Diploma Thesis

Morphologic and morphometric analyses of cardiac tissue samples in cardiovascular and cancer diseases

- a retrospective autopsy study -

Submitted by

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Graz, August 2013 (Signature)

Declaration

I hereby declare that I, Miss Stella Maria Bonschak, have written this diploma thesis independently and unassisted by third parties. Furthermore my research has been conducted exclusively with the sources, which are recorded within the document. Concepts and conclusions directly or indirectly acquired from existing publications are distinguished as such.

Graz, August 2013 *Signature*

Preface

Cardiovascular and cancer diseases represent the most common causes of death worldwide.
As the opportunity arose to investigate the question of their effects on the “engine” organ of the human body - the heart - at the Institute of Pathology at the Medical University of Graz, there was no hesitating to launch an interesting study.
As I have absolved several valuable weeks of clinical training at this Institute in the last few years, I have on the one side been in the fortunate position to fortify my knowledge of macroscopic anatomy and topography of the human organs, on the other side pathological organ alterations and their direct causal or indirect role in deaths have been revealed to me in a memorable way.
This study offered the additional opportunity to independently examine a scientific research question based on the working methods of pathologists. Aside from provided insights into pathological workflows by personal assistance - starting from postmortem inspection of the decedents through preservation and processing of tissue samples to histological assessment - a comprehensive literature research also served for immerging deeper into the subject matter.

At the end of this study we gained some interesting results with regard to macro- and microscopic alterations of the human heart in cancer, metabolic and cardiovascular patients, which should be an inspiration and a good working base for future studies.

Before going into the medias res now I would like to cite, that this thesis was generated in cooperation with a fellow student, Ms. cand.med. Nina Wolf, whose special part was the more precise analysis of metabolic diseases (Wolf N. Morphologische und morphometrische Analyse von Herzen bei kardiovaskulären und metabolischen Erkrankungen - Eine retrospektive Autopsiestudie [diploma thesis]. Graz (Austria): Medical University of Graz; 2013).

Our two diploma theses shall be reciprocal supplements, each treating a different main focus.

Acknowledgment

Let me start this way: This thesis represents the final step of long and challenging, but also fulfilling and joyful medical studies, which have come to an end.
Unaided by my beloved parents, Franz and Martha, and my wonderful sister Carmen (with Gina) I would not be in the position to write these words today. Times were pretty hard now and then, but nevertheless they strongly supported me from afar, which I did not take for granted. Thank you so much!

At professional level, I would like to highly acknowledge my first supervisor and at the same time head of department of Pathology at the Medical University of Graz, Univ.-Prof. MD Gerald Höfler, for offering me the possibility to carry out this thesis under his supervision.
My high gratitude is furthermore addressed to my second supervisor, Sen.-Scientist MD Ariane Aigelsreiter, for all her help, professional support, motivation and friendly advice throughout this project.
I would also like to thank all employees of the Institute, who were as kind to support this work. Special thanks to the gentlemen of dissecting room, Mr. Augustin Donnerer and fellows. It was a real pleasure to meet and work next to these people.

Last but definitely not least, I would like to mention two special great friends of me, Ariane Mansuri and Mag. psych. Martina Kronberger. Thank you very much for proofreading this thesis, supporting me with statistical analysis and above all motivating me.

This thesis is dedicated to all deceased people, who were examined within this study.

„Man soll vor allem Mensch sein und dann erst Arzt„
Voltaire (François-Marie Arouet)

Zusammenfassung

Hintergrund: Die Todesursache Nr. 1 wird vertreten durch kardiovaskuläre Erkrankungen (KVE), dicht gefolgt von bösartigen Tumorerkrankungen. Eine ausschlaggebende Komponente dieser KVE stellen einerseits die koronare Herzkrankheit als auch das metabolische Syndrom dar; das epikardiale Fettgewebe wurde in diesem Zusammenhang als wissenschaftlich erwiesener Risikofaktor für kardiovaskuläre und metabolische Erkrankungen identifiziert. Eine Untergruppe der KVE wird durch die arrhythmogene rechtsventrikuläre Dysplasie/Kardiomyopathie (ARVD/C) vertreten. Diese wurde im Allgemeinen bekannt durch einen plötzlichen Herztod, der vorwiegend bei jungen männlichen Sportler aufgetreten ist. Tumorerkrankungen können zu einem schwerwiegenden Mangelernährungszustand führen, genannt das „Anorexie-Kachexie-Syndrom". Diese Studie wurde durchgeführt um Veränderungen makro- und mikroskopischer Parameter des menschlichen Herzens im Bezug auf kardiovaskuläre, metabolische und bösartige Tumorerkrankungen zu untersuchen; die Resultate wurden zwischen den Gruppen verglichen, wobei der ARVD/C und dem epikardialen Fettgewebe besondere Aufmerksamkeit zuteil wurde.

Materialien und Methoden: Über einen Zeitraum von 18 Monaten wurden im Rahmen klinisch-pathologischer Autopsien 41 verstorbene Patienten/innen (Durchschnittsalter 67.8 Jahre ± 14.8; 16 Frauen; 25 Männer) der Studie zugeführt. Herzgewebsproben wurden entnommen, ebenso wie konsekutiv eine anonymisierte Dokumentation der Krankengeschichte, Merkmale des Körperbaus und herzspezifische anatomische Parameter jedes/r Patienten/in erstellt wurde. Die gewonnenen Proben wurden konserviert, aufbereitet (Hämatoxylin-Eosin-Färbung) und lichtmikroskopisch untersucht, ergänzt durch eine computergestützte morphometrische Analyse. Die Studienpopulation wurde in 4 Gruppen aufgeteilt: Tumor, Kardiovaskuläre, Metabolische und Kontrollgruppe. Auf Basis des gesamten gewonnenen Datensatzes wurden statistische Analysen durchgeführt und die Ergebnisse zwischen den Studiengruppen verglichen.

Ergebnisse: Die makroskopische Untersuchung ergab einen signifikanten Unterschied der Herzgewichte ($p = .05$) im Gruppenvergleich; die Werte metabolischer und kardiovaskulärer Patienten waren erhöht. Die Auswertung der koronaren Arteriosklerose ergab lipoide (45% aller Patienten/innen), verkalkte

(22.5%) und hochgradig vulnerable (30%) Plaques, die in allen Gruppen vorkamen; die Letzteren waren jedoch vorwiegend in der kardiovaskulären und metabolischen vertreten. Der mikroskopisch ermittelte Umbau des Myokards durch fibrös-fettiges Gewebe (ARVD/C) wurde in 60% aller histologischen Schnitte entdeckt; von diesen war der rechte Ventrikel am häufigsten (34%), die Vorhöfe gleichmäßig (jeweils 25%) und der linke Ventrikel am wenigsten häufig (16%) betroffen. Die morphometrische Analyse enthüllte einen statistisch signifikanten Unterschied ($p = .03$) zwischen den Studiengruppen im Bezug auf die Myokardbreite des Areals B (rechter Ventrikel); die kardiovaskuläre Gruppe hatte deutlich erhöhte Werte im Vergleich zur metabolischen und Tumorgruppe. Überraschenderweise war die Epikard-Myokard-Ratio in demselben Areal in der Tumorgruppe signifikant erhöht im Vergleich zur kardiovaskulären und Kontrollgruppe.

Konklusion: Die ARVD/C-charakteristischen mikroskopischen Veränderungen des Myokards wurden in allen Studiengruppen gefunden, vorwiegend im rechten Ventrikel, weswegen wir vermuten, dass diese Erkrankung häufiger sein könnte als bisher angenommen. Die Tumorpatienten/innen dieser Studie lieferten die höchste Epikard - Myokard-Ratio; dieser Ergebnis verlangt nach weiteren Untersuchungen, da die Auswirkungen von Krebserkrankungen auf das menschliche Herz bisher noch nicht ausreichend untersucht worden sind. Die Daten, die in dieser Studie gewonnen wurden, sollen weiteren Studien als Grundlage dienen.

Schlagwörter: Kardiovaskuläre Erkrankungen, Metabolisches Syndrom, Epikardiales Fettgewebe, Arrhythmogene rechtsventrikuläre Dysplasie/Kardiomyopathie (ARVD/C), Anorexie-Kachexie-Syndrom (CACS) Epikard-Myokard - Ratio.

Abstract

Background: No. 1 cause of death is represented by cardiovascular diseases (CVDs), straightly followed by malignant growth. The metabolic syndrome (MetS) is a crucial component of CVDs as well as coronary artery disease; epicardial adipose tissue (EAT) is a scientifically proven risk factor for CVDs and MetS. Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) represents a subgroup of CVDs; it became known as sudden cardiac death, predominantly occurring in young athletic men. Cancer diseases can lead to a severe state of malnutrition, called “cancer anorexia cachexia syndrome” (CACS). This study was undertaken to determine alterations in macroscopic and microscopic parameters of the human heart in cardiovascular disease, metabolic syndrome and cancer diseases and to evaluate differences between these study groups. Special emphasis was put on EAT, ARVD/C and cancer cachexia.

Materials and methods: Over a period of 18 months 41 deceased patients (mean age 67.76 ± 14.84 years; 16 female; 25 male) were conducted to the study while clinical-pathological autopsies. Tissue samples were taken from human hearts as well as anonymised documentations of medical history, body composition features and heart-specific anatomical parameters of each patient were consecutively created. Obtained tissue samples were preserved, processed (hematoxylin-eosin stain) and reviewed by light microscope assessment, supplemented by computer-assisted morphometric analysis. The study population was divided into 4 study groups: tumor, cardiovascular, metabolic and control group. Based on all collected data brought together, statistical analysis was performed and the results were compared between the study groups.

Results: Macroscopic assessment revealed a significant difference in heart weight comparison among study groups ($p = .05$); metabolic and cardiovascular group showed increased heart weights in contrast to tumor and control group. With regard to coronary atherosclerosis (LAD) lipoid (45% of all patients), calcified (22.5%) and severe ruptured (30%) plaques were present in all study groups, whereby the latter was predominantly found in cardiovascular and metabolic patients. Microscopically fibro-fatty replacement of myocardial tissue (ARVD/C) was found in 60% of all histological slices; right ventricle was the most (34%) affected, whereas the atria were equally (each 25%) and left ventricle was the

least (16%) affected. Morphometric analyses indicated significant differences between the study groups regarding the myocardial thickness of the right ventricle ($p = .03$); cardiovascular group had increased values in contrast to metabolic and tumor group. With regard to the epicardium – myocardium ratio in the right ventricle ($p = .04$) tumor group offered a significant higher ratio in opposition to cardiovascular and control group.

Conclusion: As ARVD/C-characteristically histological alterations of the myocardium were found within all study groups - predominantly in the right ventricle - we propose that this disease could be more common than previously assumed. Cancer patients offered the highest epicardium-myocardium ratio within this study; this result calls for further investigations, as the impact of cancer diseases on the human heart is not explored well yet. The data obtained here should act as baseline data for further studies.

Keywords: Cardiovascular diseases, metabolic syndrome, epicardial adipose tissue, arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), cancer anorexia cachexia syndrome (CACS), epicardium-myocardium-ratio.

Table of contents

Glossary and abbreviations

AC	Arrhythmogenic cardiomyopathy
AICR	American Institute for Cancer Research
ARDS	Acute respiratory distress syndrome
ARVD/C	Arrhythmogenic right ventricular dysplasia/cardiomyopathy
ATGL	Adipose triglyceride lipase
ATP III	Adult Treatment Panel III
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CABG	Coronary artery bypass graft
CAC	Cancer associated cachexia
CACS	Cancer anorexia cachexia syndrome
CAD	Coronary artery disease
CHD	Coronary heart Disease
CVDs	Cardiovascular diseases
DM	Diabetes mellitus
DSC-2	Desmocollin-2
DSG2	Desmoglein 2
DSP	Desmoplakin
EAT	Epicardial adipose tissue
ECG	Electrocardiography
FFA	Free fatty acids
HSL	Hormone sensitive lipase
IDF	International Diabetes Federation
IL-1β	Interleukin-1 Beta
IL-6	Interleukin-6
JUP	Plakoglobin
LAD	Left anterior descending, Ramus interventricularis anterior
LBM	Lean body mass
LC	Lipomatosis cordis
LIF	Leukaemia inhibitory factor
LPL	Lipoprotein lipase
LV	Left ventricle

LVH	Left ventricular hypertrophy
MCI	Myocardial infarction
MetS	Metabolic syndrome
MHC	Myosin heavy chain
MTA	Medical laboratory assistant
NF-κB	Nuclear factor-kappa B
oxLDL	Oxidized low density lipoprotein
PKP2	Plakophilin-2
PTCA	Percutaneous transluminal coronary angioplasty
RV	Right ventricle
RYR2	Ryanodine receptor
St.p.	Status post
TAG	Triacylglycerol
TFC	Task Force Criteria
TNF-α	Tumour necrosis factor alpha
TPR	Total peripheral resistance
VLDL	Very low density lipoprotein
VT	Ventricular tachycardia
WAT	White adipose tissue
WCRF	World Cancer Research Fund
WHO	World Health Organization

Study groups:

Con	Control group
CV	Cardiovascular group
Met	Metabolic group
TU	Tumor group

Table of figures

List of tables

1 Introduction

No. 1 cause of death in Austria and also globally is represented by cardiovascular diseases (CVDs), straightly followed by malignant growth. (1) Demonstrating this ranking in latest official figures from the year 2011, Statistics Austria has registered 76,479 deceased persons, comprised of 36,539 male and 39,940 female decedents. 32,374 persons in Austria died in consequence of CVDs, corresponding to 42.3% of all death cases; in the second place 19,992 deaths were attributed to malignant growth, corresponding to 23.6% of all death cases. (2) Exactly these two leading causes of death represent the focus of the study:

The intention was on the one hand to investigate most common macro- and microscopic pathologies concerning the heart in correlation with cardiovascular and tumor diseases. On the other hand additional scanning on ARVD/C-characteristic morphological changes was performed; arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), a subgroup of CVDs and an apparently uncommon heart muscle disease, affects particularly young athletic men, on whom the primal manifestation at worst occurs in the form of sudden cardiac death. Other just as seriously consequences could be severe disorders of cardiac conduction system. Its actual prevalence is still unidentified and was estimated to vary from 1:1,000 to 1:2,500. (3, 4) Another aspect of the study therefore was the detection of early stages of ARVD/C respectively a potential occurrence of its characteristic morphological changes in our study population.

