Diplomarbeit

Discrimination of clinical stages in non-small cell lung cancer patients by serum HSP27 and HSP70

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unter der Anleitung von

Univ.-Doz. Dr. med. univ. Hendrik Jan Ankersmit

eingereicht von

Matthias Zimmermann

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…….. ……..

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1. Zusammenfassung

Das Lungenkarzinom ist die häufigste Karzinomentität in Bezug auf Inzidenz und Mortalität weltweit. Eine Zunahme der Inzidenz bei gleichbleibend schlechter Prognose unterstreicht die Relevanz von Lösungsansätzen zur Verbesserung sowohl der Primärprävention, der frühzeitigen Erkennung als auch der Diagnose des Lungenkarzinoms. Hinsichtlich Lungen assoziierter Biomarker haben wir erst kürzlich erhöhte Heat Shock Protein-Serumwerte bei Patienten mit einer manifesten chronisch obstruktiven Lungenerkrankung nachgewiesen. Basierend auf diesen Kenntnissen erstellten wir die Hypothese, dass Heat Shock Proteine auch beim Lungenkarzinom eine entscheidende Rolle spielen.

HSP27, phosphoHSP27 und HSP70 Serumwerte wurden in Patienten mit nicht-kleinzelligem Lungenkarzinom (NSCLC) im Anfangsstadium (Stage I-II, n=37) bzw. im fortgeschrittenen Stadium (Stage III-IV, n=72), in gesunden Rauchern ohne klinische Zeichen eines Lungenkarzinoms (n=24) und gesunden Nichtrauchern ohne klinische Zeichen eines Lungenkarzinoms (n=33) bestimmt.

Gesamt HSP27 Serumwerte waren in beiden Gruppen (frühes und fortgeschrittenes Stadium) mit nicht-kleinzelligem Lungenkarzinom im Vergleich zu beiden gesunden Kontrollgruppen erhöht (Anfangsstadium: 3647 [Mittelwert] vs. 1648 bzw. 2346 pg/ml, $p<0.0001$ bzw. $p=0.0022$; fortgeschrittenes Stadium: 5364 vs. 1648 bzw. 2346 pg/ml, $p<0.0001$). Statistisch signifikante Unterschiede konnten desweiteren zwischen den beiden Gruppen mit nicht-kleinzelligem Lungenkarzinom gefunden werden ($p=0.0021$). Dementsprechend zeigten auch die Serumwerte des phosphorylierten HSP27 einen signifikanten Anstieg zwischen den im Krankheitsverlauf wenig und weit fortgeschrittenen NSCLC Patienten (315 [Median] vs. 447 pg/ml, $p=0.0153$). HSP70 Serumwerte waren erhöht in Patienten mit wenig bzw. weit fortgeschrittenem Karzinom im Vergleich zu beiden gesunden Kontrollgruppen (Anfangsstadium: 603 [Mittelwert] vs. 305 bzw. 321 pg/ml, $p=0.0028$ bzw. $p<0.0001$; fortgeschrittenes Stadium: 798 [Mittelwert] kontra 305 bzw. 321 pg/ml, $p<0.0001$). Statistisch signifikante Unterschiede zwischen den beiden Karzinomgruppen konnten keine festgestellt werden. In univariaten Regressions-Modellen mit gesunden Probanden und Patienten mit nicht-kleinzelligem Lungenkarzinom zeigte das

HSP70 einen AUC (area under the curve)-Wert von 0.779 (p<0.0001), HSP27 einen AUC-Wert von 0.870 (p<0.0001).

HSP27 Serumwerte sind signifikant erhöht in Patienten mit nicht-kleinzelligem Lungenkarzinom, korrelieren mit den klinischen Krankheitsstadien und weisen somit auf ihre potentielle Rolle als Biomarker zur Früherkennung von Patienten mit einem Nicht-kleinzelligen Lungenkarzinom hin. Darüber hinaus proklamieren die gewonnenen Daten einen möglichen Einsatz der HSP27 bzw. phosphoHSP27-Bestimmung zur Unterscheidung der verschiedenen Krankheitsstadien, welche dadurch zu einer wichtigen Hilfe in der Therapieentscheidung werden könnten.

1. Abstract

Lung cancer is the most common cancer in terms of mortality worldwide. An increase in lung cancer at a remaining poor prognosis shows the importance of appropriate approaches to primary prevention, early detection and diagnosis. In respect to lung associated biomarkers, we have recently described that patients with manifest COPD evidence increased serum levels of heat shock proteins (HSPs). Based on these data, we hypothesized that HSPs play a pivotal role in lung cancer as well.

Serum levels of phospho-HSP27, total HSP27 and HSP70 in patients with non-small cell lung cancer (NSCLC) diagnosed at an early (stage I-II, n=37) or an advanced (stage III-IV, n=72) stage, in healthy smoking volunteers without any clinical signs (n=24) and in healthy volunteers without any smoking history or clinical signs (n=33) were determined by using ELISA.

Serum levels of total HSP27 were elevated in patients with NSCLC diagnosed at an early or at an advanced stage when compared with both healthy control groups (early: 3647 [mean] vs. 1648 or 2346 pg/ml, p<0.0001 or p=0.0022; advanced: 5364 vs. 1648 or 2346 pg/ml, p<0.0001). Furthermore statistically significant differences were found between the early and advanced stage NSCLC patients (p=0.0021). Correspondingly, serum levels of phosphoHSP27 showed a significant increase between early and advanced stage NSCLC patients (315 [median] vs. 447 pg/ml, p=0.0153). Serum levels of HSP70 were elevated in

patients with NSCLC diagnosed at an early or at an advanced stage when compared with both healthy control groups (early: 603 [mean] vs. 305 or 321 pg/ml, p=0.0028 or p<0.0001; advanced: 798 [mean] vs. 305 or 321 pg/ml, p<0.0001). Statistically significant differences in HSP70 serum levels between the two carcinoma groups could not be detected. In univariate logistic regression models including healthy subjects and patients with NSCLC, HSP70 had an area under the curve (AUC) of 0.779 (p<0.0001) and HSP27 showed an AUC of 0.870 (p<0.0001).

HSP 27 serum levels are significantly increased in patients with NSCLC and correlate with the clinical stage of the disease suggesting HSP27 as a potential biomarker to identify patients with early NSCLC. Moreover, these data propose serum HSP27 and phospho-HSP27 measurement as a possible tool in the discrimination between different NSCLC stages that may provide a basis for therapy decision.

2. Introduction

2.1 Background

2.1.1 Lung Cancer

2.1.1.1. Mortality and Survival

Lung cancer is the most commonly diagnosed malignant disease in men and the fourth most frequently malignancy in women worldwide. The incidence rate is estimated at 1.2 million, the mortality-rate at 1.1 million cases per year worldwide. It is by far the most common cancer of men and the second most malignant tumor of women after breast cancer in terms of mortality , with the highest rates observed in Europe and North America [2].

The geographical distribution and the higher incidence in men closely reflect the history of tobacco smoking.

In the United States smoking prevalence in men increased from 1920 to the end of the 30s, plateaued until 1955 and then began to decline progressively. Lung cancer mortality showed a similar progress: at first it slowly rose, then more rapidly until 1980, thereafter slowed and peaked in 1990. The latency between the straight line increase in the two curves was approximately thirty years and was confirmed by the same interval between the beginning of decline in the two curves [3].

Similar curves for women show the same overall relationship except for later onset of smoking prevalence and lung cancer death rates.

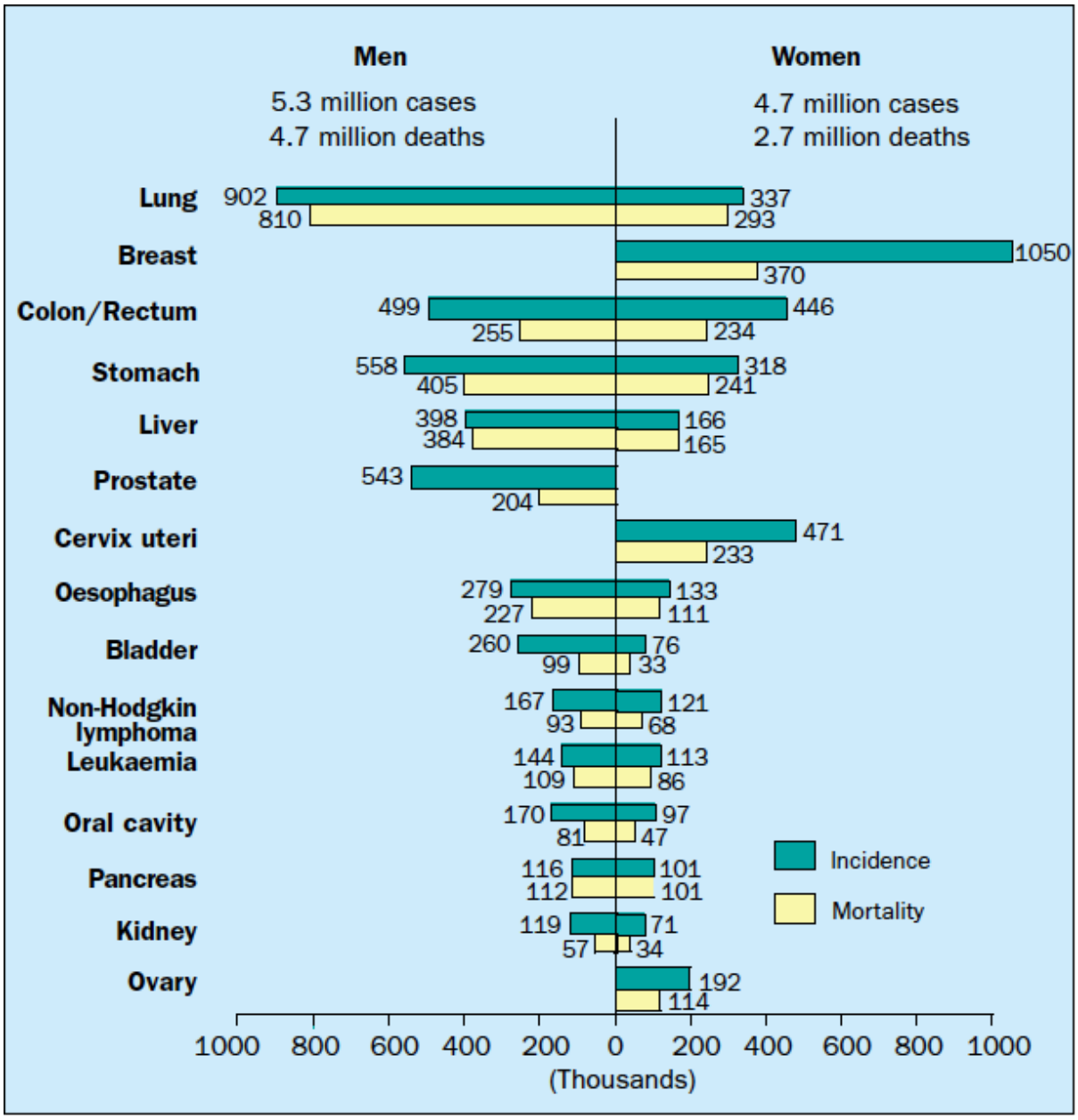

Figure 2.1: Numbers of new cases (incidence) and deaths (mortality), by sex and site [2]

In Austria a comparable appearance can be observed. In 2007 a total of 2246 men (35.3 per 100 000) and 1184 women (14.7 per 100 000) died of this tumor. The lifelong risk of dying from lung cancer is 3.7% in men and 1.6% in women. The development of age-standardized death rates shows different patterns. In men, the mortality rate has decreased from 1987 (52.9 per 100 000) up to 2007 (35.3 per 100 000) by 33%. In contrast, in women the death rate increased from 1987 (10.2 per 100 000) up to 2007 (14.7 per 100 000) by half.

Luftröhre, Bronchien und Lunge (C33-C34) - Krebsmortalität (Sterbefälle pro Jahr), Österreich ab 1983

Jahr	absolute Zahlen			altersstandardisierte Raten[1])			kumulative Raten[2])		
	Insgesamt	Männer	Frauen	Insgesamt	Männer	Frauen	Insgesamt	Männer	Frauen
1983	3.167	2.518	649	26,8	55,4	8,9	3,0	5,9	1,0
1984	3.330	2.584	746	28,4	57,3	10,1	3,1	6,1	1,0
1985	3.153	2.461	692	26,6	54,1	9,2	2,8	5,7	0,9
1986	3.073	2.418	655	26,3	53,3	9,1	2,9	5,6	1,0
1987	3.160	2.420	740	26,8	52,9	10,2	2,9	5,6	1,1
1988	3.169	2.400	769	26,8	52,1	10,7	2,9	5,4	1,1
1989	3.133	2.416	717	26,5	52,0	9,7	2,9	5,6	1,0
1990	3.179	2.427	752	26,5	51,8	10,0	2,9	5,6	1,0
1991	3.278	2.461	817	27,4	52,1	10,9	3,0	5,7	1,1
1992	3.181	2.375	806	26,2	49,7	10,6	2,9	5,3	1,1
1993	3.306	2.498	808	27,3	51,6	11,0	3,0	5,7	1,1
1994	3.193	2.329	864	26,2	47,5	11,7	2,9	5,3	1,2
1995	3.156	2.323	833	25,6	46,8	11,0	2,8	5,2	1,1
1996	3.241	2.373	868	26,2	47,0	11,7	3,0	5,2	1,3
1997	3.264	2.335	929	26,0	45,4	12,3	2,9	5,1	1,3
1998	3.323	2.399	924	26,2	45,9	12,3	3,0	5,1	1,3
1999	3.247	2.296	951	25,4	43,1	12,4	2,9	4,8	1,3
2000	3.269	2.285	984	25,3	42,1	13,1	2,9	4,7	1,4
2001	3.195	2.258	937	24,3	40,8	12,1	2,8	4,5	1,3
2002	3.419	2.393	1.026	25,4	41,9	13,4	2,8	4,4	1,4
2003	3.332	2.339	993	24,3	40,0	12,7	2,7	4,2	1,3
2004	3.388	2.319	1.069	24,6	39,0	13,7	2,7	4,3	1,4
2005	3.348	2.317	1.031	23,8	38,1	12,9	2,6	4,1	1,3
2006	3.413	2.354	1.059	23,8	37,8	13,1	2,6	4,1	1,4
2007	3.430	2.246	1.184	23,7	35,3	14,7	2,6	3,7	1,6

Table 2.1: 1) in each case per 100.000 people / men / women; 2) mortality risk up to the age of 75 [4]

The analysis of mortality by age groups and birth cohorts shows a continuous decrease in over 55 year old men, but an increase in younger birth cohorts. In these older age groups, the decline of harmful substances in cigarettes and the trend toward ex-smokers had positive impact. In younger cohorts, there is a rising trend. In women, lung cancer mortality increased in all age groups with younger cohorts outstanding.

