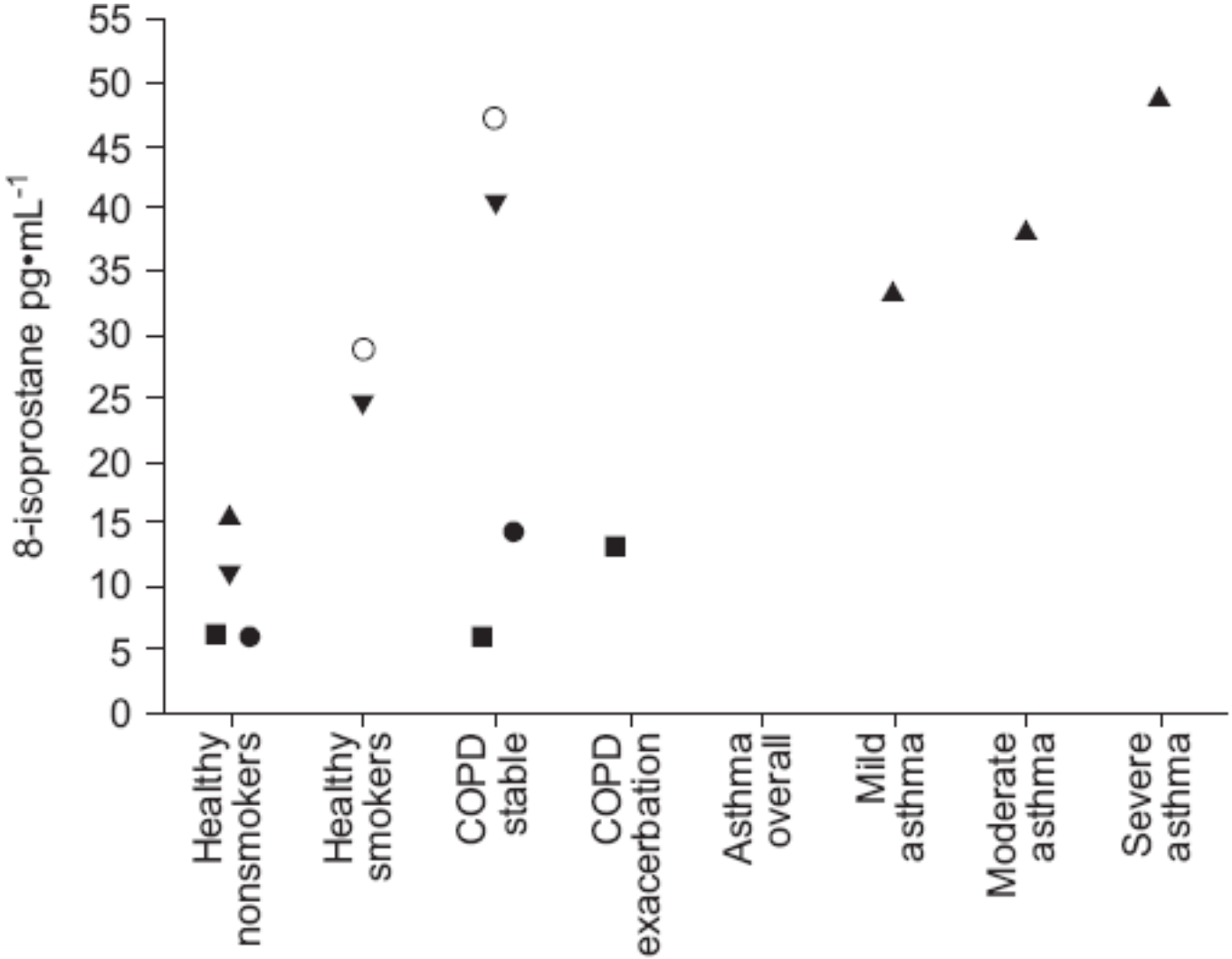
8- Isoprostane

- Produced by free radical peroxidation of arachidonic acid
- Supposed marker of oxidative stress in lungs
- Measured by ELISA
- Mainly elevated in COPD & asthma
- Baseline across similar clinical profile is variable in different studies
- Hence repeatability & standardization are difficult to achieve



