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Discrimination of Clinical Stages in Lung Cancer Patients by Serum HSP27 and HSP70

Diplomarbeit
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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

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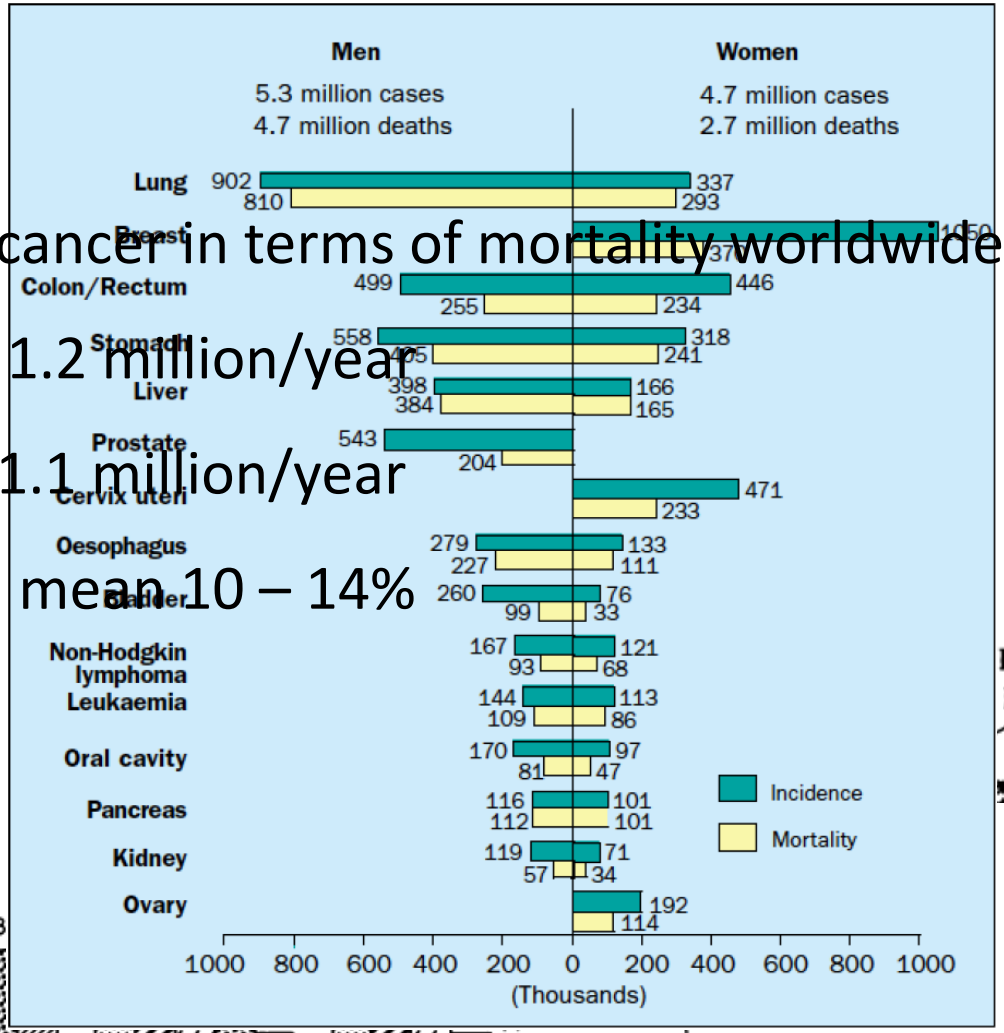
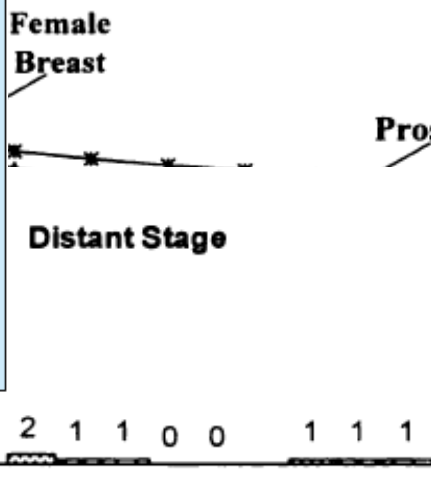
Background

Lung Cancer - Epidemiology

- most common cancer in terms of mortality worldwide
- incidence rate: 1.2 million/year
- mortality rate: 1.1 million/year
- 5-year survival: mean 10 – 14%





Parkin DM. Lancet Oncol 2(
Sugimura H et al, Chest 20(
Wingo PA et al, Cancer Epi