On 11,838 decedents a postmortem examination was carried out in Austria in the year 2011, thereof 9,397 pertain to the category of clinical-pathological autopsies. (5) The latter served as methodological fundament of our study according to sample collection; over a period of 18 months clinical-pathological autopsies have been attended at the Institute of Pathology (Medical University of Graz, Austria). In this framework 41 decedents could be conducted to the study. Tissue samples were taken from human hearts while autopsies as well as an anonymised documentation of medical history, body composition features and heart-specific anatomical parameters was consecutively created, utilizing an independently created autopsy protocol. Based on criteria formulated in this protocol, the study

population could be divided into 4 study groups: tumor, cardiovascular, metabolic and control group.
Obtained tissue samples were preserved in 4% buffered formalin, paraffin-embedded, cut with a microtome, stained with hematoxylin-eosin dye and were fixated at the end. After the period of sample-taking about 160 histological slices were reviewed by light microscope assessment in a blind fashion. The database created so far was finally extended by computer-assisted assessment of the histological slices, which enabled a more precise morphometric analysis of epicardium and myocardium. Based on all collected data brought together statistical analysis was performed; the results were compared between the study groups, distributional patterns were displayed in graphic and tabular forms and established hypotheses were verified.

The aim of this first chapter is to illustrate the current state of research in relation to CVDs and cancer anorexia – cachexia syndrome.
As the metabolic syndrome (MetS) was seen as the crucial component of CVDs and especially coronary artery disease in this work, its main risk factors chronic arterial hypertension, diabetes mellitus and overweight/obesity were elaborated in greater detail. Furthermore the issue of epicardial adipose tissue (EAT), a special reflector of visceral obesity and scientifically proven risk factor for CVDs and MetS, was taken up. A separate subchapter of CVDs was also dedicated to arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C).
The last section of this introduction chapter describes the phenomenon of cancer anorexia – cachexia syndrome. This term stands for a severe state of malnutrition in cancer patients, which can deteriorate long-term prognosis dramatically.

Summarizing the purpose of the study was to discover new knowledge of macroscopic, microscopic, morphometric and in addition ARVD/C- typical cardiac structure alterations in tumor and cardiovascular diseases.

1.1 Cardio-vascular diseases (CVDs)

Definition pursuant to the World Health Organization (WHO):

„*Cardiovascular disease is caused by disorders of the heart and blood vessels, and includes coronary heart disease (heart attacks), cerebrovascular disease (stroke), raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. The major causes of cardiovascular disease are tobacco use, physical inactivity, an unhealthy diet and harmful use of alcohol."* (6)

CVDs still are and also in the future will remain the *number one cause of death* all over the world: In the year 2008 an estimated number of 17, 3 million people died in consequence of this disease based on a broad range of underlying disorders. WHO anticipates a further rise of this number to 23, 3 million by the year 2030. (1) These figures demonstrate impressively the importance of research in this field.

1.1.1 Metabolic syndrome

Currently there are several discordant definitions of the metabolic syndrome (MetS), proposed by major scientific associations like International Diabetes Federation (IDF) or Adult Treatment Panel (ATP) III. The points in common comprise *abdominal obesity*, *dyslipidaemia*, *hypertension* and *hyperglycemia*. (7)
Occurrence can be single or combined, whereby each component of itself represents an independent risk factor for the development and increased severity of CVDs, especially coronary artery disease (CAD). One of the hallmarks of MetS is *oxidative stress*, which is highly increased in this setting. It plays a central role in atherosclerosis, that's why it is not surprising that patients with MetS often suffer from advanced atherosclerosis. (8)
An extensive meta-analysis performed by Galassi et al. showed a 61% increased risk for CVDs in patients with metabolic syndrome, as well as an increased mortality from all causes, coronary heart disease (CAD) and stroke. Comparing genders women had a higher relative risk of CVD. (9)

As you will see on the pages that follow, risk factors of MetS often occur concomitant, which enhances the topic's complexity many times over. It can be therefore, that some issues may overlap subchapter-spanning.

1.1.1.1 Chronic arterial hypertension (CAH)

One main independent risk factor for CVDs is represented by CAH. It is characterised by *chronic systolic blood pressure* ≥ *140 mmHg*. The classification of hypertension is presented in *Table 1*.

Blood pressure	Systolic blood		Diastolic blood
	(mmHg)		(mmHg)
Normal	< 120	and	< 80
Pre-hypertension	120 – 139	or	80 - 89
Stage 1	140 – 159	or	90 – 99
Stage 2	≥ 160	or	≥ 100

Table 1: Classification of hypertension (10)

CAH is a multifactorial disease, depending on various aetiologies like systolic blood pressure, age, sex, ethnic origin, body fat mass and its distribution, RAAS-system (renin-angiotensin and adrenergic system), etc.

Hypertension is frequently observed in obese and overweight subjects. Normotensive obese individuals were shown to be at a greater risk for hypertension than lean subjects, vice versa hypertensive patients seem to predispose obesity. Although there is an obvious connection between hypertension and obesity, underlying hemodynamic mechanisms are as different as cardiac adaption per se: *Essential hypertension* is accompanied by increased total peripheral resistance (TPR) and consequently *high afterload*, which the heart has to overcome. The physiological response of compensation consists of *concentric* left ventricular hypertrophy (LVH). Indeed it occurs very early in CAH; about 50% of juveniles with only borderline-pressure-levels were verified to already have hypertrophic left ventricles. In comparison, subjects with increased body mass show elevated left ventricular (LV) filling pressure and expanded intravascular volume to meet the metabolic requirements. TPR and renovascular resistance are lower. Another adaption of the heart in *obese subjects* is an *eccentric* LVH and chamber dilatation due to *increased preload*, both developing

independently from hypertension. Commonly appearing *combination* of CAH and obesity is thus associated with *elevated pre- and afterload* and all their impacts on the heart just mentioned above. (11)
Long term complications are heart failure and cardiac death as the final instance. That is why the crucial role in CAH is played by LVH, which implies increased 2 to 4-fold independent risk of cardiovascular death or nonfatal complications like impaired coronary flow reserve, increased incidence of CAD, increased electrical instability with high grade ventricular arrhythmias and reduced pumping capability. (13)

1.1.1.2 Coronary artery disease (CAD)

Coronary artery disease - also known as coronary heart disease (CHD), ischemic heart disease, etc. - emerges on the pathophysiological process of *atherosclerosis*. Formation and over a period of years taking accumulation of plaques in the tunica intima of coronary vessel's wall can lead to limited distal blood flow and hence lack of oxygen supply to myocardial cells, bearing a risk for ischemia, infarction and tissue destruction. The pathological correlate can be seen in macroscopic assessment as atherosclerotic plaque deposits inside of coronary arteries; they reduce the cross-section of the lumen, named "stenosis".
Patients with MetS have increased disposition for CAD development and severity, linked to worsened long-term prognosis. Moreover, therapeutic revascularisation measures have shown a higher procedural risk as well as poorer post procedural long-term outcomes. (8) To mention examples, the inflammatory response after percutaneous transluminal coronary angioplasty (PTCA) was higher than in healthy or solely diabetic patients, as well as thromboses of endovascular stents was 6 times higher in patients with hyperglycaemia combined with insulin resistance. (13, 14) The aforementioned constellation is also likely to double the probability to die of CAD in comparison to subjects with just CAD; full picture of MetS including all components enhances the likeliness even up to ~ 4 fold. Another impact of MetS on CAD is severely impaired coronary collateral growth, which is a physiological compensatory mechanism of the heart due to chronic hypoxia. The precise attribution of the components and their combination variants

to risk profiles is unresolved yet. Abdominal obesity paired with low HDL and elevated oxLDL are discussed to be the greatest risk factors for male, hyperglycaemia the one for female subjects. (8)

1.1.1.3 Diabetes mellitus (DM)

Diabetes mellitus is a metabolic disease characterised by *chronic hyperglycaemia*. According to International Diabetes Federation (IDF) it affected an estimated number of 371 million people worldwide in the year 2012, about 4,8 million people thereof died in consequence of it. (15)

Both type I and II DM are associated with a higher risk of CVD, especially CAD.

Various studies have proven that there is both an increase in prevalence of coronary plaques and a more frequent plaques vulnerability with higher risk for rupture and predisposition for thrombus formation (=acute coronary event) in patients with DM or even prediabetic status in comparison with non-diabetics. (16, 17) Interestingly there is a *sexual dimorphism* to the *disadvantage of female diabetic patients*; a meta-analysis performed by Lee et al. concluded that the relative risk of coronary death due to DM was 2.58 for women and 1.85 for men. (18) Another recently published population-based retrospective cohort study, performed by M. Roche et al., substantiates this unequal risk distribution: over a period of 10 years a total number of 73,783 patients (diabetic males: n = 7,751; diabetic females: n = 7,401) have been under examination. Besides the already confessed higher risk for mortality and morbidity related to all-causes - shorter survival times and more frequent hospitalisation of diabetic patients in contrast to non-diabetics - they also found a significant distinction within the diabetic subjects: Female patients were not only at a higher risk for CVD but also exposed to higher impacts and more severe courses than their male counterparts, especially when DM was diagnosed at a later stage. (19)

Diabetic patients have twofold risk for myocardial infarction and stroke, additionally long-term outcomes after an acute coronary event are also worse.

It is also often accompanied by hypertension, which creates the further risk of renal disease, retinopathy and CVD. The recommended goal blood pressure in patients with DM is 130/80 mmHg. (20)

1.1.1.4 Overweight/Obesity

Overweight and obesity are considered as cause of certain diseases like diabetes mellitus type II, hypertension, metabolic disorder, fatty liver, coronary heart disease, etc.

The impacts on the heart are *left ventricular hypertrophy* (vide *chapter 1.1.1.1*, "Chronic arterial hypertension", *page 4*) as well as possible right ventricular hypertrophy and left atria enlargement, triggered by concomitant hypertension (affects ~ 40% of obese people) and insulin resistance. (21)

Furthermore obesity is associated with increased risk of several cancer types, shown by a systematic review of the World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR). (22)

Most common method for determination of overweight/obesity is the body mass index (BMI). It is an indirect measure of body fat mass describing the relation between body weight and height. Its big disadvantage is not considering muscular individuals like athletes, who may be ranged in overweight class without being excessively fat. (23) BMI is calculated as weight (kg)/ height squared (m^2).

According to the international classification generated by WHO, overweight in adult people is defined as a body mass index of ≥ 25 kg/m^2, obesity starting at values of ≥ 30 kg/m^2. (24) Normal distribution of body fat ranges between 10-20 % in male and 20 -30 % in female, but it can also reach only a few percent in athletes or even over half of the body weight in morbidly obese subjects. (25)

It's very likely that not the total amount of body fat but its distribution, especially the one of *visceral adipose tissue*, is an important predictor for CVD and metabolic syndrome. (21)

Hence the recommendation is to evaluate waist circumference or waist-to-hip-ratio instead of BMI, substantiated by evidence of Yusuf. et alumni's study, which demonstrated a highly significant association with myocardial infarction risk and waist-to-hip-ratio. (26)

Further methods to constitute body fat distribution are more precise and elaborate techniques like bioelectrical impedance analysis (BIA), isotope dilution, ultrasonography, magnetic resonance spectroscopy, etc. (27)

For this study selected skinfold measurement, a reasonable and easy-to-use technique, served for estimation of percentage body fat, basing on good reliability and validity. (28)

1.1.2 Epicardial adipose tissue (EAT)

In principle total body fat distribution can be divided in subcutaneous (~ 80%) and internal adipose tissue (~ 20%). Counting to the latter epicardial fat tissue is a *form of visceral fat*, which in turn is one of the contributors to central (abdominal) obesity. (25) When referring to EAT the cardiac fat tissue layer between myocardium and visceral pericardium is meant. It is surrounding coronary arteries, another predilection sites are free wall of right ventricle (RV), the apex of left ventricle (LV) and atria areas. EAT has *several functions* in the human heart; it is able to produce and secret local cytokines as well as pro- and anti-inflammatory adipokines, which are specific fat tissue hormones. One the one hand they may have atherogenic potential, on the other hand they operate in a cardioprotective way, especially anti-atherogenic adipokines adrenomedullin and adiponectin. (29)
EAT is furthermore an important modulator of *fatty acid homeostasis*: Free fatty acids (FFA) are the main energy substrate for the myocardium. Increased levels can lead to arrhythmia, that's why the heart counteracts by rapid absorption of FFA, whereas in times of high energy demand it can serve as a local resource. (21)
Recent studies say that anti-atherogenic adipokines are downregulated in CAD as well as pro-inflammatory adipokines and cytokines reach higher levels. High EAT-volumes in interaction with imbalance of adipokines are suggested to be a strong determinant for CAD. (30)
In this context a meta-analysis performed by Xu Y. (n = 2,872 patients) also showed a significant increase in EAT thickness as well as volume in patients with CAD compared to non-CAD group. (31) Another recent meta-analysis performed by Pierdomenico S.D. et al. compared EAT thickness in metabolic (n = 1,030) and non-metabolic patients (n = 997) by using echocardiography. They came to the conclusion that EAT thickness significantly reflects visceral adiposity and also correlates with the MetS. (32) These findings were confirmed by a large number of studies.

Increased EAT is also independently associated with markers of insulin resistance, a key component of the MetS, as well as changes in left and right ventricle mass and diastolic function. Hypertrophy of the LV was shown to be related to a proportionate increase in EAT, not depending on age and obesity. (33)
In hypertensive patients these results were confirmed by equal outcome, but also complemented by independence from BMI, waist circumference, weight, systolic and diastolic blood pressure. (34)
Another elevation in EAT mass volume can be found in DM II- patients, where it seems to participate in the pathogenesis of diabetic CAD and therefore contribute to its aggravated risk and severity. (35)
Iacobellis et. al. evaluated the effect of weight loss on EAT in severely obese subjects (n = 20; BMI = 45 ± 5 kg/m^2), who underwent a 6-month very low calorie diet weight loss program. The result was a significant reduction of EAT (- 32% of baseline EAT, from 12.3 ± 1.8 to 8.3 ± 1 mm) due to significant weight loss (on an average -20% of original body weight). Interestingly the effect of fat depletion was higher and quicker on EAT than on BMI and waist circumference, meaning it can be proportionally higher than overall adiposity decrease. (36)
However, no agreement has been made on correlation between EAD and age or sexual dimorphism yet.

1.1.3 ARVD/C – Arrhythmogenic Right Ventricular Dysplasia/ Cardiomyopathy

ARVD/C is a heart disease characterised by *progressive structural alterations of myocardium* counting among hereditary arrhythmia syndromes. The latter were found within a study in more than 50% of all initially unexplained cases of sudden cardiac death in young people, 30% thereof were ascribed to ARVD. (37) However, the disease's actual prevalence is still not identified, estimations range from 1:1,000 to 1:2,500. (4, 5) It is likely that it exists for a long time; the first clinical more precise case report was described in the year 1982. (38)
Although ARVD/C obviously doesn't occur very often its consequences could be severe disorders of cardiac conduction system, at worst sudden cardiac death manifests as first clinical symptom of ARDV/C, most often in young athletic men.

