UMEÅ UNIVERSITY

HEART AND LUNG FUNCTION - IN HEALTH AND DISEASE

Methodological Studies in Clinical Physiology

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“Dela din kunskap. Det är ett sätt att uppnå odödlighet.”
(Dalai Lama)

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Abstract

The human heart and lungs constitute an intricate and dynamic system. Various clinical physiological examinations can be used to evaluate cardio-pulmonary function and identify abnormalities. Thus, it is important to understand how normal physiology presents, to be able to identify pathological findings. To distinguish normal from abnormal findings in a patient population compared to healthy controls, adequate, accurate and up-to-date reference materials are required.

There is currently a lack of well-established sex and age specific reference materials that clearly state boundaries of normality for electrocardiography (ECG) variables. For lung function examinations there are several different reference materials available, being discordant between ethnicities. In addition, the relation between lung function, age, sex, and height has generally been difficult to model in an optimal way. This highlights the need for more adequate sex-specific models regarding age- and height-dependency of spirometry variables.

Heart rate variability (HRV) is a method for evaluating the autonomic nervous system (ANS) and its influence on heart rate and blood pressure. Autonomic disturbances are characterized by an imbalance between the sympathetic and the parasympathetic nervous systems. It is well known that decreased HRV is associated with increased mortality. Autonomic imbalances are also associated with various pathological conditions, of which rheumatoid arthritis (RA) and ischemic heart disease (IHD) are studied in this thesis.

The purpose of this thesis was to describe the properties of different clinical physiological examinations and to investigate reference values relating to cardiovascular and pulmonary function in healthy individuals regarding age and sex. In addition, the aim was to assess the relationship between HRV, RA and CVD both cross sectionally and longitudinally.

In a subjectively healthy population (n=219) of varying age, there were age and sex-dependent differences in ECG examinations. This emphasizes former findings and supports the need to establish age- and sex-specific reference values in the future.

Lung function examinations in subjectively healthy persons (n=285) support and emphasize that the reference values presented by the Global Lung function Initiative (GLI) underestimate the pulmonary function in the adult Swedish population. The study showed that the model used in GLI can be updated with new values that are specific for the Caucasian population in Sweden.

Patients with RA (n=50) presented with lower HRV than healthy controls (n=100) during autonomic provocation tests, both at baseline examinations and after five years. This indicates a cardiac autonomic imbalance. Furthermore, increased systolic blood pressure was associated with reduced HRV, thus a decrease in HRV could be a risk marker for developing arterial hypertension in this patient group.

Females with IHD (n=197) presented with lower HRV compared to controls (n=141) at baseline, and a higher mortality rate after 15 years. The higher mortality rate was only present in females < 60 years of age. For measurements obtained in the upright position, HRV was higher in females that died during follow-up compared to those who were alive.

This thesis emphasizes the importance of validated and updated sex- and age- specific reference materials, and models that are well suited for different clinical physiological examinations. Additionally, HRV examinations exposed changes in the ANS related to RA as well as IHD, where findings were shown to be persistent over time and particularly pronounced during provocations. In the future, HRV assessment could be a useful tool to identify the increased risk of developing hypertension in patients with RA, or to customize treatment based on ANS response as the field of personalized medicine continues to evolve.

Abbreviations

AI	Artificial Intelligence
ANS	Autonomic nervous system
ATS	American thoracic society
AV-node	Atrioventricular node
BMI	Body mass index
CAD	Coronary artery disease
COPD	Chronic obstructive pulmonary disease
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
DAS28	Disease activity score based on 28-joint count
DBP	Diastolic blood pressure
ECG	Electrocardiography
ENS	Enteric nervous system
ESR	Erythrocyte sedimentation rate
ERS	European respiratory society
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GLI	Global lung function initiative
HF	High frequency
HRV	Heart rate variability
IHD	Ischemic heart disease
ICD	Implantable cardioverter defibrillator.
IL-6	Interleukin 6
LBBB	Left bundle branch block
LF	Low frequency

LLN	Lower limit of normality
OLIN	Obstructive lung function in northern Sweden
PAC	Premature atrial contraction
PEF	Peak expiratory flow
PSVT	Paroxysmal supraventricular tachycardia
PVC	Premature ventricular contraction
RA	Rheumatoid arthritis
RBBB	Right bundle branch block
SA-node	Sino atrial node
SBP	Systolic blood pressure
SVT	Supraventricular tachycardia
rLMS	acronym for the rederived LMS model
VC	Vital capacity
VT	Ventricular tachycardia

Sammanfattning på svenska

Människans hjärta och lungor utgör ett invecklat system. Olika klinisk fysiologiska undersökningar kan användas för att utvärdera funktionen hos detta system, och det är viktigt att känna till den normala fysiologin för att kunna bedöma om något är friskt eller sjukt. För att urskilja onormala fynd i en patientpopulation behövs friska kontroller och adekvata, uppdaterade referensmaterial för att fastställa korrekta diagnoser.

Idag saknas väletablerade köns- och åldersspecifika referensmaterial som tydligt anger normala gränser för elektrokardiografiska (EKG) variabler. För lungfunktionsundersökningar finns flera olika referensmaterial, och förhållandet mellan lungfunktion, ålder, kön och längd har generellt varit svårt att modellera på ett optimalt sätt, speciellt för olika etniciteter. Detta gör det svårt att veta vilket referensmaterial som är lämpligast att använda, och det finns ett behov av bättre könsspecifika modeller av ålder och längdberoende i spirometrivariablerna.

Hjärtfrekvensvariabilitet (HRV) är en metod för att utvärdera det autonoma nervsystemet (ANS) och dess påverkan på hjärtfrekvens och blodtryck. Autonoma störningar kännetecknas av en obalans mellan det sympatiska och parasympatiska nervsystemet. Det är välkänt att minskad HRV är associerad med dödlighet. Autonoma obalanser är också associerade med olika patologiska tillstånd, däribland reumatoid artrit (RA) och ischemisk hjärtsjukdom (IHS) vilka är de sjukdomarna som ingår i denna avhandling.

Syftet med denna avhandling var att beskriva egenskaperna hos olika klinisk fysiologiska undersökningar, avseende ålder och kön, för att korrekt bedöma hjärt- och lungfunktion hos friska individer. Ytterligare ett syfte var att bedöma förhållandet mellan HRV, RA och kardiovaskulär sjukdom, både i tvärsnittsstudie och genom longitudinell uppföljning.

I en subjektivt frisk population bestående av försökspersoner i varierande åldrar (n=219) fanns ålders- och könsberoende skillnader i ett flertal EKG-variabler. Detta understryker tidigare resultat och stödjer behovet av att fastställa ålders- och könsspecifika referensvärden i framtiden.

Lungfunktion undersöktes hos subjektivt friska personer (n=285). Resultaten betonar att de referensvärden som presenterats av Global lung function initiative (GLI) underskattar lungfunktionen hos den svenska vuxna befolkningen. Resultaten visade också att modellen som används i GLI kan uppdateras med nya värden som är specifika för den kaukasiska befolkningen i Sverige.

Patienter med RA (n=50) uppvisade lägre HRV än friska kontroller (n=100) under autonoma provokationstester, både under undersökning vid studiestart och vid uppföljande undersökningar efter fem år. Detta indikerar en kardiell autonom obalans hos patienter med RA. Resultatet visade även att ett ökat systoliskt blodtryck var associerat med minskad HRV. Detta indikerar att en minskning av HRV kan vara en riskmarkör för att utveckla högt blodtryck hos patienter med RA.