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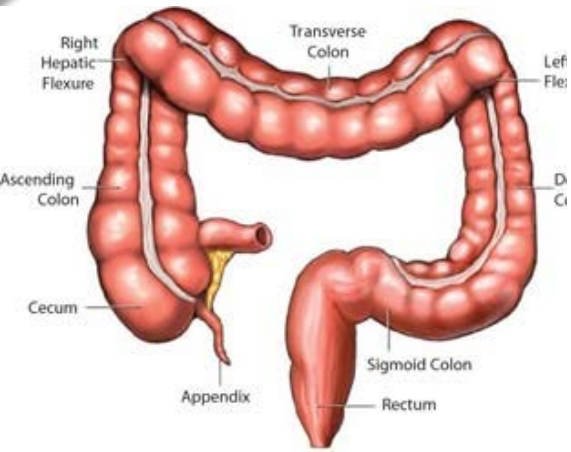
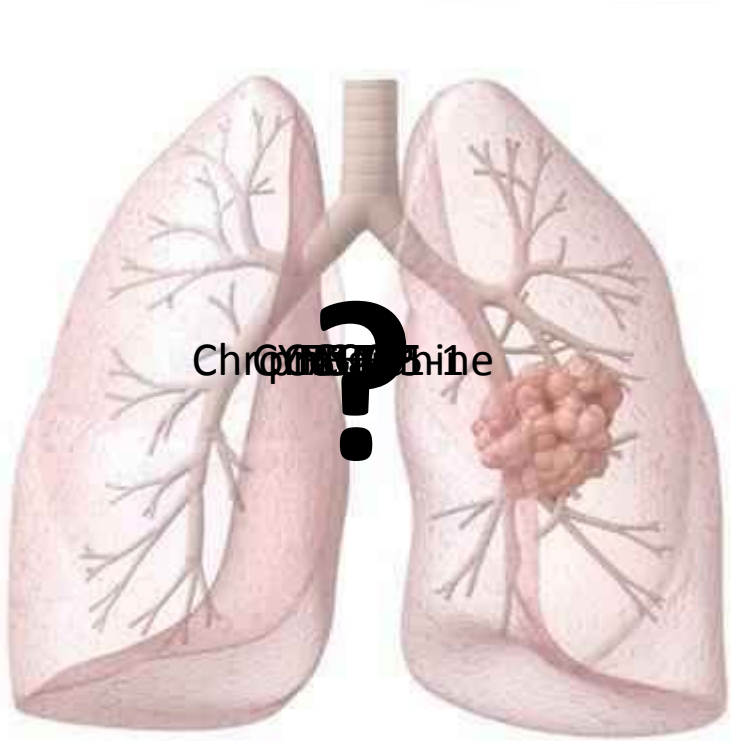
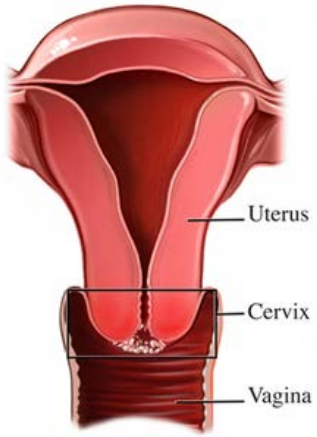
Background
Screening Programmes







http://www.uchospitals.edu/online-library/content=CDR62955
http://topnews.net.nz/content/217097-qantas-joins-prostate-cancer-drive
http://www.rho.org/ap/learn-screening.htmhttp://www.pillfreevitamins.com/coloncleanse.htm
http://www.pillfreevitamins.com/coloncleanse.htm
http://www.mylungcancerguide.com/lung-cancer-pictures.htm

