Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Increased levels of serum heat shock protein 27 as a possible marker for incipient obstructive pulmonary disease in a risk cohort

Diplomarbeit
Stefanie Nickl

ausgeführt an der
Universitätsklinik für Chirurgie
Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration

unter der Anleitung von
Ass.-Prof. Univ.-Doz. Dr. Hendrik Jan Ankersmit

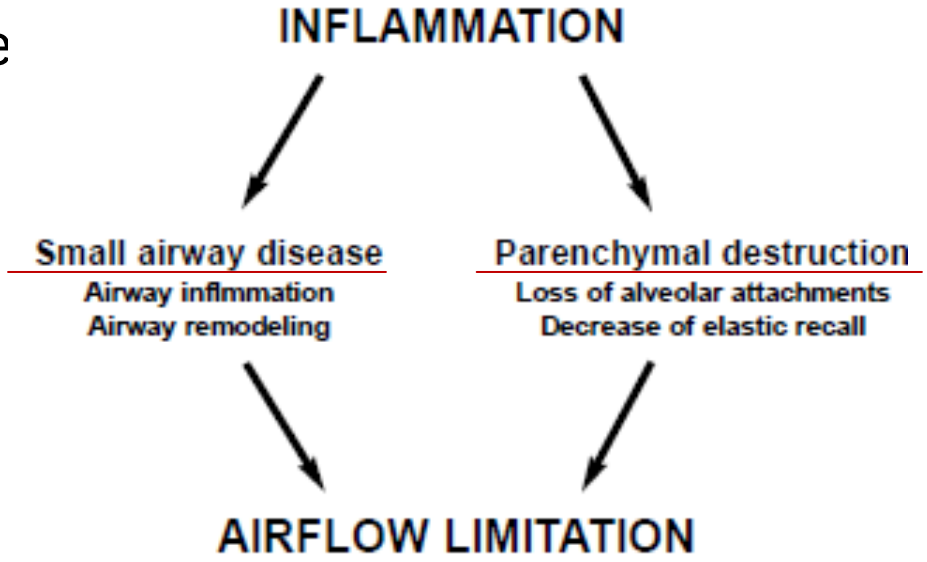
Wien, 28. Juni 2011

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Background
COPD

- progressive airflow limitation
- abnormal inflammatory response of the lung
- diagnosis and classification by means of spirometry
  - FEV1/FVC<0.7
  - FEV1%
  - GOLD I-IV (former 0-IV)
- *preventable* and *underdiagnosed*



GOLD. Global Stragey for the Diagnosis, Management, and Prevention of Chronic obstructive Pulmonary disease 2007. http://www.goldcopd.org/Guidelineitem.asp?l1=2&l2=1&intId=1816

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Background
COPD – Epidemiology

- 5th leading cause of death
  - 5-year mortality GOLD IV: 73%
- prevalence 5 – 22% (≥GOLD II)
- burden in terms of disability and impaired QOL
  - comorbidities
- financial burden (ERS: €38.7 billion/year)

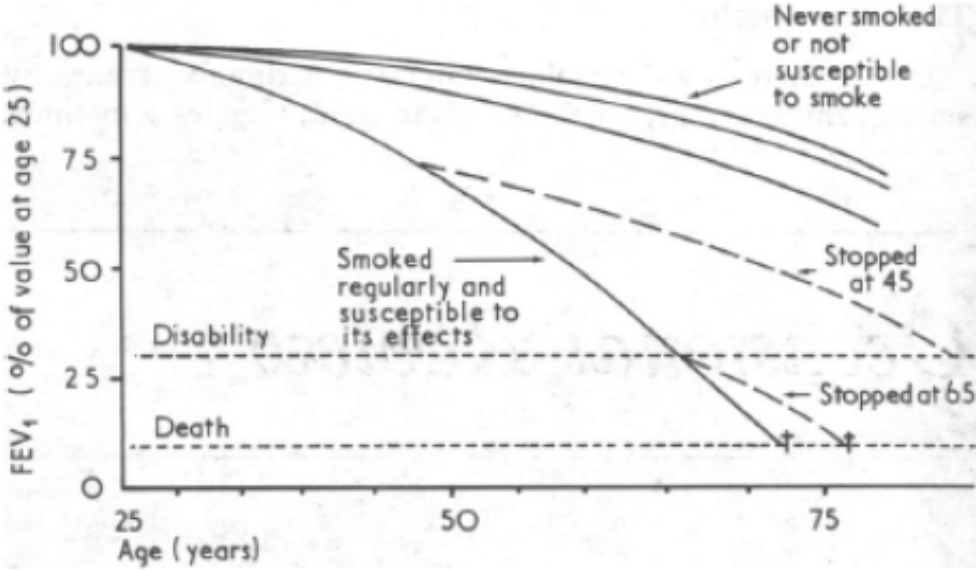
Lopez AD et al. ed. Washington DC: World Bank; 2006.
Menezes AM et al. Lancet 2005;366(9500)1875-1881.
Buist AS et al. Lancet 2007;370(9589):741-750.
Loddenkemper R et al. Eur Respir J 2003;22(6):869.

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Background
COPD – Risk Factors

- <u>tobacco smoking</u>
- environmental risk factors
- infections
  - adenovirus, rhinovirus, EBV
  - bacterial infections
    - → exacerbations
- genetic risk factors
  - severe alpha1-antitrypsin deficiency
  - many possible „target genes“