Kvinnor med IHS, (n=197) uppvisade lägre HRV jämfört med kontroller (n=141) vid inklusion, och en ökad dödlighet efter 15 år. Den högre dödligheten fanns endast hos kvinnor <60 år. HRV- analys vid lutning till upprätt läge visade att kvinnorna med IHS som avlidit under observationstiden hade högre HRV jämfört med de som levde.

Denna avhandling betonar vikten av validerade och uppdaterade könsspecifika referensmaterial och modeller för olika undersöknings-metoder inom klinisk fysiologi. Vid analys av HRV sågs förändringar i ANS relaterade till RA såväl som IHS, där fynden visade sig vara ihållande över tid och särskilt uttalade under provokationer. I framtiden skulle HRV-undersökningar kunna vara ett användbart verktyg för att identifiera den ökade risken för att utveckla högt blodtryck hos patienter med RA, och för att skräddarsy behandlingar baserat på ANS-respons i takt med att området för precisionsmedicin fortsätter att utvecklas.

Original papers

The thesis is based on the papers listed below, and the papers will be referred to in the text by their Roman numerals (I-IV).

I **Erelund, S.,** Karp, K., Wiklund, U., Hörnsten, R., Arvidsson, S. Are ECG changes in heart-healthy individuals of various age related to cardiac disease 20 years later?
Upsala Journal of Medical Sciences. 2021 1;126:e6064. doi: 10.48101/ujms.v126.6064.

II **Erelund, S.,** Karp, K., Arvidsson, S., Johansson, B., Sundström, N., Wiklund U. Pulmonary function in a cohort of heart-healthy individuals from Northern Sweden- a comparison with discordant reference values.
BMC Pulmonary Medicine. 2023;23:110.
doi;10.1186/s12890-023-02403-w.

III **Erelund, S.,** Södergren, A., Wiklund, U., Sundström, N. Heart Rate Variability and Cardiovascular risk factors in patients with Rheumatoid Arthritis: A longitudinal study.
Autonomic Neuroscience: Basic and Clinical 2023;249: 103119 doi.10.1016/j.autneu.2023.103119.

IV **Erelund, S.,** Slunga Järvholm, L., Nordendahl, M., Johansson, B., Wiklund, U., Sundström N. Heart Rate Variability and Long-term Survival in Females with Ischemic Heart Disease.
(Manuscript).

Personal introduction

In 2009 I started my career as a Biomedical Scientist in Clinical Physiology at the University Hospital of Umeå. My first years was mainly spent interpreting long-term ECG recordings and participating in the arrhythmia laboratory with invasive electrophysiology and pacemaker/implantable cardioverter defibrillator (ICD) implantations. A couple of years later, my now good friend and colleague Rolf Hörnsten began to attract me into the wonderful world of research. He helped me to complete my master's degree and then he found other great friends who wanted to join my journey towards the doctor's hat.

During my years at the department of Clinical Physiology, I have performed and analyzed many examinations on patients referred to the department. In my clinical work, comparisons, and interpretations in relation to reference values are inevitable. Often, individuals exhibit deviations from these values, that made me think about what are these deviations due to? Are the reference values accurate and solid? How are they determined? How do disease states affect the measured values?

The focus of my research has therefore been to deepen my overall knowledge in the field, regarding the methods electrocardiography (ECG), spirometry and heart rate variability (HRV) since methodology is my area of expertise. This thesis has specifically focused on methodological differences based on age, sex, ethnicity, and different medical conditions.

I have always been inquisitive and liked to immerse myself in different methods and to learn their details and intricacies, thus contributing to scientific knowledge and develop has been a fantastic experience during these years. Nowadays I am employed as a teacher for the biomedical laboratory science program at Umeå University and it is a joy every day to share my knowledge, and to be involved with the education of the future Biomedical Scientists in Clinical Physiology.

I hope you find this thesis interesting and valuable, and maybe you can learn something new!

Sofia Erelund, Umeå, October 2023

Introduction

Clinical physiology is a medical specialty focusing on functional diagnostics, in particular investigating, and assessing, both normal physiological and pathophysiological processes. This specialty is unique to parts of Europe, especially in the Nordic countries. Pulse curves were one of the first physiological methods studied in the early twentieth century. Since then, many new methods and approaches have been established in the field. In this thesis, properties important for correct assessments in health and in disease by different clinical physiological examinations will be investigated. The first method is ECG, where the function of the heart is investigated by measuring the electrical activity generated with each heartbeat. The second is spirometry, where the function of the lungs is studied by measuring air flows and volumes during breathing, and finally the third method is HRV, where the function of the autonomic nervous system (ANS) is assessed by analyzing the variation of the heart rate.

Dysfunction in the ANS is associated with various pathological conditions, of which ischemic heart disease (IHD) and rheumatoid arthritis (RA) are studied in this thesis.

RA is a chronic autoimmune disease that primarily affects the joints. Today, while there is no cure for RA, advances in medical sciences and treatment options have significantly improved the quality of life for patients with RA. Research is ongoing and trying to expand our understanding of this complex autoimmune disease.

Cardiovascular disease (CVD), coronary artery disease (CAD) and IHD, are related terms that are often used correspondingly. However, there are differences and separate meanings between them. Cardiovascular disease is a broad term that involves a range of conditions that affect the heart and blood vessels, such as stroke, heart failure, myocardial infarction, hypertension, pulmonary embolism etc. Cardiovascular disease is a specific type of heart disease that cover the spectrum of atherosclerosis in the coronary arteries. Over time CAD can lead to IHD. Ischemic heart disease is a specific subtype of CVD that implies reduced blood flow to the heart muscle, leading to ineffective oxygen supply. Ischemic heart disease

results from atherosclerosis and the most common symptom are angina pectoris.

Historical background

Clinical physiology

In the year 1900, a new medical specialty named Clinical Physiology, was established in Sweden. Professor Salomon Henschen founded the first laboratory at the Serafimer hospital situated on Kungsholmen in Stockholm, Sweden, including three different rooms: bacteriology, chemistry, and physiology. A colleague at Serafimer and future professor Nanna Svartz was commissioned to plan the laboratory for the upcoming hospital, Karolinska University Hospital in Stockholm, Sweden. In 1940, the new hospital was completed with the same set of specialties as the laboratory at the Serafimer Hospital. Physician Torgny Sjöstrand became director for the laboratory and contributed strongly to the development of the specialty of Clinical Physiology. In 1955 the Swedish medical board (Medicinalstyrelsen) decided to establish the specialty in all University hospitals and later in other hospitals in the country (1).

In the 1950s, there was a central chemical laboratory in Umeå where ECG was performed. In 1957, an independent clinical physiology laboratory was established with Hans Dahlström as director. In 1959, a professorship was established and it was awarded to Håkan Linderholm, under who's leadership the department was developed (1)

The department of Clinical Physiology at the University Hospital of Umeå currently employs about 50 people including physicians, biomedical scientists, nurses, and assistant nurses. They perform cardiac and vascular sonography, 12-lead and long-term ECG measurements and interpretations. Stress test, lung function measurements, investigations of renal function, muscle and rectal pressure measurements, and esophageal and pH measurements in both adults and children.

Previously, invasive, and non-invasive electrophysiological diagnostics and treatment were included in the department, however several years ago these areas were separated to form their own unit, the department of Arrhythmia.

Electrocardiography

Pulse curves were the first physiological assessment studied at Serafimer Hospital and this was mainly for scientific research and experiments. In 1908, ECG at rest was introduced in Lund, Sweden (2). Almost 20 years earlier the first recording of electrical activity in a human heart was performed by Augustus D Waller. Waller illustrated that electrical activity could be documented from the chest wall using a mercury capillary electrometer, this was the ancestor of today´s electrocardiograph (3). Nobel prize winner Willem Einthoven continued to develop what Waller had started.

