

Characterization of a Cytolytic CD4+ T-Cell Subset in Patients with COPD

Stefan Hacker

Supervisor: Hendrik J Ankersmit

Department of Surgery, Medical University of Vienna

COPD - Epidemiology

- 2.38 million deaths in low-income countries
- 0.30 million deaths in high-income countries (Lopez Lancet 2006)
- No. 3 leading cause of death by 2020 (Murray Lancet 1997)
- 23.9 billion \$ direct +indirect costs (US alone) (Sullivan Chest 2000)
- Enormous effects of co-morbidities

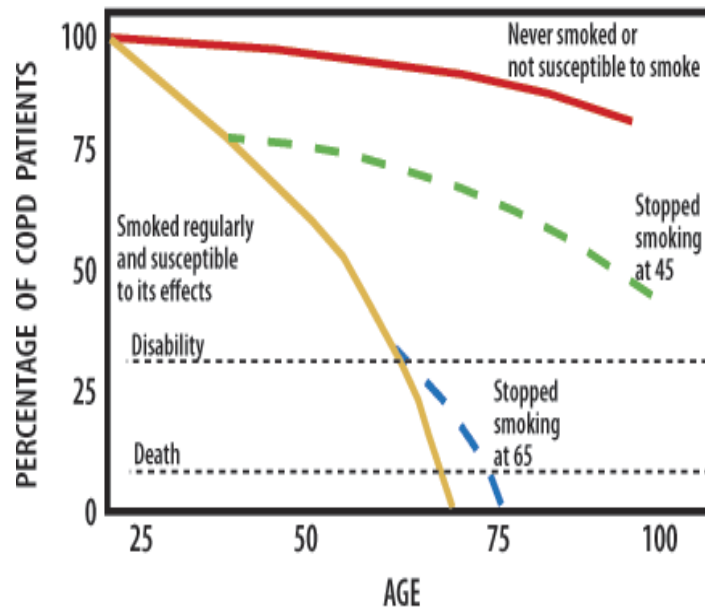
COPD - Risk Factors

- Multifactorial disease
- **Tobacco Smoking**
- Environmental Risk Factors
 - Indoor air pollution, socio-economic status
- Infections
 - Viral (esp. during childhood)
 - Bacterial (Exacerbations!)
- Genetic Factors
 - Severe AATD (1-3% of all COPD) (Hersh Thorax 2004)
 - Susceptibility Factors?