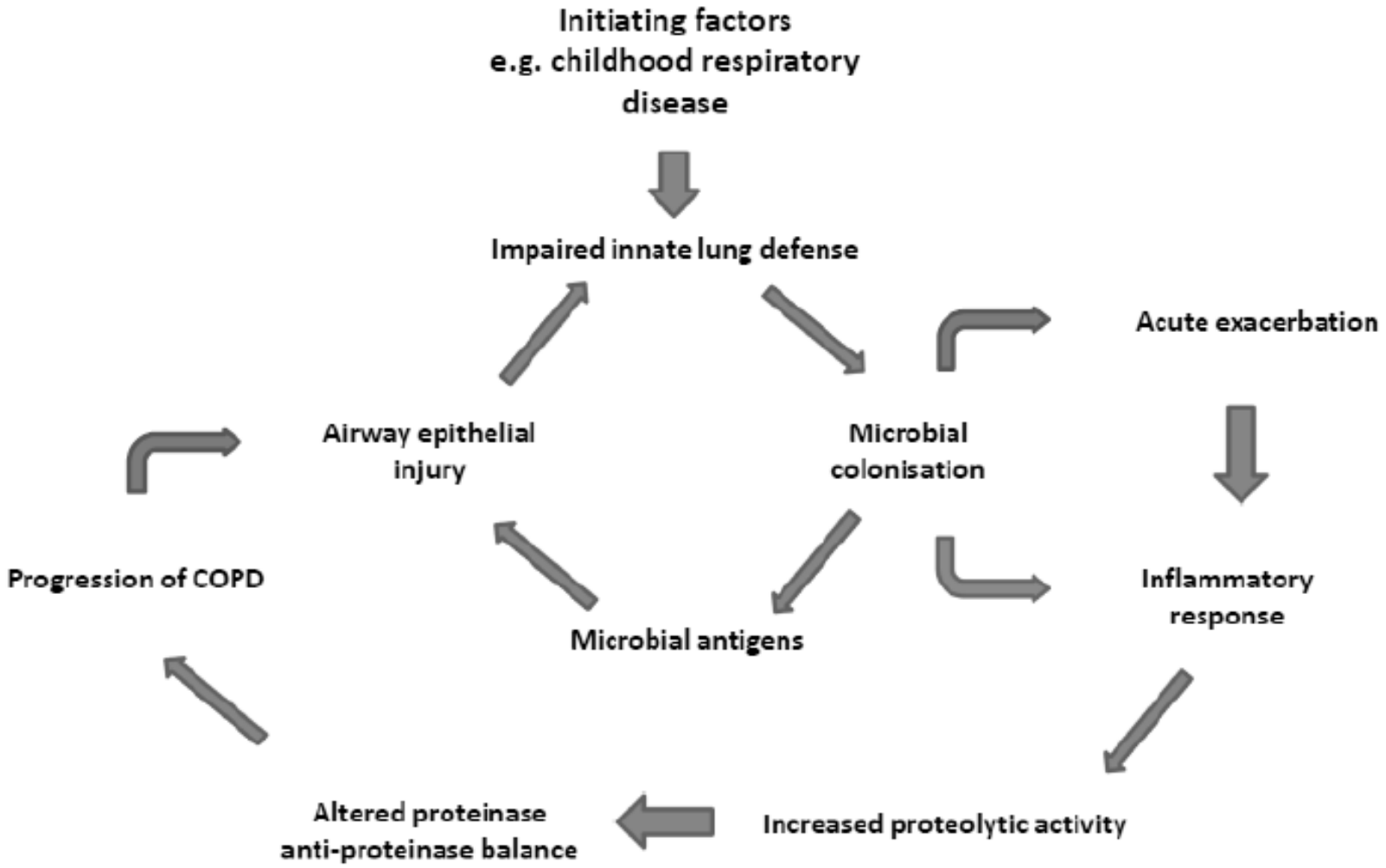


Fletcher C et al. Br Med J 1977;1(6077):1645-1648.
Sethi S et al. Chest 2000;117(5 Suppl 1):286S-291S.
Silverman EK et al. Genet Epidemiol 1992;9(5):317-331.
Marciniak SJ et al. Thorax 2009;64(4):359-364.

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Background
COPD – Risk Factors



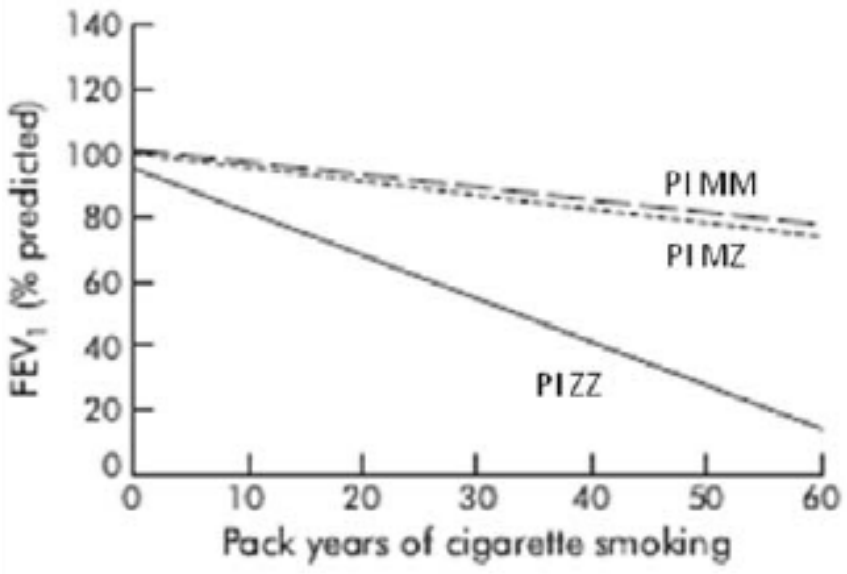
Fletcher C et al. Br Med J 1977;1(6077):1645-1648.
Sethi S et al. Chest 2000;117(5 Suppl 1):286S-291S.
Silverman EK et al. Genet Epidemiol 1992;9(5):317-331.
Marciniak SJ et al. Thorax 2009;64(4):359-364.

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Background
COPD – Risk Factors

Symbol	Name	Locus	Function
ELN	Elastin	7q11	Matrix
FBLN4	Fibulin 4	11q13	Matrix
FBLN5	Fibulin 5	14q32	Matrix
FBN1	Fibrillin	15q21	Matrix
ATP7A	Copper transporter	Xq13	Matrix
TGFB1	Transforming growth factor β1 (TGFβ1)	19q13	Matrix
LTBP4	Latent TGFβ binding protein 4	19q13	Matrix
SERPINA1	α_1-Antitrypsin	14q32	Anti-protease
SERPINE2	Serpin E2	2q33	Antiprotease
TIMP2	Tissue inhibitor of metalloproteinase 2	17q25	Antiprotease
MMP1	Metalloproteinase 1	11q22	Protease
MMP9	Metalloproteinase 9	20q11	Protease
MMP12	Metalloproteinase 12	11q22	Protease
EPHX1	Epoxide hydrolase 1	1q42	Detoxification
GST-P1	Glutathione S-transferase P1	11q13	Detoxification
GST-M1	Glutathione S-transferase M1	1p13	Detoxification
HMOX1	Heme oxygenase 1	22q13	Detoxification
SOD3	Superoxide dismutase 3	4p15	Detoxification
TNF	Tumour necrosis factor	6p21	Inflammation
GC	Group specific component	4q12	Inflammation