Einthoven believed that by developing a more precise method of recording the electrical activity of the heart, he could uncover vital information about the hearts function. After years of hard work, he came up with the string galvanometer that consisted of a fine quartz string, pending within a strong magnetic field. The string was connected to a mirror, which reflected its movement. When placed in contact with the skin, the device could detect the electrical currents generated by the heart´s muscle contractions. Einthoven´s genius lay in his ability to amplify and record the movements of the string with exceptional precision. He achieved this by combining a system of forces and weights that magnified the string´s deflections. He was able to record clear electrocardiograms and he named the different waves in each contraction of the heart P, Q, R, S and T (4) as seen in Figure 1.

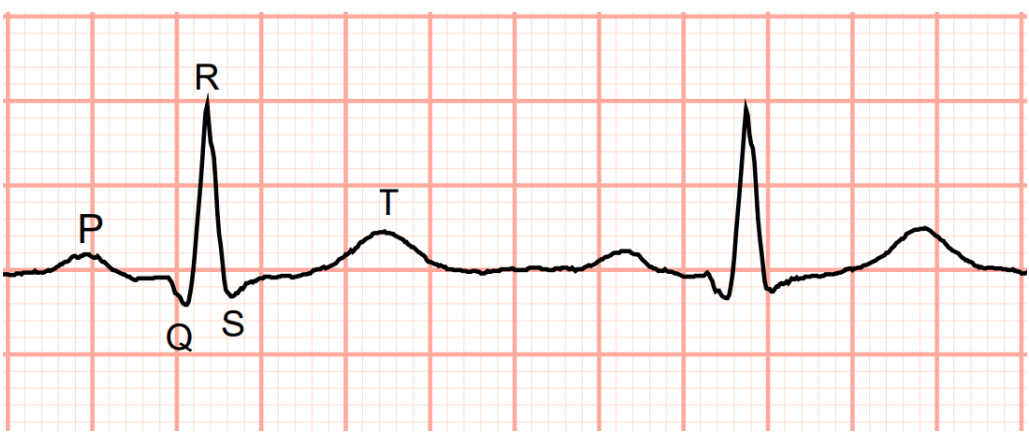

Figure 1. Illustration of a normal ECG curve with marking of the characteristic waves. Y-axis: electrical voltage, X-axis: time.

Overtime, technological development allowed for smaller, and more portable, ECG devices. In 1947 Norman Jeffery Holter was the first to develop the 24-hours portable ECG recorder, he used transistors and a tape recorder to record the ECG trace. He argued for continuous ambulatory ECG recordings by comparing the collection of ECG data with the work of a mining engineer “one does not assay a mountain of ore by testing one rock” (5). A single sample cannot reveal the richness of the mountain, nor can one rule out a suspected heart rhythm disorder by taking one 12-lead ECG at rest in an asymptomatic patient, as a few seconds of a 12-lead ECG recording may not be able to catch a transient symptomatic arrhythmia. Holter made it possible to record the electrical activity of the heart for a longer time in a non-invasive way.

The first Holter monitor system weighed 38 kg and was strapped to the back like a backpack. The patents on this invention were sold to Del Mar Engineer Laboratories and it was through Bruce Del Mar´s support and investment that Holter was able to lead the development of smaller units, firstly to a size that could fit in a briefcase weighing about 1 kg, and eventually to a unit that could fit inside a coat pocket. Instead of being bound to a bed in the hospital, patients could go home and be monitored during normal daily activities (6). Portable ECG devices have become increasingly popular in clinical settings due to their convenience and flexibility. These devices are designed to record a patient's cardiac activity over an extended period, allowing healthcare providers to monitor heart function outside of clinical settings. These monitors enable early detection of arrhythmias and facilitate more personalized and effective treatment plans.

Dynamic spirometry

In 1846 the English physician John Hutchinson presented the first water spirometer measuring vital capacity (VC) of the lungs. His device consisted of a calibrated clock that moved very easily in a water cylinder, a pneumatic tube was connected to the clock and on breathing, the watch oscillated vertically and a measurement value could be obtained (7). Some small changes were made and since then his device, the Bernstein spirometer, has been used until the development of the pneumotachometer.

About one hundred years later Tiffeneaue and Pinelli showed that forced expiratory volume (FEV) in the first second (FEV_1) is a measure of the ventilation capacity(7), and in 1958 the flow/volume curve was presented by the American cardiologist Robert E Hyatt. Hyatt showed that there is a maximum flow that can be accomplished at a given lung volume (7).

Today, the most widely used spirometer is the pneumotachometer. It is electronic and based on a flow meter. The pneumotachometer measures the pressure drop over a tube containing capillaries of pleated aluminum or a sheet of mesh(8).

Heart rate variability

Albert von Haller (1708-1777) is believed to be the first to note a beat to beat variation in the heart rhythm(9), and in 1733 Stephan Hales reported associations between spontaneous oscillations in blood pressure, inter-beat interval and the respiratory cycle (10). In 1847 this was verified by Carl Ludwig, and respiratory sinus arrhythmia became an accepted concept (9) (Figure 5). Among the first clinical applications of HRV, based on ECG recordings, was its use in obstetrics, with the first article published on this topic in 1965 by Hon and Lee (11). The authors found that fetuses with reduced or absent HRV suffered from fetal distress. Subsequently, applications within cardiology and neurology were added.

Like the development of the ECG, the progress of HRV analysis has been extensive. In the 1980´s, when more advanced computers became widely accessible, HRV analysis developed significantly. Since the 1990´s two different methods have been used to perform HRV analysis: Short-term recordings (approx. 20 min) performed on a tilt table in a laboratory with a single-channel ECG and continuous blood pressure measurement, and long-term recordings with ambulatory ECG recordings up to 24 hours. Analysis of the HRV recordings can be performed in different ways, using non-linear methods, time or frequency (spectral) domain analysis. Time domain analysis involves measuring statistical properties of the RR intervals and frequency domain analysis divides HRV into different frequency components using advanced mathematical methods. Non-linear methods include more advanced mathematical methods and are often based on 24-hour recordings, which are not included in this thesis.

Physiology of the heart and lungs

One of the most vital organs is the size of the owner´s clenched hand, weighs about 250-300 grams, is shaped like a cone, and located in the mediastinum. This organ is the human heart (12). The heart consists of two atria and two ventricles. Each heartbeat is initiated in the sinoatrial (SA) node, which is located in the right atrium. The cells within the SA-node are known as pacemaker cells or cardiac autorhythmic cells, i.e., they can initiate each cardiac cycle through spontaneous depolarization. Normally, the SA-node triggers 60-100 action potentials per minute. These spread through the atrio-ventricular (AV) bundle to reach the top of the septum between the atria and the ventricles. On both sides of the septum there are branches that gradually depolarize, and finally the signals reach the ventricles (12). Since the muscle cells in the atria and ventricles are electrically linked to each other, the electrical activity is coordinated, and relatively large currents arise. These currents are conducted within the body fluids and can be derived as potential differences between different points on the body surface. The recording of these signals is called an ECG. The action potentials that arise in individual myocardial cells have an amplitude of about 100 mV. This amplitude represents the change in voltage across the myocardial cell membranes. The potential differences recorded between different points on the body surface are significantly smaller, approximately 1-2 mV(13).

The activity in the cardiac conduction system can indirectly be assessed through the ECG. The electrical signal from the SA-node travels through the atrium, thus generating the atrial contraction, which is represented as the P-wave on an ECG. When the AV-node is activated, the signal spreads down through the branches until the cardiac apex is reached, and then the signal turns up through the ventricular walls into the Purkinje fibers where it subsequently activates the ventricles leading to ventricular contraction. This creates the QRS complex, subsequent repolarization of the ventricles generates the T-wave (12).