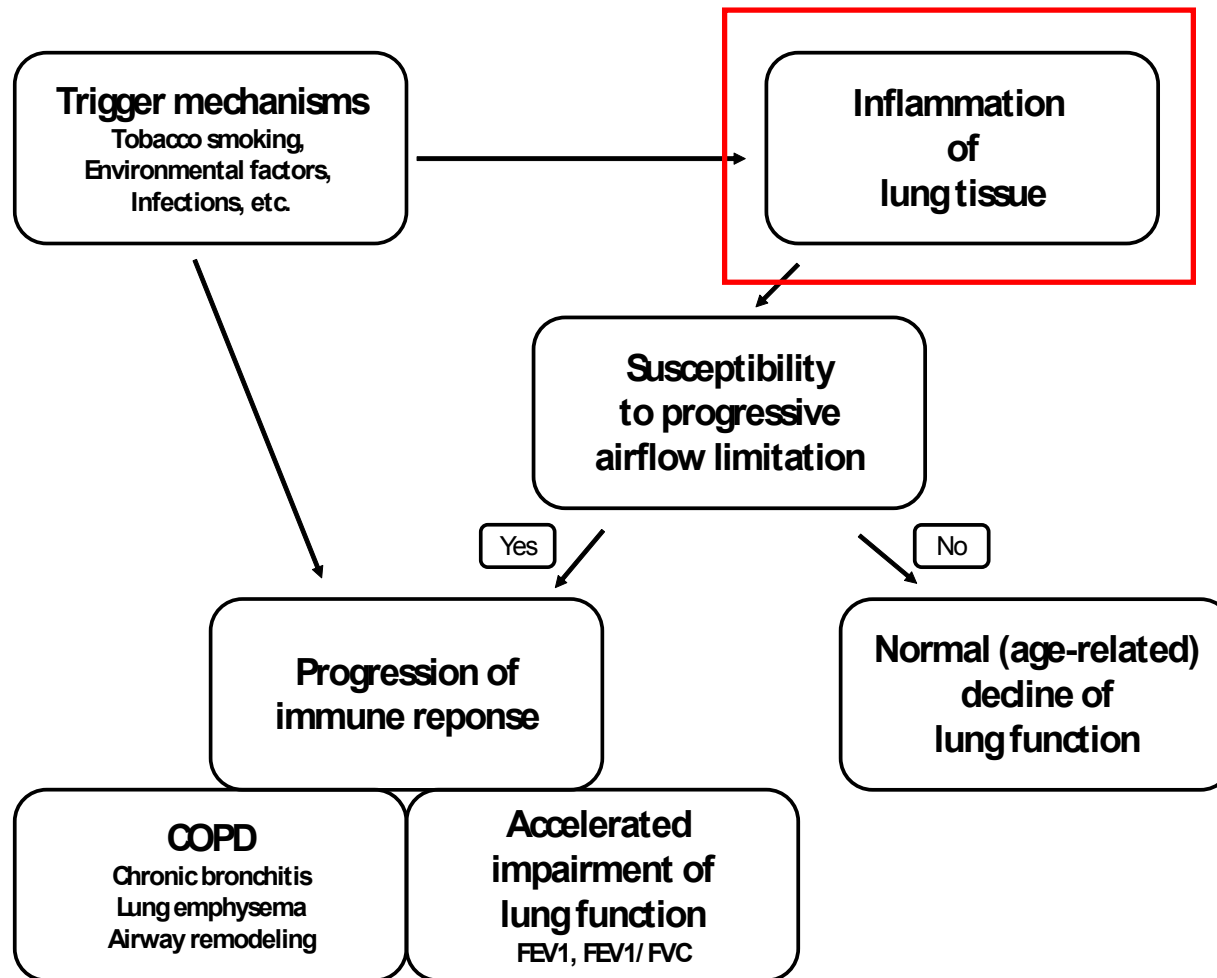
Pathogenesis

- Airflow limitation
- Not fully reversible
- One clinical complex – different pathways
- Main diagnostic tool = spirometry

Figure 1. Effects of smoking and stopping smoking on forced expiratory volume in 1 second (FEV_1)

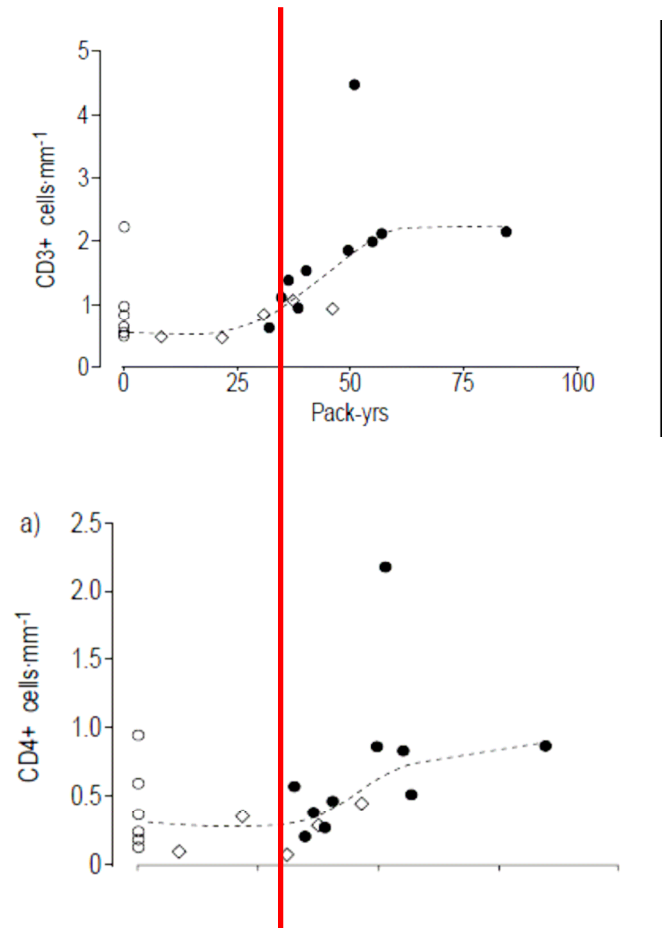


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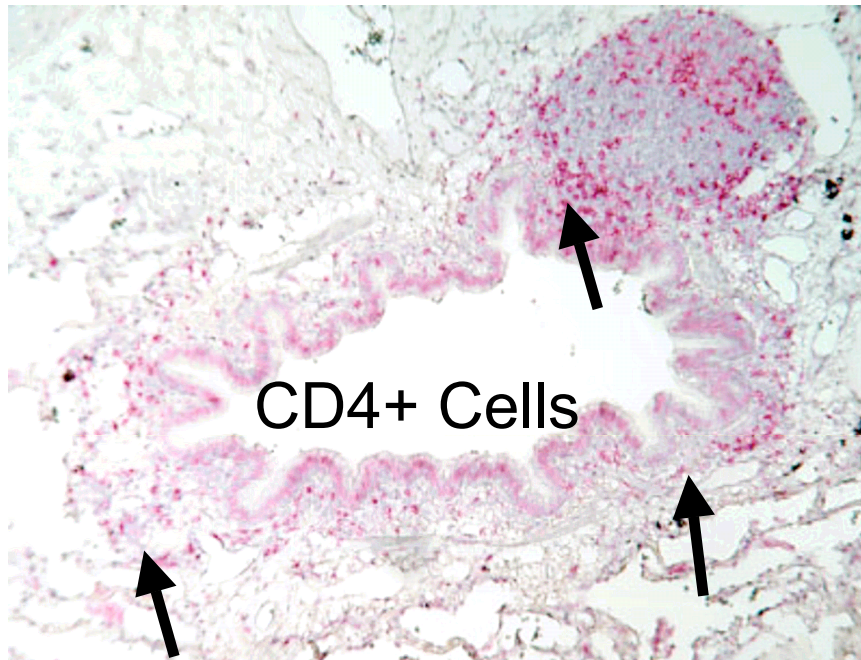


Adaptive Immunity & COPD

- Total Lymphocytes \uparrow in COPD lungs
- CD4+
- Increase develops after min. of 30 pack years
- Threshold dose? (Majo Eur Resp J 2001)