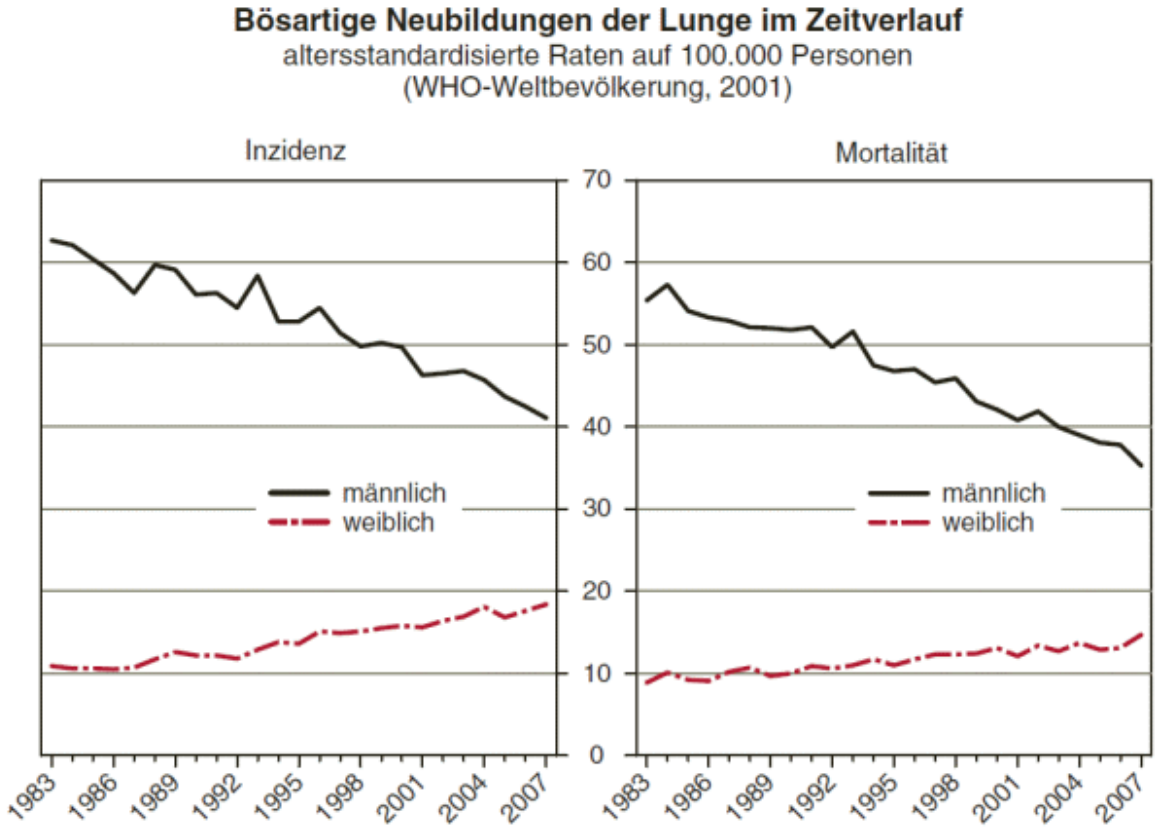

Figure 2.2 Malignant diseases of the lung in time response, Source: Statistic Austria

The prognosis of lung cancer has improved little in the last 20 years and a large proportion of newly discovered cancer cases still takes a fatal course. The 5-year survival is currently 10% in men and 14% in women [5], but heavily depends on the time of diagnosis. 5-year survival rates of 29-43% in local stage face therefore survival rates of 11-16% in regional stage and 1-2 % in distant stage [6].

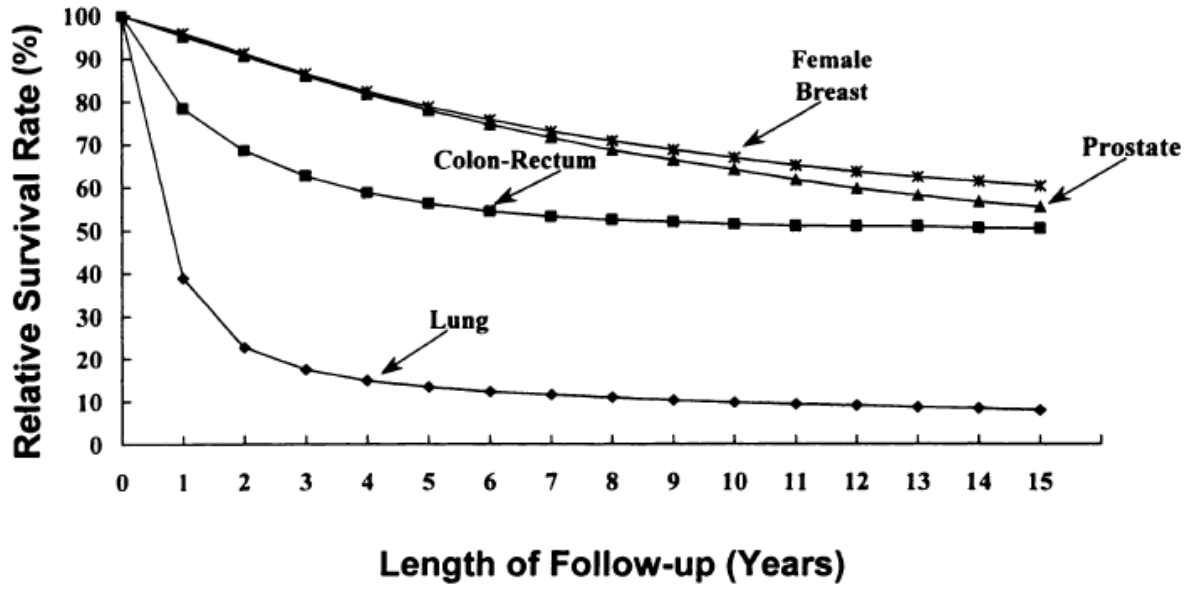

Figure 2.3: Length of relative survival by primary cancer site, all stages combined, SEER 1974 -1991 [6]

Table 4 Long-term survival[a] after lung cancer diagnosis, SEER 1974–1991

Lung cancer	5-Year		10-Year			15-Year		
	Observed survival rate %	Relative survival rate %	Observed survival rate %	Relative survival rate %	Conditional relative survival rate[b] %	Observed survival rate %	Relative survival rate %	Conditional relative survival rate[c] %
White males								
Total	10	12	5	9	61	3	7	70
Stage								
Local	29	37	16	28	67	9	23	71
Regional	11	14	6	9	57	3	8	70
Distant	1	1	1	1	40	0.3	0.6	62[d]
Black males								
Total	8	10	4	7	55	2	5	65
Stage								
Local	25	31	13	21	58	7	17	72[d]
Regional	9	11	5	7	51	2	5	61[d]
Distant	1	1	1	1	61[d]	0.13	0.29	25[r]
White females								
Total	14	16	9	12	63	6	10	73
Stage								
Local	43	49	29	39	71	20	34	76
Regional	16	18	9	11	55	5	8	64
Distant	2	2	1	1	46	1	1	61[d]
Black females								
Total	12	13	7	9	57	4	7	71[d]
Stage								
Local	36	41	23	31	65	16	26	80[d]
Regional	14	15	7	9	49	3	5	50[r]
Distant	2	2	1	1	49[r]	1	1	53[r]

[a] Based on follow-up through 1992.
[b] Restricted to 5-year survivors.
[c] Restricted to 10-year survivors.
[d] SE 5–10%.
[r] SE >10%.

Table 2.2: Long term survival after lung cancer diagnosis, SEER 1974-1991 [6]

2.1.1.2. Etiology

The etiology of lung cancer can be divided into modifiable and unmodifiable risk factors. The unmodifiable risks include gender and genetic predisposition. The modifiable factors include exposure to tobacco smoke, environmental tobacco smoke, occupational lung carcinogens, air pollution and airflow obstruction.

Unmodifiable Risk Factors

Gender

The male predominance of lung cancer is a result of the substantial smoking habits of males compared to females [7]. When differences in smoking initiation, duration and intensity are adjusted, male and female lung cancer rates are more comparable. Studies suggest that women who have never smoked (25.3 per 100 000 person years) might be at increased risk of lung cancer compared with men who have never smoked (20.3 per 100 000 person years) and reveal an hazard ratio of 1.3 [8].

Whether men and women have different susceptibilities to the carcinogens in cigarette smoke with respect to lung cancer remains the focus of considerable controversy, with researchers debating the merits of the use of absolute risks (incidence or mortality rates in smokers) or relative risks because of smoking to make this comparison [9-13]. Few studies have presented both absolute risk and relative risk. Some, but not all, case-control and cohort studies have suggested that smoking causes a significantly larger relative increase in lung cancer risk in women than in men [12, 14-17].

Whereas findings from cohort studies generally show similar incidence and mortality rates in men and women with comparable smoking histories [8-9, 18].

Genetic Predisposition

A genetic predisposition to lung cancer is suggested by the observation that between 10 and 15% of smokers develop lung cancer. Therefore, considerable investigative efforts have explored genetic predisposing risk factors. Familial clustering of bronchogenic carcinoma occurs. Comparison of patients with lung cancer to age-matched community controls showed an odds ratio for lung cancer of 1.8 (95% CI: 1.3-2.5) for those with at least one first-degree relative with lung cancer [19-20].

A genetic prevalence of lung cancer also applies to lifetime nonsmoking patients. After controlling for confounding factors like second-hand smoke, several studies have concluded that the risk of lung cancer in nonsmoking patients increases with the number of first-degree relatives with cancer [20-22].

Modifiable Risk Factors

Smoking

Tobacco smoking is by far the most important risk factor for lung cancer. Evidence of the harm done by smoking has been accumulating for around 200 years. It was first thought to apply only to cancers of the lip and mouth but is now known to cause vascular disease and lung cancer [23]. The supportive evidence for this association has been reviewed many times by different scientific groups and institutions [24-28].

Cigarette smoke is an aerosol composed of volatile agents in the vapor phase and semi- and nonvolatiles in the particulate phase [29]. 95% of the smoke of non filtered cigarettes is composed of 400-500 individual compounds in the gas phase. The remaining 5% of cigarette smoke is tar, which is composed of over 3.500 individual components in the particulate phase. When tobacco burns, the residue that forms is the tar. Although tar contains many known carcinogens, polynuclear aromatic hydrocarbons (PAH) and tobacco-specific N-nitrosamines (TSNA) are believed to be the leading causes of lung cancer [29-30].

At the beginning of the twentieth century, when tobacco smoking was a rare habit, lung cancer was uncommon. In his paper published in 1912, Adler reported that less than 0.5% of patients with cancer who had an autopsy in major hospitals in the USA and western Europe, had lung cancers [31].

The sudden increase in occurrence of this form of cancer started between the two world wars; first in England, where cigarette smoking has come in vogue at about the turn of century under King Edward VII. Because of automated mass production, smoking got comparatively cheap and affordable for every citizen.

One of the first, who suspected a link between cigarette smoking and lung cancer was Lickint in Germany [32]. With the beginning of modern epidemiology in the fifties this hypothesis was first verified in large-scale studies [33-34]. By 1985, lung cancer was the most common

malignant disease and about 85-90% of lung cancer cases were attributed to tobacco smoking [35].

Epidemiological studies in the last decades have demonstrated a number of factors that influence the disease risk of cigarette smoking:

- The risk of lung cancer increases with the duration of smoking, the number of cigarettes smoked per day and the tar content [23, 36-37].
- The average smoker has a 15-fold increased risk of developing lung cancer. The risk for heavy smokers is 20-40 times that of a non-smoker for developing lung cancer [1, 38].

Duration	Average < 10				Average 10–19				Average 20–29				Average ≥ 30			
	Ca	Co	OR[1]	(95% CI)	Ca	Co	OR[1]	(95% CI)	Ca	Co	OR[1]	(95% CI)	Ca	Co	OR[1]	(95% CI)
All centers (men)																
Non-smokers	120	1,953	1.00		120	1,953	1.00		120	1,953	1.00		120	1,953	1.00	
<20 yrs	62	564	1.63	(1.18–2.26)	79	437	2.26	(1.66–3.07)	55	229	2.87	(2.01–4.10)	8	27	4.86	(2.09–11.30)
20–29 yrs	62	288	3.36	(2.41–4.70)	312	505	8.13	(6.41–10.32)	289	298	11.59	(9.00–14.93)	28	54	7.83	(4.62–13.28)
30–39 yrs	177	340	7.86	(6.03–10.24)	767	723	14.30	(11.53–17.74)	716	382	24.37	(19.39–30.63)	99	46	31.61	(20.42–48.92)
40+ yrs	357	484	13.06	(10.14–16.81)	1,558	992	29.66	(23.94–36.75)	1,215	591	44.86	(35.73–56.32)	131	54	46.40	(29.72–72.46)
All centers (both genders)																
Non-smokers	84	1,246	1.00		84	1,246	1.00		84	1,246	1.00		84	1,246	1.00	
<20 yrs	50	441	1.65	(1.14–2.39)	74	361	2.72	(1.94–3.81)	51	201	3.25	(2.20–4.78)	8	27	5.00	(2.15–11.66)
20–29 yrs	53	195	4.05	(2.76–5.92)	276	383	9.57	(7.27–12.59)	260	258	12.74	(9.56–16.98)	28	52	8.29	(4.85–14.17)
30–39 yrs	153	257	8.85	(6.51–12.02)	697	526	17.91	(13.91–23.06)	639	288	29.22	(22.35–38.20)	98	44	33.41	(21.34–52.31)
40+ yrs	281	334	14.71	(10.81–20.01)	1245	608	36.75	(28.41–47.56)	905	369	50.98	(38.65–67.24)	130	53	48.72	(30.78–77.13)
All centers (women)																
Non-smokers	467	1,601	1.00		467	1,601	1.00		467	1,601	1.00		467	1,601	1.00	
<20 yrs	53	202	1.00	(0.72–1.39)	49	83	2.34	(1.59–3.46)	18	42	1.91	(1.06–3.45)	1	6	0.75	(0.09–6.45)
20–29 yrs	54	77	2.81	(1.91–4.13)	104	80	4.97	(3.56–6.94)	68	29	9.63	(5.97–15.53)	11	2	32.98	(6.82–159.56)
30–39 yrs	51	84	2.51	(1.71–3.68)	153	70	8.65	(6.29–11.90)	115	24	21.48	(13.42–34.36)	16	3	28.89	(8.14–102.57)
40+ yrs	95	71	6.59	(4.57–9.51)	178	63	11.57	(8.33–16.07)	123	24	29.48	(18.05–48.14)	18	3	36.43	(10.28–129.08)
All centers (both genders)																
Non-smokers	310	979	1.00		310	979	1.00		310	979	1.00		310	979	1.00	
<20 yrs	42	142	1.09	(0.75–1.61)	30	49	2.23	(1.35–3.68)	8	15	2.26	(0.91–5.64)	0	4	0	
20–29 yrs	45	60	2.85	(1.85–4.41)	70	57	4.38	(2.91–6.58)	40	13	11.42	(5.75–22.69)	8	2	26.59	(4.98–141.99)
30–39 yrs	45	65	2.81	(1.83–4.31)	121	56	8.22	(5.69–11.88)	76	16	19.44	(10.86–34.81)	10	1	64.50	(7.87–528.48)
40+ yrs	89	65	7.11	(4.81–10.51)	151	51	11.40	(7.87–16.52)	90	19	27.79	(15.72–49.14)	10	2	36.02	(7.40–175.37)

Table 2.3: Effect from duration of smoking by average number of cigarettes and by sex; Ca = Cases, Co=Controls [38]

- Lung cancer risk is inversely related to the age of smoking initiation [30, 39].
- Smokers of pipes and cigars seem to be at lower risk than cigarette smokers, but experience an increased risk of lung cancer as compared with non-smokers. This difference can either reflect a lower carcinogenic potency of smoke from cigars and pipes, as compared with cigarettes, or result from confounding by aspects of the smoking habit, such as the average amount smoked, duration of consumption, degree of inhalation, and age at start. Boffetta et al. [40] suggest with their multicenter-study that the lower overall risk might be due to lower consumption of tobacco in the former groups of smokers. The slopes of the dose-response relations that were estimated for duration of use and for average and cumulative consumption of cigars, cigarillos and pipe tobacco are comparable to those for cigarette smoking.