The mode of inheritance is usually *autosomal dominant* with variable penetrance, although recessive forms were also detected in association with cutaneous phenotypes like “Naxos disease”. This syndrome is accompanied by woolly hair at the time of birth, formation of palmoplantar keratoderma in the first year of life and ARVD/C-characteristic electrocardiographic abnormalities and symptoms starting during adolescence. (39)

In about 40% of patients affected by ARVD/C a mutation can be found in one of the *genes for desmosomal proteins*. (37)

In the year 2008 Hugh Calkins described 11 different genetic variants of ARVD, which have mutations in plakoglobin (JUP), desmoplakin (DSP), plakophilin-2 (PKP2), desmoglein 2 (DSG2), cardiac ryanodine receptor (RYR2) and desmocollin-2 (DSC-2). (40)

Desmosomes are important cell junctions providing tissue integrity, particularly found in skin and heart, where they usually have to fulfil the requirements of high mechanical stress. Thus it doesn’t surprise that disease onset and/or exacerbation occurs prevalently within athletic subjects. (41)

As consequence altered desmosomal function might lead to lost of cell-cell-contacts between adjacent cardiomyocytes. Destructed tissue integrity in turn might induce decreasing contractility of the myocardium and hence an impaired response to mechanical stretch, especially relating areas under high strain such as right ventricular outflow tract, apex and sub-tricuspid areas, also known as the “triangle of dysplasia”. (39)

Another effect might be *fibro-fatty cardiomyocyte replacement* preceded by mutation-related desmosomal changes, which might contribute to electrical uncoupling, although the underlying relationship is still very much unknown.

The resulting activation delay and conduction block entails re-entrant mechanisms and, accordingly, ventricular tachycardia (VT). It is the routine 12-lead ECG which is one of the most important as well as facilely accessible diagnostic tool in all stages of the disease. (42)

In the year 1994, an International Task Force drafted criteria (TFC) for the clinical diagnosis of ARVD/C. (43)

They were based on global or regional dysfunction and structural alterations, tissue characterisation of the wall, re- and depolarisation abnormalities, arrhythmias and family history. Since the TFC were found to be highly specific but

sensitivity was worthy of improvement – in particular for early and familial diseases – a modification was carried out in the year 2010, maintaining subdivision into major and minor categories of above mentioned features. (44)
Initially it was considered a right ventricle disease; recent studies indicated that there can also be similar histopathologic changes in the left ventricle or moreover in both ventricles. (45)
In addition the interventricular septum was also shown to be involved at molecular level, whereas imaging studies and microscopic evaluation could not confirm that finding. In any event, the discovery of further heart areas involvements lead to the approach of the new broader term “AC – Arrhythmogenic Cardiomyopathy”. (42)
The change of nomenclature is supported by great scientific research advances with regard to comprehension of ARVD/C respectively AC in the last 20 years, disproving the original concept of ARVD/C as a congenital heart defect of the RV myocardium (“Uhl’s anomaly”,1952). The disease is nowadays characterised by progressive loss of cardiomyocytes by apoptosis or necrosis and its replacement by fibrofatty tissue, beginning at epicardium and spreading towards the endocardium. *Different clinico-pathological patterns* have been described: a “silent” early subclinical phase with structural changes in the RV and sudden cardiac death as first manifestation; an “overt electrical” phase with diffuse RV involvement and symptomatic for VTs; progressive loss of myocardium with severe RV dilatation and impaired systolic function; “endstage” biventricular progressive heart failure also affecting septum, mimicking dilated CMP and in some cases requiring heart transplantation as a last resort. In early stages of the disease significant structural changes may be absent, whereas arrhythmias already may appear. (46)

In gross macroscopic examination right ventricle shows augmented transmural fat deposition, in microscopic assessment myocardium can be found replaced by interstitial fibrous and fatty tissue, often accompanied by inflammatory infiltrates. (47) A certain amount of intramyocardial fat in the right ventricle is considered as common in the normal heart, it usually increases with body weight and age. (48, 49) That’s why single fatty replacement does not count for ARVD/C-diagnostic investigation.

An important distinction has to be made between myocardial replacement and general degeneration of the heart by fatty tissue, called lipomatosis cordis (LC).
It is probably some kind of fatty metaplasia of the myocardial connective tissue, mainly found in the right ventricle. Predisposing aetiologies are obesity and differently caused damages of myocardium, for example chronic alcoholism. In LC there is an accentuated subepicardial infiltration of lipoma-like fatty strands, which penetrate the myocardium like prongs of a comb. A crucial difference to ARVD/C is the lack of dystrophic heart muscle fibres in the remaining original tissue. (50)

If the disease is suspected, the option of a *genetic test* already exists; for example it is recently offered by the clinic for cardiology at the University Hospital in Zurich, Switzerland. This "USZ-ARVC"-genetic test, which is currently in the process of gaining accreditation, includes the analysis of DSC-2, DSG2, DSP, JUP and PKP2. These genes seem to account for 90 % of all mutations within ARVD/C.
One little drop of bitterness is the test sensitivity of 55 % and a false-negative rate of 0.5 – 2%. (51)

The recommendation for persons affected by ARVD/C are implantation of a defibrillator or catheter ablation for VT suppression and shock reduction, beta-blockers as drug therapy and last but not least avoidance of competitive sports. (52)

1.2 Cancer Anorexia – Cachexia Syndrome (CACS)

Cancer diseases and its therapeutic measures like chemotherapy, surgery or radiotherapy can lead to a severe state of malnutrition called the "*Cancer Anorexia Cachexia Syndrome*". It has significant impair on quality of life as well as response to cancer therapy and mortality. Unfortunately it can't be circumvented by adaption of nourishment or food supplements, what complicates the therapy of CACS and makes it very difficult by now.
About 50 % of cancer patients are affected from *cachexia*; it is held responsible for up to 20% of all cancer deaths. (53, 54)

While often, but not necessarily, co-existing *anorexia* is defined as *loss of appetite*, the hallmark of *cancer cachexia* ("wasting syndrome") is an *excessive loss of lean body mass* (LBM) – in contrast to starvation and/or anorexia, where LBM is preserved and primarily adipose tissue fat stores are depleted.(55, 56)
Indeed, in cachexia a more or less equal loss of adipose tissue and skeletal muscle mass can be found, visceral protein is preserved or even increased. (52)
The *frequency of weight loss* and therefore cachexia depends on *tumor site* and *stage*. Upper gastrointestinal or pancreatic cancer patients suffer from weight loss in about 80%, whereas for example breast cancer patients are affected in 10 – 35%. *Anorexia* has been noticed in 13 – 55% in patients with cancer at the time of diagnosis, in terminally stages it is seen in approximately 65% of cases. (57)
The occurring weight loss is correlated to the prognosis, which means that the mean survival time as well as life quality and also mobility drop with increasing loss of weight. In particular physical impairment followed by muscle atrophy and loss of function creates the high risk that weakened patients get confined to bed and develop *hypostatic pneumonia*. 48% of death cases in cancer patients can be traced back to the subsequent *respiratory death*. (52)

1.2.1 Definition and classification of cancer cachexia

An international panel of experts in clinical cachexia research agreed on the latest definition and classification of cancer cachexia: According to that consensus it is "*a multifactorial syndrome characterised by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment."*
Three relevant stages were defined: *Precachexia, Cachexia* and *Refractory cachexia*. Diagnosis requires weight loss < 5% (*Precachexia*); > 5% loss of stable body weight over the past 6 months or a BMI< 20 at any degree or skeletal muscle depletion (sarcopenia) in combination with weight loss > 2% (*Cachexia*);
Refractory cachexia reflects very advanced stages or very aggressive cancer, not responding to anticancer therapy. Life expectancy in this 3rd stage is below three months. (58)

1.2.2 Pathophysiology/ Pathogenesis of CACS

The complex pathogenesis of CACS isn't completely enlightened yet.
Underlying mechanisms are on the one side increased production of pro-inflammatory *cytokines*, on the other side a decrease of anti-inflammatory ones. (55) Mediating pro-inflammatory cytokines in cachexia, which have been shown to be produced by human tumor cells, are interleukin-1 beta (IL-1β), interleukin-6 (IL-6), leukemia inhibitory factor (LIF) and tumor necrosis factor alpha (TNF-α). In *cardiac cachexia* patient's increase of IL-6 and TNF-α were determined as the strongest predictors for inadequate reduction of weight. They seem to contribute to cachexia by *acting in* the *periphery* as well as in the *brain*: IL-1β and TNF-α lead to increased activation of muscular nuclear factor-kappa B (NF-κB), which prevents muscle repair and promotes its degeneration. In addition protein turnover is shifting towards catabolism. Peripheral inflammation in turn stimulates the brain to respond with a central inflammation for its part, indeed the hypothalamus seems to be the critical site of action. Neuronal tissue within the hypothalamus reacts by inducing local production and release of cytokines, originating from ingestive behavior regulating nuclei. (59)
These cytokines seem to cause an inappropriate reaction of the hypothalamus to peripheral blood-borne signals about decreasing adiposity and energy status, namely a *hyperactivation of anorexigenic neurons*. Tumor growth also entails increased energy expenditure, possibly emerging due to an increased thermogenesis in skeletal muscle; tumor's own production of *proteolysis*-inducing (skeletal muscles are degrade to amino acids) and *lipid-mobilizing*-factor (adipose tissue is degraded to free fatty acids) is another contributing factor in a complex interaction. (54)

A recently published review, authored by Suman K. Das and Gerald Hoefler (Institute of Pathology, Medical University of Graz), underlined the importance of *triglyceride lipases* as a *major metabolic pathway* in cancer associated cachexia (CAC): The major component of natural fat is triacylglycerol (TAG), which can be found in the main fat storage in humans – the white adipose tissue (WAT). TAG is stored in vacuoles of adipocytes, comprised of esterified glycerin and fatty acids (FA). TAG-transporting circulating lipoproteins (VLDL, chylomicrons) are able to provide these substrates for intracellular lipogenesis: therefore endothelial cell

surfaced *lipoprotein lipase* (LPL) divides the lipoprotein complexes via TAG-hydrolysis. Further enzymes for lipolysis and now *intracellular* TAG-degradation are *hormone sensitive lipase* (HSL) as well as *adipose triglyceride lipase* (ATGL), 90% of TAG-hydrolysis can be ascribed to them.
In cancer cachexia TAG depletion, underlying the mechanisms of *decreased lipogenesis* and in particular *increased lipolysis* was found to be the reason for loss of adipose tissue. In this context LPL of WAT was significantly decreased in several studies, a possible explanation might be its impairment by increased levels of TNF-α and LIF. In consequence or to the confirmation of downregulated activity *increased serum levels* of FA, glycerol, TAG, VLDL and low-density lipoproteins were found in animal models of CAC as well as tumor patients.
Equally HSL and ATGL showed increased activities in cachectic cancer patients.
Reverting to TNF-α and IL-6, their roles in CAC must be complemented by also inducing increased lipolysis, but it is not entirely clear by which mechanisms.
TNF-α additionally inhibits LPL activity and therefore impairs lipogenesis und FA uptake. The high levels of FA mobilized as a result of deregulated lipid metabolism lead to an excess offer to other organs - especially to skeletal muscle, which is one of the main sites of lipid uptake. Lipotoxicity, including insulin insensitivity, dysfunction of mitochondria and apoptosis in myocytes can be the consequence, already seen in CAC patients. Interestingly skeletal muscle is affected later than WAT, what leaves the question about interrelation open for further studies. (60)

1.2.3 Cardiac alterations

The impact of cachexia on the heart is not well understood yet, whereas the mechanisms of skeletal muscle atrophy have been well explored.
A mouse model study was performed by Min Tian et al. to elucidate this current lack of knowledge. Therefore five-week-old male CD2F1 mice were inoculated with colon-26 adenocarcinoma cells to induce cancer cachexia. After 14 days echocardiography showed a significantly reduced heart rate as well as fractional shortening in tumor-mice in comparison to control mice. Following light and transmission electron microscopy assessment at day 17 revealed increased interstitial cardiac fibrosis, disrupted alignments of sarcomeric structure and randomly dispersed mitochondria with altered shapes and sizes. At molecular level

alterations of gene expression for contractile proteins like troponin I (decreased by 38%) and myosin heavy chain (MHC; “adult” isoform MHCα decreased by 33%, “fetal” isoform MHCβ increased by 93-fold) emerged. Summing up contractile function was impaired, but not dependent on reduced food intake. Structural changes may be the explanatory underlying mechanism. As the evaluated mice were terminally ill it was suggested that *heart failure* is a *slow process in cancer cachexia*. (61)

Besides in an ensuing study Min Tian et al. identified an according *decreased cardiac wall thickness* in mice with cancer cachexia. (62)

LV atrophy also appeared in context to caloric restriction in C57Bl6 mice. The control group showed a significantly higher weight and volume of the LV than in caloric restricted mice at 7 days and a hint to stronger body weight loss than in LV weight. (63)

2 Methods

2.1 Study population – Study groups

Our study population was composed of 41 subjects - 16 female and 25 male - with a mean age of 67.8 years, ranging from youngest person aged 27.9 to oldest person aged 93.5 years. Due to the fact that ARVD/C epidemiologically accumulates in adolescent persons the minimum age for study was determinated to be 10 years, there was no limit for maximum age. Apart from that we basically had no inclusion and exclusion criteria.

While analysis of clinical data the collected patients' data were separated to four study groups: *cardiovascular*, *metabolic*, *tumor* and *control* group.
The *supreme criterion* for group classification was an underlying of tumor disease, whether it was consecutive cause of death, a secondary disease or has already formed metastases. *Table 13: Tumor group; distribution of sex, nutritional status, organ involvement of cancer, metastases and underlying cause of death* (vide appendix, *page 60)* shows an overview of this study group.
Secondarily we selected individuals with a documented clinical history of Diabetes Mellitus Type I or II. An additional inclusion criterion for this *metabolic group* was atherosclerosis or hypertension in medical history. However, the ones without Diabetes did not decease due to a sudden cardiac event, but to pneumonias/ARDS.
Tertiary our *cardiovascular group* included individuals, who had been diagnosed with coronary heart disease, cardiac valve diseases and/or aortic aneurysm. Furthermore we accepted anybody with cardiac arrhythmia and status post surgical intervention on the heart like CABG or cardiac valve replacement. Death occurred in these cases as a result of cardiac failure.
The remaining individuals, who did not met the abovementioned criteria, represented our *control collective*.