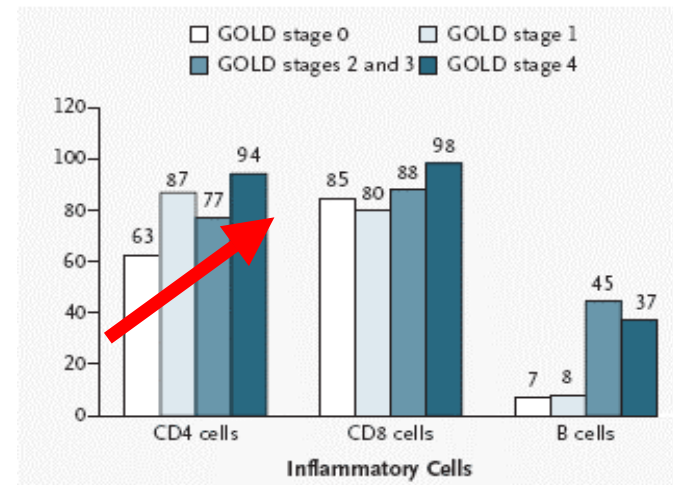


Role of CD4 Cells



(Hogg NEJM 2004)

- CD4+ lymphocytes
 - in small airways
- Numbers of patients with CD4+ cells
 - increased with disease severity



Autoimmune Aspects

Hypothesis: Does COPD have an autoimmune component?

A Agustí, W MacNee, K Donaldson, M Cosio

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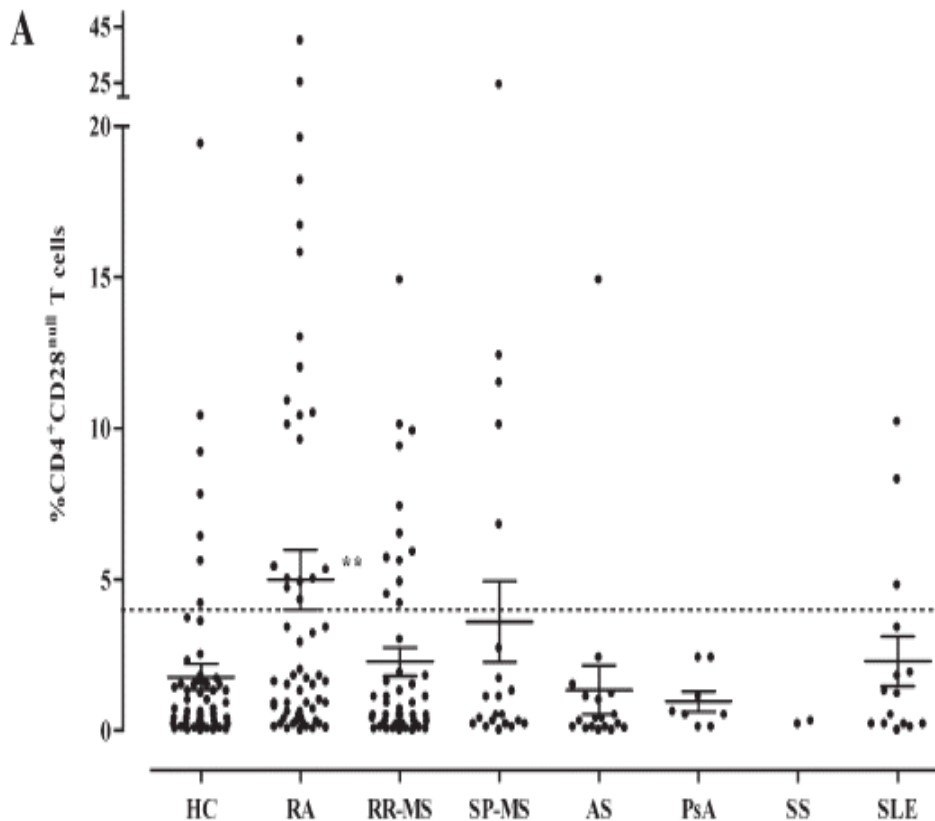
A new hypothesis that considers the role of the immune system in the pathogenesis of COPD is explored which, if true, will generate new therapeutic opportunities in this condition.

- Why do only some smokers develop COPD?
- Why does inflammation persist after quitting smoking?
- What are possible antigens?
- What leads to the creation of a possible auto-antigen?

Role of CD4+CD28null Cells

- CD28 on T-cells - for co-stimulation
- Chronic antigen stimulation
 - downregulation of CD28
 - phenotypical changes
 - CD4+CD28null cells are senescent!
 - Chronically stimulated cells are apoptosis-resistant (Debantini J Immunol 2003)
- CD4+CD28null cells
 - express Natural killer cell receptors (NKR)
 - produce IFN-gamma, perforin, granzyme B, etc.