- The risk decreases with increasing years of smoking cessation. Although efforts to change from cigarettes to other types of tobacco or from smoking substantial to smaller numbers of cigarettes seems to confer only limited benefit, stopping smoking confers striking benefit. Even people who stop smoking at 50 or 60 years of age avoid most of their subsequent risk of developing lung cancer and that those who stop at 30 years of age avoid more than 90% of the risk attributable to tobacco of those who continue [1].

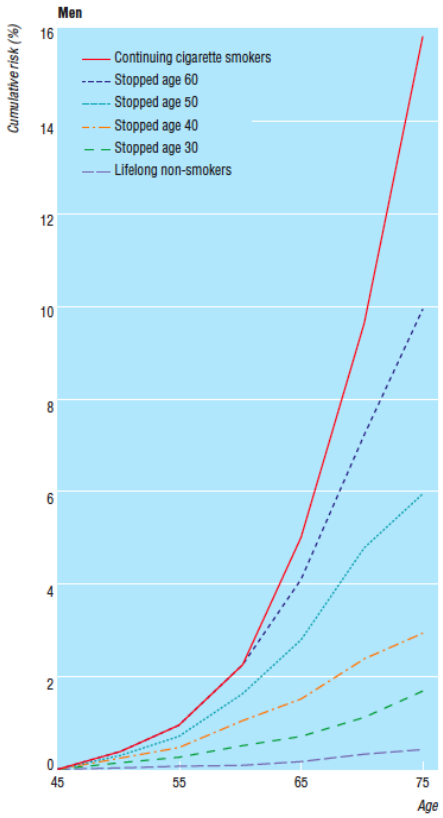

Figure 2.4: Effects of stopping smoking at various ages on the CR (%) of death from lung cancer up to age 75, at death rates for men in United Kingdom in 1990 [1]

Environmental Tobacco Smoke (ETS)

ETS consists of side-stream smoke and exhaled mainstream smoke. Side-stream smoke, which is generated from the end of the cigarette, is unfiltered and contains nicotine in the gaseous phase. Mainstream smoke is filtered and contains a particulate phase [41]. ETS is a mixture of nearly 5000 chemical compounds, including 43 known human or animal carcinogens [42]. It is not surprising that ETS causes the same diseases as active smoking, but the risk is reduced in proportion to the dilution of the smoke in the environment.

The relation between passive exposure to smoke and lung cancer was first shown by Hirayama at the beginning of the 1980s [43] and soon thereafter by two other groups [44-45]. Subsequently, this association has been examined in more than 50 investigations.

Over the past 20 years, review groups have repeatedly and consistently concluded that exposure to ETS causes lung cancer in never smokers.

In 1997, Hackshaw and colleagues [46] carried out a comprehensive meta-analysis, which included 39 published studies and estimated an excess risk of lung cancer for never smokers married to smokers as 23% (95% CI: 13-34%). Adjustment for potential bias and confounding by diet did not alter the estimate. A subsequent IARC meta-analysis [47] including 46 studies and 6.257 cases yielded similar results: 24% (95% CI: 14-34%).

These calculations illustrate that exposure to ETS must be considered an important cause of lung cancer death from a public health perspective. Exposure is involuntary and not subject to control.

Occupational Exposure

Although cigarette smoking causes the majority of bronchogenic carcinomas, occupational exposures account for between 3% and 17% of lung cancers.

Asbestos

Asbestos is a group of naturally occurring fibers used for centuries in the production of many domestic products. Some examples include insulation, ceiling tiles, brake linings, floors, textiles and fireproofing.

Asbestos has long been known to be a cause of lung cancer. In a prospective cohort study, Hammond et al. [48] found a lung-cancer mortality ratio of 5.17 for nonsmoking asbestos workers, 10.85 for smoking non exposed controls and 53.24 for asbestos workers who were smokers. Although other studies may not support a relative risk of such magnitude, there appears to be a synergistic effect between tobacco smoke and asbestos fibers. Four comprehensive reviews document the relation between these two factors [49-52].

Radon

Radon, long established as a respiratory carcinogen, is not only of concern for underground miners but for the population generally, as a ubiquitous contaminant of indoor air. Radon is an inert gas, produced naturally from radium in the decay series of uranium.

Radon decays with a half-life of 3.82 days into a series of solid, short-lived radioisotopes that collectively are referred to as radon daughters, progeny, or decay products. As the biologic basis of respiratory carcinogenesis was analyzed and the lung dosimetry of radon and its short-lived progeny were described, it was recognized that alpha-particle emissions from inhaled radon progeny, not from radon itself, cause lung cancer [53]. Two of those decay

products, polonium-218 and polonium-214, emit alpha particles, which are high-energy and high-mass particles that cause DNA base mutations and chromosomal strand breaks. The energy of these particles is invariant with concentration of radon progeny so that the potential for passage of alpha particles to damage target cells is the same at high and low concentrations. When the alpha emissions take place within the lung as inhaled and deposited radon progeny decay, the DNA of cells lining the airways is damaged and lung cancer may ultimately result.

Experimental studies have documented the occurrence of permanent damage to a cell from just one hit by an alpha particle [53]. This experimental finding suggests that assuming a linear non threshold relationship between exposure and risk at the levels found not only in mines but indoors is biologically appropriate, supporting concern that indoor radon represents a significant public health problem.

Radon was the first identified environmental cause of lung cancer. As early as the 1920s, the elevated risk of lung cancer in miners in Eastern Europe working in mines with high levels of radon had led to the hypothesis that radon was the causal agent. Approximately 20 different subsequent epidemiological studies of miners, including 11 studies that provide quantitative information on the exposure-response relationship, showed a strong association of radon progeny exposure with lung cancer risk and that the increase in the relative risk (RR) is approximately linear in exposure, as estimated by cumulative Working-Level Months [54-56].

A recent concern is whether radon exposure might also cause lung cancer in the general population. Beginning in the 1970s, there was a widespread recognition that radon is present in indoor environments, including homes where people spend the majority of their time. Case-control studies of radon and lung cancer risk in the general population were carried out to quantify the risk as a basis for risk management. Numerous studies, most involving measurement of radon in the current and previous residences, were initiated. These studies have now been completed, the findings of individual studies reported and two pooled analyses completed [57-58].

The risk of lung cancer increased by 8.4 (95% confidence interval 3.0% to 15.8%) per 100 Bq/m3 increase in measured radon (p=0.0007). In the absence of other causes of death, the absolute risks of lung cancer by age 75 years at usual radon concentrations of 0, 100, and

400 Bq/m3 would be about 0.4%, 0.5%, and 0.7%, respectively, for lifelong non-smokers, and about 25 times greater (10%, 12%, and 16%) for cigarette smokers.

Airflow obstruction

The relationship between airflow obstruction and lung cancer was first described in 1986 by Skillrud and colleagues [59]. Since then, a lot of authors argued that reduced lung function is another important risk factor for lung cancer, but several epidemiological questions remained unanswered: Since the individuals with reduced lung function frequently have a significant smoking history, it was not certain whether the relationship between lung function and lung cancer is real or simply confounded by the effects of smoking.

Wasswa-Kintu and coworkers addressed this question and conducted a systematic review and meta-analysis of population based studies of the above mentioned relationship. Their results show that a reduced forced expiratory volume in one second (FEV1) is strongly associated with lung cancer. Although the relationship is severity dependent, even a relatively modest reduction in airflow is a significant predictor of lung cancer. Furthermore, reduced FEV1 is strongly associated with mortality in advanced non-small cell lung cancer [60].

2.1.1.3. Techniques for the Diagnosis of Lung Cancer

Because of the lack of clinical symptoms, early detection of lung cancer is often a mere accidental finding. Lung tumours can grow to large sizes in an asymptomatic patient because the lung parenchyma lacks innervations for pain perception. Usually, a mass is not discovered until it invades some other structure, such as blood vessels, a cough receptor, a pleural pain receptor or a distant site. Accordingly, with the exception of solitary pulmonary nodules seen incidentally in body-imaging (chest x-ray, spiral computed tomography), the majority of patients with lung cancer present with symptoms and signs of the tumour. Common symptoms are anorexia, weight loss, cough, haemoptysis, chest-wall or bone pain, fever, hoarseness, shortness of breath and pleuritic pain. Physical findings include localized wheezing, which indicates local bronchial obstruction and decreased breath sounds and dullness over one portion of the lung, signifying effusion, tumour or collapse. Any or several of these symptoms and signs stimulate a radiographic search for the cause. Usually a mass, adenopathy, obstructive pneumonia or pleural effusion are seen on a chest X-ray or

computed tomography (CT). The clinician must then match a reliable, relatively safe diagnostic technique to the patient's risk profile and tumour anatomy to obtain a histological specimen. A staging procedure examining the involvement of lymph nodes, local invasion and distant metastases completes the diagnostic evaluation.

The techniques available to confirm diagnosis and pathologically stage lung cancer are:

- **Sputum Cytology:** The role of sputum cytology in the diagnosis of the solitary pulmonary nodule is controversial, because its use is limited to tumors that extend into the airways and the yield is lower than that of bronchoscopy and needle aspiration. Data of two randomized controlled trials are suggestive of a very modest benefit of sputum cytology screening [61].
- **Bronchoscopy Techniques:** The results of fiberoptic bronchoscopy are somewhat dependent on the nodule`s size, pathology, location and relationship to a bronchus. Beside of defining the proximal extent of the tumor lymph node invasion can be evaluated using transbronchial biopsy or transbronchial needle aspiration. Gasparini et al. [62] compared those two techniques in a series of 1.027 solitary pulmonary nodules and obtained and overall diagnostic yield for malignancy of 69% for transbronchoscopic needle aspiration, 54% for transbronchial biopsy and 75% for both combined. The advantages of the transbronchial approach are that it allows an examination of the transbronchial anatomy, transbronchial biopsy and staging of lymph nodes by transbronchial needle aspiration with a lower incidence of complications.
- **Bronchoalveolar lavage (BAL):** Bronchoalveolar lavage is a useful diagnostic tool in diffuse or disseminated lung malignancies that do not involve the bronchial structures visible by endoscopy. It increases the sensitivity of fiberoptic bronchoscopy by attempting to sample the distal bronchioles and the alveolar surface [63].
- **Percutaneous Needle Aspiration:** In case of more peripheral lesions percutaneous needle aspiration is more useful and safe. Computed tomography (CT) is the guidance modality of choice and allows much more accuracy for percutaneous needle aspiration.

- **Video-Assisted Thoracic Surgery:** Video-assisted thoracic surgery (VATS) replaces thoracotomy as a diagnostic tool more and more. Full thoracotomy incision and injury to the ribs are avoided. Beside the diagnostic aspect it also allows rapid treatment of benign and malignant nodules at the same time. According to much higher recurrence rates when tumors were removed by wedge resection compared to lobectomy, if the diagnosis of malignancy is made by VATS, procedure is usually converted to thoracotomy. The major role of VATS is to evaluate lesions not easily reached by the bronchoscope or percutaneous needle aspiration.
- **Spiral Computerized Tomography (CT)**: A CT scan may be performed on the chest, abdomen and / or brain to determine spread of lung cancer. The advantage of CT scans is that they are more sensitive than standard chest X-rays and may identify metastatic cancer in the liver, adrenal glands, the brain or the bones.
- **Positron Emission Tomography (PET) scanning:** malignant nodules are more likely to enhance after intravenous contrast during CT scanning and have an increased uptake of 2-deoxy-2-[fluorine-18]fluoro-D-glucose (^{18}F-FDG). Sensitivity, specificity and accuracy of ^{18}F-FDG PET/CT for the depiction of malignant nodes are 85%, 84% and 84% [64]. Some benign processes, including several types of infectious lesions can simulate cancer with this test. For this reason, biopsy is required to confirm the diagnosis.
- **Pulmonary function tests:** These tests determine lung capacity and respiratory reserve. Knowing the lungs' strength allows doctors to determine if the patient can safely tolerate surgery or radiation treatment.

2.1.1.4 Screening Methods

Failing early clinical symptoms, early detection of lung cancer can be achieved only by screening tests. Advances in imaging technologies currently nourish the hope of reducing lung cancer mortality similar to what has been achieved for cancer of the breast, colon, prostate and cervix. Spiral computed tomography and autofluorescence bronchoscopy can detect lung cancer down to the submilimeter range and offer unprecedented sensitivity to detect lung cancer, but are also associated with very low specificity [65-66]. Better selection of individuals at highest risk of lung cancer using biomarkers in sputum or blood may

improve their positive predictive values as well as reduce screening costs. Unfortunately, lung cancer-screening programmes with biomarkers are presently in the research realm [67]. Multiple studies have proposed blood-based biomarkers as an attractive filter. Some lung-specific serum tumour-markers, such as CEA, CA-125, CYFRA21-1, SCC, NSE, proGRP, Chromogranine, and TPA have been evaluated in patients with NSCLC, as well as with small cell lung cancer. Despite extensive studies, few have turned out to be useful in clinic [68-71].

2.1.1.5. Histopathology

This chapter describes the histopathologic classification and morphologic features of the major types and variants of lung carcinoma. The pathologic diagnosis of lung cancer can be established by examination of cytological or surgical pathology specimens and is based on the tumors light microscopic features, as seen in routine hematoxylin and eosin-stained sections. Immunohistochemistry and other special stains are usually not required for routine diagnosis of classification, but may be helpful in certain situations (neuroendocrine tumors, pleural involvement from primary epithelial mesothelioma).

The current World Health Organization (WHO) classification for lung carcinomas is presented in Table 2.4. In contrast to the clinical trend of simplifying the classification for treatment purposes the histopathologic classification of lung carcinomas continues to evolve. The major histological subtypes of lung cancer include squamous cell carcinoma, adenocarcinoma, SCLC and large cell carcinoma, but because mayor differences exist in the therapeutic approach to patients with SCLC versus NSCLC, the major question often asked is whether a lung cancer is a SCLC or a NSCLC. NSCLC comprise all types of lung cancer (squamous cell carcinoma, adenocarcinoma, large cell carcinoma) except small cell lung carcinoma (SCLC). The following description of the four main categories is based on the textbooks “Cancer of the Lung” [72] and “Lung Cancer” [73].