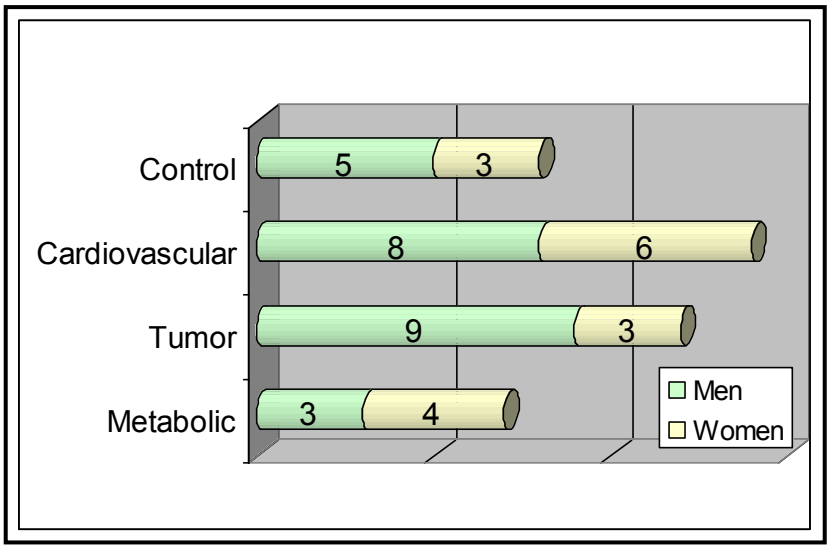

Figure 1: Distribution of male and female individuals across study groups

Figure 1 shows the distribution of male and female patients across the study groups.

2.2 Ethics

The study was approved by local ethics commission of the Medical University of Graz in April 2012. One year later the vote was extended for another year after we submitted a written presentation of an interim report, describing previous progress of the study. Ethical Committee Approval is represented as *Figure 11* (vide appendix, *page 62 - 63*).

2.3 Macroscopic Assessment - Routine conducted autopsies

Within an 18-months-period 41 patients, who have passed away while hospitalization at the University Hospital of Graz (LKH Graz) and were performed a clinical-pathological autopsy on, could be conducted to this study.
On the one side macroscopic assessment while autopsy was essential for assignment of patients into control groups, on the other side we gained a first impression of pathologies of the heart, which were likely to be present.

Sampling was carried out after heart dissection following Rokitansky's method; full-thickness sections were taken of each patient in a uniform fashion 1cm above and below atrioventricular valves out of cardiac wall of the left and right atrium as well as left and right ventricle.

2.3.1 Autopsy protocol

To assure organized collection and documentation a standardized consecutively numbered protocol (vide appendix, *page 61*) - modeled after the currently used autopsy protocol of the Institute of Pathology at Medical University of Graz - has been independently created and supplemented by the relevant characteristics according to the study and clinical data of each patient. To avoid direct inference of obtained samples or data to the decedents the protocol was drawn up anonymized just as interpretation of the study proceeded consecutively in completely anonymized manner. The data base constructed this way included following points for each patient *(Figure 2):*

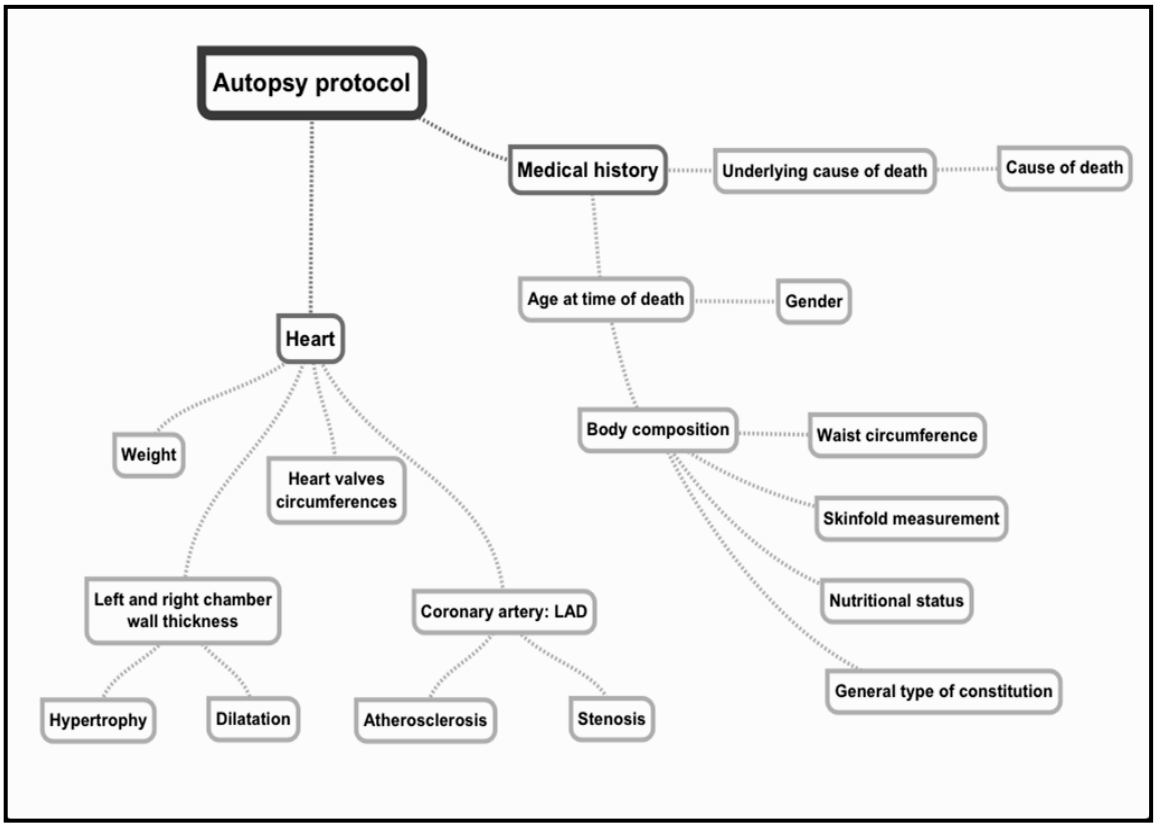

Figure 2: Points of macroscopic assessment based on the autopsy protocol

On the following pages the main points of investigation are described in detail.

- *Skinfold measurement and waist circumference*

We used a usual pressure skinfold caliper to measure 3 skinfolds (cm) of each patient's right body side. The results were used to calculate body fat mass (%) according to 3-site-skinfold test, developed by Jackson and Pollock: (64, 65)

Men: Chest, Abdominal, Thigh

S = sum of 3 skinfolds (cm)

A = Age (years)

$d = 1.1093800 - 0.0008267*S + 0.0000016*S^2 - 0.0002574*A$

Body fat [%] = 495 / d - 450

Women: Tricep, Abdominal, Suprailiac

S = sum of 3 skinfolds (cm)

A = Age (years)

$d = 1.089733 - 0.0009245*S + 0.0000056*S^2 - 0.00012828*A$

Body fat [%] = 495 / d – 450

Reflected in *Table 2* you can see the utilized classification, based on the "Principles and Labs for fitness & wellness", which have been released by Hoeger W. and Hoeger S. According to them normal range of body fat proportion lies between 20 -25 % within female and 15 -20 % within male subjects. (66)

This measurement underlined the metabolically adverse distribution of body fat in some patients. In accordance to the findings of determination of nutritional status and general type of constitution, we were able to identify the subjects belonging to metabolic study group.

	Women		Men	
Age (years)	**Overweight** (%)	**Obesity** (%)	**Overweight** (%)	**Obesity** (%)
≤ 19	27,1 – 32,0	≥ 32,1	22,1 – 27,0	**≥ 27,1**
20 – 29	28,1 – 33,0	≥ 33,1	23,1 – 28,0	**≥ 28,1**
30 – 39	29,1 – 34,0	≥ 34,1	24,1 – 29,0	**≥ 29,1**
40 – 49	30,1 – 35,0	≥ 35,1	25,1 – 30,0	**≥ 30,1**
≥ 50	**31,1 – 36,0**	**≥ 36,1**	**26,1 – 31,0**	**≥ 31,1**

Table 2: "*Body Composition Classification according to percent body fat*" (66)

Waist circumference was measured by standard tapeline, the measuring point was located midway between lower rib margin an iliac crest. Measurement of correct values was not practicable in some cases due to natural decay processes and/or increased abdominal accumulation of fluid, like for example ascites, at the time of post-mortem and as a consequence they were not included into statistical calculations concerned. However, the aim was to indirectly determine visceral adiposity, which is known to be a relevant predictor of CVDs and MetS. The classification scheme used is presented in *Table 3.*

Waist circumference		
Risk for CVD	**Women**	**Men**
Normal	< 80 cm	< 94 cm
Elevated	80 - 88 cm	94 - 102 cm
High	> 88 cm	> 102 cm

Table 3: Classification scheme for waist circumferences

- *Nutritional status and general type of constitution*

These two parameters were assessed subjectively while inspection of the decedent's body, supporting the classification of patients to our study groups.

Figure 3 demonstrates the four possible types of each body constitution and nutritional status.

General type of constitution:		*Nutritional status:*	
Mixed type	☐	Well-nourished	☐
Pyknic	☐	Obese	☐
Leptosome	☐	Mal-nourished	☐
Athletic	☐	Cachexia	☐

Figure 3: Classification scheme for „General type of condition" and "Nutritional status"

- *Underlying causes of death*

These involved any types of previous diseases, which consecutively led to the decease of the person in question. Knowledge of DM, CAH, cancer diseases, metastases and dyslipidaemia was essential for group classification. They were determined by medical history or detected whilst autopsy.

- *Causes of death*

This term describes the immediate organic failures leading to death, which is comprehended as the last consequence of the underlying diseases mentioned above.

Nutritional status, general type of constitution, underlying cause of death as well as actual cause of death was assessed by autopsy performing pathologist.

- *Myocardium: Hypertrophy, dilatation and thickness of chamber walls*

In order to get representative values of the thickness of left and right chamber wall for further investigations, a right angle cut was performed at a distance of 1cm below the atrioventricular valves, after macroscopic value measurement by a usual ruler was performed at the same position.

Dilatation was evaluated subjectively by the experienced autopsy performing pathologist; an indication for a present dilatation was, inter alia, an already macroscopic visible deviation from usual Gothic configured cardiac apex to a rounded Romanesque shape. (67) Standard values of chamber wall thicknesses are 13 – 15 mm for the left and 3 – 5 mm for the right ventricle. (67) In our study we used the following classification scheme (*Table 4*):

	Hypertrophy		Dilatation
Classification	**Left Ventricle**	**Right Ventricle**	**Left + Right Ventricle**
Non-present	13 – 15 mm	3 – 5 mm	0
Low-grade	16 – 17 mm	6 – 7 mm	+
Moderate	18 -19 mm	7 – 8 mm	++
High-grade	> 20 mm	> 9 mm	+++

Table 4: Classification scheme for hypertrophy and dilatation of the left and right ventricle

Hypertrophy as well as dilatation were additionally assessed by light microscope (vide *chapter 2.5.1*, “Microscopic Assessment”, *page 25*).

- *Heart weight and heart valves circumferences*

Enlarged values of *heart valves circumferences* were considered as a reference for valve insufficiency, substantiating the suspicion or diagnosis of cardiac dilatation or hypertrophy. Standard values are shown in *Table 5*. (67)

Valve	Circumference
Tricuspid	12,5 cm
Mitral	10 cm
Aortic	7,5 cm
Pulmonary	8,5 cm

Table 5: Standard values of heart valves circumferences

The range of normal *heart weight* is 250 – 300 g in women and 300 – 350 g in men. Starting from the value of 500 g one can speak of “critical heart weight”, according to an extensive hypertrophy of myocardium. (67) Heart weights were recorded and compared with this classification.

- *Coronary arteries*

We selected the left anterior descendens (LAD; RIVA) as the coronary artery of interest for our study, since it is one of the preferred predilection sites for coronary atherosclerosis. (68)
Macroscopic assessment based on a classification of atherosclerosis and stenosis, as you can see in *Table 6* each subject matter was divided into 4 grades.

Grade	Stenosis:	Sclerosis:
0	none	none
1	25 – 50 %	lipoid
2	50 – 75 %	calcified
3	75 – 100 %	ruptured

Table 6: Classification scheme for stenosis and sclerosis of LAD

2.4 Preservation and processing of tissue samples

The tailored tissue samples (~2 x 1,5 x 0,5 cm) were inserted into tissue embedding-cassettes and fixed in 4% buffered formalin immediately in order to avoid rapid autolysis processes.
After one day wait time they were embedded in paraffin independently, the resulting tissue blocks were passed on to institute’s medical-laboratory assistants (MTAs) to further cut them into wafer-thin layers with a microtome, transfer them to a microscope slide, stain them with hematoxylin-eosin dye and finally fixate them.
The histological tissue embedding-cassettes as well as the microscope slides were labeled with the corresponding areas the tissue samples were taken from as

well as a unique number, which enabled a later attribution to the corresponding patient.

2.5 Analysis of tissue samples

2.5.1 Microscopic Assessment

The database created so far was extended by results from light microscope assessment. About 160 slices - according to 4 areas of the heart - were therefore reviewed in a blind fashion, without prior knowledge of macroscopic findings or medical history.
The points of interest and its classifications are shown in *Table 7*; complementary findings like fluctuations in nuclei of myocytes, inflammatory infiltrates, necroses, calcifications, general fatty degeneration of the heart (lipomatosis cordis) and nature of fat tissue (white and brown fat tissue cells) were also recorded.

Area	Hypertrophy	Dilatation	Myocardial Replacement	Grade of Replacement
A = right atrium	0 = none	0 = none	0 = no fat	1 = rare, focal
B = right ventricle	1 = mild	1 = mild	1 = fatty	2 = diffuse (>40%)
C = left atrium	2 = moderate	2 = moderate	2 = fibrous	3 = massive; only nests of residual myocytes
D = left ventricle	3 = high-grade	3 = high-grade	3 = fibro-fatty	

Table 7: Points of interest and its classification in microscopic assessment

Detailed descriptions of the microscopic structure of the heart in general and for this study relevant histological assessment criteria can be read in the associated diploma thesis, already mentioned in the “preface” of this work.

2.5.2 Computer-assisted Assessment

The histological slices were examined by using a microscope, which operated with special software ("NIS- Elements D") for their documentation and analysis.
A section of each slice was photographed, displayed as a live image on computer screen and marked four times at each epicardium (EAT) and myocardium. In this way the average thickness of the tissue layers was measured and regarded as representative for the entire area (region A: right atrium; region B: right ventricle; region C: left atrium; region D: left ventricle) the tissue sample was taken from while autopsy. The unit of measurement was non-metric; the measured values solely served the purpose of description of correlations between study groups and study areas. Mean values as well as epicardium-myocardium ratio were calculated in SPSS for further statistical analysis, including an overall result and the comparison among study groups.