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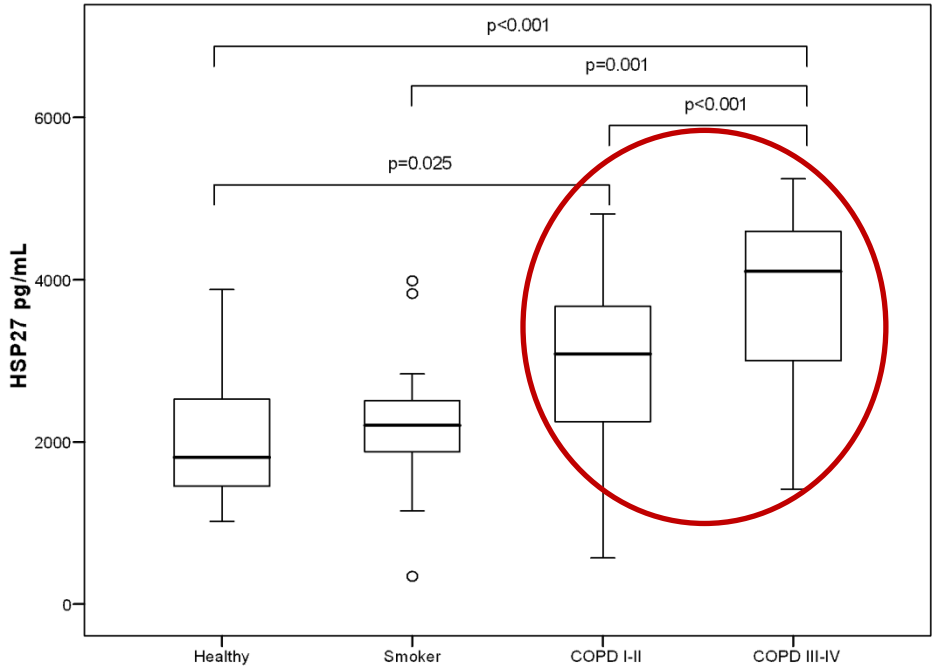
Background

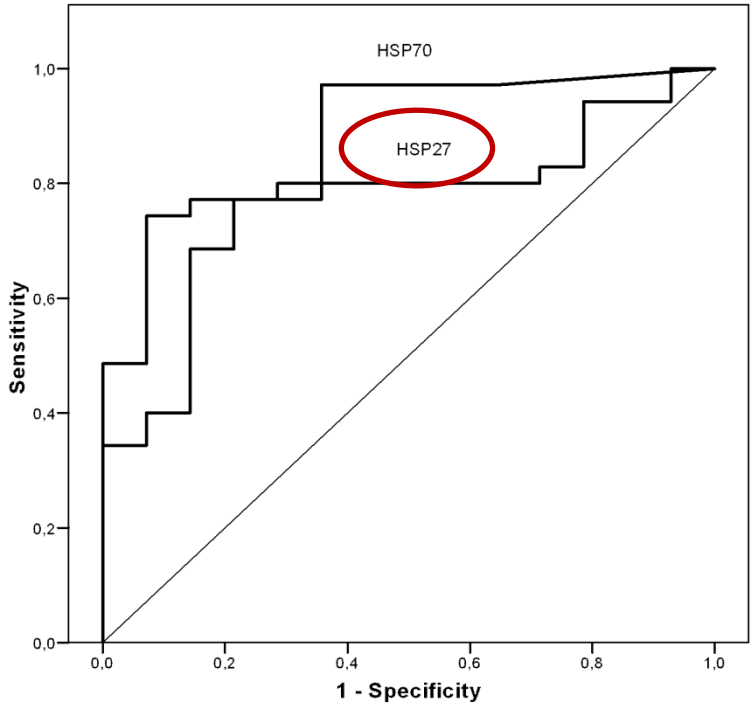
Heat Shock Proteins in COPD

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.