Role of CD4+CD28null Cells

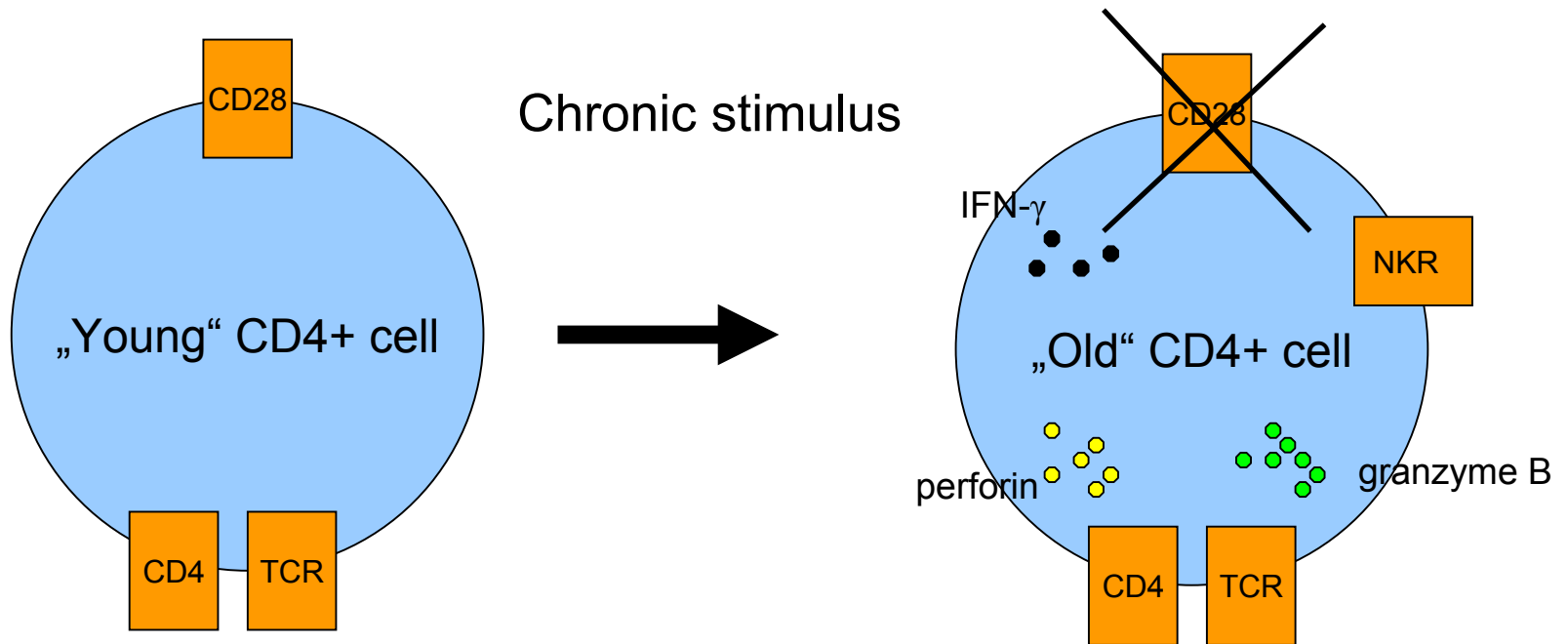


(Thewissen Clin Immunol 2007)

- Percentage of CD4+CD28null cells in the peripheral blood in different disease entities

- HC, healthy controls
- RA, rheumatoid arthritis
- MS, multiple sclerosis
- AS, ankylosing spondylitis
- PsA, psoriatic arthritis
- SS, Sjögren Syndrome
- SLE, systemic lupus erythematosus

Role of CD4+CD28null Cells



Role of Heat Shock Proteins

- Usually intracellular chaperons
- Released after stress, trauma, etc.
- Extracellular „danger signals“
- Modulate immune response
 - HSP-antigen-complexes

Table 1
Intracellular and extracellular properties of HSP and GRP

Chaperone	Protein function	
	Intracellular	Extracellular
Hsp27	Chaperone anti-death	Anti-inflammatory
Hsp60	Chaperonin	Pro-inflammatory
Hsp70	Chaperone anti-death	Immunoregulatory pro-inflammatory neuronal agonist
Hsp90	Chaperone evolutionary modulator	Pro-immune
Hsp110	Chaperone co-chaperone	Pro-immune

(Calderwood Ann NY Acad Sc 2007)

Hypothesis

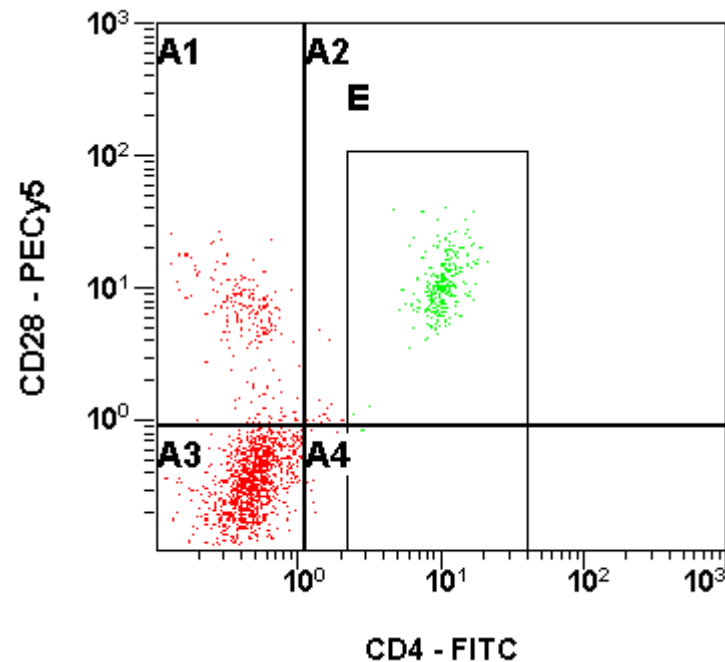
- Patients with COPD have increased numbers of CD4+CD28null cells in systemic blood flow
- CD4+CD28null cells in COPD express Natural Killer Cell Receptors (NKR) – CD94, CD158
- CD4+CD28null cells in COPD produce increased amounts of cytokines upon stimulation
- HSPs are increased in the serum of COPD patients
- Levels of CD4+CD28null cells and serum HSP can be used to diagnose COPD