2.6 Statistical analysis

All statistics were done with commercially available Software (IBM SPSS Statistics 20, Microsoft Office Excel 2003). Descriptive analysis was performed; differences between mean values of study groups (tumor, cardiovascular, metabolic and control group) were generally calculated by single factor variance analysis (one-way ANOVA). Homogeneity check was done by Levene Test to ensure equality of variances in different study groups. If these prerequisites were not fulfilled, nonparametric Kruskal Wallis Test was used for comparison of mean values of study groups. $P < .05$ was considered statistically significant. Numeric data were presented as mean ± standard error of mean (SEM). Distribution of classification grades across study groups and areas (right and left atria, right and left ventricles) were displayed in form of tables and figures and were also verbally described.
We refrained from analysing age dependency of results, as 35 out of a total of 41 study patients were aged above 50 years.
Skinfold measurement data were compared with other determinant parameters of body composition - waist circumference, general type of constitution and nutritional status. Waist circumference measurement of the decedents was not practicable in

some cases; abdominal swelling due to natural decay processes and/or increased abdominal accumulation of fluid, like for example ascites, at the time of post-mortem inspection inhibited measurement of correct values. Nonetheless in case of doubt waist circumference values were prioritized over subjectively determined nutritional status. To our disappointment skinfold measurement with a calliper seems to be an error-prone method, that is why some measured values were not sufficient for further statistical analysis.
As a consequence 16 patients had to be excluded from statistical analysis, since their data were obviously not in compliance with each other. The remaining data records were analysed for correlations between study groups with the Kruskal-Wallis-test.

2.7 Hypotheses

1. Macroscopic measurement shows different results in patients with cancer in contrast to metabolic, cardiovascular and control group.

2. Microscopic measurement shows different results in patients with cancer in contrast to metabolic, cardiovascular and control group.

3. Morphometric analysis of histological slices shows differences between tumor patients and cardiovascular, metabolic and control patients.

4. ARVD-characteristic alterations of myocardium (fibro-fatty tissue) can also be found in patients with cancer.

3 Results

3.1 Macroscopic Assessment

As macroscopically evaluated “general type of constitution”, “underlying cause of death”, “cause of death” and “waist circumference” mainly served as criteria for study group classification they were not explicitly cited.
An overview of macroscopic findings is given in *Table 14* (vide appendix, *page 64*). For pragmatic reasons study groups will be given in following abbreviated forms, occurring in following tables and figures:

Study group	Abbreviation
Tumor	Tu
Cardiovascular	CV
Metabolic	Met
Control	Con

3.1.1 Distribution of age and gender

The study population was composed of 41 patients - 16 female and 25 male - with a mean age of 67.8 (± 14.8) years across all study groups, ranging from youngest person aged 28 to oldest person aged 93 years. The mean age within tumor group was 68.3 (± 12.2) years, cardiovascular and metabolic group individuals had an average age of approximately 72 years (± 10.9 vs. 15.4), whereas control collective patients showed a mean age of 56 (± 19.7) years. As the majority of study subjects were male patients they analogous predominated the number of group members in each study group – except metabolic group. An outstanding gender imbalance was although only present within tumor group, partitioned into 9 male and only 3 female subjects.

3.1.2 Skinfold measurement, waist circumference and general type of constitution

25 patient's skinfold measurement data showed no differences between the study groups, analysed with the Kruskal-Wallis-test (χ^2_3, n = 25 = 5.75, p = .13). Correlations (Spearman) between skinfold measurement data and heart weight (r = .28, p = .18) as well as left chamber wall thickness (r = .30, p = .14) could not be detected, whereas in view of right chamber wall thickness (r = .39, p = .06) a tendency was proven, but without being significant.

3.1.3 Nutritional status

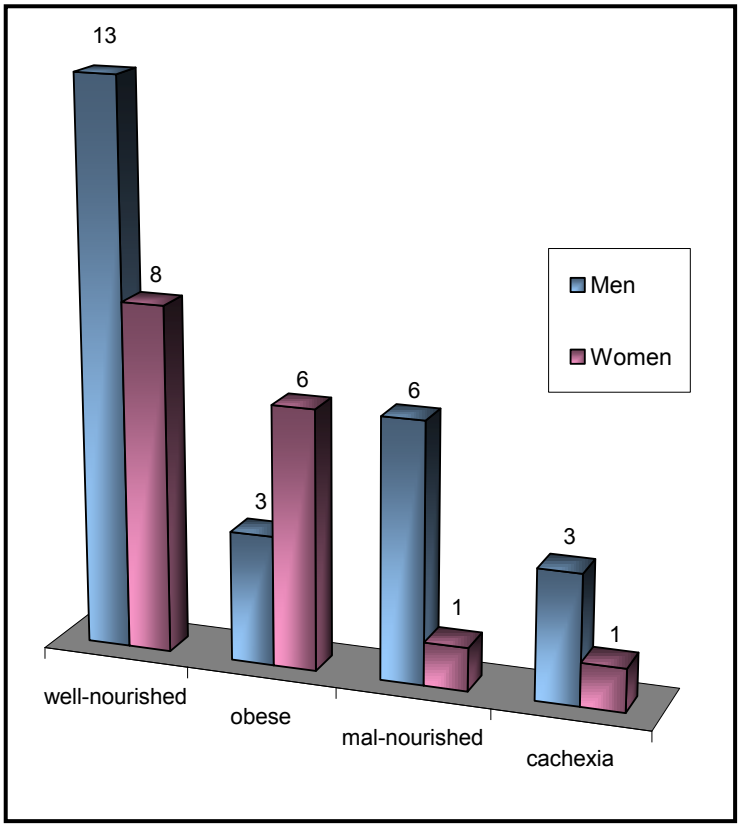

Figure 4: Distribution of nutritional status in men and women

As you can see in *Figure 4* the lion's share of patients (n = 21) was well-nourished at the time of death. Apart from that obesity (n = 9) was found twice as much in female than in male subjects, whereas in mal-nourished (n = 7) and cachectic (n = 4) status male gender prevails.

Interestingly, all female patients of *tumor group* (n = 3) were assigned to well-nourished and obese grade, whereas all male patients (n = 9) of this study group showed a mal-nourished (n = 6) or even cachectic (n = 3) state of nutrition. The residual 2 patients referring to these 2 grades of undernourishment are each female subjects of metabolic group. An emphasis of tumor group is displayed in *Figure 5* below*:*

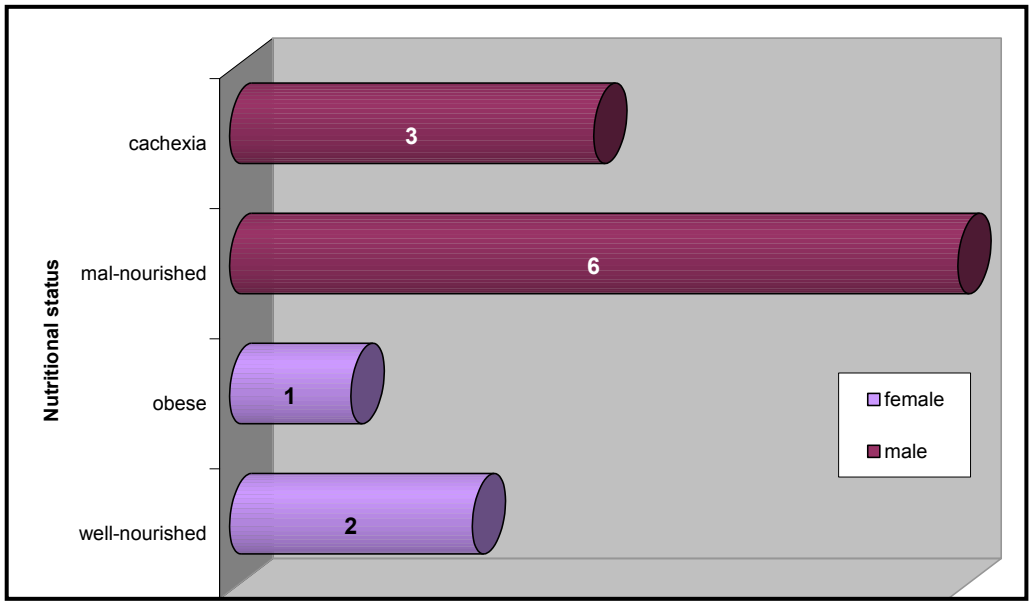

Figure 5: Distribution of nutritional status across tumor group, male:female

All eight patients pertaining to control group (5 male, 3 female) were well-nourished. None of the cardiovascular patients (n = 14) was mal-nourished or cachectic. A tabular presentation of underlying data is given in *Table 8*.

State of nutrition	**Tu**	**CV**	**Met**	**Con**	**Male: Female (∑)**
Well-nourished	0:2	6:3	2:0	5:3	**13:8 (21)**
Overweight/ Obese	0:1	2:3	1:2	-	**3:6 (9)**
Mal-nourished	6:0	-	0:1	-	**6:1 (7)**
Cachectic	3:0	-	0:1	-	**3:1 (4)**
	12	**14**	**7**	**8**	

Table 8: State of nutrition across study groups
(Values are presented as ratio of male:female)

3.1.4 Myocardium: Hypertrophy, dilatation and thickness of chamber walls

Mean left chamber wall thickness was 15.2 mm (± 2.1 mm), ranging from minimum value of 12 to maximum value of 20 mm. *Mean right* chamber wall thickness was 4.5 mm (± 1.0 mm), ranging from 3 to 7 mm.

In statistic calculations of macroscopic assessed values for *left* ($F_{(3, 36)} = 1.80$; $p = .17$; $\eta^2 = .13$) and *right* ($F_{(3, 36)} = .09$; $p = .97$; $\eta^2 = .01$) chamber wall thickness no correlations between the study groups were found.

Table 9 displays the list of classification of hypertrophy of left and right ventricle, also based on *macroscopically* measured values. This is important to emphasise because of additional *microscopically* obtained values for hypertrophy as well as dilatation, which can be found in chapter *3.2 (*"Microscopic Assessment", *page 34*). Nevertheless, the first thing to be noticed in *Table 9* is that there were hardly any patients without a normal thickness of the right ventricle; only five patients out of all (n = 40) had low-graded hypertrophy, they were entirely members of the cardiovascular group.

Grade of Hypertrophy	**(mm)**	**Tu**	**CV**	**Met**	**Con**
Left ventricle					
Atrophy	< 13	1	-	-	1
Normal	13 - 15	10	7	5	2
Low	16 - 17	-	3	1	1
Middle	18 -19	1	3	1	3
High-grade	> 20	-	1	-	-
Right ventricle					
Atrophy	< 3	-	-	-	-
Normal	3 - 5	12	9	7	7
Low	6 - 7	-	5	-	-
Middle	7 - 8	-	-	-	-
High-grade	> 9	-	-	-	-

Table 9: Distribution of grades of left and right ventricular hypertrophy across study groups

Relating to the left ventricle the situation is quite different: two values below normal thickness were found, corresponding to atrophy. The majority of patients (n = 24) again had normal graded ventricle thickness, but there were also sporadic findings of pathological values across all study groups.

3.1.5 Coronary arteries: Atherosclerosis and stenosis of LAD

No sign of atherosclerosis (grade 0) was found only in one patient of the control collective. Lipoid (grade 1; 45% of all patients), calcified (grade 2; 22.5%) and ruptured (grade 3; 30%) plaques were present in all study groups, whereby cardiovascular and metabolic patients had the highest emergence of very severe grade 3 atherosclerosis. Distributional pattern of LAD stenosis showed a manifold picture; no lumen constriction was found in 15% (n = 6) of a total of 40 patients, in the majority (37.5%; n = 15) grade 1 stenosis was found, whereas almost each a quarter of assessed coronary arteries showed grade 2 (n = 10) and grade 3 (n = 9) classification. *Table 10* indicates the underlying data of the results just described.

	Study groups					
	Tu	**CV**	**Met**	**Con**	**∑**	**%**
Stenosis (Lumen constriction)						
0 = none	2	0	1	3	**6**	**15,0**
1 = 25 – 50%	7	5	2	1	**15**	**37,5**
2 = 50 – 75 %	2	4	2	2	**10**	**25,0**
3 = 75 -100%	1	5	2	1	**9**	**22,5**
					40	**100,0**
Atherosclerosis (Quality of plaques)						
0 = none	0	0	0	1	**1**	**2,5**
1 = lipoid	7	5	3	3	**18**	**45,0**
2 = calcified	3	3	1	2	**9**	**22,5**
3 = ruptured	2	6	3	1	**12**	**30,0**
					40	**100,0**

Table 10: Distribution of LAD stenosis and atherosclerosis across study groups

3.1.6 Heart weights and heart valves circumferences

Heart weights were recorded and mean values were compared between the study groups. The values ranged from minimum 180 g (tumor patient) to maximum 770 g (metabolic patient). Mean heart weight was overall 414.6 g (n = 39; ± 122.1 g), 362.5 g (n = 12; ± 109.8 g) in tumor, 416.2 g (n = 13; ± 104.6 g) in cardiovascular, 453.3 g (n = 6; ± 162.2 g) in metabolic and 363.8 g (n = 8; ± 92.0 g) in control group.
Statistical analysis revealed a significant difference in heart weight comparison among study groups ($F_{(3, 35)} = 2.86$; $p = .05$; $\eta 2 = .20$).

Heart weight (g)	**Men** (n =)	**Women** (n =)	**%**
< 250		2	**5**
250 - 300		-	**0**
300 - 500		12	**31**
< 300	2		**5**
300 - 350	5		**13**
350 - 500	12		**31**
> 500	5	1	**15**

Table 11: Deviations from normal heart weight, divided by gender

As you can see in *Table 11*, 62% of patients had increased heart weights (n = 39), evenly distributed between men and women. Two women and two men even showed decreased (< 250 or < 300 g), whereas one female and 5 male subjects showed exceedance of critical heart weight (> 500g). Normal heart weight was only found in 5 male and no female patients.

The mean value of circumferences of pulmonary heart valve was 8.7 cm (n = 40; ± 0.6 cm), statistical comparison of study groups also in view of tricuspid (n = 41; $\bar{x}$ = 12.8 cm; ± 1.1) and mitral (n = 38; $\bar{x}$ = 10.8 cm; ± 1.0) heart valve circumferences did not indicated noteworthy results.

However, analysis of aortic heart valve circumferences (n = 40; $\bar{x}$ = 8.2 cm; ± 0.7) showed a tendency toward differences between study groups ($F_{(3, 36)} = 2.45$; $p = .08$; $\eta^2 = .17$). Mean values amounted 8.4 cm (± 0.8) in tumor (n = 12), 8.4 cm (± 0.7) in cardiovascular (n = 13), 7.9 cm (± 0.5) in metabolic (n = 7) and 7.8 cm (± 0.6) in control group (n = 8).

Considering standard values for heart valve circumferences (vide *Table 5*, *page 23*), just described mean values of pulmonary, tricuspid and mitral valve exceed by only a few millimeters. In this regard aortic heart valve circumferences were obviously extended in cardiovascular and tumor group and only minimal in metabolic and control group.

3.2 Microscopic Assessment

An overview of microscopic assessment findings is given in *Table 15 (*vide appendix*, page 65*).

As degeneration of myocardium as well as fluctuations in nuclei of myocytes were found in each slice, they were not listed separately. The same applies to the distribution of the nature of fat tissue, since all histological slices showed only white fat tissue; there was no finding of brown fat tissue.