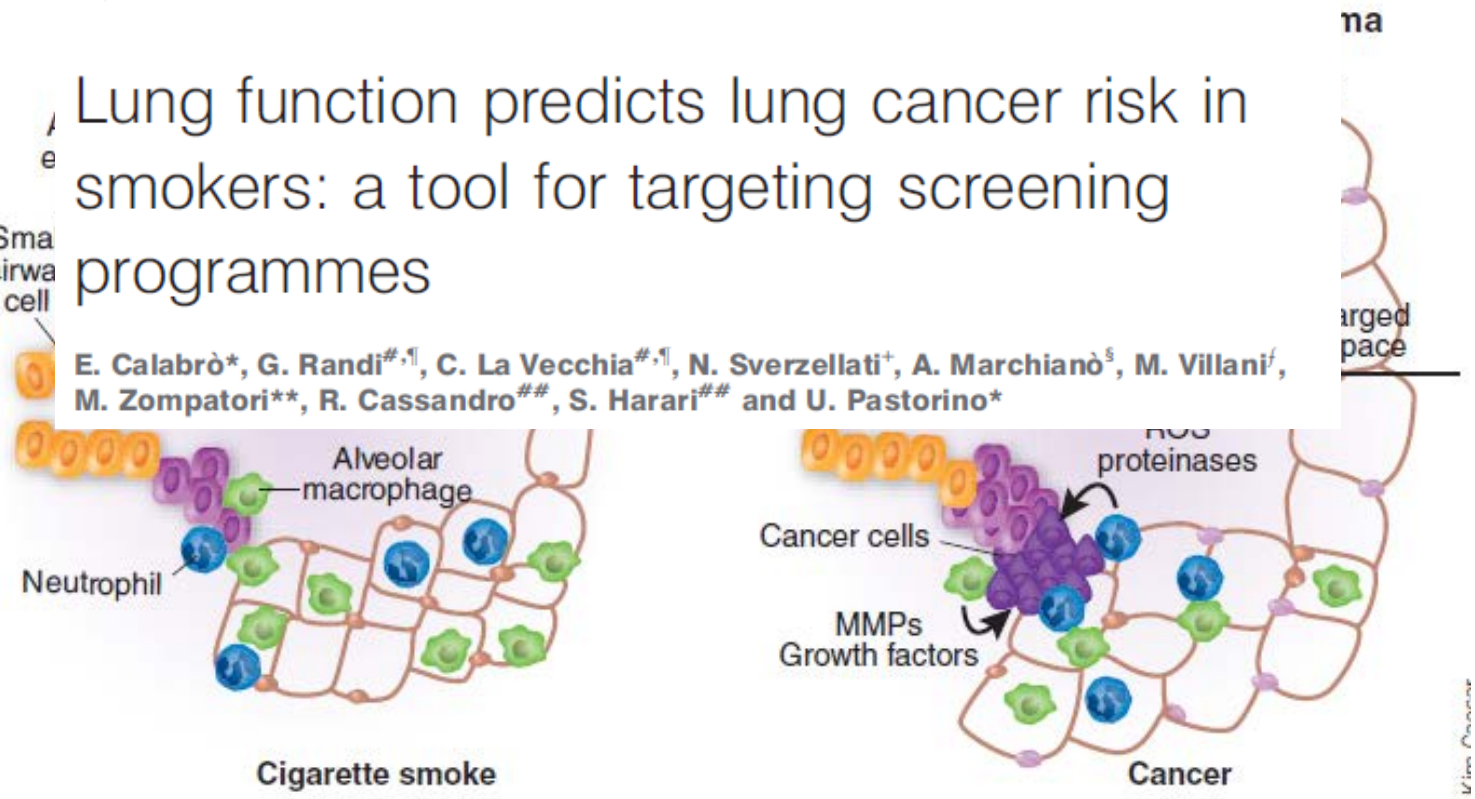
Background
Lung Cancer & COPD

- smo
- envi
- gene
- airw
  lung
- 40-7

Eur Respir J 2010; 35: 146–151
DOI: 10.1183/09031936.00049909

Lung function predicts lung cancer risk in smokers: a tool for targeting screening programmes

E. Calabrò*, G. Randi#,¶, C. La Vecchia#,¶, N. Sverzellati+, A. Marchianò§, M. Villani', M. Zompatori**, R. Cassandro##, S. Harari## and U. Pastorino*



Houghton A et al., Nat Med 2008;14:1023-24

Houghton A et al., Nat Med 2008;14:1023-24
Kishi et al., Eur Respir J 2002;19:1093-8.
Calabro et al., Eur Respir J 2010;35:146-151.
Petty TL. Chest 2005;128:1895-7.

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Background
Heat Shock Proteins

- Induced by different kinds of stress (heat, irradiation, oxidative stress,…)
- classified according to their molecular mass

  (HSP10, HSP27, HSP 40, HSP 60, HSP70, HSP90 and HSP110)
- strong cytoprotective effect
  - molecular chaperones (protein holding and folding)
  - inhibition of key effectors of the apoptotic machinery
  - Participation in proteasome-mediated degradation of proteins under stress conditions
- usually intracellular chaperones
- release after cell stress and trauma → extracellular „danger signal“
- modulation of the immune response

Stress Protein	Intrac
Hsp27	Chape
Hsp60	Chape
Hsp70	Chape
Hsp90	Chape

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

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Background
Heat Shock Proteins

- Properties of the HSPs are co-opted during malignant progression
- HSPs are overexpressed in a wide range of malignant cells and tissues
- increased transcription of HSPs due to loss of p53 function and to higher expression of proto-oncogenes HER2 and c-Myc
- promoting autonomous cell proliferation
- Inhibiting death pathways