The heart itself is a muscular pump, the right side of the heart receives deoxygenated blood from the body and pumps it to the lungs, while the left side receives oxygenated blood from the lungs and pumps it out to the body. Also of importance is the elasticity of the arterial tree, the pumping effect of skeletal muscles on the veins and the respiratory variation of the

thoracic pressure and abdominal cavity, which all aid in circulation. In total, about 10,000 liters of blood are pumped every day. This large amount is needed to meet the different tissues' need for oxygen and nutrient supply as well as for the removal of carbon dioxide and metabolites (14).

Functionally, we can divide the airways into conduction structures and respiratory structures. The conduction structures consist of a series of interconnected cavities and tubes that transport the inhaled air first from the nose or oral cavity, further through the throat down to the bronchi and finally to the bronchioles. The task of these structures is to bring air to the parts of the lungs that are responsible for the gas exchange, but they also have the task of filtering, heating, and humidifying the inhaled air on its way to the lungs. After the air has passed through the bronchi, it eventually ends up in the respiratory zone, where actual exchange of oxygen and carbon dioxide between the air and blood takes place (14).

The human heart and lungs make up a complex dynamic system, and thus knowledge of their normal physiology is important to determine whether an individual's condition is healthy or not, and what changes belong to the normal aging process and what are pathological.

Differences in heart structure between the sexes

There are some differences in the structure of the heart between females and males(15), primarily driven by the influence of physiological factors, genetics, and sex hormones. The size and weight of the heart are related to overall body size and muscle mass; thus, females tend to have slightly smaller hearts. Females generally have a higher heart rate, a difference that is likely influenced by hormones and the ANS, and the coronary arteries tend to be smaller (16). The latter difference may affect the risk and presentation of CAD. Left ventricular mass index and relative wall thickness are two echocardiographic measures that tend to be smaller in females (17). It is important to emphasize that these structural differences do not necessarily imply better or worse health in one sex over the other. Additionally, the significance of these structural differences may vary from person to person. The risk factors and presentation of heart disease may differ between sexes, and thus diagnostic criteria need to account for these differences.

Differences in lung function between the sexes

Differences in lung function between the sexes are common and can partly be derived from anatomical and physiological differences in the lungs and respiratory muscles. In general, males have larger lung volumes than females. This is because the male body are generally larger, and they therefore have larger lungs. Compared to females, males have a greater total lung capacity, vital capacity and FEV_1. Females have lower percentage of muscle mass and higher percentage of body fat than males and therefore an increased chest volume can affect lung function in males (18).

The autonomic nervous system

The heart rate is normally controlled by the firing frequency of the SA-node, i.e., how closely together in time the action potentials arise in these cells. The SA-node, in turn, is under the influence of the ANS.

The central nervous system regulation of the heart originates in the *medulla oblongata*. This part of the brainstem receives signals partly from sensory receptors but also from higher centers in the brain such as the cerebral cortex, limbic system, and hypothalamus. The cardiovascular center subsequently sends on suitable nerve impulses by modifying the impulses from sympathetic and parasympathetic pathways within the ANS (14). The ANS consists of three main components: the sympathetic nervous system (SNS), the parasympathetic nervous system (PNS) and the enteric nervous system (ENS).

The variability in the heart rhythm is part of ANS regulation of the cardiovascular system, which also influences cardiac stroke volume, venous and arterial blood pressure. The autonomous regulation includes both the force of the contractions of the heart muscle and how often heart beats are triggered by action potentials from the SA-node. The SNS has an increasing effect on the SA-node impulse formation while PNS has an inhibitory effect. Increased PNS activity decreases the heart rate, but also delays the conduction of action potentials through the AV-node. The PNS can make quick fine-tuning of the timing of the next heartbeat while SNS is a slower-acting system(19). The heart rate is determined by the balance of activity in these two branches of the ANS. At rest, the PNS usually

dominates, but this decreases as SNS activity increases as the heart rate rises during physical work and stress.

The SNS acts as the gas pedal of the heart and arises from the *medulla oblongata* into the spinal cord. At the level of thorax, cardiac accelerator nerves extend into the SA-node, the AV-node and most of the myocardium. Impulses in the accelerator nerve of the heart produce and secrete the hormone noradrenaline, which binds to β-1 receptors in the myocardial fibers and increases the frequency of depolarization at the SA-node, which in turn results in an increased heart rate. Noradrenaline also stimulates an increased output of Ca^{2+} ions from the contractile myocardiocytes, which increases contractility and thus increases stroke volume (12).

The PNS is the heart's brake. The activity in the PNS also originates from the *medulla oblongata*, acts via branches of the vagus nerve, and innervate the SA-node and the AV-node in the atria. The vagus nerve produces the neurotransmitter acetylcholine that causes reduced frequency of depolarizations in the autorhythmic myocardiocytes. This consequently leads to a lower heart rate (12).

The ENS is a complex network of neurons located within the walls of the gastrointestinal tract, extending from the esophagus to the anus. It plays a crucial role in regulating digestive processes, including peristaltic, secretion of digestive enzymes, and absorption of nutrients. While the ENS can function independently, it also receives input and influence from both the SNS and PNS to coordinate digestive activities (20).

The limbic system in the brain senses stressful situations, for example during competition or fight, even before the physical exertion begins and affects the ANS by sending nervous signals to the *medulla oblongata*. The *medulla oblongata* in turn, via the SNS increases the heart rate. Sensory receptors, such as proprioceptors, help increase and maintain heart rate. Proprioceptors are activated when moving voluntarily controlled muscles, i.e., skeletal muscles. This accounts for both the preemptive increase in heart rate and respiratory rate at the beginning of a physical activity (21).

In the arterial system there are baroreceptors which senses the blood pressure. These receptors are located in the *sinus caroticus* and in the aortic sinus. The *sinus caroticus*, is located in the bifurcation of the carotid artery. The aortic sinus is located in the aortic arch and is innervated by

afferent fibers of the vagus nerve(12) . Blood pressure normally oscillates. When blood pressure increases, the action potentials triggered by the baroreceptors act as inhibitors on the vasomotor center and result in reduced heart rate and weaker contraction force, caused by increased vagal tone and decreased sympathetic tone. When blood pressure decreases, the baroreceptors will trigger changes so that the blood pressure normalizes again (14, 22).

What is normal aging?

Normal aging is the natural process by which the body gradually undergoes physiological changes over time. It is a normal and irreversible part of human development and can include a range of changes that affect the body´s function and appearance. However, there is no simple definition of physiological aging. Even if we could manage to avoid all the world´s diseases and ailment, biological aging will still result in our vital organ systems being unable to maintain all the processes of life. With increasing age, we experience effects on both the central and peripheral cardiovascular system. Most often, this is due to the collagen rendering stiffer tissues, with high blood pressure as a result. This in turn can lead to compensation and changes in, for example, the heart rate(17). Another example is the effect of aging in lung function. From age 30 to 80 years lung capacity decreases by 30-50 % and the compliance of the lungs decreases (23). We are getting older and older and at the same time CVD is the most common cause of death among the elderly in Sweden today. It is therefore important to understand that normal aging involves certain physiological changes in the cardiovascular system, while pathological cardiovascular aging may involve the development of serious CVD.