3.2.1 Replacement of the myocardium

With regard to *ARVD/C*-characteristic replacement of myocardium by *fibro-fatty* tissue, we found out that not only subjects subordinated to tumor group, but also cardiovascular, metabolic and control group meet the chosen criterion. Indeed, as you can see in *Figure 6*, 60% of all examined histological slices showed fibro-fatty, 38% fibrous and only 2% fatty alterations in myocardial tissue.

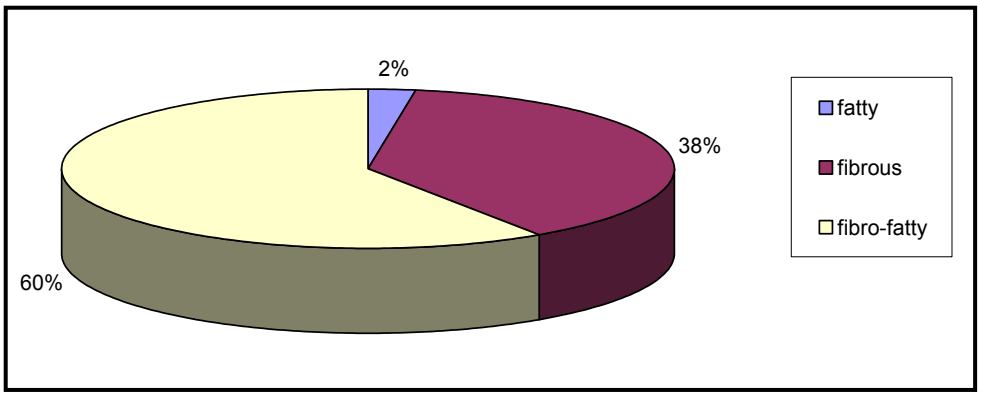

Figure 6: Distribution (%) of fatty, fibrous and fibro-fatty myocardial replacement across all samples

The former 60% were subsequently divided into the 4 areas and 4 study groups. As you can see in *Figure 7* the right ventricle was the most (34%), whereas the atria were equally (each 25%) and left ventricle was the least (16%) affected. The distribution of study groups across the areas turned out to be fairly even.

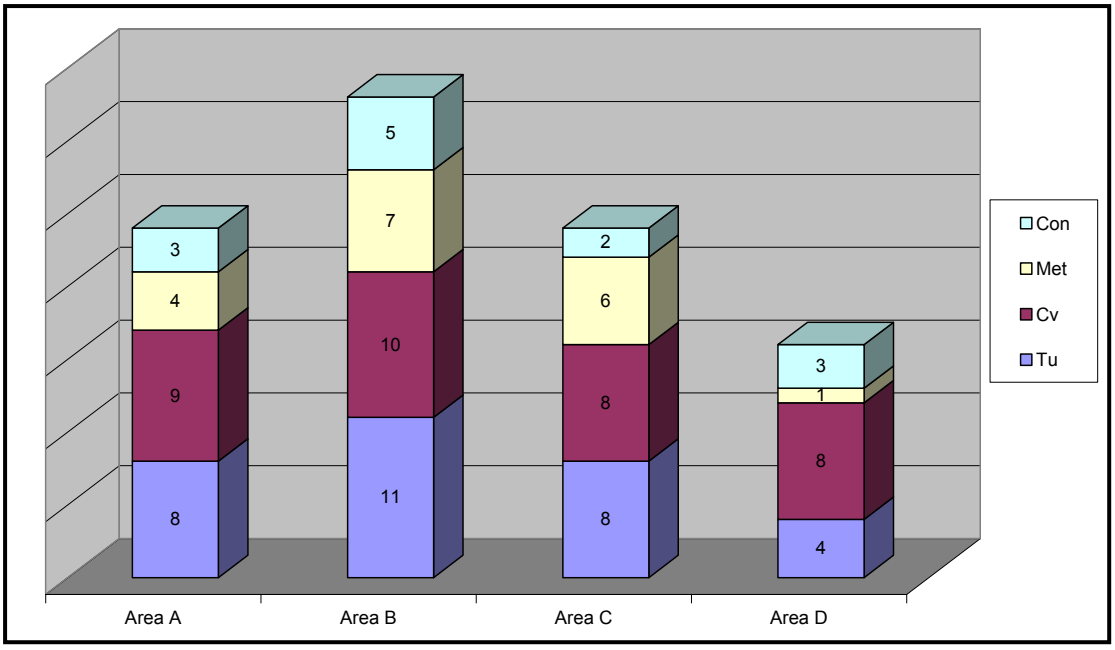

Figure 7: Distribution of fibro-fatty myocardial replacement across areas

(Area A= right atrium; Area B= right ventricle; Area C= left atrium; Area D= left ventricle)

Calculations on the grade of myocardial replacement across all subjects displayed a 49% (79 slices) share of focal changes, diffuse (more than 40% of tissue affected) replacement was found in 39% (64 slices) and massively extended

myocardial involvement with only nests of residual myocytes constituted the minority with only 12% (20 slices), depicted in *Figure 8*. This percentage division principally correspondents with the distribution across areas and study groups, meaning that for example focal changes are predominantly seen in each area A,B,C,D as well as in each study group. The only exceptions are mainly diffuse replacement in area B (right ventricle; 21 out of a total of 41 slices) and also in cardiovascular study group (28 out of a total of 55 slices).

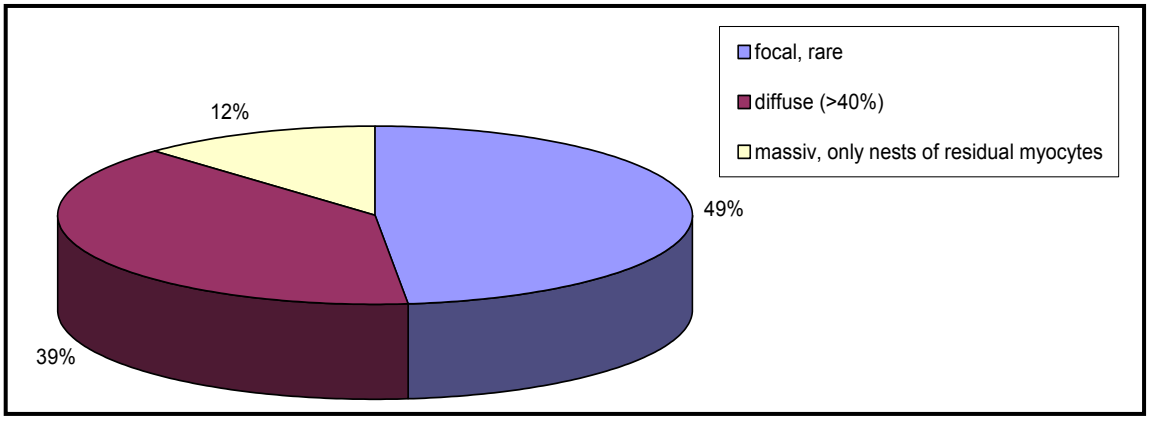

Figure 8: Distribution of grade of myocardial replacement across all samples

3.2.2 Hypertrophy and dilatation

Hypertrophy and dilatation were also compared across areas and study groups; Graphical presentation can be seen in *Figure 9*, corresponding values can be obtained from *Table 12*. In general, high-grade dilatation (n = 12; 7%) as well as high-grade hypertrophy (n = 5; 3%) was found in the minority of all patient's histological slices (n = 163). Over half (n = 85; 52%) of all cases didn't show any and almost each a quarter had mild (n = 38; 23%) or moderate (n = 36; 22%) graded hypertrophy.

Looking at hypertrophy's distribution across areas and study groups, it becomes apparent that only patients with cardiovascular diseases (n = 56 slices) had accumulated occurrence of mild (n = 20) and moderate (n = 22) grades. In metabolic and control group there were no cases of high-grade hypertrophy.

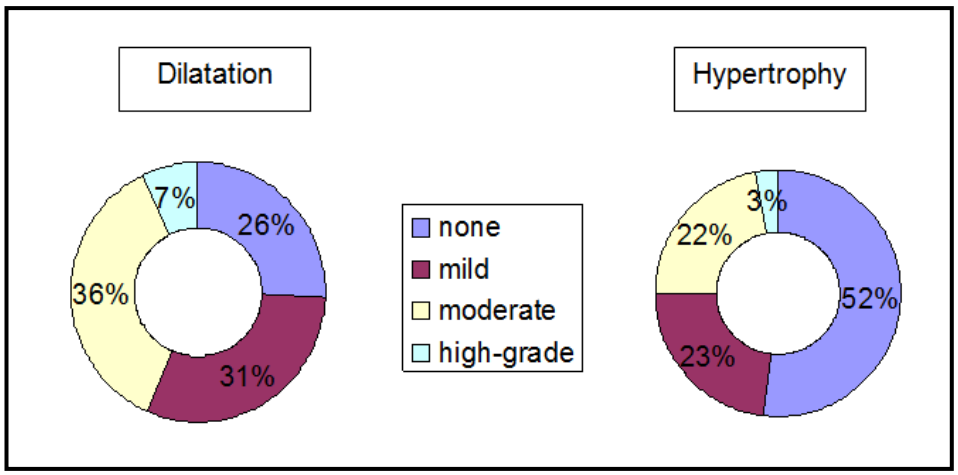

Figure 9: General distribution of grades of dilatation and hypertrophy

With regard to dilatation it is striking that again in cardiovascular study group mild and moderate grades were present predominantly. Half of metabolic patient's slices showed moderate dilatation. Severe dilatation was distributed equally with the number of patients across study groups (each 4), except for control collective, which did not show any case.

			Area					Study Group			
Classification	Dilatation										
	Grades		**A**	**B**	**C**	**D**	**∑**	**Tu**	**CV**	**ME**	**CO**
	none	**0**	10	9	13	10	**42**	18	0	10	14
	mild	**1**	15	12	13	11	**51**	15	24	0	12
	moderate	**2**	13	16	14	16	**59**	11	28	14	6
	severe	**3**	3	4	1	4	**12**	4	4	4	0
	Hypertrophy										
	none	**0**	22	19	24	20	**85**	33	10	18	24
	mild	**1**	11	11	8	8	**38**	8	20	8	2
	moderate	**2**	7	10	8	11	**36**	6	22	2	6
	severe	**3**	1	1	1	2	**5**	1	4	0	0

Table 12: Distribution of hypertrophy and dilatation across areas and study groups

None of the examined areas was particularly affected by the single classification grades of hypertrophy as well as dilatation.

3.3 Computer-assisted Assessment

An overview of computer-assisted findings is given in *Table 16* (vide appendix, *page 66*). The following values correspond to non-metric units, serving the purpose of description of correlations between study areas and study groups.
For an easier understanding beforehand again the heart anatomical correlations of examined areas:

Area A	Right atrium
Area B	Right chamber
Area C	Left atrium
Area D	Left chamber

3.3.1 Epicardium – EAT

Overall mean epicardial thickness was 1351.4 (± 1002.7) in *area A* (n = 34), 1581.2 (± 865.6) in *area B* (n = 35), 1495.0 (± 719.1) in *area C* (n = 37) and 1336.8 (± 844.9) in *area D* (n = 33).
Kruskal Wallis test did not show any correlation (χ^2_3, n = 34 = .56, p = .91) between the study groups in reference to epicardial fat thickness (EAT) in *area A*.
The same results were achieved for *area B* ($F_{(3, 31)}$ = 2.13; p = .12; η2 = .17), *area C* ($F_{(3, 33)}$ = .54; p = .66; η2 = .05) and *area D* ($F_{(3, 29)}$ = .61; p = .61; η2 = .06), analysed by ANOVA.

3.3.2 Myocardium

Mean thickness of myocardium was 2023.3 (± 1072.5) in *area A* (n = 41), 2540.2 (± 981.7) in *area B* (n= 41), 1907.8 (± 892.6) in *area C* (n = 39) and 2977.3

(± 1234.7) in *area D* (n= 40) across all study groups.
Statistical analysis revealed significant differences between the study groups regarding myocardial thickness of *area B* ($F_{(3, 37)}$ = 3.20; p = .03; η2 = .21);
Metabolic group had a mean value of 2020.7 (± 974.2) in contrast to cardiovascular group with 3125.2 (± 771.4) and tumor group with 2225.2 (± 1033.9). The mean thickness of control collective's right ventricle myocardium was 2443.2 (± 879.6). It is therefore concluded, that there are differences between metabolic as well as tumor group and cardiovascular group.
Comparing myocardium of *area A* ($F_{(3, 37)}$ = .92; p = .44; η2 = .07), *area C* (Kruskal Wallis test: χ^2_3, n = 39 = .34, p = .95) and *area D* ($F_{(3, 36)}$ = .50; p = .68; η2 = .04) did not provide statistical significant results.

3.3.3 Ratio Epicardium – Myocardium

Mean ratio of epicardium – myocardium was 0.78 (± 0.51) in *area A* (n = 34), 0.72 (± 0.45) in *area B* (n = 35), 1.0 (± 0.57) in *area C* (n = 37) and 0.47 (± 0.27) in *area D* (n = 33) across the whole study population.
Statistical significant differences between the study groups were already proven in myocardium of *area B*, accordingly identical outcome was found with regard to the epicardium – myocardium ratio in *area B* (Kruskal Wallis Test: χ^2_3, n = 35 = 8.10, p = .04); tumor group offered a higher ratio (n = 11; $\bar{x}$ = 0.98; ± 0.29) in opposition to the mean values of cardiovascular (n = 11; $\bar{x}$ = 0.54; ± 0.25) and control group (n = 8; $\bar{x}$ = 0.53; ± 0.38). The mean ratio of metabolic group (n = 5) was 0.84 (± 0.84).
Statistical analyses of epicardium-myocardium-ratio of *area A* ($F_{(3, 30)}$ = .70; p = .56; η2 = .07), *area C* ($F_{(3, 30)}$ = .70; p = .56; η2 = .07) and *area D* ($F_{(3, 29)}$ = .54; p = .66; η2 = .05) didn't show any significant results.

3.4 Hypotheses

1. *Macroscopic measurement shows different results in patients with cancer in contrast to metabolic, cardiovascular and control group.*

Statistical analysis revealed a *significant difference* in *heart weight* comparison among study groups ($F_{(3, 35)} = 2.86$; $p = .05$; $\eta 2 = .20$). Metabolic and cardiovascular group showed increased heart weight in contrast to tumor and control group.

Skinfold measurement showed a tendency ($r = .39$, $p = .06$) toward correlation with right chamber wall thickness. The same applies to analysis of aortic heart valve circumferences ($F_{(3, 36)} = 2.45$; $p = .08$; $\eta^2 = .17$). The values were obviously extended in cardiovascular and tumor group and only minimal in metabolic and control group.

In calculations for values of *left* and *right chamber wall thickness* no correlations between the study groups were found.

Lipoid (grade 1; 45% of all patients), calcified (grade 2; 22.5%) and ruptured (grade 3; 30%) plaques were present in all study groups, whereby cardiovascular and metabolic patients had the highest emergence of very severe grade 3 atherosclerosis.