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

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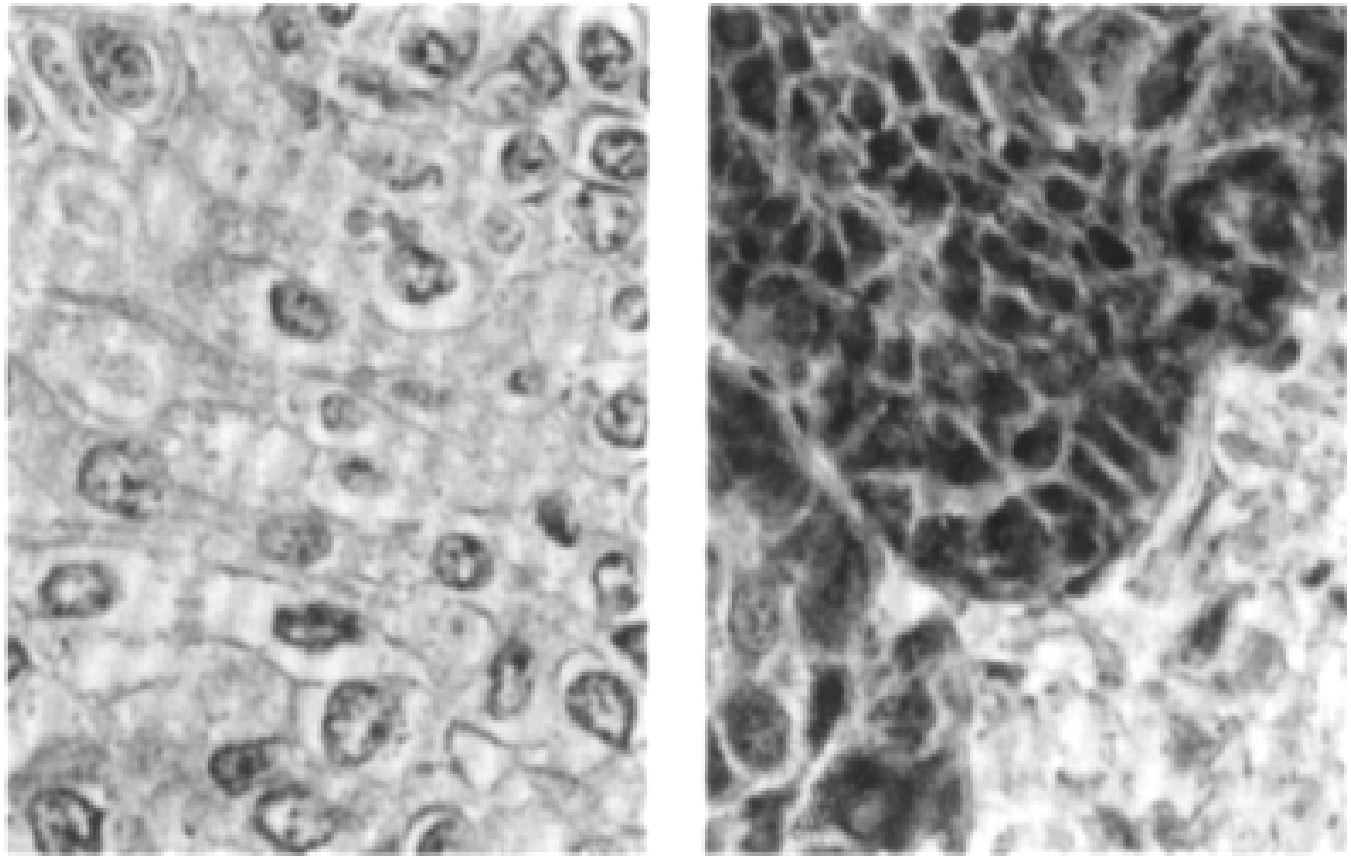
Heat Shock Proteins in Lung Cancer



Immunohistochemical staining of non-small cell lung carcinomas with antibody to heat shock protein 70. Left: negative tumor; right: positively stained tumor.

Volm S et al., Cancer Lett. 1995;95:195-200.