Table 2.4: The 1999 World Health Organization/International Association for the Study of Lung Cancer Histological Classification of Lung and Pleural Tumours

1. Epithelial Tumours

- 1.1. Benign
  - 1.1.1. Papillomas
    - 1.1.1.1. Squamous cell papilloma
    - 1.1.1.2. Glandular papilloma
    - 1.1.1.3. Mixed squamous cell and glandular papilloma
  - 1.1.2. Adenomas
    - 1.1.2.1. Alveolar adenoma
    - 1.1.2.2. Papillary adenoma
    - 1.1.2.3. Adenomas of salivary-gland type
    - 1.1.2.4. Mucinous cystadenoma
    - 1.1.2.5. Others
- 1.2. Preinvasive lesions
  - 1.2.1. Squamous dysplasia / Carcinoma in situ
  - 1.2.2. Atypical adenomatous hyperplasia
  - 1.2.3. Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia
- 1.3. Malignant
  - *1.3.1. Squamous cell carcinoma*
    - 1.3.1.1. Papillary
    - 1.3.1.2. Clear cell
    - 1.3.1.3. Small cell
    - 1.3.1.4. Basaloid
  - *1.3.2. Small cell carcinoma*
    - 1.3.2.1. Combined small cell carcinoma
  - *1.3.3. Adenocarcinoma*
    - 1.3.3.1. Acinar
    - 1.3.3.2. Papillary
    - 1.3.3.3. Bronchioalveolar carcinoma
      - 1.3.3.3.1. Non-mucinous
      - 1.3.3.3.2. Mucinous
      - 1.3.3.3.3. Mixed mucinous and non-mucinous or intermediate cell type
    - 1.3.3.4. Solid adenocarcinoma with mucin
    - 1.3.3.5. Adenocarcinoma with mixed subtypes
    - 1.3.3.6. Variants
      - 1.3.3.6.1. Well-differentiated fetal adenocarcinoma
      - 1.3.3.6.2. Mucinous (“colloid”) adenocarcinoma
      - 1.3.3.6.3. Mucinous cystadenocarcinoma
      - 1.3.3.6.4. Signet-ring adenocarcinoma
      - 1.3.3.6.5. Clear cell adenocarcinoma
  - *1.3.4. Large cell carcinoma*
    - 1.3.4.1. Large cell neuroendocrine carcinoma
      - 1.3.4.1.1. Combined large cell neuroendocrine carcinoma
    - 1.3.4.2. Basaloid carcinoma
    - 1.3.4.3. Lymphoepithelioma-like carcinoma
    - 1.3.4.4. Clear cell carcinoma
    - 1.3.4.5. Large cell carcinoma with rhabdoid phenotype
  - *1.3.5. Adenosquamous carcinoma*
  - *1.3.6. Carcinomas with pleomorphic, sarcamatoid or sarcamatous elements*
    - 1.3.6.1. Carcinomas with spindle and / or giant cells
      - 1.3.6.1.1. Pleomorphic carcinoma
      - 1.3.6.1.2. Spindle cell carcinoma
      - 1.3.6.1.3. Giant cell carcinoma
    - 1.3.6.2. Carcinosarcoma
    - 1.3.6.3. Pulmonary blastoma
    - 1.3.6.4. Others
  - *1.3.7. Carcinoid tumour*
    - 1.3.7.1. Typical carcinoid
    - 1.3.7.2. Atypical carcinoid
  - *1.3.8. Carcinomas of salivary-gland type*
    - 1.3.8.1. Mucoepidermoid carcinoma
    - 1.3.8.2. Adenoid cystic carcinoma
    - 1.3.8.3. Others
  - *1.3.9. Unclassified carcinoma*

Squamous-Cell Carcinoma (SCC)

SCC accounts for approximately 30% of all lung cancers [74]. Two thirds of SCC are central, involving large bronchi, but the incidence of peripheral SCC is increasing [75]. The tumor may grow to a large mass and then cavitate. Most cavitating lung cancers are squamous cell carcinomas. SCCs are commonly believed to arise from a progression of squamous metaplasia or basal cell hyperplasia with atypia, through dysplasia, carcinoma in situ and microinvasive carcinoma [76].

It is a malignant epithelial tumor with the microscopic morphological characteristics of squamous epithelium or epidermis. Exfoliated cells in sputum and local symptoms are more common than in other types and these tumors have earlier symptoms and a lower stage at presentation. Squamous differentiation is recognized by the presence of intracellular bridging, squamous pearl formation and keratinization of tumor cells. The cut surface is gray-white or yellowish and is often dry and flaky, reflecting the degree of keratinization.

Secondary changes associated with bronchial obstruction and infections are common in the tumor, as well as in the surrounding lung.

The histological subtypes of squamous cell carcinoma include papillary, clear cell, small cell and basaloid variants [77].

Adenocarcinoma

Adenocarcinomas account for nearly 40% of all lung cancers and is hence the predominant histological subtype of lung carcinoma in many countries [78].

It is a malignant epithelial neoplasm with glandular differentiation or production of epithelial mucin by the tumor cells. Although subtypes of adenocarcinoma recognized by the WHO include acinar, papillary, bronchioloalveolar, solid with mucous formation and mixed adenocarcinoma, this diverse group usually show considerable histological overlap and varies widely in the degree of differentiation. Hence, most are adenocarcinoma with mixed subtypes.

The cut surface is gray-white, with frequent hemorrhage and necrosis, anthracotic pigment and often a central scar.

The concept of scar carcinoma was proposed for those lung cancers associated with dense fibrotic scars. Although scars can be found in association with any histological type of lung cancer, most of these tumors are adenocarcinomas [79].

Bronchioloalveolar Carcinoma (BAC)

BAC is usually separated from the rest of the group because of its distinctive gross and microscopic morphology as well as its clinical presentation and more indolent course. It is recognized on the basis of its histological pattern, which consists of uniform columnar cells growing in a single file, the so called lepidic or “picket fence” arrangement, over the surfaces of intact alveoli. The new WHO definition of BAC requires exclusion of stromal, vascular and pleural invasion.

Small Cell Carcinoma (SCLC)

SCLC accounts for 20% of all lung cancers. They are typically situated in a peribronchial location with infiltration of the bronchial submucosa and peribronchial tissue. Approximately two-thirds present a perihilar mass. Bronchial obstruction is usually caused by circumferential compression. The cut surface is usually white-tan, soft, friable and shows extensive necrosis.

By light microscopy, SCLC consists of tumor cell characterized by small size, around fusiform shape, scant cytoplasm, finely granular nuclear chromatin and absent or inconspicuous nucleoli.

When a component of NSCLC is identified in an otherwise typical SCLC, the tumor is classified as combined small-cell carcinoma. Combined SCLC does not differ clinically or in survival from pure SCLC.

Large Cell Carcinoma (LCC)

LCC has declined in number relative to the rising incidence of adenocarcinoma, falling to under 10% [80]. LCC is a poorly differentiated carcinoma that does not have features of squamous cell carcinoma, adenocarcinoma or SCLC. Thus it is a diagnosis of exclusion and a spectrum of morphology is encompassed by this histological subtype. Histologically, most tumors consist of large cells with abundant cytoplasm and large nuclei with prominent nucleoli and/or vesicular nuclei [77].

They usually form large, well demarcated masses that may be centrally or more often peripherally located, are soft and grayish-white with anthracotic pigment and show extensive hemorrhage, necrosis and cavitations. Acute and chronic inflammation is seen to some extent in most tumors and granulomatous and eosinophilic infiltrates have also been described.

Several variants are described in the WHO/IASCL histological classification of lung cancer, including large cell neuroendocrine carcinoma (LCNEC), basaloid carcinoma, lympho-epithelial-like carcinoma, clear cell carcinoma and large cell carcinoma with rhabdoid phenotype.

LCNEC is defined by tumor cell configuration like an LCC, light microscopic features commonly associated with neuroendocrine tumors, a high mitotic rate, frequent necrosis and neuroendocrine features by immunohistochemistry or electron microscopy. Virtually all patients are cigarette smokers and most have a great number of pack years. LCNEC is a very aggressive malignancy with a very dismal prognosis.

2.1.1.6. Molecular Pathology

It has been shown that multiple genetic changes are found in clinically evident lung cancers and involve several dominant oncogenes as well as known and recessive oncogenes (tumor suppressor genes) [81-82]. Many growth factors or regulatory peptides and their receptors are overexpressed by cancer cells and adjacent normal-appearing cells in the lung and thus provide a series of autocrine and paracrine growth stimulatory loops in this neoplasm.

Studies of large numbers of lung cancers have demonstrated different patterns of molecular alterations between the two major groups of lung carcinomas (SCLC vs. NSCLC) and among the two major histological types of NSCLC.

Table 2.5: Molecular differences between non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) [81-87]

Molecular abnormalities	SCLC	NSCLC
Oncogenes		
EGFR TK domain mutations	No	10-40%
EGFR gain copy number	No	25 – 50%
HER2 mutations	Not studied	4%
HER2 gain copy number	No	10%
RAS mutations	<1%	15 – 20%
MYC amplification	18 – 31%	8 – 20%
NKX2-1 (TITF-1) amplification	Not studied	14%
BCL-2 ICH	75 – 95%	10 – 35%
Tumor suppressor genes		
TP53 abnormalities		
LOH	90%	65%
Mutation	75%	50%
p53 IHC	40 – 70%	40 – 60%
RB abnormalities		
LOH	67%	31%
rb abnormalities (IHC)	90%	15 – 30%
$P16^{Ink4}$ abnormalities		
LOH	53%	66%
Mutation	<1%	10 – 40%
p16 IHC	0 – 10%	30 – 70%
LKB1 Mutation or deletion	Not studied	26%
PTEN/MMAC1 loci LOH	91%	41%
TSG101 abnormal transcripts	100%	0%
DMBT1 abnormal expression	100%	43%
3p LOH various regions	>90%	>80%
8p21-23 LOH	80-90%	80-100%
Other specific LOH regions	1q23, 9q22-32, 10p15, 13q34	13q11, Xq22.1
Promoter hypermethylation		
RASSF1 gene	>90%	40%
RARβ gene	72%	41%

LOH loss of heterozygosity; *IHC* immunohistochemistry; *%* percent of tumors that have the abnormality

2.1.1.7. Staging

A stage is a formal classification that signifies the extent of the cancer, where it is located, if or where it has spread and whether it is affecting the functions of another organ.

The generally admitted staging of lung cancer acts in accordance with the seventh edition (2009) of the TNM classification system of the International Association for the Study of Lung Cancer (IASCL) and determines the type of treatment recommended [88].

The stage of both small cell lung and non-small cell lung cancer is described by a number: zero (0) through four (IV). In general, a lower number of stage is associated with a better outcome, attributing the fact a complete surgical resection can only be achieved in stages I and II.

Table 2.5: TNM staging system of the IASCL Lung Cancer Staging Project (7th edition) proposal for 2009 [88]

T – Primary Tumor	
TX	Primary tumor cannot be assessed or tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 3 cm or less in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus
T1a	T ≤ 2 cm
T1b	2 cm < T ≤ 3 cm
T2	Tumor with any of the following features of size or extent: ▪ More than 3 cm in greatest dimension ▪ Involves main bronchus, 2 cm or more distal to the carina ▪ Invades visceral pleura ▪ Associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung
T2a	3 cm < T ≤ 5 cm
T2b	5 cm < T ≤ 7 cm
T3	Tumor is larger than 7 cm; or invades any of the following structures: chest wall (including superior sulcus tumors), diaphragm, mediastinal pleura, parietal pericardium; or tumor in the main bronchus less than 2 cm distal to the carina, but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung or additional nodule(s) in the same lobe of the primary tumor
T 4	Tumor of any size that invades any of the following: mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina, separate tumor nodule(s) in another ipsilateral lobe

N – Regional Lymph Nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in ispilateral peribronchial and / or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2	Metastasis in ipsilateral mediastinal and / or subcarinal lymph node(s)
N3	Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene or supraclavicular lymph node(s)

M – Distant Metastasis	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1a	Separated tumor nodule(s) in the contralateral lung; tumor with pleural nodules or malignant pleural (or pericardial) effusion
M1b	Distant metastasis

G – Histopathologic Grading	
GX	Grade of differentiation cannot be assessed
G1	Well differentiated
G2	Moderately differentiated
G3	Poorly differentiated
G4	Undifferentiated

Stage Grouping			
Occult Carcinoma	TX	N0	M0
Stage 0	Tis	N0	M0
Stage IA	T1a, T1b	N0	M0
Stage IB	T2a	N0	M0
Stage IIA	T1a, T1b, T2a	N1	M0
	T2b	N0	
Stage IIB	T2b	N1	M0
	T3	N0	M0
Stage IIIA	T3	N1	M0
	T1-3	N2	M0
	T4	N0-1	M0
Stage IIIIB	T4	N2	M0
	Any T	N3	M0
Stage IV	Any T	Any N	M1

2.1.1.8. Treatment of Non Small Cell Lung Cancer (NSCLC)

Lung cancer treatment is based on the type and stage of tumor, and the patient's general medical condition. Treatment options include surgery, radiation, chemotherapy or a combination of these treatments.

Surgery

Surgical resection remains the single most consistent and successful option for cure for patients diagnosed as having lung cancer. For this option to be feasible the cancer must be completely resectable and the patient must be able to tolerate the proposed surgical intervention. It is the principle form of treatment for patients with stage I or stage II NSCLC and may be recommended for patients with very limited stage IIIA disease. If the resected margins are found to be close to or involved with the tumour during surgery, additional treatment in form of radiotherapy is recommended [89].

Surgery for lung cancer can be performed via several accesses and procedures: thoracoscopy, median sternoscopy mediastinoscopy, video assisted thoracic surgery (VATS), wedge resection, segmentectomy, lobectomy, sleeve resection, pneumonectomy. Which procedure is being chosen depends on the site and extent of the cancer, as well as the patients' constitution. Randomized controlled trials showed an increased long-term survival and decreased local recurrence in patients undergoing lobectomy compared with those undergoing limited resections (i.e. wedge resection or segmentectomy) [90].

Minimal-access surgical procedures are expanding the applicability of surgical resection to patients of marginal operability by providing a less invasive method with a similar long-term survival rate [91].

A complete resection (R0) has to be achieved in any case for potential cure.

Radiotherapy

Radiotherapy is used for the treatment of NSCLC in various ways. In unresectable disease, it is the primary modality and is often given with chemotherapy. In the postoperative setting, it is used as an adjuvant treatment to reduce the rate of tumor regrowth. Radiotherapy is also frequently used for the palliation of advanced and metastatic lung cancer.

Chemotherapy

Adjuvant Chemotherapy is generally indicated for patients with resected stages IIA through IIIA NSCLC because of the high risk of relapse. Two meta-analysis using updated data on patients from 52 respectively 34 randomized clinical trials compared surgery plus adjuvant chemotherapy versus surgery alone. It showed a benefit of adding chemotherapy after surgery with an absolute increase in survival of 4% after 5 years (from 60% to 64%). Additionally, a benefit of adding chemotherapy to surgery plus radiotherapy could be shown [92-93].

Since a very large proportion of patients with lung cancer presents with locally advanced or metastatic disease at the time of diagnosis, chemotherapy is beneficial for palliation for those patients. Many phase 3 studies have shown the superiority of systemic chemotherapy over best supportive care in patients with locally advanced and metastatic lung cancer.

Treatment according to stage:

- **Stage I and II:** Treatment for these stages is typically surgical removal. If surgery is not an option, radiation therapy with or without chemotherapy is often recommended [94-95].
- **Stage IIIA:** Patients with stage IIIA represent a relatively heterogeneous group, which lies between the generally resectable stage I and II tumors and unresectable stage IIIB patients. Surgical removal of the tumor is probably only reasonable in patients with an occult single-station mediastinal node metastasis that is recognized at thoracotomy and when a complete resection of the nodes and primary tumor is technically possible. In patients with unresectable, bulky N2 Disease a combination of radiotherapy and chemotherapy should be administered [96].
- **Stage IIIB:** Generally, patients in this stage receive chest radiation treatment combined with chemotherapy. Surgery may be indicated only for very carefully selected T4N0M0 patients [97].
- **Stage IV:** Cancers in this stage are not treated with surgery. Chemotherapy is the main treatment option for stage IV disease. Radiation therapy may be recommended as well and targeted to areas that cause pain or other problems [98].