Diagnostic methods and models in this thesis

Electrocardiography

A 12-lead ECG at rest should be performed on several indications e.g., chest pain of unknown cause, arrhythmia, preoperative assessment, diagnosis of infarction and for screening(13). To evaluate the heart rhythm over a longer period of time, different types of monitoring systems are available today. The choice of system is based on the patient's symptoms. Indications for performing long-term ECGs are palpitations, presyncope, syncope, episodes of fatigue, dizziness, and chest discomfort (24). A full 12-lead ECG at rest, is after auscultation of the heart, the most common survey in healthcare, therefore 12-lead ECG at rest is one of the most widely used laboratory analyses in cardiac diagnosis.

The effects of sex and aging on the ECG

The physiology and structure of the heart is different between the sexes, and this is reflected in an individual's ECG (25). The ECG changes with age, and the largest changes occur from the time of birth, through childhood up to puberty and young adolescence. Later, in healthy adolescents, the differences are more pronounced based on sex, which probably is due to the interaction between factors such as autonomic function, body size, anatomy, hormones, and genetics (25, 26). Aging is a normal physiological mechanism and is a known major independent risk factor for cardiovascular events (17). To detect abnormal findings in a patient population, healthy controls of both sexes covering a wide spectrum of ages are required.

Earlier studies in which healthy adults have been screened with ambulatory ECG recordings have shown that arrhythmias are common, especially in an elderly population (27, 28, 29, 30). Although most of the ECG changes found have been considered as benign, other studies have shown that a deviating ECG, is associated with complications later in life (31, 32, 33, 34, 35). Thus, there are different factors such as rhythm, conduction disorders, ischemic coronary disease, and other conditions (e.g., drug effects, electrolyte imbalance, Long QT Syndrome to consider when interpreting ECGs. There are currently no established reference materials that clearly state boundaries for normality of all ECG variables in both sexes and across a wide age range (15).

Dynamic spirometry

The indications for spirometry vary depending on the purpose. The American Thoracic Society/European Respiratory Society (ATS/ERS) has published guidelines for indications of spirometry and those are divided into four groups; diagnostic, monitoring, disability/impairment evaluations and public health (36).

Measurement of lung function has two main applications; as part of the assessment of overall lung function, or assessment of respiratory or other diseases that can affect the respiratory system. The first is to determine in what way and to what degree the lung function is impaired. The second is for follow-up of e.g., effect of treatment, lung/airway disease progression, or for early detection of changes in lung function that may occur because of disease or therapy. Spirometry should be performed on patients seeking primary care for shortness of breath, wheezing in the chest, and/or prolonged cough (36).

To assess the flow resistance of the airways, dynamic spirometry is used. Figure 2 shows a typical recording of exhaled air flow and the calculated volume during a forced expiration. Three important parameters are determined.

1. FEV_1 = forced expiratory volume in one second, i.e., volume that has been exhaled at the end of the first second at forced expiration (Figure 2).
2. FVC = forced vital capacity, i.e., the largest volume a person can exhale forcefully after a maximum inhalation.
3. FEV_1/FVC = The ratio between FEV_1 and FVC.

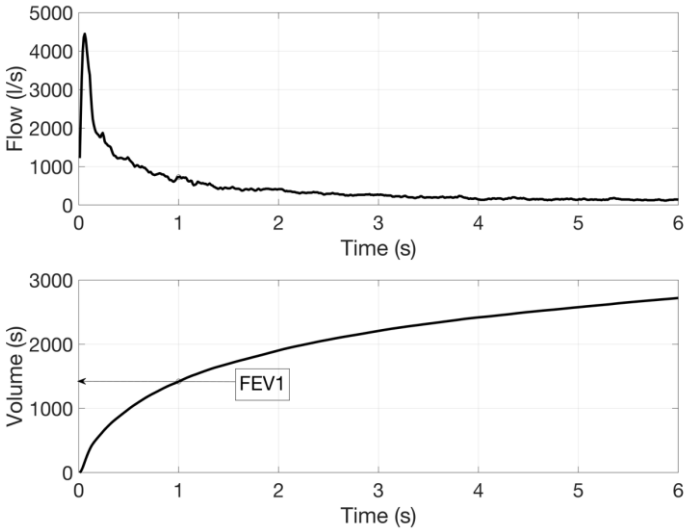

Figure 2. Illustration of a flow and a volume curve during dynamic spirometry. FEV_1=Forced expiratory volume in one second.

Impaired lung function can largely be divided into be obstructive or restrictive (37). A normal and an obstructive pattern with a very low FEV_1 are illustrated in Figure 3.

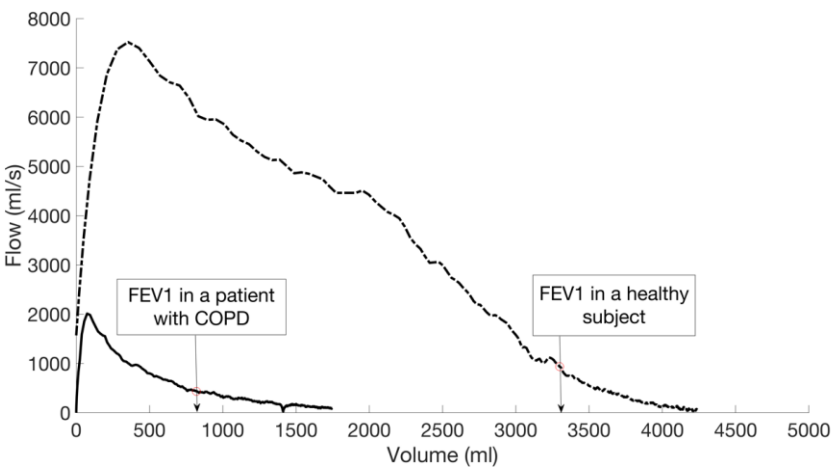

Figure 3. Flow/volume curves from dynamic spirometry on a healthy subject and a subject with chronic obstructive pulmonary disease (COPD). FEV_1=Forced expiratory volume in one secund. Arrows show the FEV_1 value, determined by the value on the x-axis.

Dynamic spirometry data must be interpreted in conjunction with appropriate reference materials, i.e., reference materials which consider the differences in sex, ethnicity, age, and height of the individual. Several reference equations for lung function have been previously published. However, the statistical models often do not meet the requirements for diversity between populations, are based on too few subjects, or built on too simple mathematical methods (8). There is no standardization of the use of reference material in Sweden today. In general, reference materials from Hans Hedenström (38, 39), Erik Berglund, European Coal and Steel (ECS) and ERS have been used for adults (40, 41), while Polgars, Solymar/Bjures or Zapletals have been used for children (42, 43, 44).

Using separate reference materials for children and adults cause discontinuity when a near 18-year-old is compared to one material or the other. To overcome this problem, new reference values based on spirometry data from several thousand infants, children and adults of Caucasian origin were published in 2008 and were presented as for "all ages" (8, 45). In 2009 further updates were made to this material and reference equations were based on both body size and age using the generalized additive model for location, scale, and shape (GAMLSS) technique (46).

In 2012 another update of "all ages" was presented by The Global Lung Function Initiative (GLI), which now includes ages between 3-95 and covers several different ethnicities (47). Thus, international guidelines recommend the use of population-specific reference values to eliminate the influence of ethnic variation in lung function. However, Backman et al. showed in 2015 that GLI reference values can give erroneous values regarding airway obstruction in Swedish females (48). In the study Obstructive Lung disease in Northern Sweden (OLIN) the authors also showed that lung function in the population of northern Sweden is better than predicted by GLI, since both the predicted mean values and lower limit of normality (LLN) for FEV_1 and FVC were lower in GLI compared to OLIN, and the ratio FEV_1/FVC was higher in both sexes and for average height across the whole life span. Their conclusion was that, when accessible, population-specific reference values may be better (49).