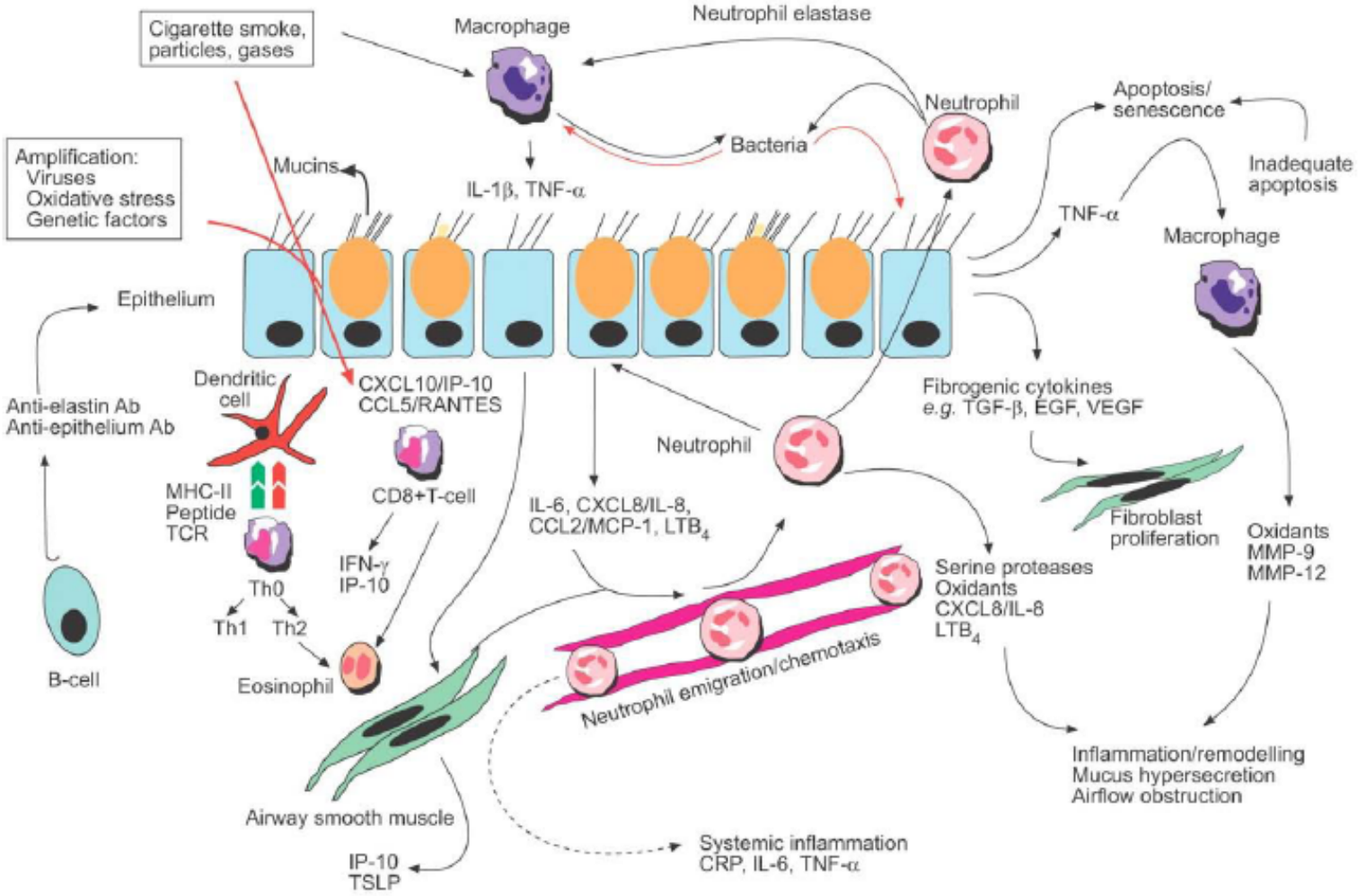


Fletcher C et al. Br Med J 1977;1(6077):1645-1648.
Sethi S et al. Chest 2000;117(5 Suppl 1):286S-291S.
Silverman EK et al. Genet Epidemiol 1992;9(5):317-331.
Marciniak SJ et al. Thorax 2009;64(4):359-364.

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Background
COPD - Pathogenesis



Chung KF et al. Eur Respir J 2008;31(6):1334-1356.

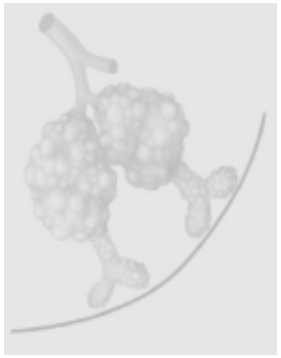
Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

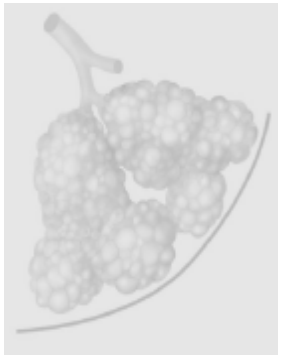
MEDIZINISCHE UNIVERSITÄT WIEN

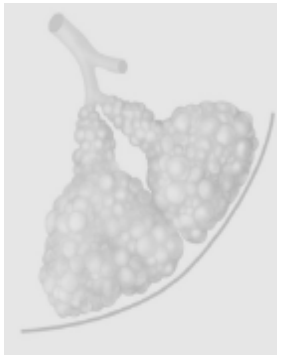
Background

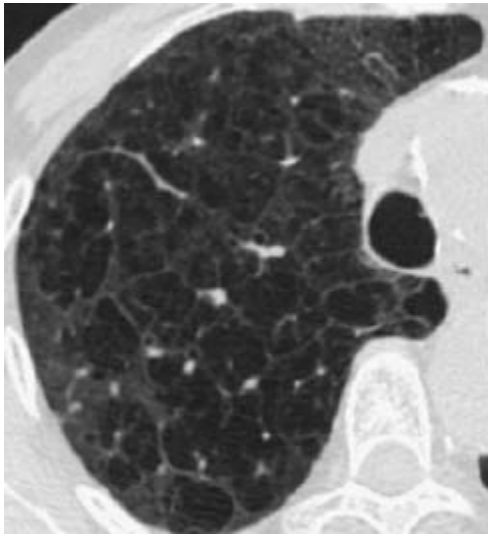
COPD – Morphologic Changes I

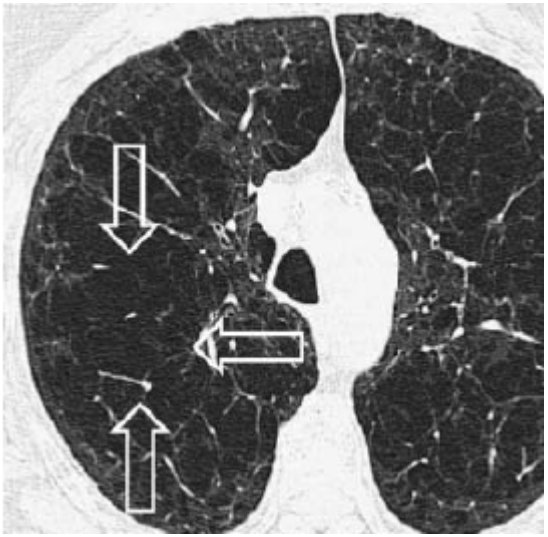
- pulmonary emphysema
  - centrilobular
  - panlobular
  - paraseptal

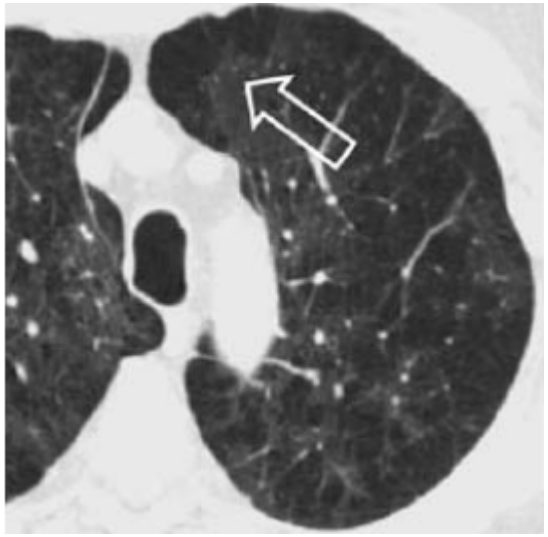










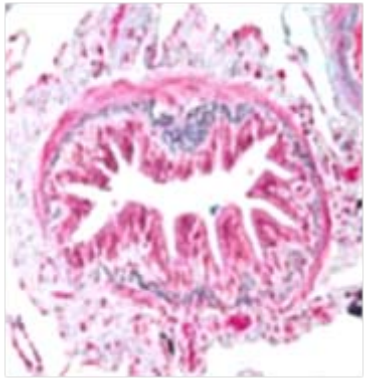


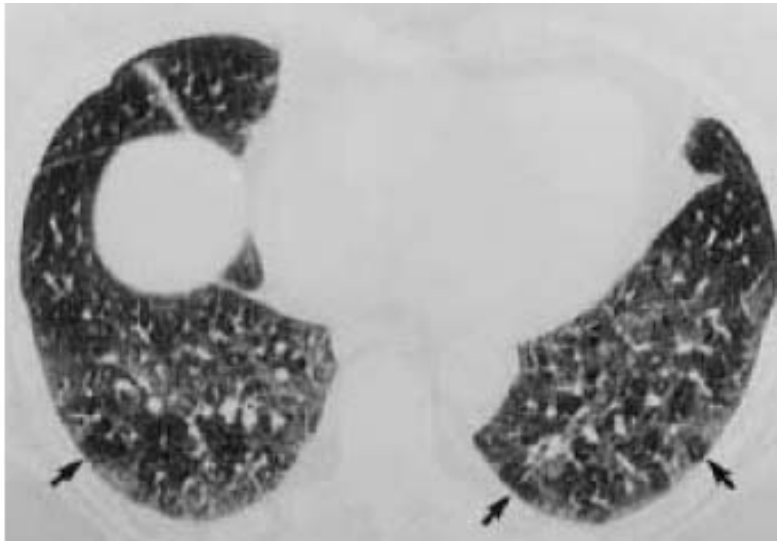
Takahashi M et al. Int Jchron Obstruct Pulmon Dis 2008;3(2):193-204.
Litmanovich D et al. Eur Radiol 2009;19(3):537-551.