Materials and Methods

- 4 study groups (n = 64)
 - COPD – GOLD Stage I-II
 - COPD – GOLD Stage III-IV
 - Smokers without COPD
 - Healthy controls
- Exclusion criteria:
 - Acute exacerbation within 14 days before study entry
 - Steroids within 14 days before study entry
 - History of asthma, autoimmune diseases or other relevant lung diseases (e.g., lung cancer, known α 1-antitrypsin deficiency)
 - Coronary artery disease, peripheral artery disease, and carotid artery disease

Materials and Methods

- Flow Cytometry Analysis
 - CD4, CD28
 - CD94, CD158 (NKR)
 - Perforin, Granzyme B (intracellular)
- Stimulation of PBMCs
 - with anti-CD3 or phytohemagglutinin (PHA)
 - Cytokines in supernatants
- ELISA
- Statistical analysis

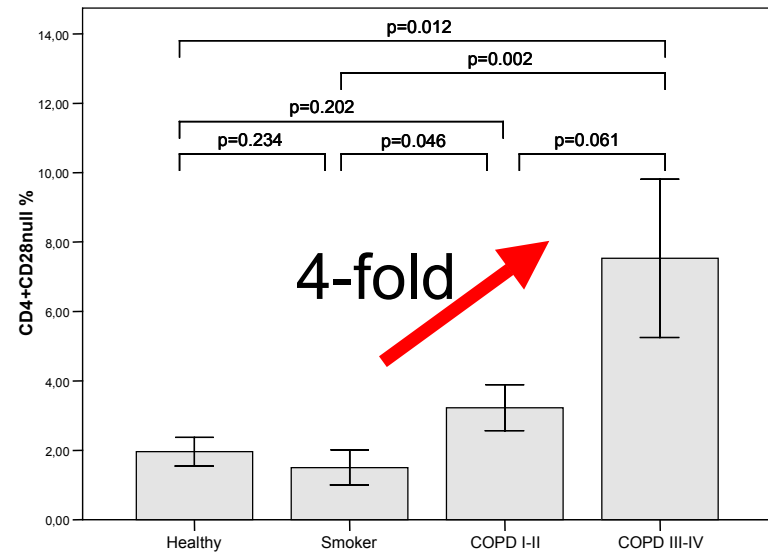


Patient Characteristics

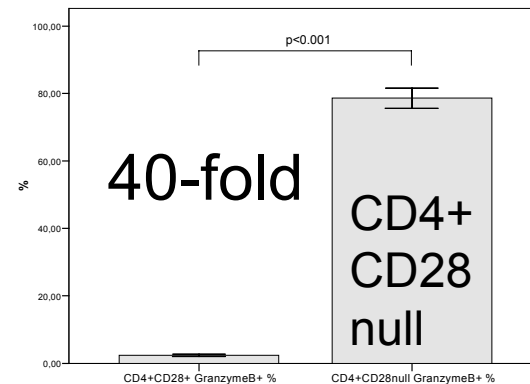
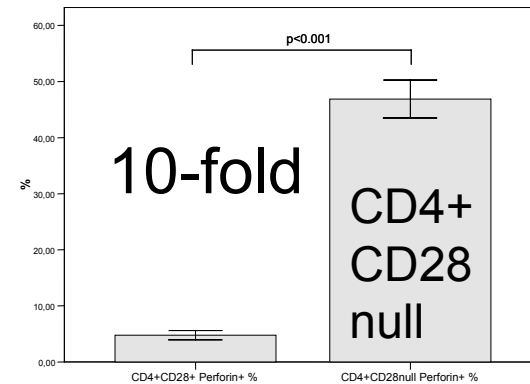
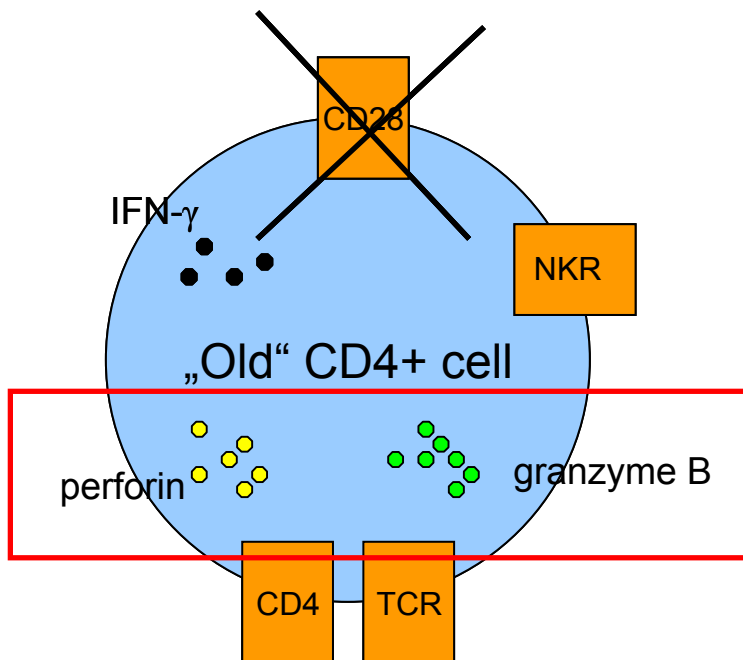
Subject Category	Healthy Non-Smokers	Healthy Smokers	COPD GOLD I&II	COPD GOLD III&IV
N	15	14	19	16
Male/Female	10/5	7/7	10/9	10/6
Age	57.20 (12.50)	56.64 (9.17)	60.68 (7.39)	58.31 (8.75)
Body Weight (kg)	71.6 (13.9)	76.4 (8.6)	79.7 (16.7)	81.1 (27.2)
Body Height (cm)	172.7 (10.9)	168.7 (8.1)	167.7 (12.1)	171.2 (7.9)
Lung Function				
FVC (L)	4.55 (0.94)	3.84 (0.66)	3.33 (1.06)	2.14 (0.70)
FEV1 (%)	105.37 (17.11)	94.40 (11.96)	70.21 (13.33)	30.67 (12.66)
FEV1/VC (%)	76.80 (7.85)	75.95 (3.99)	61.74 (8.36)	37.80 (15.33)
MEF 50 (%)	100.67 (28.92)	87.64 (21.45)	39.42 (15.93)	11.93 (6.60)
MEF 25 (%)	103.53 (33.89)	75.71 (31.33)	37.37 (16.19)	20.00 (5.94)
Smoking History				
Never-smoker	15	0	0	0
Ex-smoker	0	3	4	3
Current-smoker	0	11	15	13
Pack Years	0	34 (25.2)	47.3 (29.7)	44.0 (32.6)