2.1.2. Heat Shock Proteins and Lung Cancer

Heat shock proteins (HSPs) belong to a highly conserved protein family and normally act as intracellular molecular chaperones which maintain protein homeostasis. When cells are exposed to stressful conditions, HSP synthesis gets massively triggered in order to fold heat-denatured proteins and block caspase-dependent apoptosis, permitting repair and thwarting death [99]. However, HSPs can also be released into the circulation, where they are able to interact with the immune system in a number of contexts. HSPs can act as proinflammatory mediators and lead to cytokine transcription and release. Through their ability to bind antigenic peptides during antigen procession, they can further act as stimulants of the adaptive immune response. Thus, anti-inflammatory and immunosuppressive patterns of HSPs are also described, depending on the biological microenvironment [100-102]. Especially HSP70, either as serum protein or cellular component, has been studied extensively in various inflammatory diseases [103-105].

There is growing evidence in literature that the expression of HSPs is increased in various human cancers. For example, HSP27 was found to be strongly expressed in breast cancer [106], hepatomas and well differentiated hepatocellular carcinomas [107], brain tumours [108] and prostatic carcinoma [109]. The increased transcription of HSPs in tumour cells is due to loss of p53 function and to higher expression of the proto-oncogenes HER2 and c-Myc and is crucial to tumorigenisis [110]. HSP 27 and 70 are highly cytoprotective proteins and their folding properties might be co-opted during malignant progression when the HSPs become expressed at high level to facilitate tumour cell growth and survival [111]. An essential role in the emerging malignant cell is supposed and might permit it to evade both fast (PCD) and slow (senescence) pathways of cell inactivation. Consistent with this theory, increased expression of both HSPs is correlated with resistance to chemotherapy [112].

However, a growing list of contradictory data is emerging regarding expression patterns of HSPs in lung cancer tissues and serum/plasma samples of lung cancer patients. Michils and coworkers [113] evaluated the quantitative expression of low molecular weight (ubiquitin and HSP27) and high molecular weight (HSP60 and HSP70) HSPs in tumour- and healthy lung tissue. They concluded that HSP60 and HSP70, but not HSP27 expression, was increased in cell lysates of NSCLC tissue. In line with this conclusion is a publication authored by Huang et al [114]. They have studied 60 NSCLC cancer patients and were able to demonstrate that

clinicopathological features of NSCLC correlated with tissue expression of HSP70, but not with the expression of HSP27. No statistical significance was observed in histological types and gender with respect to both HSP70 and HSP27 expression.

In contrast, Malusecka et al [115] reported that cytoplasmic immunostaining for HSP27 was positive in a high amount (70%) of samples obtained from patients with NSCLC. They further found a positive correlation between expression levels of HSP27 and HSP70, and a correlation between Ki-67 proliferation index and nuclear HSP70 staining. Another publication from these authors describes a significant survival advantage in patients overexpressing HSP27 in NSCLC tissue. Furthermore, a significantly decreased survival was observed in those patients that were HSP70 tissue negative [116]. Several years before, Volm and coworkers [117] described a wide range of HSP70 levels in human NSCLCs processed for immunostaining, possibly reflecting different biological stressors. Moreover, they found a strong correlation between the number of daily smoked cigarettes and HSP70 expression in NSCLC tissue. 75% of tumours from smoking patients showed high HSP70 expression, whereas only 57% of non-smokers presented with high HSP70 expression in the tumour samples [118]. The first attempt to investigate the relationship between serum HSP70 levels and lung cancer was by Susuki K et al [119]. They detected a significant association between elevated serum HSP70 levels and increased lung cancer risk among Japanese males. However, no association between lung cancer risk and HSP70 levels in female subjects could be found. A recent study investigated the expression of HSP27 and HSP70 in coal-mine workers [120]. This group investigated the association between plasma levels of HSP27/70 in coal-mine dust exposed miners with or without lung cancer and in healthy controls. Interestingly, those miners exposed to coal dust without cancer evidenced a significant increment of plasma HSP27 as compared to control groups.

2.1.3. Chronic obstructive pulmonary (COPD) disease and Lung Cancer

Although chronic obstructive pulmonary disease (COPD) is long known to be associated with lung cancer risk [59], it is not yet known which aspects of this risk can be attributed to shared genetic susceptibility and/or activation of the same pathways. Cross sectional studies have evidenced that the prevalence of COPD is 40-70% among those diagnosed with lung cancer [121-123].

Several mechanisms have been proposed to explain the link between lung cancer and COPD. Both diseases share tobacco smoking as the most important etiologic factor. Smoke contains high concentrations of reactive oxygen species together with thousands of particles that are potentially carcinogenic and may induce a chronic inflammatory state in lung tissue. However, it remains unclear why there are different responses with some individuals having the hallmarks of cancer (uncontrolled cell proliferation, lack of cellular apoptosis, tissue invasion and angiogenesis), others the hallmarks of COPD (increased apoptosis, matrix degeneration, ineffective tissue repair, inflammation and lack of angiogenesis), while the majority remains disease free [124]. Petty hypothesized in an editorial [125] that COPD and lung cancer could have common origins based on the same inflammatory disease process. Since transactivation of inflammation related genes appear in both COPD and lung cancer and these events occur to some extent in all smokers, genetic predisposition seems to play a pivotal role. These interindividual differences in activation of genes that control genomic integrity and those that control tissue injury may distinguish between lung cancer, COPD or disease-freeness.

Intuitively, these hypotheses pertain to airflow obstruction and emphysema, two overlapping manifestations of chronic lung disease related to cigarette smoking.

COPD is characterized by a largely irreversible obstruction of the small airways due to aberrant inflammatory response and airway remodeling [126]. Chronic bronchitis and lung emphysema are pathologic characteristics of COPD and both conditions result from progressive inflammatory destruction of the lung parenchyma. Recently, COPD was accepted as a disease entity featuring immunological alterations seen in autoimmune disease. These reports demonstrated alterations in CD8+ and CD4+ T cells as a part of the adaptive immune

system [127-132]. We have recently demonstrated that levels of systemic CD4+CD28null T cells, a cell population described in various rheumatologic diseases, were increased in COPD patients and correlated with severity of COPD GOLD (Global Initiative for Obstructive Lung Disease) classification [133]. Moreover, we were able to evidence that manifest COPD is associated with increased systemic release of apoptosis-specific proteins as markers for increased cellular turnover as compared to controls [134]. Remarkably, increased serum levels of heat shock protein (HSP) 27 and HSP70 evidenced a high sensitivity and specificity as diagnostic marker for manifest COPD [103]. In a further attempt, we were able to show that increased levels of HSP27 positively correlate with the presence of air trapping and emphysema in subjectively healthy smokers with normal lung function [135].

Recently, CT-based screening programs have clearly shown that emphysema and chronic airway inflammation are associated with the risk of lung cancer [136-137].

2.2. Hypothesis

Based on the obvious relationship between COPD pathogenesis and lung cancer immunology, we hypothesized that HSP27 and HSP70 levels are increased in patients with manifest NSCLC. To test this hypothesis, peripheral blood HSP27 and HSP70 levels of early (IA-IIB) and advanced (IIIA-IV) stage NSCLC patients were compared to those of age-, sex-, and smoking status-matched controls by ELISA.

3. Materials and Methods

Study Subjects

The study protocol has been approved by the Ethics Committee of the Medical University of Vienna, Austria (EC-No.: 449/2008). Informed and written consent was obtained from each subject included in the study and all clinical and laboratory tests were performed in accordance with the Declaration of Helsinki and the guidelines for Good Clinical Practice of the Medical University of Vienna.

A total number of 166 NSCLC patients and healthy controls were included in this case control study. Healthy smoking volunteers without any clinical signs of cancer (n=24), healthy volunteers without any smoking history or any clinical signs of cancer (n=33), patients with NSCLC diagnosed at an early stage (*n*=37) and patients with carcinoma diagnosed at an advanced stage (*n*=72) were evaluated in four study groups. Diagnosis of NSCLC was confirmed through histological specimens in all cases. The cases were staged according to operative and pathologic findings based on seventh edition of the TNM staging system (2009) of the International Association for the Study of Lung Cancer (IASCL) [88]. Patients with adenocarcinoma (AC), squamous cell carcinoma (SCC), non small cell lung cancer not otherwise specified (NSCLC-NOS) and large cell carcinoma (*n*=1) were included in this study. Characteristics of the study subjects are depicted in Table 8.1.

After informed and written consent, all study subjects were asked to answer a questionnaire (Figure 8.1) regarding their smoking habits, and pulmonary function parameters (forced vital capacity [FVC], forced expiratory volume in one second [FEV1], and FEV1/FVC ratio) were obtained by spirometry. Blood samples were collected at the time of first admission to the Department of Thoracic Surgery, serum was obtained after centrifugation and aliquots were stored at -80°C until further testing. Exclusion criteria were any other known malignant or inflammatory diseases, autoimmune diseases and alpha1-antitrypsin deficiency.

Quantification of serum HSP27

Serum levels of HSP27 were determined using adapted enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). Ninety-six well microtitration plates were coated overnight at room temperature with a capture antibody against human HSP27 at a concentration of 1µg/ml. Plates were then washed and blocked with block buffer for 2 hours. Following another washing step, samples and standard protein in different concentrations were added to the wells. After a washing step, a biotin-labeled antibody was added to each well and incubated for 2 hours. After another washing step, horseradish-peroxidase-conjugate (HRP) was applied for 20 minutes. Wells were washed, and color reaction was achieved using tetramethylbenzidine (TMB) (Sigma-Aldrich Corp, St. Louis, MO, USA) and the reaction was stopped by an acid stop solution. Color development was then monitored using a Wallac Multilabel Counter 1420 (PerkinElmer, Waltham, MA, USA). The optical density (OD) values obtained at 450 nm were compared to the standard curve calculated from OD values of standards with known concentrations of antigen. Specificity was demonstrated by the manufacturer by Western blot analysis of the protein bound by the capture antibody supplied in the kit. HSP70 cross reactivity was 0.23%.

Quantification of serum phospho-HSP27 (S78/S82)

Serum levels of phosphor-HSP27 were determined using adapted enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). Ninety-six well microtitration plates were coated overnight at room temperature with a capture antibody against human HSP27 at a concentration of 2µg/ml. Plates were then washed and blocked with block buffer for 2 hours. Following another washing step, samples and standard protein in different concentrations were added to the wells. After a washing step, a biotin-labeled antibody was added to each well and incubated for 2 hours. After another washing step, horseradish-peroxidase-conjugate (HRP) was applied for 20 minutes. Wells were washed, and color reaction was achieved using tetramethylbenzidine (TMB) (Sigma-Aldrich Corp, St. Louis, MO, USA) and the reaction was stopped by an acid stop solution. Color development was then monitored using a Wallac Multilabel Counter 1420 (PerkinElmer, Waltham, MA, USA). The optical density (OD) values obtained at 450 nm were compared to the standard curve calculated from OD values of standards with known concentrations of antigen.

Quantification of serum HSP70

Serum levels of HSP70 were determined using adapted ELISA kits (R&D Systems, Minneapolis, MN, USA). Ninety-six well microtitration plates were coated overnight with a capture antibody against human HSP70 at a concentration of 2µg/ml. Plates were then washed and blocked with block buffer for 2 hours. Following another washing step, samples and standard protein in different concentrations were added to the well. After a washing step, a biotin-labeled antibody was added to each well and incubated for 2 hours. After another washing step, horseradish-peroxidase-conjugate was applied for 20 minutes. Wells were washed, and color reaction was achieved using TMB (Sigma-Aldrich Corp, St. Louis, MO, USA) and the reaction was stopped by an acid stop solution. Color development was then monitored using a Wallac Multilabel Counter 1420 (PerkinElmer, Waltham, MA, USA). The OD values obtained at 450 nm were compared to the standard curve calculated from OD values of standards with known concentrations of antigen.

Statistical Methods

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism5 (GraphPad Software, La Jolla, CA, USA) was used for data visualization. Data are given as mean ± standard deviation (SD) or median and interquartile range (IQR) if data were not Gaussian distributed. To determine Gaussian distribution, Shapiro-Wilk test was used. Pair-wise comparisons between groups were performed using Student´s *T* test. Either one-way ANOVA or, if data were not Gaussian distributed, Kruskal-Wallis tests were used to determine statistical significance between more than two study groups. Categorical variables were compared using chi2 test. Univariate logistic regression models were calculated for both HSP27 and HSP70. Receiver operating characteristics (ROC) curves with area under the curve (AUC) were plotted to demonstrate sensitivity and specificity of the evaluated serum proteins. *P-values*<0.05 were considered statistically significant.

4. Results

4.1. Heat Shock Protein 27

Serum levels of HSP27 were 1648±777 pg/ml in healthy non smoking controls, 2346±1080 pg/ml in healthy smoking controls, 3647±1613 pg/ml in patients with NSCLC diagnosed at an early stage, and 5364±2679 pg/ml in patients with NSCLC diagnosed at an advanced stage. Statistically significant differences were found between all four groups (healthy vs. early stage, healthy vs. advanced stage, and early vs. advanced stage: in each case $p<0.001$) (Fig. 4.1). Detailed results (IASCL stages I to IV) of HSP27 serum levels are given in Table 8.2.

4.2. Phospho Heat Shock Protein 27 (S78/S82)

Serum levels of pHSP27 were 315 [median] (Q1=172, Q3=527) pg/ml in patients with NSCLC diagnosed at an early stage and 447 [median] (Q1=229, Q3=1733) pg/ml in patients with NSCLC diagnosed at an advanced stage. Statistically significant differences were found between the two groups (early vs. advanced stage: $p=0.015$) (Fig.4.2).

4.3. Heat Shock Protein 70

Mean serum levels of HSP70 were 305±212 pg/ml in healthy non smoking controls, 321±316 pg/ml in healthy smoking patients, 603±386 pg/ml in patients with NSCLC diagnosed at an early stage, and 793±545 pg/ml in patients with NSCLC diagnosed at an advanced stage. Statistically significant differences were found between healthy non smoking controls and patients with NSCLC diagnosed at an early or advanced stage ($p=0.0028$ and $p<0.0001$ respectively), between healthy smoking controls and patients diagnosed at an early or advanced stage ($p=0.006$ and $p<0.0001$ respectively), but not between the two groups with NSCLC (Fig.4.3). Detailed results (IASCL stages I to IV) of HSP70 serum levels are depicted in Table 8.2.

4.4. Regression Models

In univariate logistic regression models including healthy volunteers and patients with NSCLC, HSP70 had an area under the curve (AUC) in the receiver operating characteristic (ROC) curve of 0.779 (0.707–0.851 95% confidence interval; $p<0.0001$), phospho HSP27 an AUC of 0.682 (0.580-0.783 95% confidence interval; $p=0.002$) and HSP27 showed an AUC of 0.870 (0.817–0.923 95% confidence interval; $p<0.0001$) (Fig.4.4).