Background

COPD – Morphologic Changes II

- small airway disease
  - inflammatory infiltrate
  - airway wall remodeling
  - „air trapping“





Hogg JC. Lancet 2004;364(9435):709-721.
Kauczor HU et al. Eur Radiol 2000;10(10):1539-1546.

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Background
COPD – Biomarkers

U.S. Center for Drug Evaluation and Research (CDER)

"with the exception of lung function tests, there are no well-validated biomarkers […] that can be used […] for COPD".

- Informs on the disease process and prognosis
- Reliable and reproducible in a routine clinical setting
- Inexpensive
- Measurable changes in response to intervention
- Little or no diurnal variation
- Sensitive, disease-specific, high positive and negative predictive values
- Sampling method acceptable to patients

- blood-derived biomarkers
- airway-sampled biomarkers
  - sputum
  - BAL
  - exhaled air
  - EBC

Guidance for Industry. Chronic Obstructive Pulmonary Disease: Developing Drugs for Treatment. 2007; http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm071575.pdf
Patel AR et al. Semin Respir Crit Care Med 2010;31(3):267-275.

Background
Heat Shock Proteins

	Protein Function	
Stress Protein	Intracellular	Extracellular
Hsp27	Chaperone antideath	Anti-inflammatory
Hsp60	Chaperonin	Proinflammatory
Hsp70	Chaperone antideath	Immunoregulatory proinflammatory neuronal survival
Hsp90	Chaperone cell regulation	Proimmune prometastatic
Hsp110	Chaperone co-chaperone	Proimmune

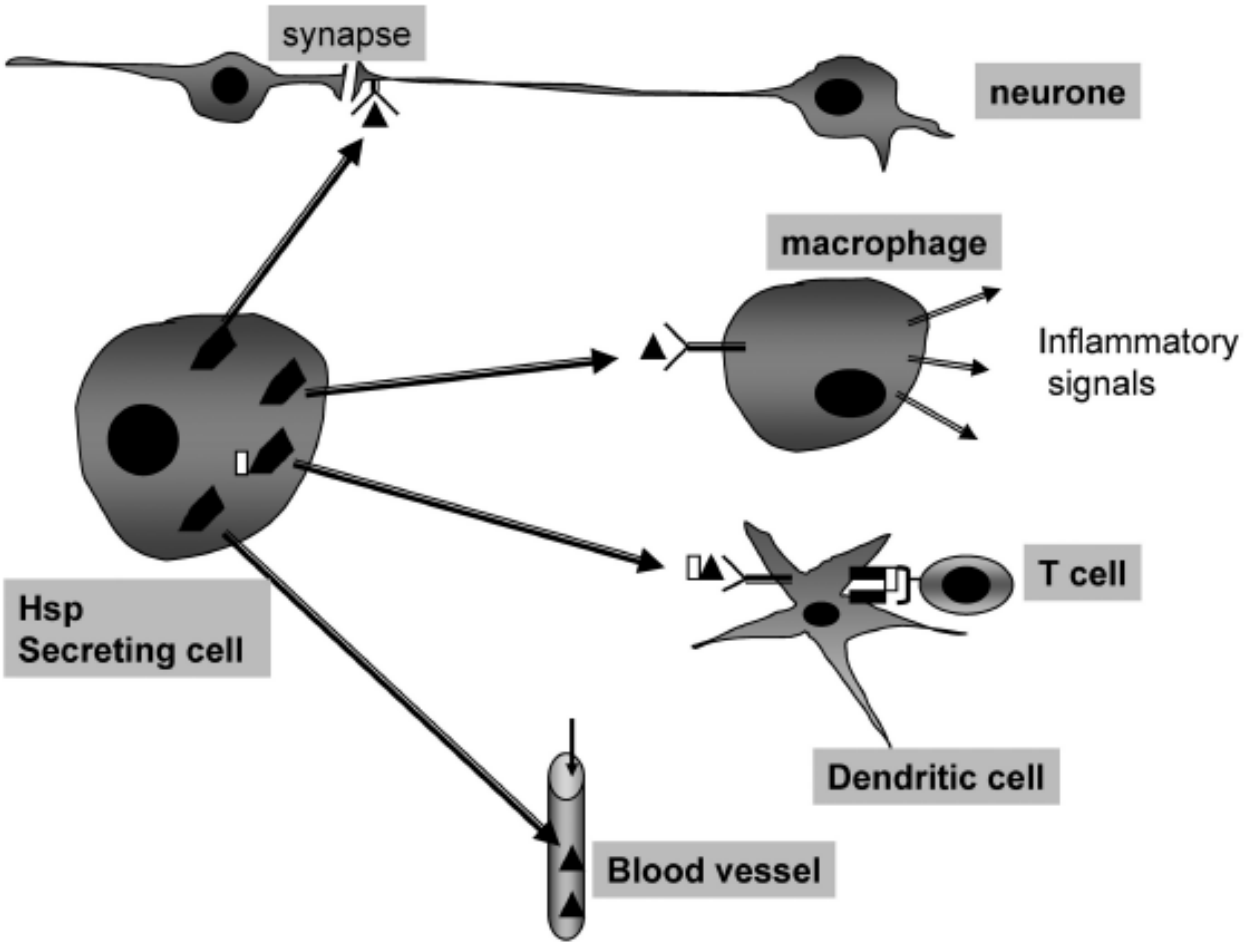
- usually intracellular chaperones
- release after cell stress and trauma → extracellular „danger signal“
- modulation of the immune response

Calderwood SK et al. FEBS Lett 2007;581(19):3689-3694.

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Background
Heat Shock Proteins



Calderwood SK et al. FEBS Lett 2007;581(19):3689-3694.