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Prostaglandins

- PGE2 is elevated in stable COPD & asthmatics who are smokers but not in non-smoking asthmatics
- TXB2 is elevated in asthmatics but not in COPD
- Profile of PG may differ in asthma & COPD
- More studies needed to establish normal levels & variability

Other EBC

- Small studies have shown increase as well as positive co-relation with disease activity for various EBC
  - Ammonia
  - Nitrates & nitrites
  - Hydrocarbons(ethane , pentane)
  - CO
- All hampered by size of study, expense, lack of reproducibility, standardization, validation & hence inability for use outside research setting

Serum biomarkers

- Ideal marker
  - Rise before clinical manifestation
  - Easy to measure
  - Help target intervention
  - High sensitivity
  - Consistent results
  - Short half life
  - Cost effective

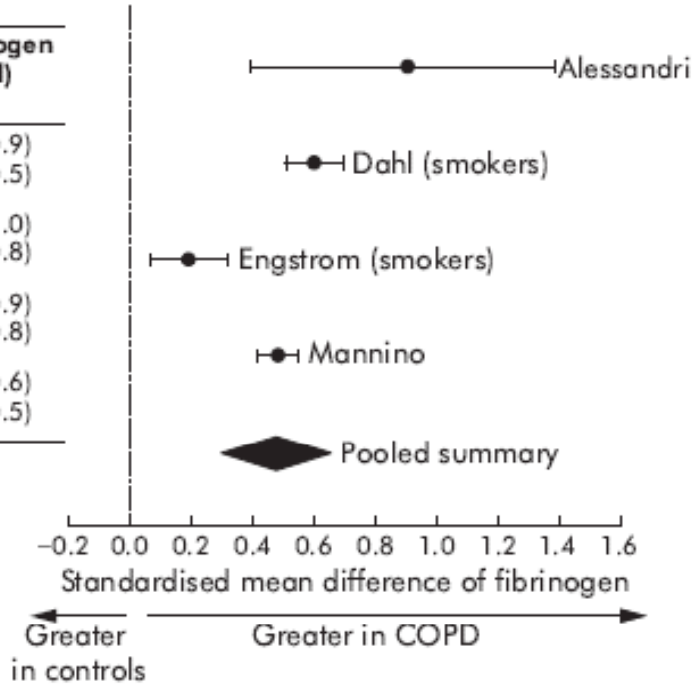
- Inflammatory biomarkers
  - CRP
  - Procalcitonin
  - S-TREM 1
  - Copeptin
  - Cytokines
- Protein biomarkers
  - CEA
  - CYFRA
  - SP –A & SP - D

CRP

- Acute phase reactant
- Increased in most forms of tissue damage, inflammation & infection
- Liver secretes it in response to IL 6
- Most extensively studied biomarker
- Evaluated in almost all subspecialties of medicine !!!
- Pulmonary diseases
  - COPD
  - Asthma
  - CAP/ VAP & sepsis

CRP in COPD

Author	Group	N	Age (yr)	FEV_1 (% pred)	Current smoker (%)	Men (%)	Fibrinogen (g/l)
Alessandri[24]	COPD	37	68 (8)	1.1 (0.5)*	19	73	3.0 (0.9)
	Control	30	58 (10)	NR	20	70	2.3 (0.5)
Dahl[25]	COPD	1427	62 (12)	66 (15)	100	51	3.5 (1.0)
Smokers	Control	785	55 (15)	117 (9)	100	43	3.0 (0.8)
Engstrom[126]	COPD	720	47 (4)‡	<85	100	NR	3.8 (0.9)
Smokers	Control	449	47 (4)‡	>105	100	NR	3.6 (0.8)
Mannino[20]	COPD	2065	67 (12)	77 (21)	31	60	3.1 (0.6)
	Control	3488	57 (13)	104 (14)	17	47	2.8 (0.5)