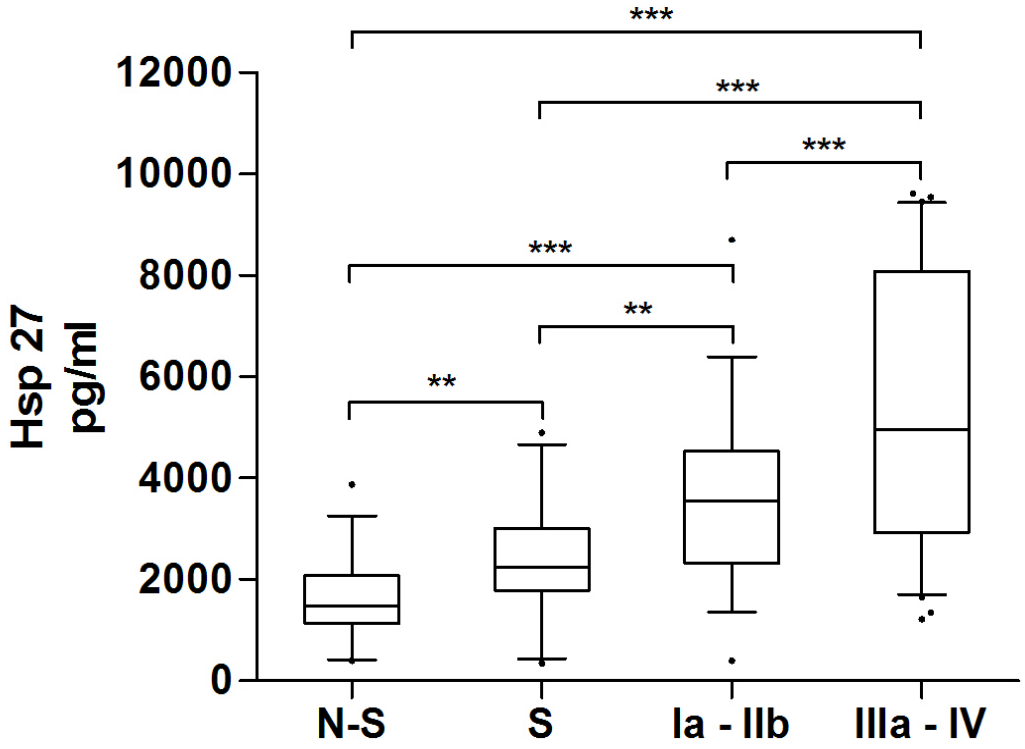

Figure 4.1: HSP27 levels are significantly elevated in patients with NSCLC diagnosed at an early (IA-IIB) or at an advanced stage (IIIA-IV) when compared with either healthy control groups (**p<0.01, *** p<0.001). Between early and advanced stage NSCLC patients (***p<0.001). N-S, health never-smokers; S, healthy smokers.

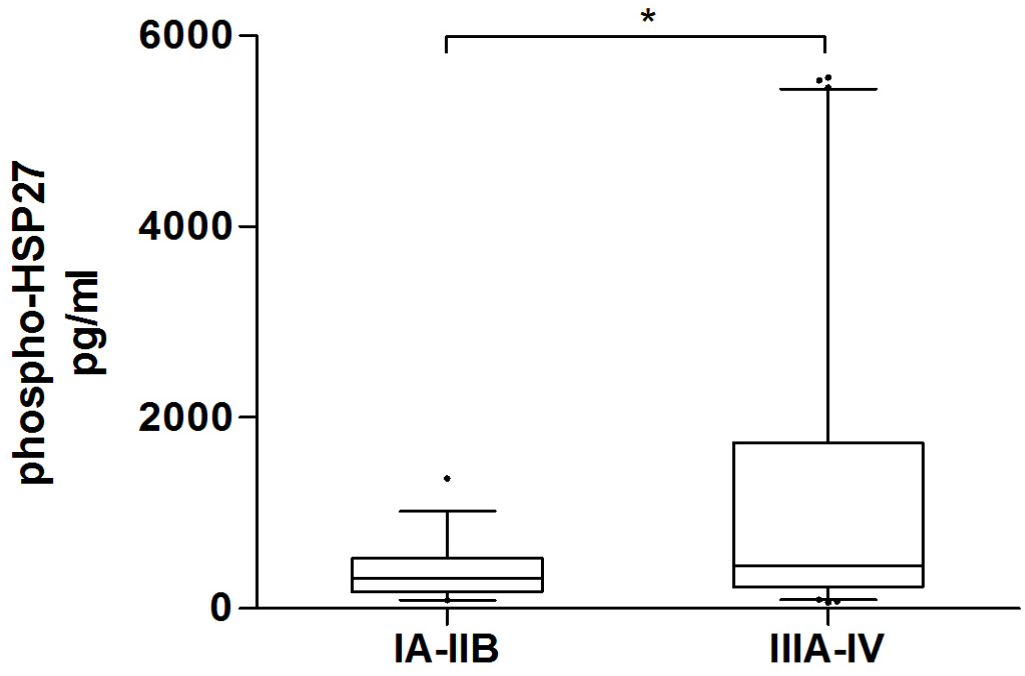

Figure 4.2: Serum levels of pHSP27 were only evaluated in early and advanced stage NSCLC patients (*p<0.05).

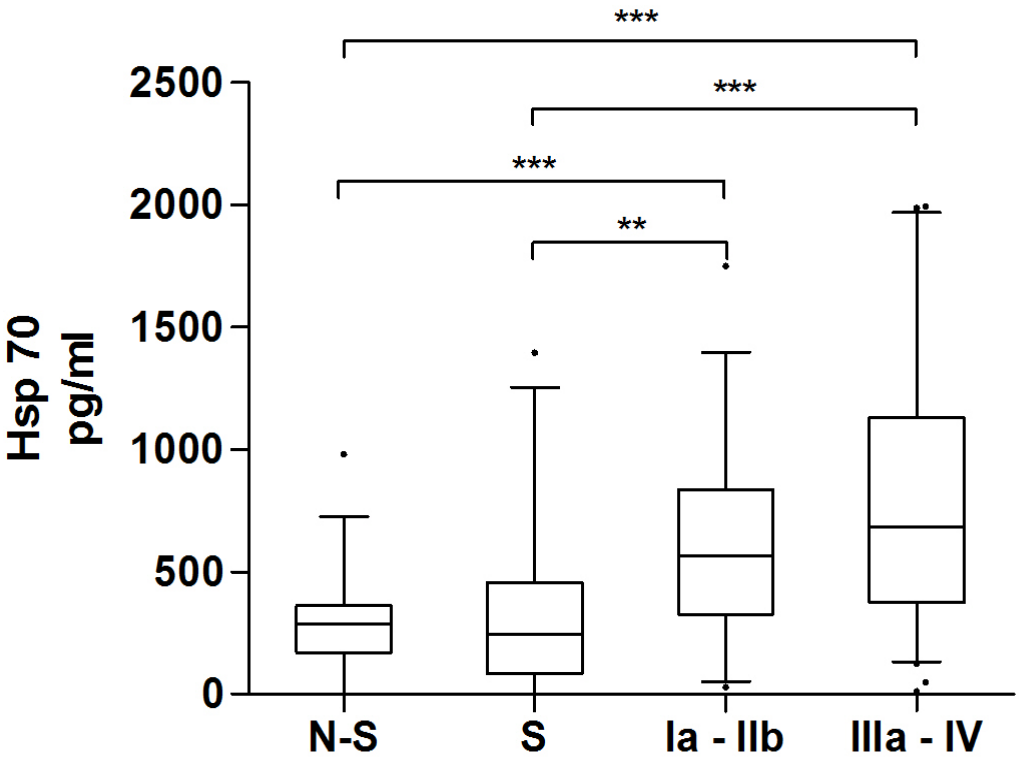

Figure 4.3: Serum levels of HSP70 are elevated in patients with NSCLC diagnosed at an early or at an advanced stage when compared with either healthy control groups, (** p<0.01, *** p<0.001). However, there is no statistically significant difference between the two NSCLC patient groups.

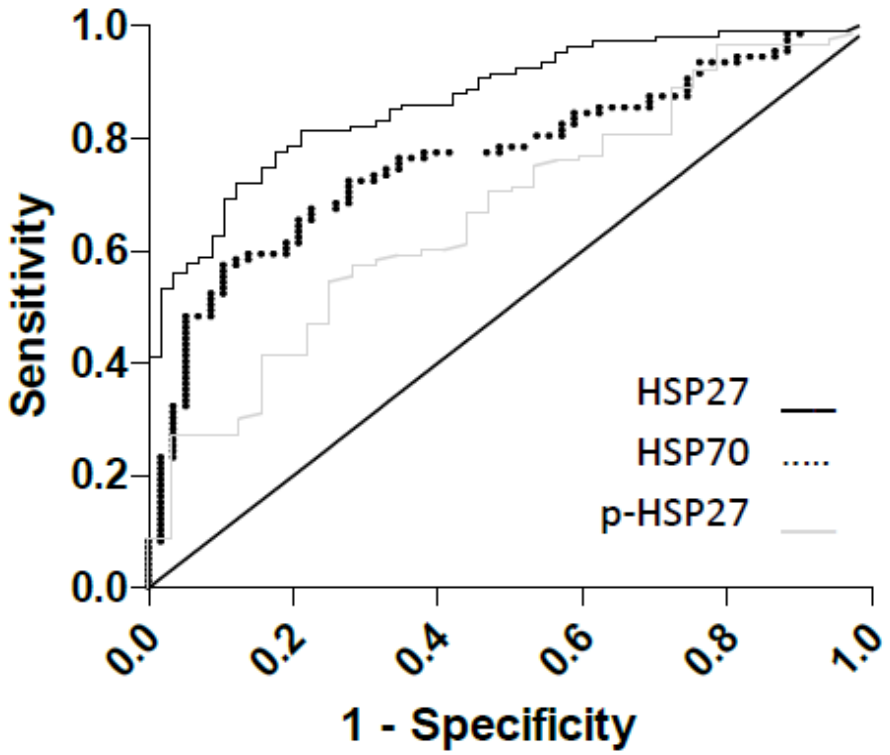

Figure 4.4: ROC curve indicating sensitivity and specificity of HSP27 (AUC= 0.870), pHSP27 (AUC=0.682) and HSP70 (AUC=0.779) in the diagnosis of NSCLC

5. Discussion

In the present study, we detected that serum HSP27 and HSP70 levels were significantly increased in a population of NSCLC patients as compared to our sample of community based controls. In addition, serum levels of HSP27 were significantly indicative for presence of early versus advanced NSCLC. We further discovered in this group analysis that impaired lung function parameters were significantly correlated with early and advanced stage NSCLC. This finding corroborates recently published studies that tied CT verified lung pathology with impaired lung function parameters [121-123].

Concluding our results, we were able to show significantly elevated serum HSP27 and serum HSP70 levels in NSCLC patients compared with healthy controls. HSP27 serum levels showed a stage-dependent increase with 2- and 3-fold higher levels in early (IA – IIB) versus advanced stage (IIIA – IV) carcinoma patients. These data indicate that augmented spillage of this stress protein into the systemic circulation occurs during disease progression, presumably caused by a continuous activation of the immune system. Further, HSP27 showed an excellent sensitivity and specificity in a regression model to distinguish between healthy subjects and NSCLC patients. With an overall area under the curve of 0.870, HSP27 is prone to serve as a possible diagnostic marker for NSCLC progression.

6. References

1. Peto, R., S. Darby, H. Deo, et al., *Smoking, smoking cessation, and lung cancer in the UK since 1950: combination of national statistics with two case-control studies.* BMJ, 2000. **321**(7257): p. 323-9.
2. Parkin, D.M., *Global cancer statistics in the year 2000.* Lancet Oncol, 2001. **2**(9): p. 533-43.
3. Weiss, W., *Cigarette smoking and lung cancer trends. A light at the end of the tunnel?* Chest, 1997. **111**(5): p. 1414-6.
4. Statistik-Austria, *Krebsinzidenz und Krebsmortalität in Österreich*. 2010.
5. Sugimura, H. and P. Yang, *Long-term survivorship in lung cancer: a review.* Chest, 2006. **129**(4): p. 1088-97.
6. Wingo, P.A., L.A. Ries, S.L. Parker, et al., *Long-term cancer patient survival in the United States.* Cancer Epidemiol Biomarkers Prev, 1998. **7**(4): p. 271-82.
7. Beckett, W.S., *Epidemiology and etiology of lung cancer.* Clin Chest Med, 1993. **14**(1): p. 1-15.
8. Freedman, N.D., M.F. Leitzmann, A.R. Hollenbeck, et al., *Cigarette smoking and subsequent risk of lung cancer in men and women: analysis of a prospective cohort study.* Lancet Oncol, 2008. **9**(7): p. 649-56.
9. Bain, C., D. Feskanich, F.E. Speizer, et al., *Lung cancer rates in men and women with comparable histories of smoking.* J Natl Cancer Inst, 2004. **96**(11): p. 826-34.
10. Blot, W.J. and J.K. McLaughlin, *Are women more susceptible to lung cancer?* J Natl Cancer Inst, 2004. **96**(11): p. 812-3.
11. Neugut, A.I. and J.S. Jacobson, *Women and lung cancer: gender equality at a crossroad?* JAMA, 2006. **296**(2): p. 218-9.
12. Henschke, C.I., R. Yip, and O.S. Miettinen, *Women's susceptibility to tobacco carcinogens and survival after diagnosis of lung cancer.* JAMA, 2006. **296**(2): p. 180-4.
13. Risch, H.A. and A.B. Miller, *Re: Are women more susceptible to lung cancer?* J Natl Cancer Inst, 2004. **96**(20): p. 1560; author reply 1560-1.
14. Khuder, S.A., *Effect of cigarette smoking on major histological types of lung cancer: a meta-analysis.* Lung Cancer, 2001. **31**(2-3): p. 139-48.
15. Schoenberg, J.B., H.B. Wilcox, T.J. Mason, et al., *Variation in smoking-related lung cancer risk among New Jersey women.* Am J Epidemiol, 1989. **130**(4): p. 688-95.
16. Zang, E.A. and E.L. Wynder, *Differences in lung cancer risk between men and women: examination of the evidence.* J Natl Cancer Inst, 1996. **88**(3-4): p. 183-92.
17. Risch, H.A., G.R. Howe, M. Jain, et al., *Are female smokers at higher risk for lung cancer than male smokers? A case-control analysis by histologic type.* Am J Epidemiol, 1993. **138**(5): p. 281-93.
18. Haiman, C.A., D.O. Stram, L.R. Wilkens, et al., *Ethnic and racial differences in the smoking-related risk of lung cancer.* N Engl J Med, 2006. **354**(4): p. 333-42.
19. Shaw, G.L., R.T. Falk, L.W. Pickle, et al., *Lung cancer risk associated with cancer in relatives.* J Clin Epidemiol, 1991. **44**(4-5): p. 429-37.
20. Gao, Y., A.M. Goldstein, D. Consonni, et al., *Family history of cancer and nonmalignant lung diseases as risk factors for lung cancer.* Int J Cancer, 2009. **125**(1): p. 146-52.
21. Poole, C.A., T. Byers, E.E. Calle, et al., *Influence of a family history of cancer within and across multiple sites on patterns of cancer mortality risk for women.* Am J Epidemiol, 1999. **149**(5): p. 454-62.