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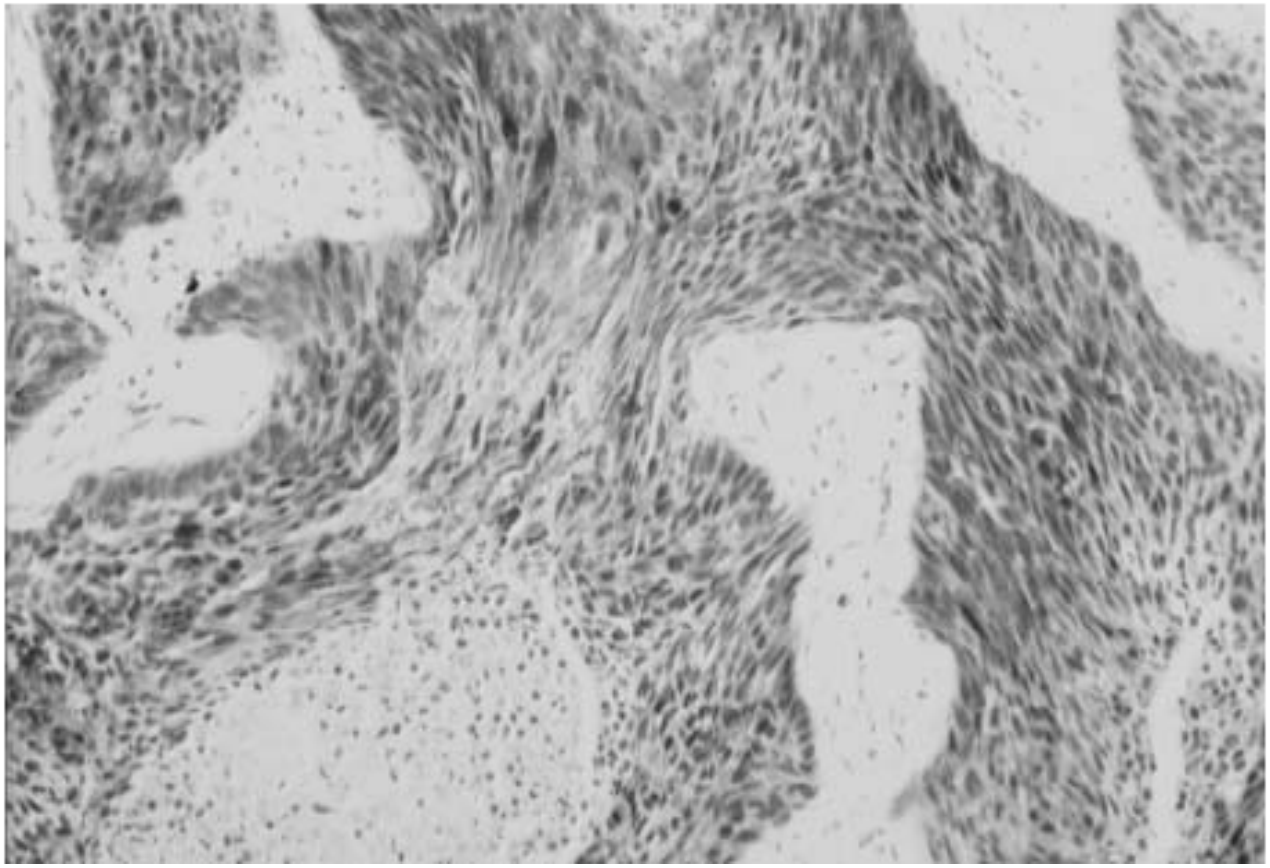

Immunohistochemical localization of HSP70i in differentiated squamous cell carcinoma

Malusecka et al., Anticancer Res. 2008;28(1B):501-6.

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Aims of the Study

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Evaluation of HSP27 and HSP70 in NSCLC patients and controls

- elevated HSP27/70 serum levels in NSCLC patients?
- discrimination between early (IA-IIB) and advanced (IIIA-IV) stage lung cancer by means of HSPs?

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Materials & Methods

Multicenter Study

Dept. of Thoracic Surgery
General Hospital Vienna

National Koranyi Institute of
Pulmonology Budapest

166 NSCLC patients and healthy controls

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Materials & Methods II

histological verification

staging according to TNM classification

early (IA-IIB) and advanced (IIIA-IV) stage NSCLC

lung function testing

acquisition of serum samples

ELISA

SPSS

GraphPad Prism 5

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Demographics

	healthy		NSCLC		total	*P*
	non smokers	smokers	early stage	advanced stage		
n	33	24	37	72	166	-
M / F %	48.5 / 51.5	37.5 / 62.5	59.5 / 40.5	56.9 / 43.1	53.0 / 47.0	*n.s.*
Age (years)	55.8 ± 7.8	56.3 ± 7.0	59.9 ± 6.2	57.8 ± 6.9	57.6 ± 7.1	*n.s.*
Smoking History %						
Current/Ex	0	100	73.0	63.9	58.4	
Never	100	0	13.5	7.0	25.9	-
No details	0	0	13.5	29.1	15.7	
Lung Function						
FVC(L)	3.73 ± 0.98	3.52 ± 0.85	3.50 ± 0.81	3.18 ± 0.91	3.44 ± 0.93	*n.s.*
FEV1(L)	2.96 ± 0.73	2.71 ± 0.67	2.46 ± 0.74	2.12 ± 0.75	2.48 ± 0.82	***
FEV1%	99.4 ± 9.5	92.1 ± 13.9	79.2 ± 20.8	71.3 ± 22.5	82.2 ± 21.8	***
FEV1/VC	0.80 ± 0.06	0.77 ± 0.06	0.70 ± 0.11	0.66 ± 0.13	0.72 ± 0.12	***