Further support for the need of population-specific reference equations for lung function assessment in the elderly was presented in 2009 by a research group in Croatia (50). Cross-Validation with existing reference

equations for the elderly showed that almost all European and American equations systematically overestimated lung function parameters in their respective populations.

In spirometry, the reference equations are often derived from healthy volunteers from selected age, sex, geographical, ethnical, and occupational groups. Volunteers with previous or current pulmonary symptoms, lung disease, injury or smokers are generally excluded. After satisfactory numbers of normal values, reference equations are developed and can be used to predict normal values for those who meet the properties in the assessed population (51). As most populations are getting older and older, there is a need to produce reference materials that accurately reflect lung function in an aging population. In doing this it is especially important to include a sufficient number of healthy, older persons of different height.

Modelling and interpretation of lung function

Several models of how spirometry variables change with age have been proposed (38, 39, 41, 52). One approach, as undertaken by Hedenström (38), is to apply multiple linear regression, where age, and possibly age^2, as well as height are included as independent variables.

$$y(age, height) = B_1 + B_2 * age + B_3 * age^2 + B_4 * height$$

Linear regression models may also be based on logarithmically transformed age and height data and/or on the interaction between these parameters, i.e., models where the dependency of height is not constant over all ages. However, these models are not ideal because predicted lung function will decay towards zero with high age (45, 49).

To overcome this problem, GLI included age-dependency in the spirometry variables (47) using the LMS method, which was also the chosen method to analyze the new data presented in this thesis. The LMS method is based on logarithmically transformed data and splines (46, 53) allowing the fluctuations of data over different ages to be described using three characteristics: the location (L), the mean value (M) and the scatter (S). The S corresponds to fluctuations around the M and is estimated by the coefficient of variation (CV), which in turn is given by the ratio between the M and standard deviation (SD). The L is an index of the skewness of the data.

The corresponding models for the age-related changes in the M and S are given by:

$$M(age, height) = \exp\left(B_0 + B_1 * \log\left(height\right) + B_2 * \log\left(age\right) + Mspline\right)$$

$$S(age) = \exp\left(B_0 + B_1 * \log\left(age\right) + Sspline\right)$$

Another model of interest in this thesis is the spline-based model suggested in the OLIN study. Upon inspection of the published model parameters (49), it can be noted that below 40 and above 80 years of age, the model consists of a quadratic dependency on age, a linear dependency on height and a linear interaction between age and height. Between 40-60 years of age and between 60-80 years of age the age dependency also includes a third order term.

Generally, assuming a normal distribution of the measured data, a measured value is considered to be normal if it falls within the 95 % confidence interval (CI 95%) limits of the reference population (corresponding to ±1.96 SD around the expected value). The lower limit of this range is referred to as lower limit of normality (LLN). In spirometry, traditionally this has not always been the case, and instead percentages of expected values have been used. This can lead to incorrect interpretations as normal values have a large variance at extreme young and advanced ages. A way to overcome this and to include the variance in the analysis is to express the measured value in terms of numbers of SDs from the expected value, also stated as *Z-score:*

Z-score = (measured value-reference value)/SD.

Opinions differ regarding where the limit of normality should be. ATS/ERS recommends that the expected value minus 1.64 SD, i.e., the 5th percentile, should be the lower limit of normality (54) while GLI considers that values that deviate from expected values more than 1.96 SD, i.e., lower than the 2.5th or higher than the 97.5th percentile, should be considered abnormal (47). Many laboratories use the mean value in the population minus 1.96 SD (2.5th percentile) as the lower limit of normality since it is considered that the 5th percentile conveys a risk of underdiagnosis of tall individuals or over-diagnosis in shorter or older individuals (55).

Figure 4 shows a comparison of the predicted lung function according to the GLI and OLIN reference values for female and male subjects of heights 164 cm and 178 cm respectively.

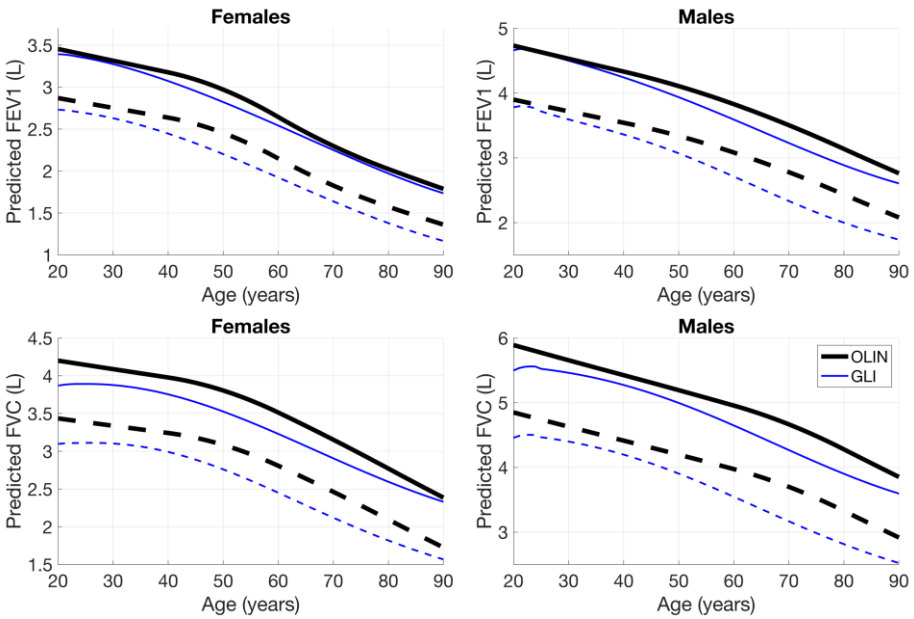

Figure 4. Reference values of FEV_1 and FVC at different ages in females (164 cm) and males (178 cm). Blue (thin) solid line represents GLI values, and black (thick) solid line represents OLIN. Dashed lines represent LLN for each model respectively. FEV_1=Forced expiratory volume for one second, FVC=Forced vital capacity.

Heart rate variability

HRV is a valuable physiological measurement that can provide insights into the ANS and cardiovascular health. HRV has extensively been used in various clinical research projects, such as for assessment of autonomic dysfunction in e.g., heart disease, diabetes, and sleep apnea. HRV has also been used for assessment of risk of sudden cardiac death or lethal arrhythmias after acute myocardial infarction and in patients with heart failure. In Umeå, HRV has also been used clinically for detecting and quantifying neuropathy in patients with hereditary transthyretin amyloidosis (ATTRv-amyloidosis).

During a normal heart rhythm (sinus rhythm), there are minor fluctuations in heart rate, i.e., the RR interval. The impulse formation by the SA-node is dependent on several physiological rhythms and the ANS has an important role in "fine-tuning" the timing of each heartbeat. One common way to further understand the information hidden in the ECG signal is to analyze HRV. HRV is based on the sequence of time intervals obtained from the detection of each heartbeat. The heart rate is induced by the ANS as well as the respirational cycle and HRV is the variation in time between consecutive heartbeats. Normal HRV is dependent on the balance between the SNS and PNS. A high variability in heart rate is normally a sign of good adaption, i.e., a healthy individual with well-functioning autonomic control mechanisms (12). An example of this is illustrated in Figure 5.