22. Brownson, R.C., M.C. Alavanja, N. Caporaso, et al., *Family history of cancer and risk of lung cancer in lifetime non-smokers and long-term ex-smokers.* Int J Epidemiol, 1997. **26**(2): p. 256-63.
23. Doll, R., *Uncovering the effects of smoking: historical perspective.* Stat Methods Med Res, 1998. **7**(2): p. 87-117.
24. Physicians, R.C.o., *Smoking or health now: a new report and summary on smoking and its effect on health from the Royal College of Physicians of London.* 1971: London.
25. *Smoking or health: the third report from the Royal College of Physicians of London*. 1977, Royal College of Physicians: London.
26. IARC, *IARC monographs on the evaluation of carcinogenic risk to humans*. 1986: Lyon.
27. USDHHS, *The health consequences of smoking: cancer. A report of the Surgeon General*. 1982: Rockville.
28. USDHHS, *Reducing the health consequences of smoking: 25 years of progress. A report of the Surgeon General*. 1989: Washington.
29. Hoffmann, D. and I. Hoffmann, *The changing cigarette, 1950-1995.* J Toxicol Environ Health, 1997. **50**(4): p. 307-64.
30. Benhamou, E. and S. Benhamou, *Black (air-cured) and blond (flue-cured) tobacco and cancer risk. VI: Lung cancer.* Eur J Cancer, 1993. **29A**(12): p. 1778-80.
31. Adler, *Primary Malignant Growths of the Lungs and Bronchi.* American Journal of the Medical Sciences, 1921. **144**(6).
32. Lickint, F., *Der Bronchialkrebs der Raucher.* Münchner Medizinische Wochenschrift, 1935. **2**: p. 1232-1234.
33. Doll, R. and A.B. Hill, *Smoking and carcinoma of the lung; preliminary report.* Br Med J, 1950. **2**(4682): p. 739-48.
34. Wynder, E.L. and E.A. Graham, *Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma; a study of 684 proved cases.* J Am Med Assoc, 1950. **143**(4): p. 329-36.
35. Parkin, D.M., P. Pisani, A.D. Lopez, et al., *At least one in seven cases of cancer is caused by smoking. Global estimates for 1985.* Int J Cancer, 1994. **59**(4): p. 494-504.
36. Service, U.P.H., ed. *The Health Consequences of Smoking*. Cardiovascular Disease. A Report of Surgeon General. 1983: Rockville, MD.
37. *The Health Consequences of Smoking for Women. A Report of the Surgeon General.* 1980, US Deparment of Health and Human Services, Office on Smoking and Health: Rockville.
38. Simonato, L., A. Agudo, W. Ahrens, et al., *Lung cancer and cigarette smoking in Europe: an update of risk estimates and an assessment of inter-country heterogeneity.* Int J Cancer, 2001. **91**(6): p. 876-87.
39. Doll, R. and R. Peto, *Cigarette smoking and bronchial carcinoma: dose and time relationships among regular smokers and lifelong non-smokers.* J Epidemiol Community Health, 1978. **32**(4): p. 303-13.
40. Boffetta, P., G. Pershagen, K.H. Jockel, et al., *Cigar and pipe smoking and lung cancer risk: a multicenter study from Europe.* J Natl Cancer Inst, 1999. **91**(8): p. 697-701.
41. Law, M.R. and A.K. Hackshaw, *Environmental tobacco smoke.* Br Med Bull, 1996. **52**(1): p. 22-34.

42. Brownson, R.C., M.P. Eriksen, R.M. Davis, et al., *Environmental tobacco smoke: health effects and policies to reduce exposure.* Annu Rev Public Health, 1997. **18**: p. 163-85.
43. Hirayama, T., *Non-smoking wives of heavy smokers have a higher risk of lung cancer: a study from Japan.* Br Med J (Clin Res Ed), 1981. **282**(6259): p. 183-5.
44. Trichopoulos, D., A. Kalandidi, L. Sparros, et al., *Lung cancer and passive smoking.* Int J Cancer, 1981. **27**(1): p. 1-4.
45. Garfinkel, L., *Time trends in lung cancer mortality among nonsmokers and a note on passive smoking.* J Natl Cancer Inst, 1981. **66**(6): p. 1061-6.
46. Hackshaw, A.K., M.R. Law, and N.J. Wald, *The accumulated evidence on lung cancer and environmental tobacco smoke.* BMJ, 1997. **315**(7114): p. 980-8.
47. IARC, *Tobacco smoke and involuntary smoking.* IARC Monograph, 2004(83): p. 1452.
48. Hammond, E.C., I.J. Selikoff, and H. Seidman, *Asbestos exposure, cigarette smoking and death rates.* Ann N Y Acad Sci, 1979. **330**: p. 473-90.
49. Vainio, H. and P. Boffetta, *Mechanisms of the combined effect of asbestos and smoking in the etiology of lung cancer.* Scand J Work Environ Health, 1994. **20**(4): p. 235-42.
50. Erren, T.C., M. Jacobsen, and C. Piekarski, *Synergy between asbestos and smoking on lung cancer risks.* Epidemiology, 1999. **10**(4): p. 405-11.
51. Liddell, F.D., *The interaction of asbestos and smoking in lung cancer.* Ann Occup Hyg, 2001. **45**(5): p. 341-56.
52. Lee, P.N., *Relation between exposure to asbestos and smoking jointly and the risk of lung cancer.* Occup Environ Med, 2001. **58**(3): p. 145-53.
53. Radon, C.o.H.R.o.E.t., *Health effects of exposure to radon (BEIR VI)*. 1999.
54. *Health effects of radon exposure. Report of the Council on Scientific Affairs, American Medical Association.* Arch Intern Med, 1991. **151**(4): p. 674-7.
55. Lubin, J.H., J.D. Boice, Jr., and J.M. Samet, *Errors in exposure assessment, statistical power and the interpretation of residential radon studies.* Radiat Res, 1995. **144**(3): p. 329-41.
56. Lubin, J.H. (1994) *Lung cancer and radon: a joint analysis of 11 underground miners studies*. US National Institutes of Health.
57. Krewski, D., J.H. Lubin, J.M. Zielinski, et al., *Residential radon and risk of lung cancer: a combined analysis of 7 North American case-control studies.* Epidemiology, 2005. **16**(2): p. 137-45.
58. Darby, S., D. Hill, A. Auvinen, et al., *Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies.* BMJ, 2005. **330**(7485): p. 223.
59. Skillrud, D.M., K.P. Offord, and R.D. Miller, *Higher risk of lung cancer in chronic obstructive pulmonary disease. A prospective, matched, controlled study.* Ann Intern Med, 1986. **105**(4): p. 503-7.
60. Lee, J.H., E.M. Song, Y.S. Sim, et al., *Forced expiratory volume in one second as a prognostic factor in advanced non-small cell lung cancer.* J Thorac Oncol, 2011. **6**(2): p. 305-9.
61. Doria-Rose, V.P., P.M. Marcus, E. Szabo, et al., *Randomized controlled trials of the efficacy of lung cancer screening by sputum cytology revisited: a combined mortality analysis from the Johns Hopkins Lung Project and the Memorial Sloan-Kettering Lung Study.* Cancer, 2009. **115**(21): p. 5007-17.

62. Gasparini, S., M. Ferretti, E.B. Secchi, et al., *Integration of transbronchial and percutaneous approach in the diagnosis of peripheral pulmonary nodules or masses. Experience with 1,027 consecutive cases.* Chest, 1995. **108**(1): p. 131-7.
63. Pirozynski, M., *Bronchoalveolar lavage in the diagnosis of peripheral, primary lung cancer.* Chest, 1992. **102**(2): p. 372-4.
64. Shim, S.S., K.S. Lee, B.T. Kim, et al., *Non-small cell lung cancer: prospective comparison of integrated FDG PET/CT and CT alone for preoperative staging.* Radiology, 2005. **236**(3): p. 1011-9.
65. McWilliams, A., C. MacAulay, A.F. Gazdar, et al., *Innovative molecular and imaging approaches for the detection of lung cancer and its precursor lesions.* Oncogene, 2002. **21**(45): p. 6949-59.
66. Henschke, C.I., D.F. Yankelevitz, D.M. Libby, et al., *Survival of patients with stage I lung cancer detected on CT screening.* N Engl J Med, 2006. **355**(17): p. 1763-71.
67. McWilliams, A. and S. Lam, *Lung cancer screening.* Curr Opin Pulm Med, 2005. **11**(4): p. 272-7.
68. Molina, R., J.M. Auge, X. Filella, et al., *Pro-gastrin-releasing peptide (proGRP) in patients with benign and malignant diseases: comparison with CEA, SCC, CYFRA 21-1 and NSE in patients with lung cancer.* Anticancer Res, 2005. **25**(3A): p. 1773-8.
69. Farlow, E.C., M.S. Vercillo, J.S. Coon, et al., *A multi-analyte serum test for the detection of non-small cell lung cancer.* Br J Cancer, 2010. **103**(8): p. 1221-8.
70. Foa, P., M. Fornier, R. Miceli, et al., *Tumour markers CEA, NSE, SCC, TPA and CYFRA 21.1 in resectable non-small cell lung cancer.* Anticancer Res, 1999. **19**(4C): p. 3613-8.
71. Molina, R., X. Filella, J.M. Auge, et al., *Tumor markers (CEA, CA 125, CYFRA 21-1, SCC and NSE) in patients with non-small cell lung cancer as an aid in histological diagnosis and prognosis. Comparison with the main clinical and pathological prognostic factors.* Tumour Biol, 2003. **24**(4): p. 209-18.
72. Weitberg, A., *Cancer of the Lung. From Molecular Biology to Treatment Guidelines*. 2002.
73. Pass, H.I., J.B. Mitchell, D.H. Johnson, et al., *Lung Cancer*. 2000: Lipincott Williams & Wilkins.
74. Parkin, D.M., P. Pisani, and J. Ferlay, *Global cancer statistics.* CA Cancer J Clin, 1999. **49**(1): p. 33-64, 1.
75. Tomashefski, J.F., Jr., A.F. Connors, Jr., E.S. Rosenthal, et al., *Peripheral vs central squamous cell carcinoma of the lung. A comparison of clinical features, histopathology, and survival.* Arch Pathol Lab Med, 1990. **114**(5): p. 468-74.
76. Carter, D., *Squamous cell carcinoma of the lung: an update.* Semin Diagn Pathol, 1985. **2**(4): p. 226-34.
77. Travis WD, C.T., Corrin B, et al., ed. *Histological typing of lung and pleural tumors*. 3rd edition ed. 1999, Springer Verlag: Berlin.
78. Travis, W.D., L.B. Travis, and S.S. Devesa, *Lung cancer.* Cancer, 1995. **75**(1 Suppl): p. 191-202.
79. Cagle, P.T., S.D. Cohle, and S.D. Greenberg, *Natural history of pulmonary scar cancers. Clinical and pathologic implications.* Cancer, 1985. **56**(8): p. 2031-5.
80. Auerbach, O. and L. Garfinkel, *The changing pattern of lung carcinoma.* Cancer, 1991. **68**(9): p. 1973-7.
81. Minna, J.D., J.A. Roth, and A.F. Gazdar, *Focus on lung cancer.* Cancer Cell, 2002. **1**(1): p. 49-52.
82. Herbst, R.S., J.V. Heymach, and S.M. Lippman, *Lung cancer.* N Engl J Med, 2008. **359**(13): p. 1367-80.

83. Wistuba, II, C. Behrens, A.K. Virmani, et al., *High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints.* Cancer Res, 2000. **60**(7): p. 1949-60.
84. Toyooka, S., K.O. Toyooka, R. Maruyama, et al., *DNA methylation profiles of lung tumors.* Mol Cancer Ther, 2001. **1**(1): p. 61-7.
85. Wistuba, II, A.F. Gazdar, and J.D. Minna, *Molecular genetics of small cell lung carcinoma.* Semin Oncol, 2001. **28**(2 Suppl 4): p. 3-13.
86. Bhattacharjee, A., W.G. Richards, J. Staunton, et al., *Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses.* Proc Natl Acad Sci U S A, 2001. **98**(24): p. 13790-5.
87. Garber, M.E., O.G. Troyanskaya, K. Schluens, et al., *Diversity of gene expression in adenocarcinoma of the lung.* Proc Natl Acad Sci U S A, 2001. **98**(24): p. 13784-9.
88. Rami-Porta, R., J.J. Crowley, and P. Goldstraw, *The revised TNM staging system for lung cancer.* Ann Thorac Cardiovasc Surg, 2009. **15**(1): p. 4-9.
89. Molina, J.R., P. Yang, S.D. Cassivi, et al., *Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship.* Mayo Clin Proc, 2008. **83**(5): p. 584-94.
90. Ginsberg, R.J. and L.V. Rubinstein, *Randomized trial of lobectomy versus limited resection for T1 N0 non-small cell lung cancer. Lung Cancer Study Group.* Ann Thorac Surg, 1995. **60**(3): p. 615-22; discussion 622-3.
91. McKenna, R.J., Jr., W. Houck, and C.B. Fuller, *Video-assisted thoracic surgery lobectomy: experience with 1,100 cases.* Ann Thorac Surg, 2006. **81**(2): p. 421-5; discussion 425-6.
92. Arriagada, R., A. Auperin, S. Burdett, et al., *Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data.* Lancet, 2010. **375**(9722): p. 1267-77.
93. Leonard, C.T., R.I. Whyte, and G.A. Lillington, *Primary non-small-cell lung cancer: determining the suitability of the patient and tumor for resection.* Curr Opin Pulm Med, 2000. **6**(4): p. 391-5.
94. Smythe, W.R., *Treatment of stage I non-small cell lung carcinoma.* Chest, 2003. **123**(1 Suppl): p. 181S-187S.
95. Scott, W.J., J. Howington, and B. Movsas, *Treatment of stage II non-small cell lung cancer.* Chest, 2003. **123**(1 Suppl): p. 188S-201S.
96. Robinson, L.A., H. Wagner, Jr., and J.C. Ruckdeschel, *Treatment of stage IIIA non-small cell lung cancer.* Chest, 2003. **123**(1 Suppl): p. 202S-220S.
97. Jett, J.R., W.J. Scott, M.P. Rivera, et al., *Guidelines on treatment of stage IIIB non-small cell lung cancer.* Chest, 2003. **123**(1 Suppl): p. 221S-225S.
98. Socinski, M.A., D.E. Morris, G.A. Masters, et al., *Chemotherapeutic management of stage IV non-small cell lung cancer.* Chest, 2003. **123**(1 Suppl): p. 226S-243S.
99. Beere, H.M., *"The stress of dying": the role of heat shock proteins in the regulation of apoptosis.* J Cell Sci, 2004. **117**(Pt 13): p. 2641-51.
100. Asea, A., S.K. Kraeft, E.A. Kurt-Jones, et al., *HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine.* Nat Med, 2000. **6**(4): p. 435-42.
101. Asea, A., M. Rehli, E. Kabingu, et al., *Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4.* J Biol Chem, 2002. **277**(17): p. 15028-34.
102. van Eden, W., R. van der Zee, and B. Prakken, *Heat-shock proteins induce T-cell regulation of chronic inflammation.* Nat Rev Immunol, 2005. **5**(4): p. 318-30.