Results I

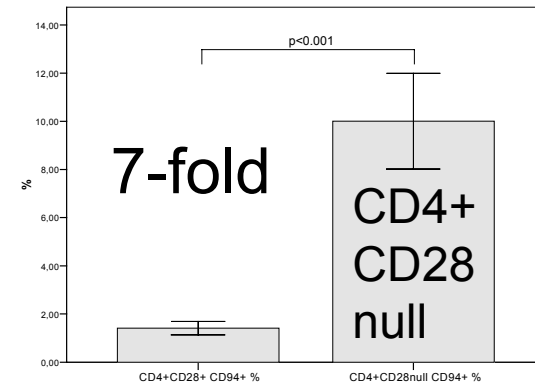
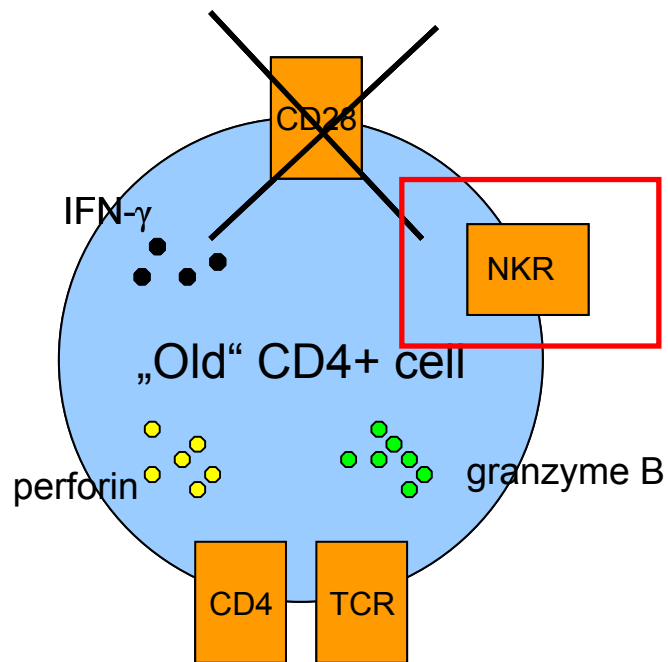
- Levels of CD4+CD28null cells
 - Healthy: 1.96%
 - Smokers: 1.50%
 - COPD I-II: 3.22%
 - COPD III-IV: 7.53%
 - (RA: 5-8%)