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

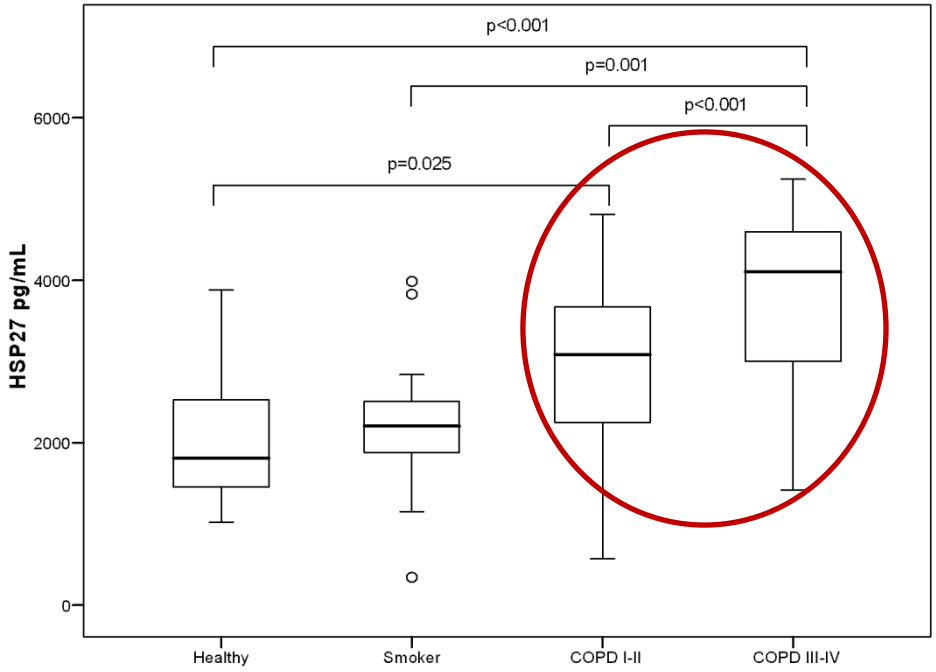
Background

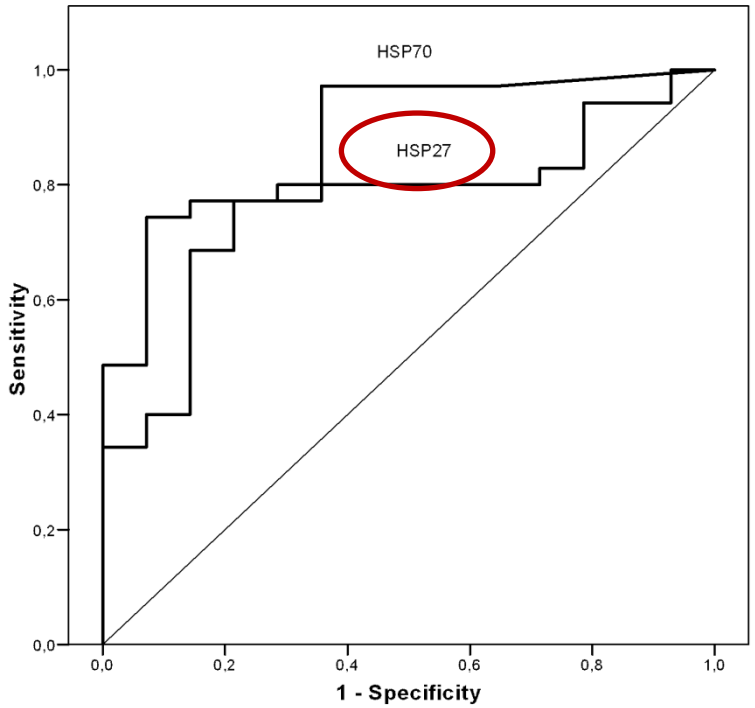
Heat Shock Proteins in COPD

Clin Lab 2009;55(1-2):31-40.

Elevated HSP27, HSP70 and HSP90 alpha in chronic obstructive pulmonary disease: markers for immune activation and tissue destruction

Hacker S, Lambers C, Hoetzenecker K, Pollreisz A, Aigner C, Lichtenauer M, Mangold A, Niederpold T, Zimmermann M, Taghavi S, Klepetko W, Ankersmit HJ.





Hacker S et al. Clin Lab 2009;55(1-2):31-40.

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Aims of the Study

- Evaluation of HSP27 serum levels in long-time smokers
  - normal lung function
  - air trapping and/or emphysema
- Are HSP27 serum values altered before lung function parameters indicating obstruction deteriorate?
- Measurement of proinflammatory, pro-angiogenic and chemotactic factors, markers for apoptosis and metalloproteinases

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Materials & Methods I

- 120 subjectively healthy smokers
  - questionnaire (smoking habits, medication)
  - lung function testing (FVC[%], FEV1[%], FEV1/FVC ratio)
  - acquisition of serum samples
  - HR-CT (optional)

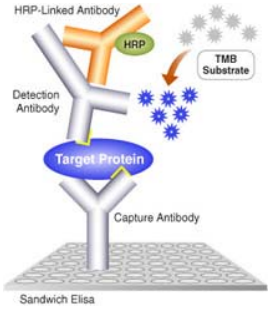
- Exclusion criteria:
  - known lung diseases
  - relevant cardiopulmonary morbidities
  - autoimmune diseases
  - use of immunomodulatory drugs within 2 weeks before study entry

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Materials & Methods II

- ELISA



- High resolution CT
  (16-detector MDCT scanner)

- SPSS / GraphPad Prism 5

http://user.meduni-graz.at/helmut.hinghofer-szalkay/II.8.htm
http://www.usedctscannersandmri.com/used-toshiba-aquilion-16-ct.html
http://www.goomedic.com/spss-tutorials-educational-videos-and-courses-materials.html
http://www.graphpad.com/prism/prism.ht

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Demographics

	total study group 100%	HR-CT study group 78.3%	*P*-value
n	120	94	-
M / F *n* M / F %	53 / 67 44.2 / 55.8	42 / 52 44.7 / 55.3	*n.s.*
Age	43.1±9.7	43.4±9.5	*n.s.*
Pack Years	17.0 (10.5/31.0)	16.7 (10.8/33.9)	*n.s.*
HSP27 (pg/ml)	3623±1552	3761±1582	*n.s.*
Lung Function			
FVC%	90.2±11.5	90.6±11.7	*n.s.*
FEV1%	83.6±11.8	83.5±12.9	*n.s.*
FEV1/FVC	0.779±0.078	0.771±0.077	*n.s.*
FEV1/FVC≤0.7	15 (12.5%)	13 (13.8%)	*n.s.*