All male patients of tumor group were mal-nourished or even cachectic.

2. *Microscopic measurement shows different results in patients with cancer in contrast to metabolic, cardiovascular and control group.*

An accumulation of cases with mild and moderate *hypertrophy* and *dilatation* was found within cardiovascular study group, patients with cancer as well as metabolic and control group didn't show any distinctive features.

3. *ARVD-characteristic alterations of myocardium (fibro-fatty tissue) can also be found in patients with cancer.*

60% of all examined histological slices showed *fibro-fatty alterations* in myocardial tissue. *Right ventricle* was the most (34%), whereas the *atria* were equally (each 25%) and *left ventricle* was the least (16%) affected from fibro-fatty replacement. The distribution across study groups and areas turned out to be fairly even, except for *mainly diffuse replacement* in *area B* (right ventricle; 21 out of a total of 41 slices) and also in *cardiovascular* study group (28 out of a total of 55 slices).

4. *Morphometric analysis of histological slices shows differences between tumor, cardiovascular, metabolic and control patients.*

There were *no significant differences* between the study groups in reference to *epicardial fat thickness* (EAT) in area *A, B, C* and *D*.

However, statistical analysis revealed *significant differences* between the study groups regarding *myocardial thickness* of *area B* ($F_{(3, 37)} = 3.20$; $p = .03$; $\eta 2 = .21$); metabolic group had a mean value of 2020.7 (± 974.2) in contrast to cardiovascular group with 3125.2 (± 771.4) and tumor group with 2225.2 (± 1033.9). The mean thickness of control collective's right ventricle myocardium was 2443.2 (± 879.6).

Accordingly, identical outcome was found with regard to the *epicardium – myocardium ratio* in *area B* (Kruskal Wallis Test: χ^2_3, n = 35 = 8.10, $p = .04$); tumor group offered a higher ratio (n = 11; $\bar{x} = 0.98$; ± 0.29) in opposition to the mean values of cardiovascular (n = 11; $\bar{x} = 0.54$; ± 0.25) and control group (n = 8; $\bar{x} = 0.53$; ± 0.38).

4 Discussion

This study evaluated the macroscopic and microscopic impacts of cardiovascular disease, metabolic syndrome and - last but not least - cancer on the human heart.

Our results show a significant difference in heart weights between the study groups; compared to standard values, metabolic patients as well as cardiovascular ones showed a considerably increased heart weight.

Furthermore, tendencies ($p = .06$) were revealed in regard of right chamber wall thickness correlating with skinfold measurement. That means that the higher percentage of body fat mass, calculated by 3-site-skinfold test, the thicker right chamber wall is. This discovery is compatible with the fact, that intramyocardial fat in the right ventricle is considered as common in the normal heart and usually increases with body weight and age. (48, 49) Obesity/overweight can also entail right ventricular hypertrophy, presumably by reason of pulmonary burden due to adverse body constitution. (21) Both conditions lead to an increase of chamber wall thickness and heart weights, which were highest within cardiovascular and second-highest within metabolic patients. This corresponds with the fact, that a heightened body weight was mainly present in these two study groups.

Another explanation could lie in the fact, that overweight or obesity are both associated with eccentric left ventricular hypertrophy and chamber dilatation, often triggered by a often co-existing chronic arterial hypertension. (11, 21) Another important aspect is a fatty degeneration of the heart, which can be expressed as pathological correlate of lipomatosis cordis. (50)
Although half of the histological slices of metabolic patients showed moderate dilatation, there were no concise striking features in relation to hypertrophy; myocardial degeneration was mainly fibro-fatty across all areas, none of the histological slices showed single fatty replacement. Our findings are therefore partially in contradiction with these facts.

The cardiovascular study group on the contrary revealed predominant mild and moderate dilatation as well as hypertrophy, primarily in the left ventricle. This result doesn't surprise, as especially concentric left ventricular hypertrophy is a known consequence of increased cardiac work due to chronic arterial hypertension, one of the main characteristics of cardiovascular disease.

A mouse model study performed by Min Tian et al. identified a decreased cardiac wall thickness in mice with cancer cachexia. (61) Left ventricular (LV) atrophy also appeared in context to caloric restriction in C57BI6 mice. The control group showed a significantly higher weight and volume of the LV than in caloric restricted mice and a hint to stronger body weight loss than in LV weight. (62)
According to these studies we expected to find decreased heart weights within cancer patients, but surprisingly tumor and control group showed an almost equal general heart weight, both slightly exceeding standard values. This discrepancy can be explained by an inhomogeneous distribution of nutritional status among study groups; 3 female patients of the tumor group were identified as well-nourished or obese, in contrary all 9 male patients of this group showed mal-nourished (n = 6) or even cachectic (n = 3) state of nutrition.
Nevertheless, an interesting outlier value of 180 g, corresponding to cardiac atrophy and minimum value among all groups, was though found in the tumor group. Furthermore one of these patients had a macroscopically visible atrophy of the left ventricle, although the majority of patients showed a normal configuration of both ventricles. Moreover, comparing mean values showed discreetly lowest left and right chamber wall thicknesses within cancer patients.
As tumor group was represented by different stages, organ involvements and types of cancer (carcinoma, leukemia, melanoma) we would strongly recommend future studies to investigate the question of heart atrophy due to cancer cachexia, based on uniform study groups and a larger number of study subjects. In our study exclusively male patients were affected by severe loss of body weight, which leads us to the question whether there is a sexual dimorphism, disadvantaging male tumor patients. Potential confirmation might have major influence on prognosis and therapy of cancer cachexia.

The slightly increased heart weight of the control group could be explained by the fact, that in general 62% (n = 24) of the study population exceeded normal values, 6 patients even exceeded critical heart weight (> 500 g), impressively demonstrating unexpectedly high values found in this study.

Coronary artery disease (CAD) is a part of the definition of cardiovascular disease and also occurs augmented in metabolic syndrome; including all of its components the likeliness to die in consequence of CAD is up to 4 fold in metabolic patients. Oxidative stress, which plays a central role in atherosclerosis, is highly increased in metabolic syndrome, that is why it's not surprising that affected patients are particularly exposed to an increase in prevalence and often suffer from advanced atherosclerosis. (8)
Our investigational findings support these statements, as cardiovascular and metabolic patients had the highest emergence of very severe grade 3 atherosclerosis in LAD (Left anterior descending/ Ramus interventricularis anterior), characterised by ruptured and vulnerable plaques. Despite this, all three classification grades were present in all study groups.
Presumably there is a similarity to normal distribution among the population, in our case represented by control collective.

Staying with the topic of CAD, a large number of studies substantiated the correlation with epicardial adipose tissue (EAT); indeed, patients with CAD were found to have increased EAT volume, which is suggested to be a strong determinant for CAD.
A recent meta-analysis performed by Pierdomenico S.D. et al. compared EAT thickness in metabolic (n = 1,030) and non-metabolic patients (n = 997) by using echocardiography. They came to the conclusion that EAT thickness significantly reflects visceral adiposity and also correlates with the metabolic syndrome. (32) These findings were substantiated by a large number of other studies. (30, 31, 35)

The results of our study could surprisingly not confirm these previous findings. We found no statistically significant difference in EAT between study groups and areas. This discordance can probably be explained by the fact, that only 3 out of 7 patients of metabolic study group were classified as obese/overweight.

However, in opposition to myocardium (epicardium – myocardium – ratio) the tumor group offered a higher ratio in Area B (right ventricle) compared to the cardiovascular and control group, equated to statistical significant result ($p = .04$). Contrary to our expectations, mean value of EAT thickness in the right ventricle was highest in the tumor group.

Iacobellis et. al. found a significant reduction of EAT due to weight loss in severely obese subjects, who underwent a 6-month very low calorie diet weight loss. (36)
Analogue consideration of weight loss in cancer diseases and their most extreme manifestation called cachexia ("wasting syndrome") leaves basic approaches open; to our knowledge, this is the first autopsy study to examine human hearts, especially EAT, in cancer patients. Within our tumor study group three patients showed cachectic, six mal-nourished, two well-nourished and one obese nutritional status. This unequal distribution reflects dependence of weight loss and hence possible cachexia on tumor site as well as stage; upper gastrointestinal or pancreatic cancer patients suffer from weight loss in about 80%, whereas breast cancer patients are affected in 10 – 35% and lung cancer patients in 50 – 66%. (55)

An important pathophysiological process in complex cancer cachexia is tumor-induced degradation of skeletal muscles to amino acids (proteolysis) as well as degradation of adipose tissue to free fatty acids (lipolysis). (54)
The latter are main energy substrates for the myocardium. In times of high energy demand adipose tissue can serve as a local resource of fatty acids, whereas increased levels of them are rapidly absorbed by the heart. (21) Tumor growth also entails increased energy expenditure, possibly emerging due to an increased thermogenesis in skeletal muscle. (54)

The impact of cancer on the heart is hardly understood yet, but it does not seem to be one of the primary organs affected;
Our results show an increased epicardium-myocardium-ratio with regard to the right ventricle, which is a known predilection site of EAT. As in tumor diseases energy expenditure is increased, it might be possible that EAT, absorbing increased free fatty acids caused by tumor-induced lipolysis, is preserved by the

heart as some kind of energy storage due to ensure essential supply of the myocardium. The mentioned discreetly lowest mean left and right chamber wall thicknesses within cancer patients could be an indication of slow and progressive cardiac muscle degradation, which is in agreement with Tian M. et al., who pointed out that heart failure seems to be a slow process in cancer cachexia.

On closer examination of all cachectic patients (vide appendix, *Table 17* on *page 67*) of this study we also found the highest values for EAT thickness in the right ventricle within them, but the epicardium-myocardium-ratio was clearly lower. This distinction is explainable by the fact, that the mean value of the right chamber wall thickness was higher than in the tumor group. If the number of all cachectic patients would not be as low, we could see this finding as another hint for subtle myocardial involvement in cancer induced cachexia – in contrast to non-cancer cachexia, where the lean body mass is usually preserved. (55, 56)

However, the statements mentioned above are only speculations, based on actual lack of knowledge and an inhomogeneous, small patient collective. Assessing the pathophysiology of cancer cachexia, triglyceride lipases were underlined as a major metabolic pathway. Lipoproteinlipase (LPL) of white adipose tissue was significantly decreased in several studies, whereas hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) showed increased activities. In consequence serum levels of free fatty acids, glycerol, triacylglycerol, VLDL and low-density lipoproteins were found. (60) It would be interesting if there is a correlation between them and EAT-thickness. Future studies will seek to determine whether cardiac involvement in tumor diseases, in special cancer cachexia, deteriorates survival time and prognosis.

Another significant ($p = .03$) outcome in relation to the right ventricle (Area B) was found in regard to myocardial thickness; the cardiovascular group showed increased values in contrast to metabolic and tumor group. This discovery mirrors macroscopically evaluated hypertrophy in right ventricle, which was only present in the cardiovascular study group. More accurate histological assessment additionally underlined as already mentioned above accumulated occurrence of mild and moderate hypertrophy as well as dilatation within these subjects. The

sum of these results is assumed as an expression of consecutive right heart involvement due to chronic left heart failure, causing a backpressure in lungs and as a consequence in right ventricle.

Complementing, analysis of heart valve circumferences showed a tendency ($p = .08$) toward differences between study groups in regard to aortic valve. The values were slightly extended in the cardiovascular and tumor group, but only minimal in the metabolic and control group. Once more we regard our assumption of chronic left heart failure within cardiovascular patients as confirmed, whereas the result in cancer patients is unanticipated. Presumably these pathologic-anatomical changes developed from adverse effects of cancer therapy like chemotherapy, which can induce cardiac dilatation as consequence of cardiotoxicity.
Apart from that, analyses of heart valve circumferences did not provide remarkable results.

Special emphasis was put on the arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C). Underlying structural alterations of myocardium, including replacement by interstitial fibrous and fatty tissue often accompanied by inflammatory infiltrates, were the features in demand. (47)
Autopsy studies performed by Tavora F et al. as well as several other studies have shown evidence of similar histopathologic changes in the left ventricle or in both ventricles, although it was initially considered a right ventricle disease. (45) This is why the sub-tricuspid area, one of the predilection sites known as “triangle of dysplasia”, was investigated as well as both atria and left chamber. (39) The interventricular septum was also shown to be involved at a molecular level, whereas imaging studies and microscopic evaluation could not confirm that finding, hence we refrained from its examination. (42)

Our results demonstrate that fibro-fatty replacement was found fairly even distributed in tumor, metabolic, cardiovascular as well as control subjects; indeed, 60% of all examined histological slices showed fibro-fatty, 38% fibrous and only 2% fatty alterations in myocardial tissue. The right ventricle was the most affected (34%), whereas atria (each 25%) and left ventricle were less (16%) affected.

Almost half of our cases showed focal changes; more than 40% of original tissue replacement (diffuse) was found in 39% of study population and massively extended myocardial involvement with only nests of residual myocytes constituted the minority with only 12%.
Outstanding features were mainly diffuse changes in cardiovascular study group and again area B, the right ventricle.
These findings could eventually support the idea that ARVD/C-characteristic histological alterations occur more often than previously assumed, not only in young athletic men, but also in elderly suffering from different diseases.

Of course, one must be very careful with this kind of statement, as there were grave limitations: First of all our patients did not decease due to sudden cardiac death without explanatory pre-existing conditions, nor did our study population consist of young athletic men – which might have been interesting due to the fact, that disease onset and/or exacerbation occurs prevalently within them. (41) Comparison of our study groups with the latter would have been interesting, probably providing substantiated results.
The most decisive limitation was lack of medical history according to cardiological preliminary findings (ECG; arrhythmias, biopsy, syncopes, familial cases, etc.). This means, that we were only able to illuminate tissue by limited perspective of histological assessment, but diagnosis of ARVD/C requires the knowledge of global or regional dysfunction, re- and depolarisation abnormalities, arrhythmias and family history – according to the International Task Force drafted criteria (TFC) for the clinical diagnosis of ARVD/C (43) and its modification carried out in the year 2010. (44)

Regardless of the critical approach to our findings, it is striking that the right ventricle repeatedly presented cumulative alterations. This could be consistent with the initial definition of ARVD/C.

Recently, genetic testing was initiated. The clinic for cardiology at the University Hospital in Zurich, Switzerland, offers a “USZ-ARVC”-genetic test, which is currently in the process of gaining accreditation. It includes the analysis of DSC-2, DSG2, DSP, JUP and PKP2. These 5 genes seem to account for 90 % of all

mutations within ARVD/C. Unfortunately the test's sensitivity is only 55 % and has a false-negative rate of 0.5 – 2%. Nevertheless, genetic testing could be an important instrument to realise a vision for the future, making ARVD/C-screening possible by simple blood sampling. (51)

Finally, a few words about our control collective: patients of this study group showed a composite picture of underlying diseases like subdural hematoma, peritonitis, encephalomyelitis, polytrauma, etc., mirroring diversity of corresponding results; atherosclerosis as well as stenosis of LAD were evenly distributed in relation to grades of classification. The same applied to histological assessed grades of hypertrophy and dilatation, except high-grade ones, which were not present within this group.
Assessment of heart weight, heart valves, right and left chamber wall thickness, epicardial adipose tissue, epicardium-myocardium-ratio as well as myocardial replacement did not show any outstanding results.