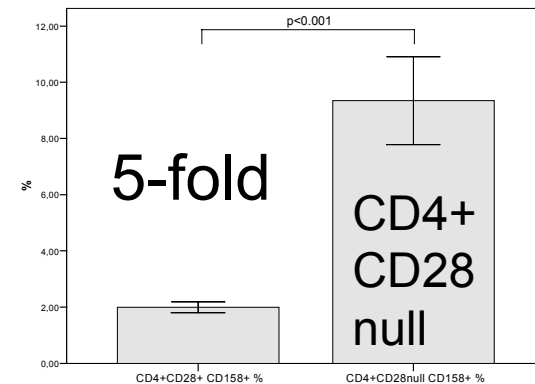
Results II



Results III

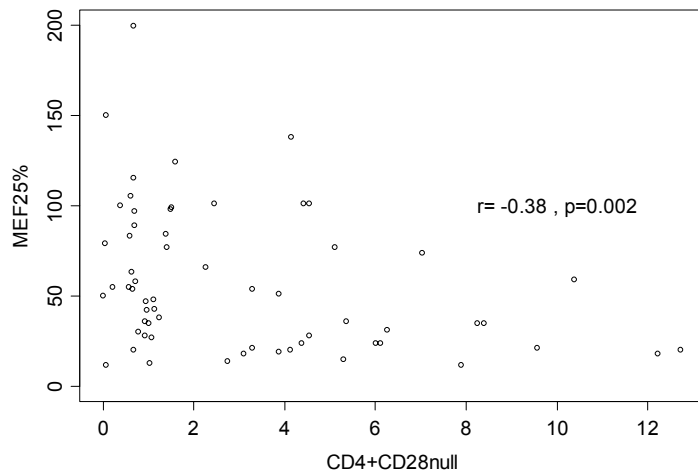
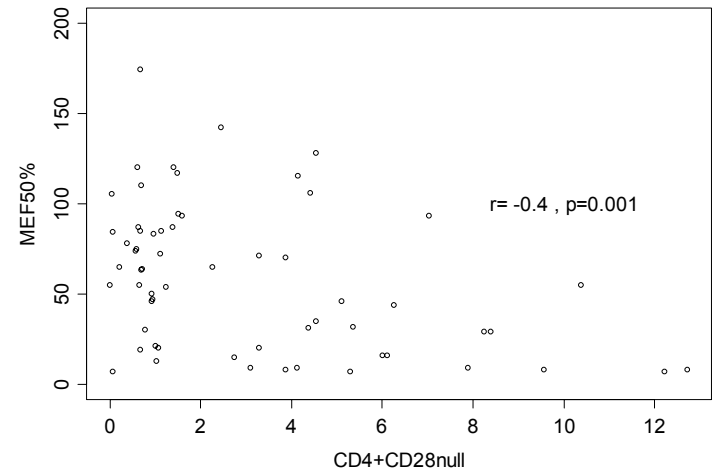
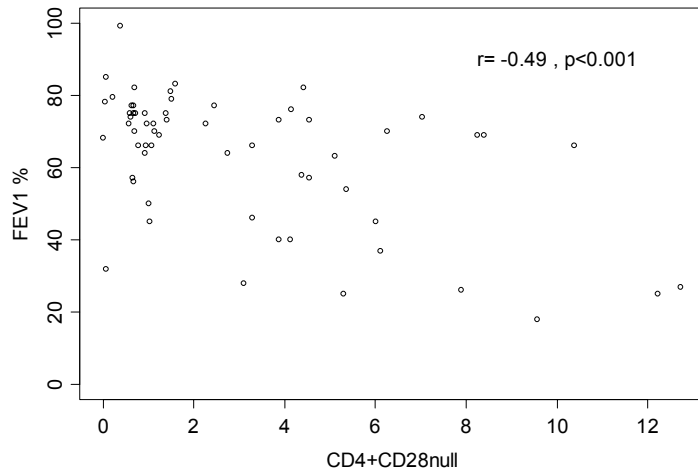


CD94



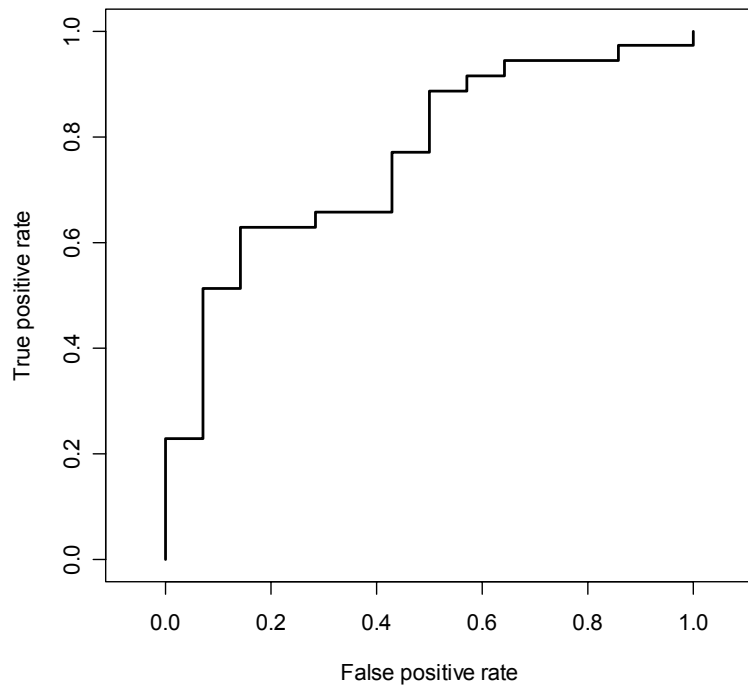
CD158

Results IV



Decreased lung function
=
higher levels
of CD4+CD28null cells

Results V

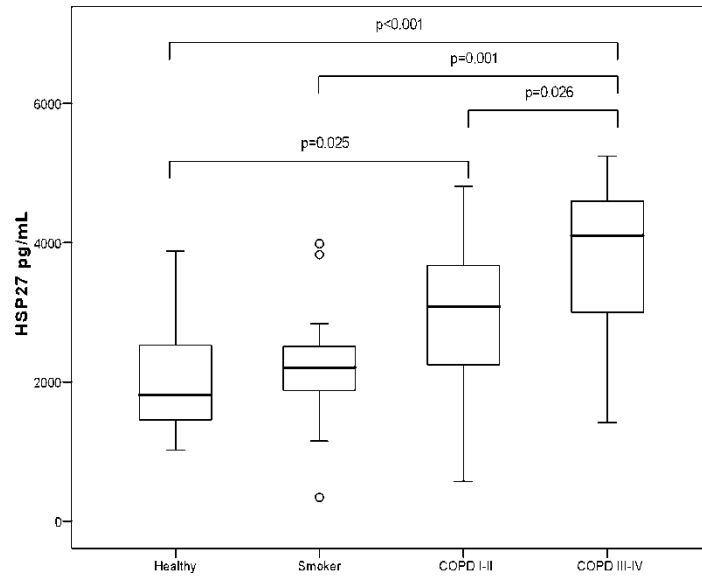


- Logistic regression model
- CD4+CD28null cells as diagnostic marker
- ROC-curve
 - AUC = 0.76
- In MCI: (Collinson Heart, 2003)
 - Troponin:
 - AUC = 0.78 (<2 hrs.)
 - AUC = 0.86 (2-6 hrs.)