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

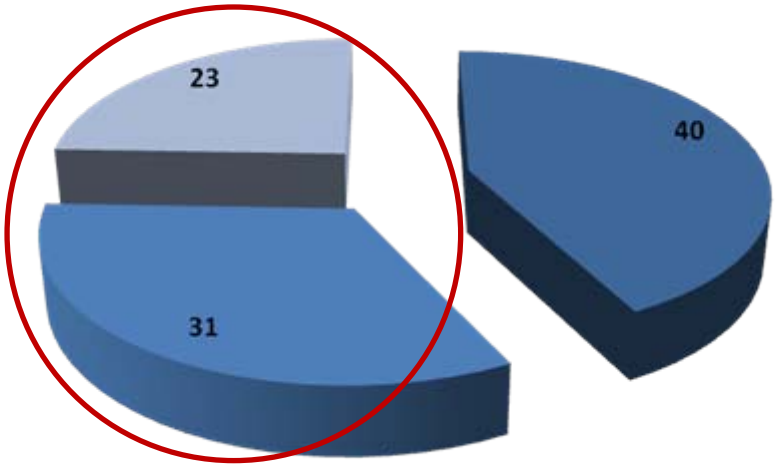
MEDIZINISCHE UNIVERSITÄT WIEN

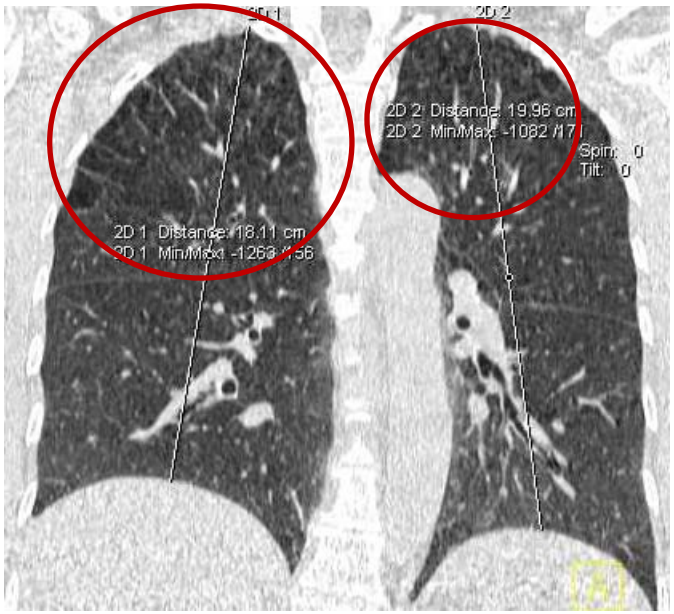
Results I

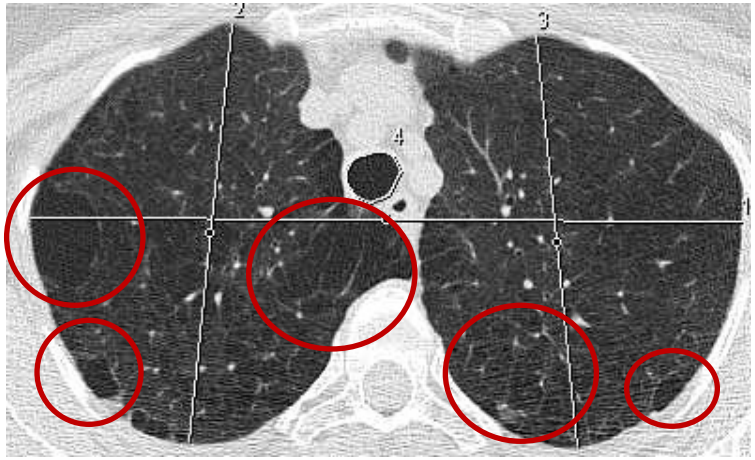
n= 54 (57.5%) HR-CT abnormalities

n= 31 (33.0%) AT

n= 23 (24.5%) AT+E







U.H., 53 yrs., >40 PYs
normal lung function
centrilobular emphysema; air trapping

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

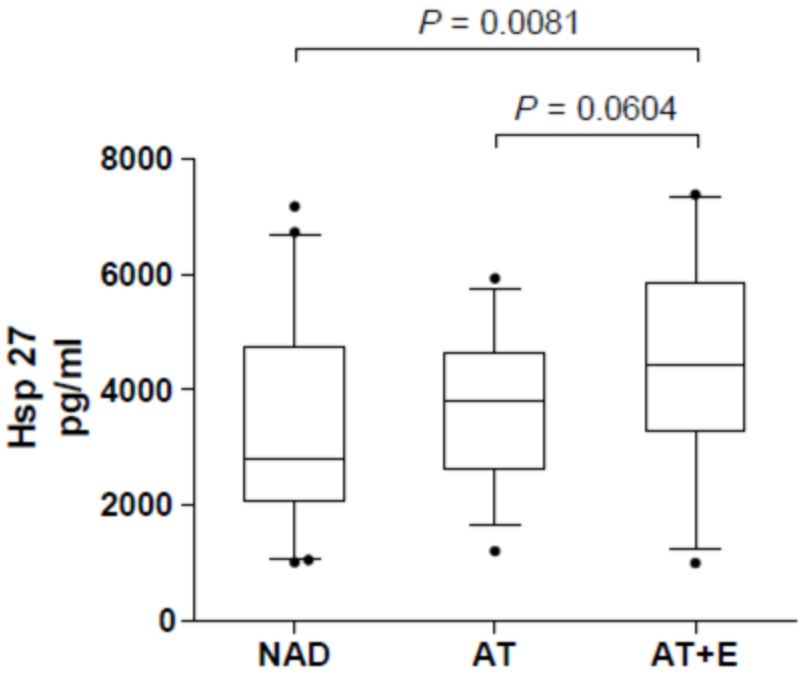
Results II

	nothing abnormal detected	air trapping	air trapping and emphysema	total	*P*-value
n	40	31	23	94	-
M / F *n* **M / F %**	19 / 21 (47.5 / 52.5)	15 / 16 (48.4 / 51.6)	8 / 15 (34.8 / 65.2)	42 / 52 (44.7 / 55.3)	*n.s.*
Age	42.1±10.0	41.9±9.5	47.7±7.5	43.4± 9.5	*0.039*
Pack Years	15.6 (10.0/29.8)	14.8 (8.6/21.3)	33.0 (15.3/40.3)	16.7 (10.8/33.9)	*0.011*
Lung Function					
FVC%	92.4±10.5	90.4±12.5	87.7±12.5	90.6±11.7	*n.s.*
FEV1%	86.6±12.9	83.2±13.1	78.4±11.3	83.5±12.9	*n.s.*
FEV1/FVC	0.78±0.07	0.77±0.06	0.75±0.11	0.77±0.08	*n.s.*
FEV1/FVC≤0.7	5 (12.5%)	3 (9.7%)	5 (21.7%)	13 (13.8%)	*n.s.*

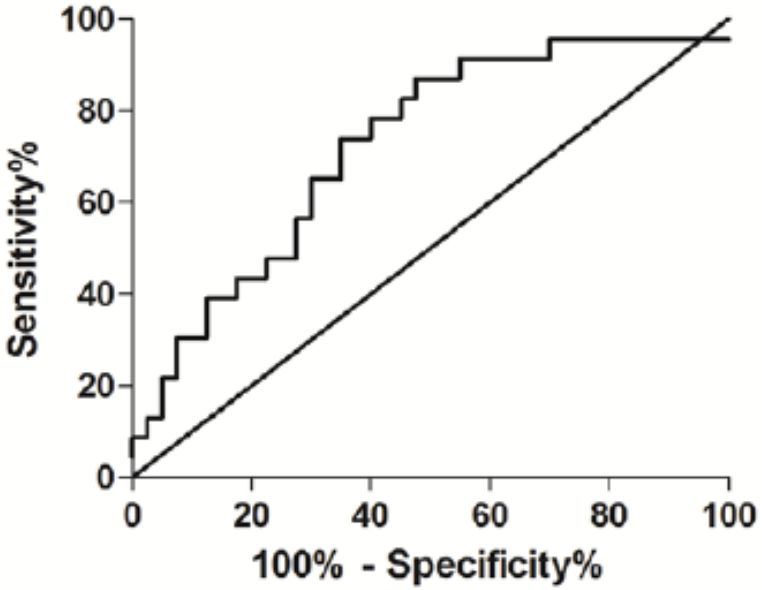
Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Results III



mean ± SD	3282 ± 1607	3744 ± 1210	4618 ± 1677



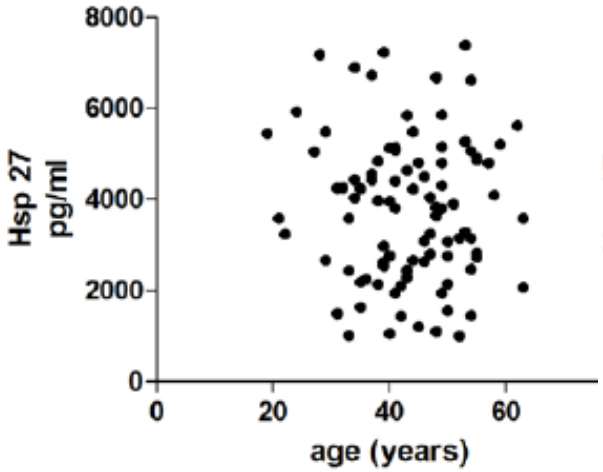
ROC-curve (AT+E)