To recapitulate, overall heart weights as well as valve circumferences exceeded normal values. We put that down to post-mortem accumulation of fluid and to sudden ending of heart function, which can explain a slight dilatation of the heart and hence of its valves.

4.1 Limitations of the study

There are several limitations to the present study, besides the specific ones that were already mentioned. The main limitation was presented by a too small patient collective. Some results showed tendencies; we assume that an expansion of the patient collective would give evidence of the clarity and plausibility of the findings.
Study groups partially had an inhomogeneous character and hence inhibited evaluation of some correlations between them.
However, one has to take into account that the study was primarily planned as an explorative study for hypotheses generating and evaluation. This is why classification of study groups was performed by predetermined attributes, leading to uneven distribution of patient collective.

Furthermore, the majority of patients were aged above 50 years, that is why our statements refer to elderly and we had to refrain from analysis of age dependency of parameters.

With regard to methodological limitations, waist circumference measurement of the decedents was not practicable in some cases; abdominal swelling due to natural decay processes and/or increased abdominal accumulation of fluid, like for example ascites, at the time of post-mortem inspection inhibited measurement of correct values. To our disappointment skinfold measurement with a calliper seems to be an error-prone method, that is why some measured values were not sufficient for further statistical analysis.

4.2 Conclusion

As ARVD/C-characteristically histological alterations of the myocardium were found within all study groups - predominantly in the right ventricle - we propose that this disease could be more common than previously assumed. Our findings are furthermore consistent with recent studies, saying that there is no explicit right-ventricular involvement.

Cancer patients offered the highest epicardium-myocardium ratio within this study; this result calls for further investigations, as the impact of cancer diseases on the human heart is not explored well yet. The data obtained here should act as baseline data for further studies.

However, heaped involvement of the right ventricle was conspicuous; therefore we suspect it as another predilection site for cardiac alterations due to cardiovascular, metabolic as well as cancer diseases – in addition to acquainted pathological changes in left ventricle, which surprisingly could not be seen as significant within our study.

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Appendix

Sex	NS	Organ involvement of cancer (primary organ)	Metastases	Underlying cause of death
m	3	Esophagus	+	Esophageal cancer Lung metastases
m	3	Lung	-	St. p. lobectomy due to aspergillom
m	4	Larynx	-	Laryngeal cancer
m	3	Thigh	-	Tumor of thigh; Lymphoma/Leukemia
m	3	Burkitt`s-Lymphoma	-	Burkitt`s-Lymphoma
m	3	Lung, Pleura	+	Adenocarcinoma of the lung Pleural carcinomatosis
m	3	Lung	+	Lung cancer Cerebral and local metastases
m	4	Colon sigmoideum	+	Sigmoid colon cancer Hepatic metastases
m	4	Lung	-	Bronchial carcinoma
f	1	Mamma	+	Breast cancer Multiple metastases
f	2	Lung	-	Lung cancer
f	1	Skin	+	Malignant melanoma Multiple metastases

Table 13: Tumor group; distribution of sex, nutritional status, organ involvement of cancer, metastases and underlying cause of death

Note: m= male; f = female;
NS = nutritional status: 1 = well-nourished, 2 = obese/overweight, 3 = mal-nourished, 4 = cachectic

Macroscopic Assessment

Patient's Nr.:		**Pathologist:**	
Anamnesis			***Underlying cause of death:***
Age:	Waist circumference: cm		
Sex: ☐ m ☐ w			
Skinfold measurement:	Abdominal (♀+♂) mm Tricep ♀ mm Suprailiac ♀ mm Chest ♂ mm Thigh ♂ mm		
			Cause of death:
General type of constitution	***Nutritional status:***		
Mixed type ☐	Well-nourished ☐		
Pyknic ☐	Obese ☐		
Leptosome ☐	Mal-nourished ☐		
Athletic ☐	Cachexia ☐		

Myocardium	Right Atrium	Right Ventricle	Left Atrium	Left Ventricle	***Comments:***
Area	A	B	C	D	
Hypertrophy					
Dilatation					
Heart valves	Mitral	Tricuspid	Aortic	Pulmonary	
Circumference					
Thickness of chamber wall (mm)	Right:		Left:		
Coronary artery	LAD		Heart weight (g):		
Stenosis	I II III				
Sclerosis	I II III				

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Figure 10: Autopsy protocol

Ethikkommission

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FOLGEVOTUM

gültig bis 14.04.2013

EK-Nummer:	23-142 ex 10/11
Studientitel:	Cardiac death in cancer, metabolic and cardiovascular diseases with special emphasis on arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) - a retrospective autopsy study
Prüfer: *)	Dr. Ariane Aigelsreiter Inst. für Pathologie
Sponsor:	(Prüfer)
CRO:	-

*) Antragsteller

Die o.a. Studie wurde von der Ethikkommission erstmals in der Sitzung 04-10/11 am 17.01.2011 behandelt.
Die Ethikkommission ist zu folgendem Schluss gekommen:

Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.

Stimmberechtigte bzw. anwesende Mitglieder bei der Behandlung waren: Siehe beiliegende Liste vom 17.01.2011.

Kommissionsmitglieder, die für diesen Tagesordnungspunkt als befangen anzusehen waren und daher gemäß Geschäftsordnung an der Entscheidungsfindung und Abstimmung nicht teilgenommen haben: keine

Zur Beurteilung vorliegende Dokumente:

Dokumente eingegangen am 15.12.2010, begutachtet in der Sitzung 04-10/11 am 17.01.2011	
Antragsformular	15.12.2010
Originalprotokoll 1.0	14.12.2010
Dokumente eingegangen am 18.02.2011 (in der nächsten Begutachtung mitbegutachtet)	
✓ Antragsformular	18.02.2011
✓ Originalprotokoll 1.1	17.02.2011
✓ Sonstiges: Layout Oduktionsprotokoll 1.0	17.02.2011
Dokumente eingegangen am 14.04.2011, begutachtet im 'expedited Review' am 14.04.2011	
✓ Sonstiges: Stellungnahme Biobank	28.02.2011
Dokumente eingegangen am 19.03.2012, begutachtet im 'expedited Review' am 23.03.2012	
✓ Zwischenbericht	15.03.2012

Datum Erstvotum: 14.04.2011

Das Votum der Ethikkommission berührt in keiner Weise die alleinige Verantwortung der Prüferin / des Prüfers / der Prüfer für die ordnungsgemäße Durchführung der Studie unter Einhaltung aller einschlägiger gesetzlicher Bestimmungen und Richtlinien.

Weiters machen wir darauf aufmerksam, dass der Kommission unverzüglich zu melden sind:

- Abweichungen vom Protokoll aus Sicherheitsgründen oder Protokolländerungen

Medizinische Universität Graz, Auenbruggerplatz 2, A-8036 Graz. www.medunigraz.at
Rechtsform: Juristische Person öffentlichen Rechts gem. Universitätsgesetz 2002. Information: Mitteilungsblatt der Universität und www.medunigraz.at. DVR-Nr. 210 9494.
UID: ATU 575 111 79. Bankverbindung: Bank Austria Creditanstalt BLZ 12000 Konto-Nr. 500 948 400 04, Raiffeisen Landesbank Steiermark BLZ 38000 Konto-Nr. 49510.

Figure 11: Ethical Committee Approval

- Änderungen, die das Risiko der Teilnehmer/-innen erhöhen oder die Durchführung der Studie wesentlich beeinflussen

- Mutmaßliche unerwartete schwerwiegende Nebenwirkungen - SUSARs (AMG-Studien ab 1.5.2004) oder schwerwiegende unerwünschte Ereignisse - SAEs (andere Studien)

- Jegliche Information über sonstige Umstände, die die Sicherheit der Teilnehmer/-innen oder die Durchführung der Studie beeinträchtigen können

Graz, 23. März 2012

Univ.Prof.DI Dr.Peter H. Rehak
Vorsitzender

Univ.Prof.DDr.Hans-Peter Kapfhammer
Stv. Vorsitzender

Achtung: Bitte bei allen das Projekt betreffende Schreiben oder telefonischen Anfragen die EK-Nummer angeben!

Medizinische Universität Graz, Auenbruggerplatz 2, A-8036 Graz. www.medunigraz.at
Rechtsform: Juristische Person öffentlichen Rechts gem. Universitätsgesetz 2002. Information: Mitteilungsblatt der Universität und www.medunigraz.at. DVR-Nr. 210 9494.
UID: ATU 575 111 79. Bankverbindung: Bank Austria Creditanstalt BLZ 12000 Konto-Nr. 500 948 400 04, Raiffeisen Landesbank Steiermark BLZ 38000 Konto-Nr. 49510.

	Study groups				
Parameter	**Tumor**	**Cardio-vascular**	**Metabolic**	**Control**	**∑ / x̄**
	n= 12	**n= 14**	**n= 7**	**n= 8**	**n= 41**
Age *(years ± SD)*	68.25 (12.16)	71.99 (10.87)	71.96 (15.44)	55.95 (19.71)	**67.76** (14.84)
Minimum	48	39	45	28	**28**
Maximum	92	84	93	82	**93**
Gender *(n =)*					
Male	9	8	3	5	**25**
Female	3	6	4	3	**16**
Heart weight *(g ± SD)*	362.50 (109.80)	476.15 (104.61)	453.33 (162.19)	363.75 (91.99)	**414.62** (122.07)
Minimum	180	350	320	200	**180**
Maximum	580	750	770	475	**770**
Thickness of chamber wall: *(mm ± SD)*					
Left	14.58 (2.31)	16.21 (1.85)	14.67 (1.21)	14.88 (2.17)	**15.23**
Right	4.42 (1.0)	4.57 (0.94)	4.50 (1.38)	4.63 (0.74)	**4.53**
Circumferences of heart valves: *(cm ± SD)*					
Tricuspid	12.96 (0.94)	12.75 (0.92)	12.41 (1.79)	12.88 (0.69)	**12.78**
Mitralis	11.17 (1.14)	10.92 (1.04)	10.25 (0.69)	10.31 (0.84)	**10.76**
Aortic	8.38 (0.80)	8.42 (0.70)	7.86 (0.48)	7.75 (0.60)	**8.18**
Pulmonary	8.75 (0.69)	8.89 (0.49)	8.50 (0.63)	8.38 (0.74)	**8.69**

Table 14: Overview of macroscopic findings

Data are presented as mean values (± SD)

	Study groups															
	Tumor				Cardio-vascular				Metabolic				Control			
Area:	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Replacement of Myocardium:																
fatty	0	0	0	0	0	1	0	0	0	0	0	0	0	1	2	0
fibrous	4	1	4	8	5	3	5	6	3	0	1	6	5	2	4	5
fibro-fatty	8	11	8	4	9	10	8	8	4	7	6	1	3	5	2	3
Grade of Replacement:																
rare, focal	4	7	6	7	6	3	6	7	3	3	3	5	6	3	4	6
diffuse	3	2	5	5	8	10	6	4	1	4	3	2	2	5	2	2
massive	5	3	1	0	0	1	1	3	3	0	1	0	0	0	2	0
Hypertrophy:																
none	9	8	9	7	3	3	2	2	5	3	6	4	5	5	7	7
mild	2	2	2	2	6	5	5	4	2	3	1	2	1	1	0	0
moderate	1	2	1	2	4	5	6	7	0	1	0	1	2	2	1	1
high-grade	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0
Dilatation:																
none	5	4	6	3	0	0	0	0	2	2	3	3	3	3	4	4
mild	4	4	3	4	8	5	7	4	0	0	0	0	3	3	3	3
moderate	2	3	3	3	5	7	7	9	4	4	3	3	2	2	1	1
high-grade	1	1	0	2	1	2	0	1	1	1	1	1	0	0	0	0

Table 15: Distribution of microscopic findings across study groups and areas

Data are presented as number of histological slices affected (n=)

	Study groups				
Area	**Tumor**	**Cardio-vascular**	**Metabolic**	**Control**	$\bar{x}$
Epicardium (EAT)					
A	1245.3 (532.8)	1419.0 (1215.6)	1618.1 (1419.8)	1097.2 (651.8)	1351.4 (1002.7)
B	2053.2 (711.5)	1536.0 (613.9)	1371.7 (1447.1)	1125.2 (747.3)	1581.2 (865.6)
C	1387.0 (919.8)	1648.6 (565.4)	1649.5 (881.1)	1297.2 (452.3)	1495.1 (719.1)
D	1216.6 (969.1)	1301.2 (840.6)	1875.7 (1035.0)	1273.8 (549.1)	1336.8 (844.9)
Myocardium					
A	2189.6 (1387.2)	2271.7 (1060.5)	1668.6 (890.0)	1649.6 (570.1)	2023.3 (1072.5)
B	2225.2 (1033.9)	3125.2 (771.4)	2020.7 (974.2)	2443.2 (879.6)	2540.2 (981.7)
C	1819.9 (742.9)	2099.1 (1266.6)	1913.0 (937.6)	1748.0 (279.2)	1907.8 (892.6)
D	2682.8 (1185.7)	3295.4 (1439.8)	2924.2 (1091.2)	2948.3 (1170.5)	2977.2 (1234.7)
Ratio Epicardium – Myocardium					
A	.79 (0.38)	.64 (0.39)	1.00 (0.85)	.83 (0.52)	.78 (0.50)
B	.98 (0.29)	.53 (0.25)	.84 (0.84)	.53 (0.38)	.72 (0.45)
C	.86 (0.56)	1.12 (0.62)	1.06 (0.66)	.80 (0.45)	.96 (0.57)
D	.47 (0.29)	.41 (0.23)	.61 (0.37)	.50 (0.25)	.47 (0.27)

Table 16: Overview of computer-assisted findings

Data are presented as mean values (± SD)

Cachectic patients			
Area	**Epicardium**	**Myocardium**	**Epi/Myo (Ratio)**
A	1471.7 (672.8) *n= 3*	2918.7 (2234.1) *n= 4*	0.87 (0.27) *n= 3*
B	2045.2 (832.0) *n= 3*	2701.6 (632.0) *n= 4*	0.67 (0.23) *n= 3*
C	1903.2 (1063.4) *n= 4*	1696.0 (412.9) *n= 4*	1.11 (0.56) *n= 4*
D	1579.4 (1729.7) *n= 3*	2153.3 (931.4) *n= 4*	0.58 (0.45) *n= 3*

Table 17: Overview of computer-assisted findings in cachectic patients

Data are presented as mean values (± SD)