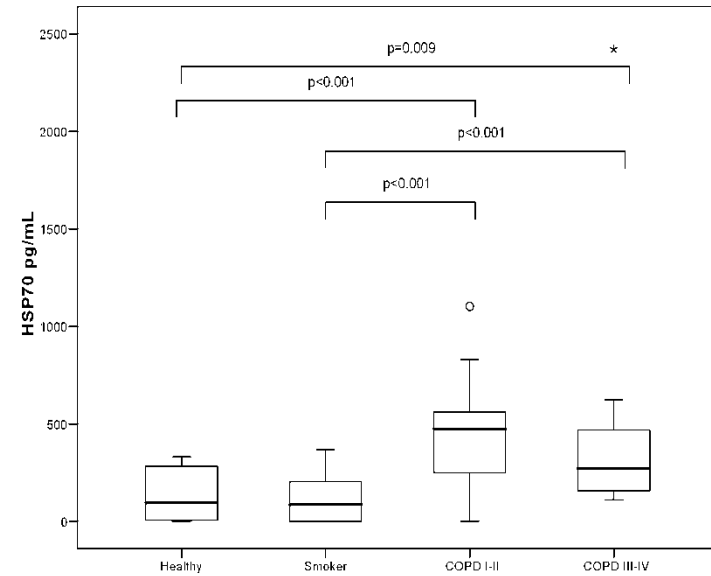
Results VI

Subject Category	Healthy	Smoker	COPD GOLD I&II	COPD GOLD III&IV
IFN- γ CD3 (pg/mL)	272	240	440	328
IFN- γ PHA (pg/mL)	116	91	375	134
TNF- α CD3 (pg/mL)	922	731	1234	1508
TNF- α PHA (pg/mL)	1096	777	2465	1144

Results VII

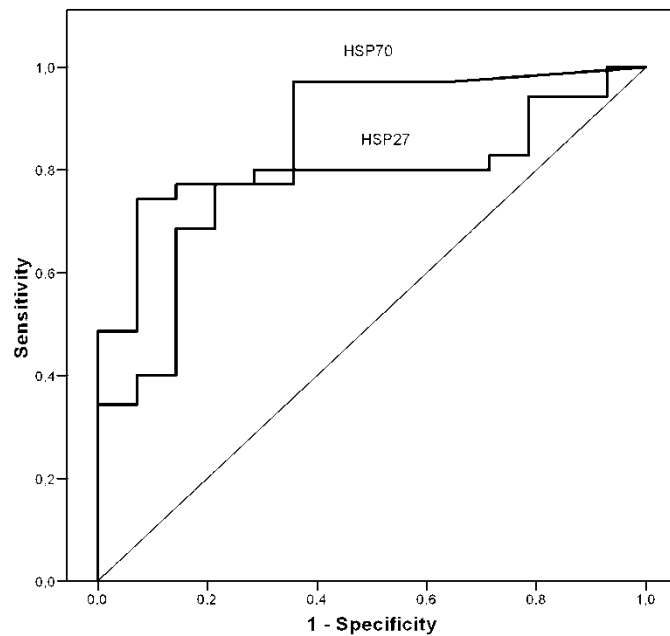


HSP27



HSP70

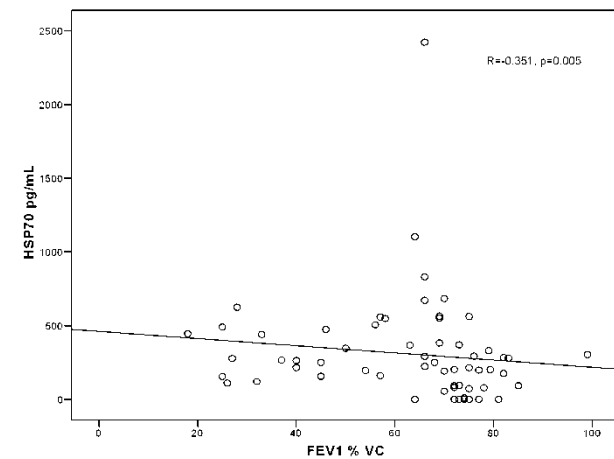
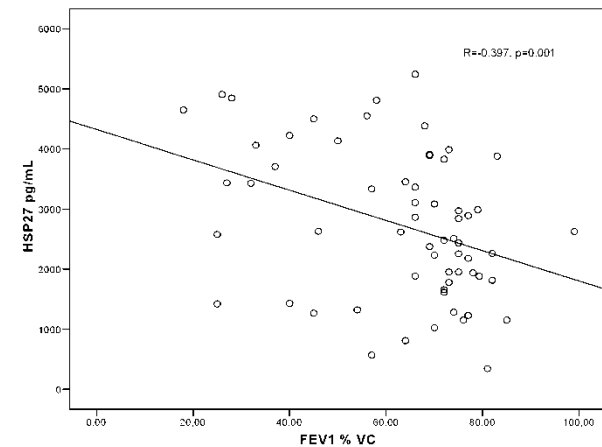
Results VIII



AUC

HSP27 = 0.763

HSP70 = 0.885



Conclusions

- CD4+CD28null cells
 - are systemically increased in COPD
 - show immunologic features of NK cells
 - contribute to immune activation (IFN- γ , etc.)
 - may be the link between lung and inflammation of other organs
- HSPs
 - are increased in serum of patients with COPD
 - may contribute to adaptive immune response
- Quantification of CD4+CD28null cells + HSPs
 - may help in the diagnostic process

The future

- Prospective study design
 - CD4+CD28null cells as risk factor?
- Describe CD4+CD28null cells in the lung tissue of patients
- Develop screening tests (cells, HSPs)

Thank you for your attention!

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