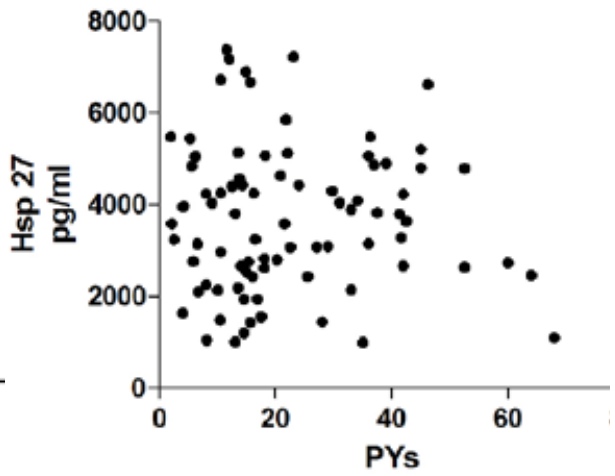
→ AUC=0.724 (*P*=0.003)

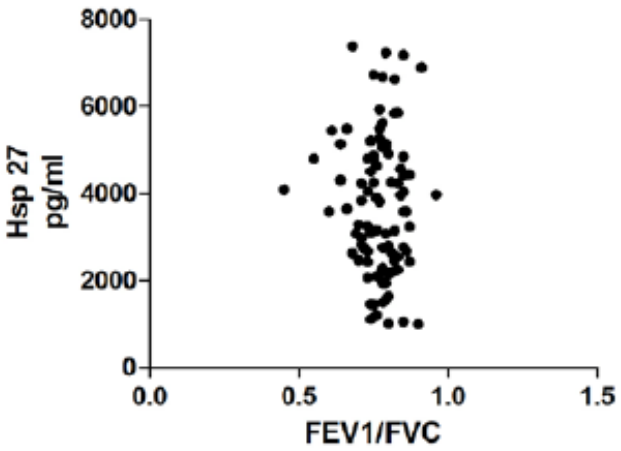
Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Results IV







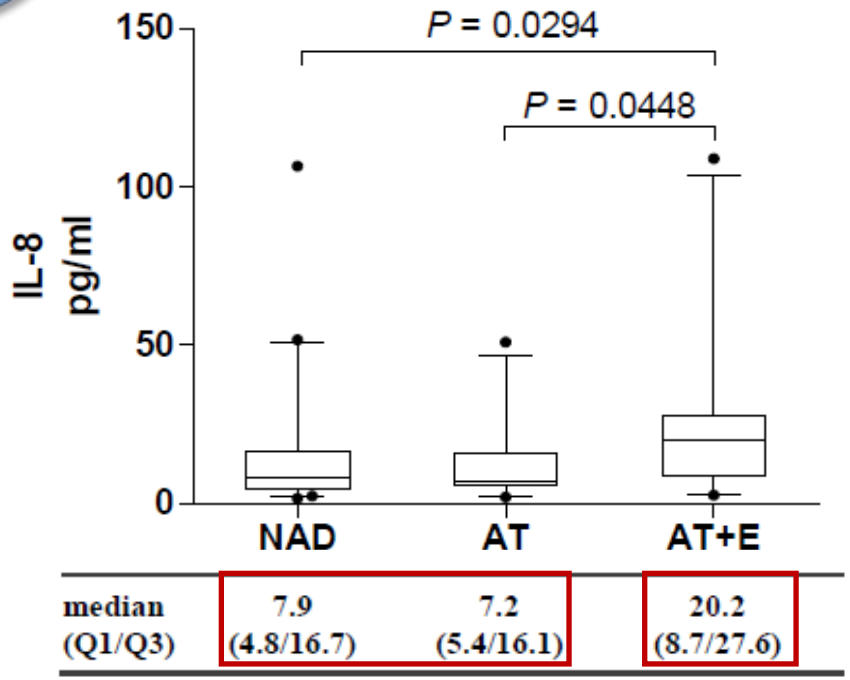
→ no correlation of serum HSP27 with other clinical parameters

Age: r=-0.056; *P*=0.595
PYs: r=-0.028; *P*=0.801
FEV1/FVC: r=-0.059; *P*=0.575

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Results V



HR-CT Findings	nothing abnormal detected		air trapping		air trapping + empyhsema		*P*-value
	Median (Q1/Q3)	Min–Max	Median (Q1/Q3)	Min–Max	Median (Q1/Q3)	Min–Max	
IL-1ß (pg/ml)	0.0 (0.0/2.67)	0.0–81.36	0.0 (0.0/0.08)	0.0–96.16	0.0 (0.0/1.95)	0.0–38.75	*0.916*
IL-6 (pg/ml)	0.0 (0.0/1.08)	0.0–76.26	0.0 (0.0/0.65)	0.0–23.15	0.19 (0.0/1.16)	0.0–46.25	*0.679*
TNF-α (pg/ml)	0.0 (0.0/0.0)	0.0–9.37	0.0 (0.0/0.0)	0.0–0.0	0.0 (0.0/0.0)	0.0–2.25	*0.132*