- Systematic review of studies showed baseline CRP is elevated in stable COPD

Current Opinion in Pulmonary Medicine 2009, 15:120–125

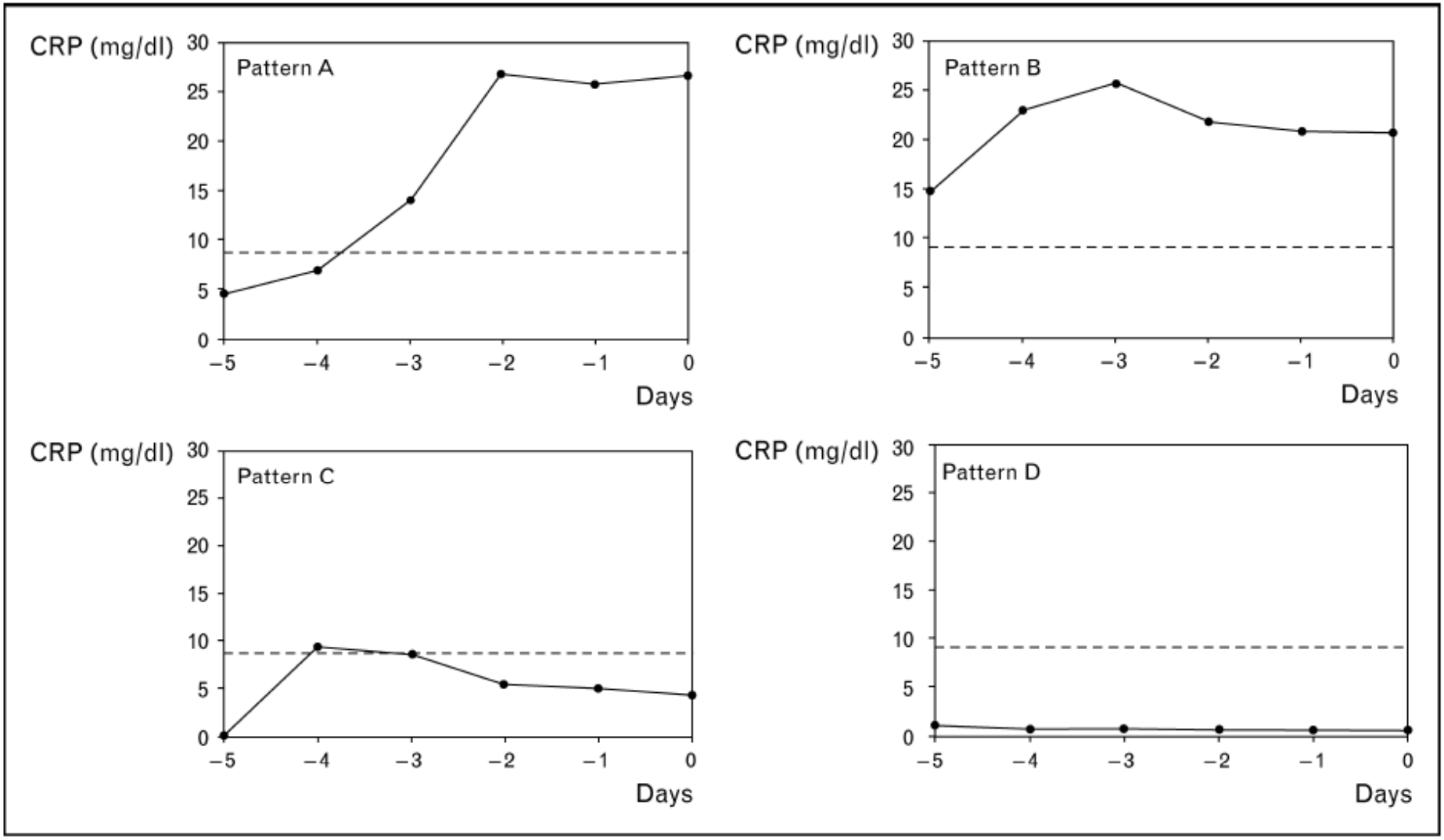
CRP in AECOPD

- CRP tends to co relate with severity of exacerbation
- Decreases in responders but data is only from observational studies
- Effect of steroids on CRP unclear