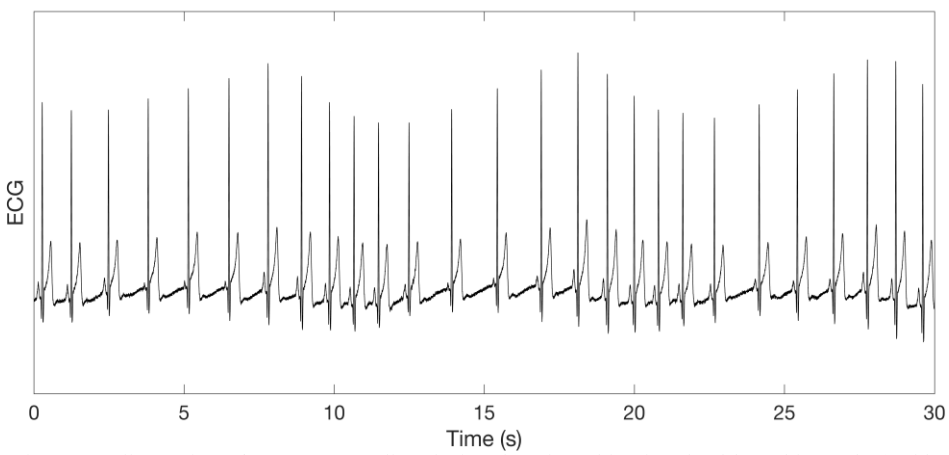

Figure 5. Illustration of an ECG recording during deep breathing in a healthy subject. The "rubber band effect", i.e., differences in length of the RR intervals, reflecting normal sinus arrythmia is visible.

However, high variability can also be a sign of cardiac arrhythmias, such as frequent extrasystolic beats and atrial fibrillation (AF), as these arrythmias can also causes large fluctuations in heart rate (56).

A low HRV is often an indicator of abnormal cardiac autonomic modulation by the ANS as presented in Figure 6 (57). Low HRV can also be due to increased heart rate during stress or exercise. An important aim of this type of analysis is to clarify how the SA-node is affected by the autonomic nerve function, and therefore, the beginning of the P-wave is logically the natural starting point in the cardiac cycle. However, the

beginning of the P-wave is very difficult to determine with sufficiently high accuracy due it's the low amplitude, thus, instead the QRS complex is used as the starting point for HRV analysis. This method is generally accepted because the length of the PR intervals is assumed to be relatively constant. It is therefore assumed that the RR intervals are also able to reflect the activity of the SA-node (10).

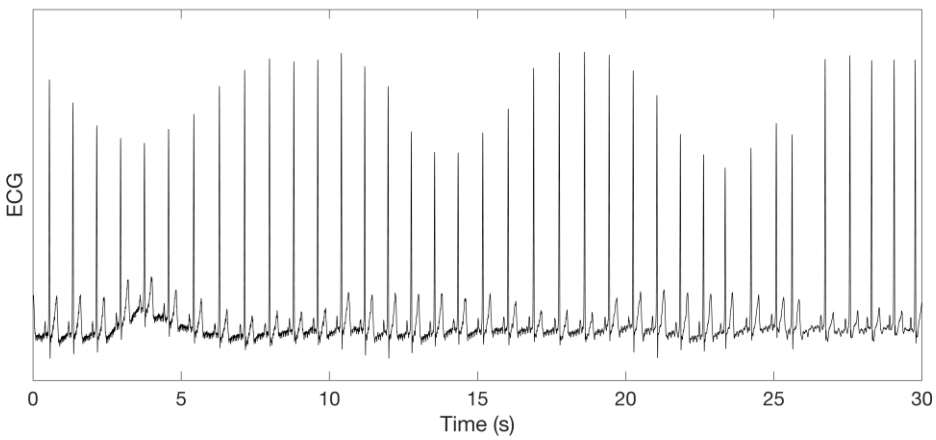

Figure 6. Illustration of an ECG recording during deep breathing in a patient with ATTRv. A pronounced respiration variation in amplitude is seen, but no "rubber band effect" in RR intervals are noted.

As previously mentioned, there are different ways to measure HRV; the time domain, the frequency domain (spectral analysis) and non-linear methods (58). The frequency domain analysis method requires mathematical transformation of the variation between RR intervals in the ECG, i.e., the time interval between two consecutive heartbeats into the corresponding power spectral density (ms^2). With spectral analysis, the rhythms that reflect how different physiological mechanisms affect the ANS modulation of the heart rate can be identified (59).

The area under the power spectrum corresponds to the total variability around the mean value, which is equivalent to the variance of the RR-intervals. In spectral analysis of HRV, the area within different frequency intervals is calculated and the power spectrum is normally divided into three spectral regions (60). The very low-frequency region (VLF, below 0.04 Hz) reflects slow variations in heart rate such as fluctuations due to

the renin-angiotensin system or thermoregulation (12). The low frequency region (LF, 0.04-0.15 Hz) is associated with blood pressure control of peripheral vascular resistance mediated by baroreceptors, and it reflects both parasympathetic and sympathetic activity (61). The high frequency region (HF, 0.15-0.40 Hz) reflects the parasympathetic activity related to normal respiration. The ratio LF/HF is used as a marker for sympathetic vagal balance and high values indicate a dominant sympathetic activity (12, 58, 62). The power in the spectrum between 0.005-0.50 Hz is defined as total power (PTOT). Figure 7 shows a typical HRV recording and power spectra in a healthy subject in the supine and upright position, respectively. In the supine position the spectrum is dominated by the HF component which is also found in the spectrum for respiration. In the upright position there is a shift of the main peak to the LF component.

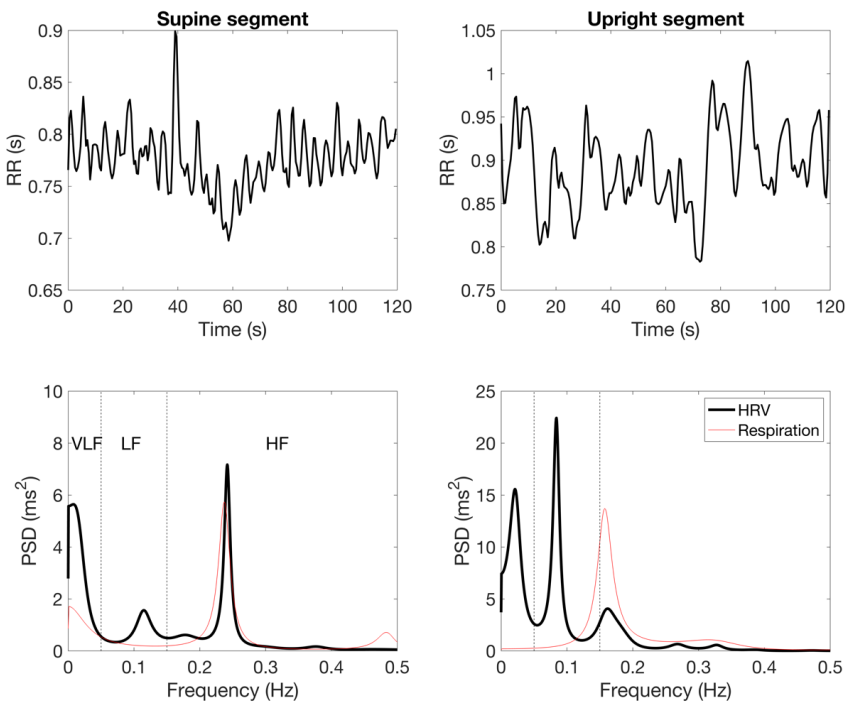

Figure 7. Examples of HRV in the supine and upright position. Upper panels show variations in RR intervals and lower panels show the corresponding power spectra for HRV and the simultaneously recorded respiration signal. Y-axis on the upper panels shows RR intervals and time (s) on the X-axis. In the lower panel the Y-axis presents the power in the spectrum (ms^2) and X-axis the frequency (Hz).