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

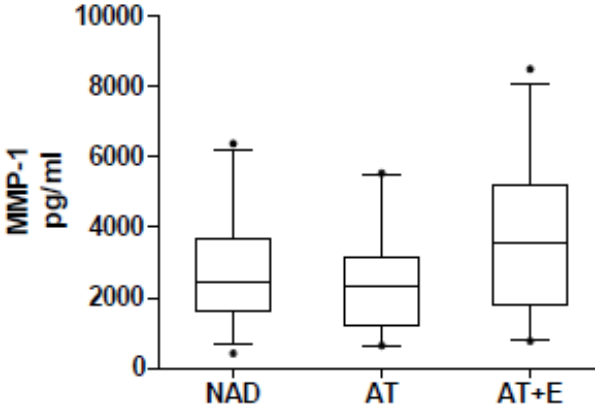
Results VI

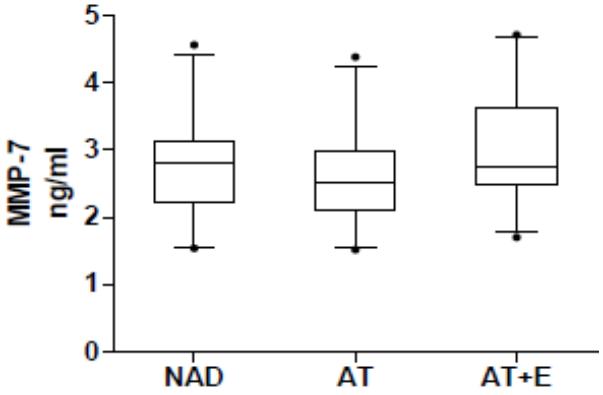
HR-CT Findings	nothing abnormal detected		air trapping		air trapping + empyhsema		*P*-value
	Median (Q1/Q3)	Min–Max	Median (Q1/Q3)	Min–Max	Median (Q1/Q3)	Min–Max	
sST2 (pg/ml)	67.24 (41.19/137.1)	3.16–810.4	60.14 (32.36/125.7)	2.86–445.2	69.6 (9.93/206.1)	0.0–623.6	*0.573*
GRO-α (pg/ml)	24.79 (8.74/45.54)	0.0–116.9	27.39 (18.76/44.70)	0.0–112.7	25.42 (11.18/44.91)	0.0–107.3	*0.700*
M30 (U/L)	147.1 (108.6/223.8)	59.13–519.0	115.5 (96.55/164.3)	43.97–712.4	168.8 (130.1/264.1)	85.4–530.9	*0.062*
HMGB1 (ng/ml)	2.21 (1.53/3.52)	0.85–7.79	2.09 (1.36/2.87)	0.57–12.23	2.15 (1.53/4.0)	1.04–8.25	*0.618*
	Mean ± SD	**95% CI**	**Mean ± SD**	**95% CI**	**Mean ± SD**	**95% CI**	
ENA-78 (pg/ml)	2664 ± 1529	2175–3153	3575 ± 1694	2954–4196	2643 ± 1322	2024–3262	*0.031*
RANTES (ng/ml)	71.29 ± 26.17	62.81–79.78	74.18 ± 16.42	68.05–80.31	75.57 ± 19.37	67.20–83.95	*0.730*
MMP-1 (pg/ml)	2812 ± 1691	2264–3360	2467 ± 1433	1922–3012	3602 ± 2082	2701–4502	*0.062*
MMP-7 (ng/ml)	2.73 ± 0.71	2.49–2.97	2.62 ± 0.69	2.36–2.88	3.01 ± 0.80	2.66–3.36	*0.157*
MMP-9 (ng/ml)	1837 ± 857.9	1562–2111	1844 ± 647.8	1607–2082	2184 ± 1078	1718–2650	*0.252*
HSP70 (pg/ml)	282.3 ± 152.1	233.7–331.0	250.6 ± 149.6	194.7–306.4	311.6 ± 172.0	237.2–386.0	*0.371*
sRAGE (pg/ml)	363.5 ± 188.5	300.6–426.3	384.9 ± 188.0	314.7–455.1	283.0 ± 221.2	182.3–383.7	*0.175*

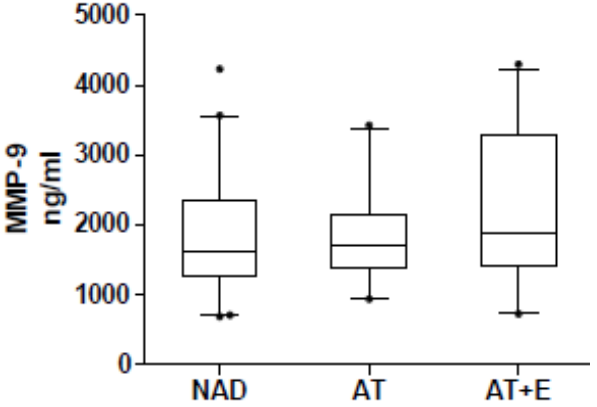
Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

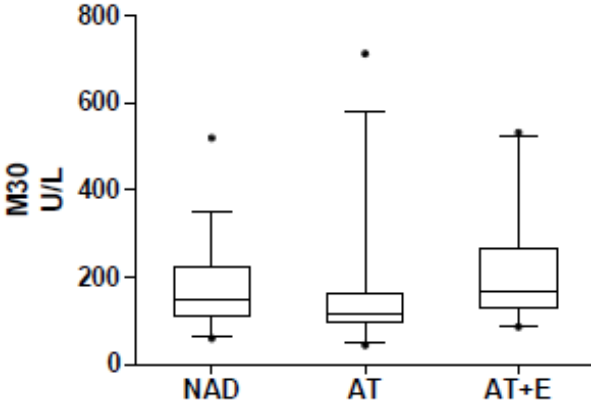
MEDIZINISCHE UNIVERSITÄT WIEN

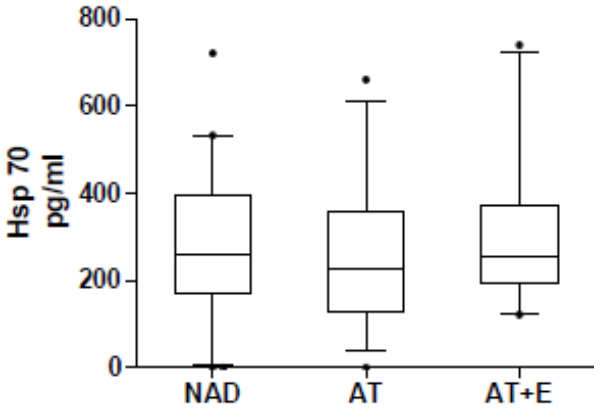
Results VI









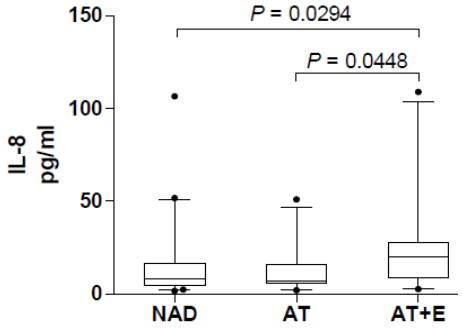


Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

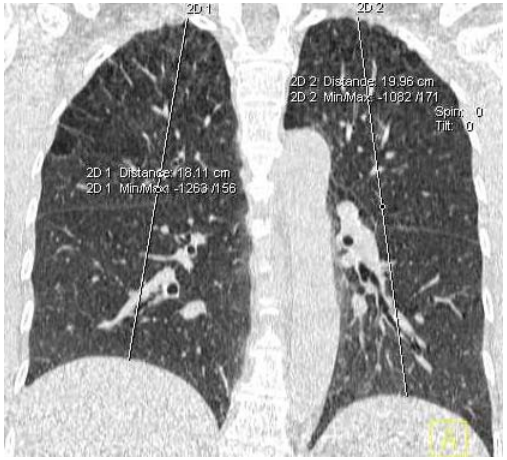
Conclusions

Chronic antigen exposure (e.g. tobacco smoke)



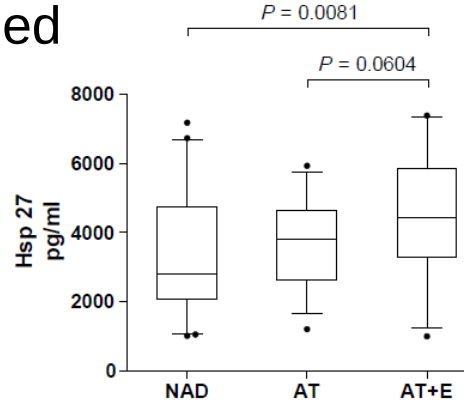
immune activation

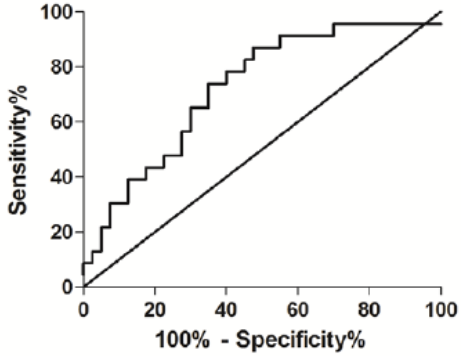
HSP27 as future marker for incipient COPD?



secretion of HSP27 into the vascular bed

COPD-associated radiological findings





Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

Special thanks to

MEDIZINISCHE UNIVERSITÄT WIEN