CRP in CAP / VAP

- Prediction of VAP / CAP
  - More so for VAP / HAP in admitted pts
  - Requires serial monitoring (possibly daily)
- Surrogate tool for diagnosis
  - Most sensitive of the available biomarkers for thoracic infections
  - In two studies was better than procalcitonin
- Monitoring therapy
  - Short half life
  - Hence shows decreasing trend in responders

Patterns of CRP course in pneumonia



Current Opinion in Infectious Diseases 2008, 21:157–162

CRP in asthma

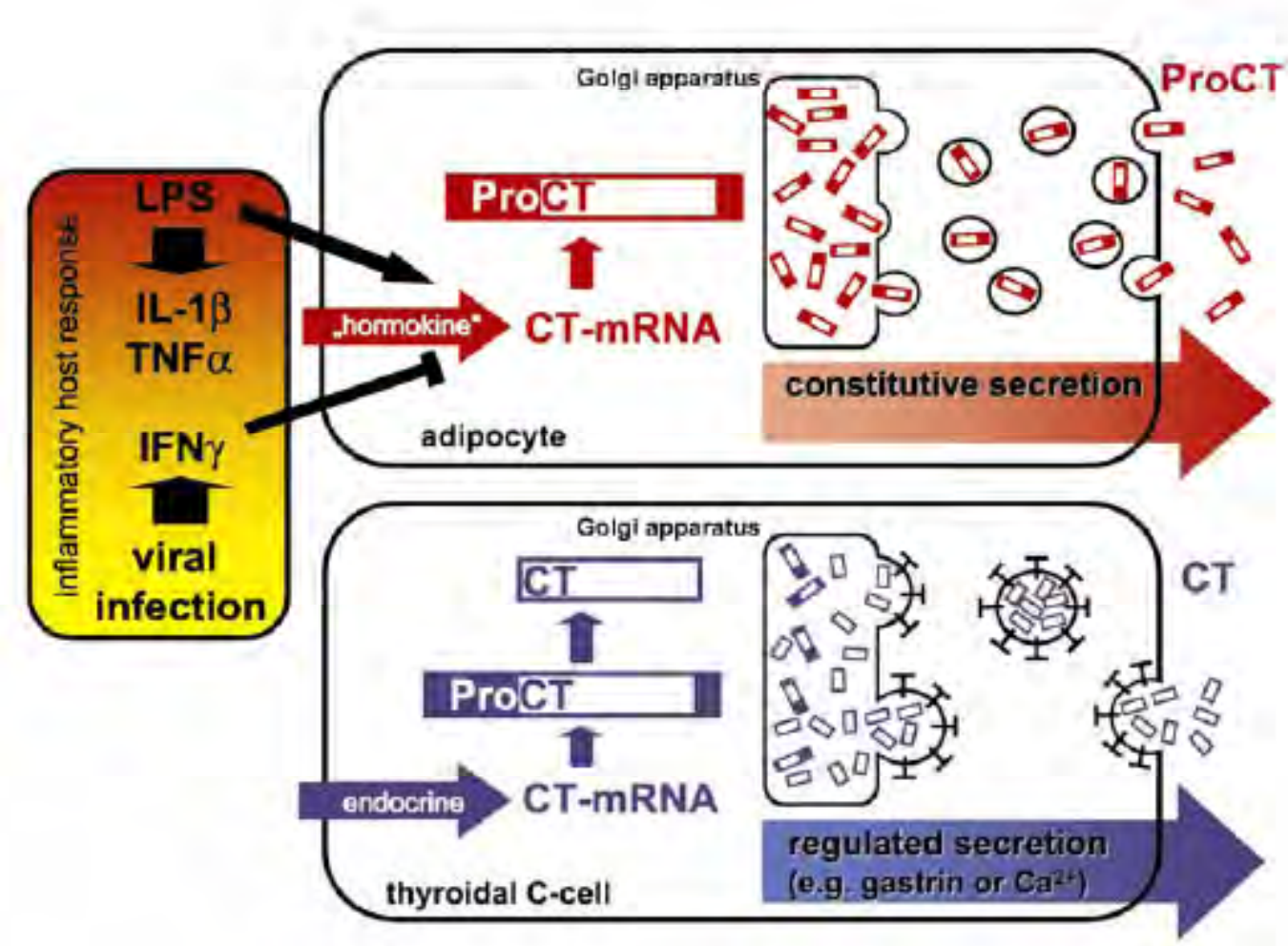
- With the advent of hs-CRP several studies have been published recently
- Including one from India
- Salient points
  - Elevated in asthma
  - Co-relate with disease severity
  - May be surrogate marker for systemic inflammation