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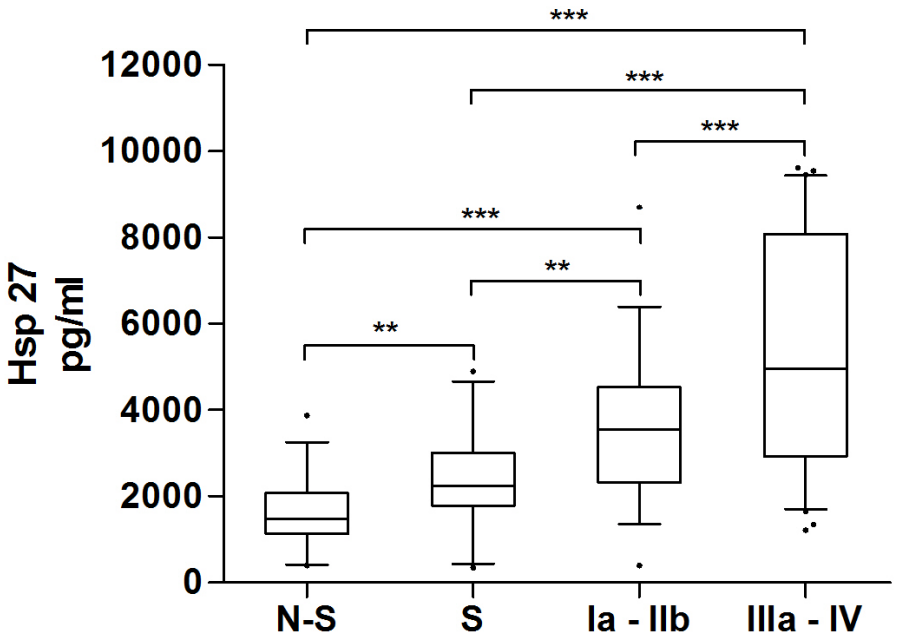
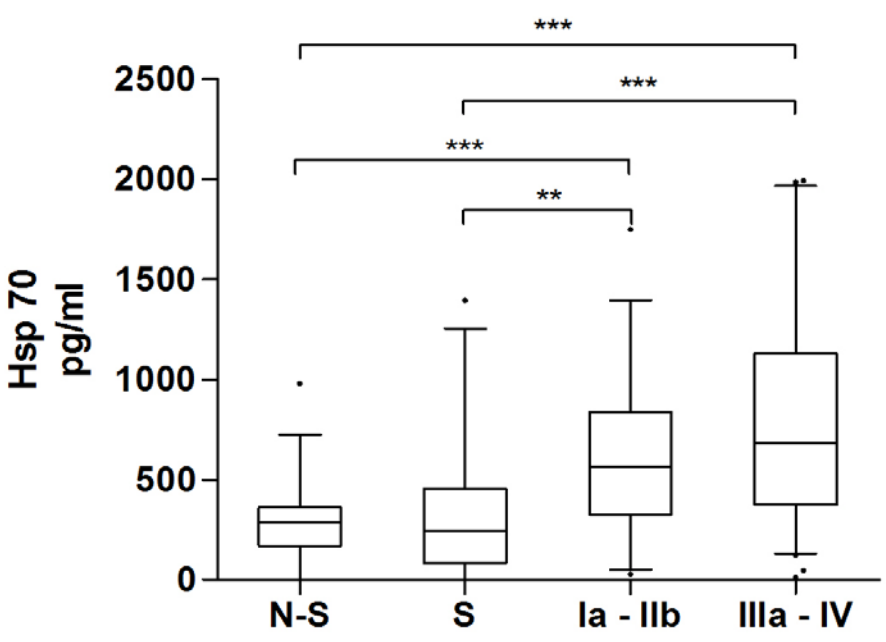
Results I



	N-S	S	Ia - IIb	IIIa - IV
	n=33	*n*=24	*n*=37	*n*=72
mean ± SD	1648 ± 777	2346 ± 1080	3647 ± 1613	5364 ± 2679



	N-S	S	Ia - IIb	IIIa - IV
	n=33	*n*=24	*n*=37	*n*=72
mean ± SD	305 ± 212	321 ± 316	603 ± 386	793 ± 545