103. Hacker, S., C. Lambers, K. Hoetzenecker, et al., *Elevated HSP27, HSP70 and HSP90 alpha in chronic obstructive pulmonary disease: markers for immune activation and tissue destruction.* Clin Lab, 2009. **55**(1-2): p. 31-40.
104. Njemini, R., C. Demanet, and T. Mets, *Inflammatory status as an important determinant of heat shock protein 70 serum concentrations during aging.* Biogerontology, 2004. **5**(1): p. 31-8.
105. Szerafin, T., K. Hoetzenecker, S. Hacker, et al., *Heat shock proteins 27, 60, 70, 90alpha, and 20S proteasome in on-pump versus off-pump coronary artery bypass graft patients.* Ann Thorac Surg, 2008. **85**(1): p. 80-7.
106. Love, S. and R.J. King, *A 27 kDa heat shock protein that has anomalous prognostic powers in early and advanced breast cancer.* Br J Cancer, 1994. **69**(4): p. 743-8.
107. Delhaye, M., B. Gulbis, P. Galand, et al., *Expression of 27-kD heat-shock protein isoforms in human neoplastic and nonneoplastic liver tissues.* Hepatology, 1992. **16**(2): p. 382-9.
108. Kato, M., F. Herz, S. Kato, et al., *Expression of stress-response (heat-shock) protein 27 in human brain tumors: an immunohistochemical study.* Acta Neuropathol, 1992. **83**(4): p. 420-2.
109. Thomas, S.A., I.L. Brown, G.W. Hollins, et al., *Detection and distribution of heat shock proteins 27 and 90 in human benign and malignant prostatic tissue.* Br J Urol, 1996. **77**(3): p. 367-72.
110. Calderwood, S.K., M.A. Khaleque, D.B. Sawyer, et al., *Heat shock proteins in cancer: chaperones of tumorigenesis.* Trends Biochem Sci, 2006. **31**(3): p. 164-72.
111. Ciocca, D.R. and S.K. Calderwood, *Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications.* Cell Stress Chaperones, 2005. **10**(2): p. 86-103.
112. Vargas-Roig, L.M., F.E. Gago, O. Tello, et al., *Heat shock protein expression and drug resistance in breast cancer patients treated with induction chemotherapy.* Int J Cancer, 1998. **79**(5): p. 468-75.
113. Michils, A., M. Redivo, V. Zegers de Beyl, et al., *Increased expression of high but not low molecular weight heat shock proteins in resectable lung carcinoma.* Lung Cancer, 2001. **33**(1): p. 59-67.
114. Huang, Q., Y. Zu, X. Fu, et al., *Expression of heat shock protein 70 and 27 in non-small cell lung cancer and its clinical significance.* J Huazhong Univ Sci Technolog Med Sci, 2005. **25**(6): p. 693-5.
115. Malusecka, E., A. Zborek, S. Krzyzowska-Gruca, et al., *Expression of heat shock proteins HSP70 and HSP27 in primary non-small cell lung carcinomas. An immunohistochemical study.* Anticancer Res, 2001. **21**(2A): p. 1015-21.
116. Malusecka, E., S. Krzyzowska-Gruca, J. Gawrychowski, et al., *Stress proteins HSP27 and HSP70i predict survival in non-small cell lung carcinoma.* Anticancer Res, 2008. **28**(1B): p. 501-6.
117. Volm, M., R. Koomagi, J. Mattern, et al., *Heat shock (hsp70) and resistance proteins in non-small cell lung carcinomas.* Cancer Lett, 1995. **95**(1-2): p. 195-200.
118. Volm, M., J. Mattern, and G. Stammler, *Up-regulation of heat shock protein 70 in adenocarcinomas of the lung in smokers.* Anticancer Res, 1995. **15**(6B): p. 2607-9.
119. Suzuki, K., Y. Ito, K. Wakai, et al., *Serum heat shock protein 70 levels and lung cancer risk: a case-control study nested in a large cohort study.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(9): p. 1733-7.
120. Wang, H., J. Xing, F. Wang, et al., *Expression of Hsp27 and Hsp70 in lymphocytes and plasma in healthy workers and coal miners with lung cancer.* J Huazhong Univ Sci Technolog Med Sci, 2010. **30**(4): p. 415-20.

121. Loganathan, R.S., D.E. Stover, W. Shi, et al., *Prevalence of COPD in women compared to men around the time of diagnosis of primary lung cancer.* Chest, 2006. **129**(5): p. 1305-12.
122. Congleton, J. and M.F. Muers, *The incidence of airflow obstruction in bronchial carcinoma, its relation to breathlessness, and response to bronchodilator therapy.* Respir Med, 1995. **89**(4): p. 291-6.
123. Buist, A.S., M.A. McBurnie, W.M. Vollmer, et al., *International variation in the prevalence of COPD (the BOLD Study): a population-based prevalence study.* Lancet, 2007. **370**(9589): p. 741-50.
124. Brody, J.S. and A. Spira, *State of the art. Chronic obstructive pulmonary disease, inflammation, and lung cancer.* Proc Am Thorac Soc, 2006. **3**(6): p. 535-7.
125. Petty, T.L., *Are COPD and lung cancer two manifestations of the same disease?* Chest, 2005. **128**(4): p. 1895-7.
126. Barnes, P.J., *Immunology of asthma and chronic obstructive pulmonary disease.* Nat Rev Immunol, 2008. **8**(3): p. 183-92.
127. Hodge, S., G. Hodge, J. Nairn, et al., *Increased airway granzyme b and perforin in current and ex-smoking COPD subjects.* COPD, 2006. **3**(4): p. 179-87.
128. Hodge, G., J. Nairn, M. Holmes, et al., *Increased intracellular T helper 1 proinflammatory cytokine production in peripheral blood, bronchoalveolar lavage and intraepithelial T cells of COPD subjects.* Clin Exp Immunol, 2007. **150**(1): p. 22-9.
129. Di Stefano, A., G. Caramori, A. Capelli, et al., *STAT4 activation in smokers and patients with chronic obstructive pulmonary disease.* Eur Respir J, 2004. **24**(1): p. 78-85.
130. Di Stefano, A., A. Capelli, M. Lusuardi, et al., *Severity of airflow limitation is associated with severity of airway inflammation in smokers.* Am J Respir Crit Care Med, 1998. **158**(4): p. 1277-85.
131. Campisi, J., *Replicative senescence: an old lives' tale?* Cell, 1996. **84**(4): p. 497-500.
132. Agusti, A.G., *Systemic effects of chronic obstructive pulmonary disease.* Proc Am Thorac Soc, 2005. **2**(4): p. 367-70; discussion 371-2.
133. Lambers, C., S. Hacker, M. Posch, et al., *T cell senescence and contraction of T cell repertoire diversity in patients with chronic obstructive pulmonary disease.* Clin Exp Immunol, 2009. **155**(3): p. 466-75.
134. Hacker, S., C. Lambers, A. Pollreisz, et al., *Increased soluble serum markers caspase-cleaved cytokeratin-18, histones, and ST2 indicate apoptotic turnover and chronic immune response in COPD.* J Clin Lab Anal, 2009. **23**(6): p. 372-9.
135. Nickl, S., *Elevated Heat Shock Protein 27 serum levels positively correlate with the presence of air trapping and emphysema in lung CT scan.* Wien Klin Wochenschr, 2010. **122**(17-18): p. A35.
136. Wilson, D.O., J.L. Weissfeld, A. Balkan, et al., *Association of radiographic emphysema and airflow obstruction with lung cancer.* Am J Respir Crit Care Med, 2008. **178**(7): p. 738-44.
137. de Torres, J.P., G. Bastarrika, J.P. Wisnivesky, et al., *Assessing the relationship between lung cancer risk and emphysema detected on low-dose CT of the chest.* Chest, 2007. **132**(6): p. 1932-8.

7. Abbreviations

AC	adenocarcinoma
AUC	area under the curve
BAC	bronchioloalveolar carcinoma
BAL	bronchoalveolar lavage
COPD	chronic obstructive disease
CT	computed tomography
ELISA	enzyme linked immunosorbent assay
ETS	environmental tobacco smoke
FEV^1	forced expiratory volume in one second
FVC	forced vital capacity
GOLD	global initiative for obstructive lung disease
HRP	horseradish peroxidase conjugate
HSP	heat shock protein
IASCL	international association for the study of lung cancer
IQR	interquartile range
LCC	large cell lung cancer
LCNEC	large cell neuroendocrine carcinoma
NSCLC	non small cell lung cancer
NSCLC-NOS	non small cell lung cancer – not otherwise specified
OD	optical density
PAH	polynuclear aromatic hydrocarbons
PCD	programmed cell death
PET	positron emission tomography
PNA	percutaneous needle aspiration
ROC	receiver operating characteristics
RR	relative risk
SCC	squamous cell carcinoma
SCLC	small cell lung cancer
SD	standard deviation
TSNA	tobacco specific N-nitrosamines
UICC	union for international cancer control
VATS	video assisted thoracic surgery
WHO	world health organization

8. Supplements

Datenerhebungsbogen

Pat. Nr. ☐☐☐

Geburtsdatum: ☐☐.☐☐.☐☐☐☐ Geschlecht: m ☐ w ☐

BMI : ☐☐.☐ Nikotinanamnese: ☐☐ Pack-Years

Aufnahmedatum: ☐☐.☐☐.☐☐☐☐

Aufnahmegrund: _____

_____

Komorbiditäten: _____

_____

Vorhandene Kopien:

- ☐ Patientenzuweisung
- ☐ Röntgen Thorax
- ☐ MRT
- ☐ CT
- ☐ Spirometrie
- ☐ Blutgase
- ☐ Labor
- ☐ Klinische Stadieneinteilung
- ☐ Histologisch-/zytologischer Befund

Histologie:

NSCLC

- Adenokarzinom ☐
- Plattenepithelkarzinom ☐
- großzellig anaplastisches Karzinom ☐

SCLC

- kleinzelliges Karzinom ☐

Karzinoid ☐

Mischform: _____ ☐

Andere: _____ ☐

Patient aufgeklärt am ☐☐.☐☐.☐☐☐☐

Serum abgenommen am ☐☐.☐☐.☐☐☐☐ von _____

Figure 8.1: Questionnaire

Table 8.1

	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	***
Histological Classification						
AC	-	-	24	52	76	*n.s.*
SCC	-	-	11	15	26	*n.s.*
NSCLC NOS	-	-	1	5	6	-
others	-	-	1	0	1	-

Table 8.2

stage		healthy non-smokers	healthy smokers	Ia	Ib	IIa	IIb	IIIa	IIIb	IV	*P*
n		33	24	*10*	*15*	*3*	*9*	*16*	*6*	*50*	
HSP27	median	1482	2242	3452	3198	2689	4377	4023	4339	5558	***<0.0001***
	IQR	1136–2071	1787–3009	1823–4347	2469–4206	2258–4074	3105–5626	3025–7355	3371–8620	2854–8125	
HSP70	median	285	244	643	517	1014	616	452	825	719	***<0.0001***
	IQR	166–345	82–456	129–847	246–806	58–1748	344–791	282–1147	318–1796	432–1105	

Detailed results of HSP27 and HSP70 serum levels in NSCLC patients (IASCL stages I to IV) and in healthy controls. *P*-values were determined using Kruskal-Wallis test.

9. Curriculum Vitae

Personal Background

Nationality:	Austria
Date of Birth:	13th July 1985, Bregenz, Austria
Parents:	Dr. Kurt and Monika Zimmermann
Residence:	Vienna, Austria

Education

Since 2008/10:	Student research fellow at the Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Medical University of Vienna
Since 2004/10:	Medical Student at the Medical University of Vienna, Austria
2003 – 2004:	Military Service, with training as paramedic
2003/06	Matura (High School Graduation)
1995- 2003	Federal Austrian High School (BG Gallusstraße) with emphasis on modern languages, Bregenz
1991- 1995	Elementary School, Höchst

Clinical Training

2010/09	Clinical Clerkship at the Department of Cardiology, Charité, Berlin
2009/07	Clinical Clerkship at the Department of Trauma Surgery, LKH Bregenz
2009/06	Clinical Clerkship at the Department of Clinical Pathology, Otto-Wagner Hospital, Vienna
2008/07	Clinical Clerkship at the Department of Internal Medicine, LKH Dornbirn
2008/02	Clinical Clerkship at the Department of Trauma Surgery, LKH Bregenz
2007/07	Clinical Clerkship at the Department of Surgery, LKH Bregenz
2006/04	Clinical Clerkship at the Department of Trauma Surgery, Hanuschkrankenhaus, Vienna
2011/03	Clinical Clerkship at the Department of Dermatology, Inselspital, Bern
2011/04	Clinical Clerkship at the Department of Oto-Rhino-Laryngologie, Inselspital, Bern

2011/04-05 Clinical Clerkship at the Department of Gynaecology, LMU, Munich

2011/05 Clinical Clerkship at the Department of Ophthalmology, LMU, Munich

Continuing Education

2010/02	Advanced Anatomy of the Central Nervous System – Preparation for Tutorial
2006/03-06	Therapy of Acupuncture
2006/03-06	Homeopathy
2006/06	Anesthesia and Intensive Therapy at Liver Transplantation
2006/04	Intubation Training
2005/10-2006/03	Basic Lecture „Anatomie Kompakt“
2006/02	Anatomical Preparation Technology

Certification

Paramedical training

Publications

Hoetzenecker K, Adlbrecht C, Lichtenauer M, Hacker S, Hoetzenecker W, Mangold A, Nickl S, Mitterbauer A, Zimmermann M, Lang IM, Klepetko W, Ankersmit HJ. **Levels of sCD40, sCD40L, TNF alpha and TNF-RI in the Culprit Coronary Artery during Myocardial Infarction**. *Labmedicine* 2009, 40 (11): 660-664

Lichtenauer M, Nickl S, Hoetzenecker K, Mangold A, Moser B, Zimmermann M, Hacker S, Niederpold T, Mitterbauer A, Ankersmit HJ. **Phosphate buffered saline containing calcium and magnesium elicits increased secretion of Interleukin-1 receptor antagonist**. *Labmedicine* 2009;40(5):290-293

Hacker S, Lambers S, Hoetzenecker K, Pollreisz A, Aigner C, Lichtenauer M, Mangold A, Niederpold T, Zimmermann M, Taghavi S, Klepetko W, Ankersmit HJ. **Elevated HSP27, HSP70 and HSP90α in Chronic Obstructive Pulmonary Disease: Markers for Immune Activation and Tissue Destruction**. *Clinical Laboratory* 2009;55(1-2):31-40

Roth G, Zimmermann M, Lubsczyk B, Pilz J, Faybik P, Hetz H, Hacker S, Mangold A, Bacher A, Krenn CG, Ankersmit HJ. **Upregulation of Interleukin 33 and soluble ST2 serum levels in liver failure.** *Journal of Surgical Research* 2010;163(2):e79-83

Lichtenauer M, Nickl S, Hoetzenecker K, Mangold A, Mitterbauer A, Hacker S, Zimmermann M, Ankersmit HJ. **Effect of PBS Solutions on Chemokine Secretion of Human Peripheral Blood Mononuclear Cells.** *American Laboratory* 2011; 43(1):30-33

Abstracts

Nickl S, Hoetzenecker K, Mangold A, Moser B, Zimmermann M, Hacker S, Niederpold T, Mitterbauer A, Ankersmit HJ, Lichtenauer M. **Heightened extracellular levels of calcium and magnesium induce secretion of chemokines and anti-inflammatory cytokines.**

Annual Meeting of the Austrian Society of Transplantation, Transfusion and Genetics, Seefeld in Tirol 2009/10; published in Abstractbook

Annual Meeting of the Austrian Society of Internal Medicine, Vienna 2009/09 WIENER KLINISCHE WOCHENSCHRIFT 2009;121 (15-16):A27-A28

Clinical-Investigation:

Sub-Investigator of the clinical investigation "Multi-center, double-blind, randomized, placebo-controlled, parallel-group study to assess the efficacy, safety and tolerability of tezosentan in patients with pre-operative pulmonary hypertension, due to left heart disease, undergoing cardiac surgery" assigned by Actelion.

Language Skills

Native German Speaker
Proficient in English
Knowledge of Spanish, Italian and French