CRP in sepsis

- It is elevated in sepsis
- Performs better than clinical parameters in predicting infection
- Low sensitivity for differentiating SIRS or non septic shock from sepsis
- Was hailed as a prognostic marker –same has been challenged in recent trials
- In general inferior to PCT as a biomarker in sepsis

Clin Chest Med 29 (2008) 591–603

Procalcitonin



Clin Chest Med 29 (2008) 591–603

In sepsis

- Advantages
  - Relatively specific marker for sepsis
  - Differentiates SIRS from septic shock
  - Absolute & more importantly persistent elevation co – relates with organ dysfunction scores & poor prognosis
  - Serial measurements have more meaning
  - < .5ng/ ml & > 2 ng/ml a/w low & high risk respectively for sepsis
- FDA has approved it for use in critically ill with emphasis on
  - Conjunction with other lab & clinical parameters
  - Serial values to be interpreted rather than a one

- Disadvantages
  - Available assays are relatively insensitive for assessment of minor daily variations
  - Though general cut offs have been defined but evidence for same are weak (based on few studies)
  - Marker has been applied over spectrum of diseases, its utility in individual pt needs clinical discretion
  - Utility in presence of renal failure not defined
  - Cost

PCT in pneumonia

- Relatively insensitive for predicting pneumonia in the absence of wide spread sepsis
- For deciding whether to start antibiotic
  - Two studies compared procalcitonin based vs. standard protocol for need for antibiotic therapy
  - Significant decrease in duration of therapy & cost with no morality difference
  - Offset by cost of serial procalcitonin

S-TREM 1

In sepsis & pneumonia

- Few single centre trials have shown
  - Sensitive marker for distinguishing sepsis from SIRS
  - Potential as a useful biomarker in sepsis
- Uncertainties
  - No real large RCTs
  - Value of serial measurement unclear
  - Conflicting reports on course of illness & plasma level co-relation

Copeptin

- Secreted along with AVP from pre-pro-vasopressin
- Stable in withdrawn blood for days
- Blood levels have been used in diagnosis of
  - Diabetes insipidus
  - Cardiovascular disease
  - Sepsis
  - Pneumonia
  - AECOPD
- Data for support of its use in pulmonary diseases is emerging

ARDS

- Long PTX 3 was found to be elevated in pts of ARDS in a single trial

- In a recently published study by ARDSnet group
  - IL 8 , neutrophil chemotactic factor & SP-D levels were found to be significant in predicting mortality when interpreted with clinical predictors

Chest 2010;137 (2):288-96

Biomarkers in Ca lung

- CEA
  - Elevated in adeno ca & LCLC
  - Limited value when used alone
  - It is used in combination with CYFRA for diagnosis
  - Can also be used to monitor therapy in NSCLC
- CYFRA
  - CYFRA 21-1 potential for monitoring Rx in NSCLC
- Both are non specific biomarker also elevated in other cancers
- ProGRP & NSE are potential tools for diagnosis & monitoring Rx in SCLC

BMB reports 2008; 41(9): 615-625

Summary

- No single biomarker is ideal
- Our understanding of most is still incomplete
- A panel of biomarkers could be more helpful with each supplementing the other
- Need for more reliable assays
- Serial monitoring would hold the key in the future in both acute & chronic pulmonary diseases