*** < 0.001

** < 0.01

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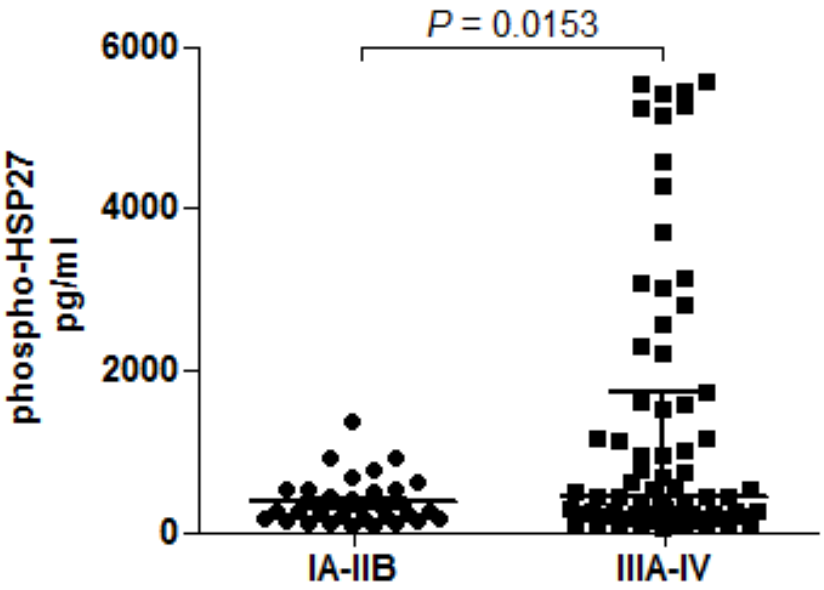
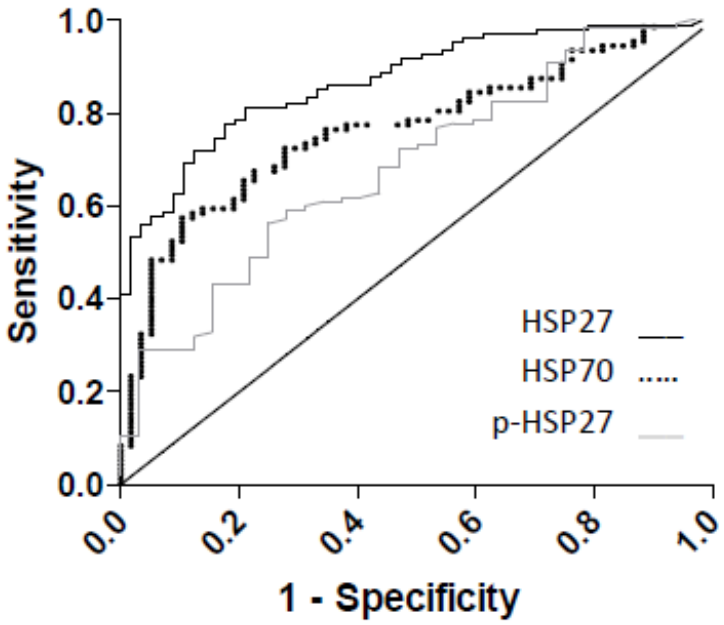
Results II



	median pg/ml	IQR pg/ml
IA – IIB	315	172 – 527
IIIA - IV	447	229 – 1733



	AUC	95% CI	*P*
HSP27	0.870	0.817 – 0.923	<0.0001
HSP70	0.779	0.707 – 0.851	< 0.0001
phospho-HSP27	0.682	0.580 – 0.784	0.0019

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Results III

healthy controls

stage		healthy nonsmokers	healthy smokers
n		33	24
HSP27	median	1482	2242
	IQR	1136–2071	1787–3009
HSP70	median	285	244
	IQR	166–345	82–456

NSCLC early stage

stage		Ia	Ib	IIa	IIb
n		*10*	*15*	*3*	*9*
HSP27	median	3452	3198	2689	4377
	IQR	1823–4347	2469–4206	2258–4074	3105–5626
HSP70	median	643	517	1014	616
	IQR	129–847	246–806	58–1748	344–791

NSCLC advanced stage

stage		IIIa	IIIb	IV	*P*
n		*16*	*6*	*50*	
HSP27	median	4023	4339	5558	***<0.0001***
	IQR	3025–7355	3371–8620	2854–8125	
HSP70	median	452	825	719	***<0.0001***
	IQR	282–1147	318–1796	432–1105	

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Conclusions

Serum levels of HSP27 and HSP70 are significantly elevated in NSCLC patients compared with healthy controls

HSP27 serum levels show a stage-dependent increase in early and advanced stage NSCLC

HSP27 serum levels show excellent sensitivity and specificity in a regression model (NSCLC vs. healthy)