In spectral analysis of HRV, short-term recordings are used, and the analysis is performed on the RR intervals and the distances between heartbeats is measured for a certain duration (e.g., 2-5 min segments).

The time domain analysis is performed using the original RR intervals. Based on those, various statistical measures are calculated, such as the mean RR interval, the standard deviation (SD) of all RR intervals (SDNN), which reflects all variability regardless of frequency (strongly correlated with PTOT), and the root mean square of successive differences (RMSSD) which is an index that reflects parasympathetic function (strongly correlated with HF) (10).

HRV is often analyzed in short-term recordings during rest or different autonomic provocations, such as deep breathing and posture changes, and the analysis is normally performed in segments with duration of up to 5 minutes. Another alternative is to analyze HRV in ambulatory ECG recordings (24-hour recordings). With 24-hour registrations, the measurements are calculated for the entire 24-hour period or for certain parts (day, night, hourly averages). The choice of protocol and analysis method varies between studies.

Normally, activity in the two branches of the ANS is a dynamic balance, changing rapidly based on changing demands on the systems such as stress, physical activity, metabolic processes, and hormone secretion. Based on various complex theories, it is believed that adaptability and stability are maintained through the dynamic processes between these systems, and that this dynamic variability is needed to maintain health (63).

Characteristic autonomic imbalances include a hypoactive PNS and hyperactive SNS. HRV measurements and analysis can assess those imbalances. The vagus nerve is a key component of the PNS and plays a crucial role in regulating heart rate and autonomic balance. Inflammation can influence vagal tone, which is the activity level of the vagus nerve. Reduced vagal tone is associated with reduced PNS influence and diminished HRV (64). Moreover, imbalances are associated with various pathological conditions, and decreased HRV is associated with mortality (9, 63).

Rheumatoid arthritis

Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease that is associated with a higher risk of mortality than the general population. Currently 0.5 to 1 % of the world's population is currently affected. Rheumatoid arthritis is a chronic polyarthritis and early RA tends to affect smaller joints first, in particularly fingers and toes, and this is commonly the main symptom (65). It is more common in females than in males and usually starts around the age of 30-50 years. Rheumatoid arthritis can affect more than just the joints, the disease can damage the skin, eyes, lungs, heart and vessels (65). Inflammation in RA is mainly focused in the synovium of the joints and is thus called synovitis. Synovitis is initiated by penetration of adaptive immune cells into the synovial lining. The synovial lining becomes hypertrophic and cells from the synovium are stimulated to produce cytokines and chemokines. This consequently causes joint destruction and creates a positive feedback mechanism which maintains the inflammation. An autoimmune response in the synovium is activated due to CD4+ T-cells interacting with B-cells (66). The exact cause of the autoimmune response in RA is not fully understood, but it likely involves a combination of genetic predisposition, environmental triggers, and hormonal factors (67).

Disease activity in RA can be measured in different ways including clinical examinations with findings such as tender and swollen joints, patients' assessment of pain using the visual analogue scales (VAS), analysis of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and disease activity scoring using the disease activity score-28 method (DAS28) (68), as well as identification of specific serological markers.

In order to determine the diagnosis of RA, examinations of symptoms are required which must be determined with a clinical examination, blood testing and X-rays. Since 1956 different classification criteria has been used in different settings. In 2010, the European league against rheumatism and the American college of rheumatology developed the criteria used today. The criteria are based on four different areas: joint involvement, serology, acute-phase reactants, and duration of symptoms (69).

Treatments for RA often focus on modulating the immune response to reduce inflammation, manage symptoms, and slow down the progression

of the disease. These treatments include disease-modifying antirheumatic drugs, biologic therapies, and targeted synthetic therapies that aim to suppress the immune systems abnormal activity (65).

Rheumatoid arthritis and cardiovascular disease

Cardiovascular disease is highly prevalent in patients with RA. Earlier studies have reported that patients with RA have a higher risk of developing CVD and the risk of mortality due to CVD is increased by up to 50 % compared to the general population (70, 71, 72, 73). There are tools to evaluate the risk of cardiovascular events. The existing ones are based on traditional risk factors which are divided into modifiable (obesity, hypertension, smoking, diabetes, increased lipids, physical activity, and alcohol consumption) and fixed (family history of CVD, age, and male sex) (74). There are also tools considering, inflammation measured as CRP or the RA-disease itself (ERS-RA). However, none of the risk assessment tools today have been shown to correctly predict true risk for CVD in patients with RA (75).

The European league against rheumatism has recommended that for the assessment of risk for cardiovascular events in patients with RA, the factor should be multiplied by 1.5 compared to the general population (76). The processes causing patients with RA to have a higher risk of CVD are not fully understood, however, apart from the traditional risk factors, inflammation and related processes may offer the explanation (77). Inflammation in combination with e.g., hypertension, has been shown to increase the risk of CVD, and the prevalence of hypertension is higher in patients with RA compared to the general population (78, 79). Disease duration, the follow-up time, and cohort types have also been shown to impact the development of CVD in patients with RA (80, 81, 82).

Ischemic heart disease

Ischemic heart disease (IHD) is a medical condition characterized by decreased blood supply to the myocardium due to stenosis or blockage of the coronary arteries. Situated on the epicardial surface, the coronary arteries form a tree of arteries, with the diameter of the vessels varying from 250 μm to 5 mm (83). Decreased coronary blood flow deprives the heart muscle of oxygen and nutrients, leading to angina, often manifesting

as chest pain, or, in severe cases, a myocardial infarction. Ischemic heart disease is common and can lead to premature death and reduced quality of life. Several complicated pathophysiological mechanisms are involved in IHD; atherosclerosis, inflammation, coronary microvascular dysfunction, and vasospasm (83). When the coronary artery is obstructed with an atherosclerotic plaque the definition for CAD is fulfilled. Additional common diagnoses are stable angina and acute coronary syndrome, which in turn are divided into ST-elevated infarction and unstable coronary artery disease. The latter includes unstable angina and non-ST elevated infarction (84).

Manifestations of IHD can range from sudden cardiac death or intensive chest pain with constitutional symptoms, to a complete absence of symptoms i.e., silent ischemia. In both females and males, the classic symptom of a ST-elevated infarction is persistent chest pain with radiation of pain to the arms, neck, or lower jaw. Depending on the location of the ischemic area, the pain can also be located in the epigastrium or the back. In comparison with males, females more commonly have atypical chest pain, fatigue, or dyspnea, as well as pain in the shoulders, neck, back and the jaw (85).

Various diagnostic tests to assess and diagnose IHD are used i.e., ECG, biomarker cardiac Troponin I and T (specific cardiac protein), stress testing, coronary angiography, and cardiac imaging. The medical history of the patient is also essential when diagnosing IHD. This applies, in particular, to the type of pain, time course and triggering factors, as well as the patient´s cardiovascular risk profile. Corresponding to the Fourth Universal Definition, the classification of a myocardial injury is used when raised cardiac troponin (detected with blood testing) values are detected, with at least one value above the upper reference limit. Furthermore, the criteria for acute myocardial infarction is used when there is myocardial injury observed, and additional clinical indications of acute myocardial ischemia, i.e., symptoms, ECG deviations, pathological findings in imaging and identifications of thrombosis by angiography (86).

Different types of myocardial infarction exist, with types 1 and 2 being the most clinically significant. Type 1 is defined as ischemic injury due to acute coronary syndrome (86). The main underlying mechanism of acute coronary syndrome is an atheromatous lipid-stored inflamed plaque that

ruptures. Platelets perceive the rupture as a vascular lesion and consequently activate the coagulation system which leads to the formations of a thrombosis. This thrombosis subsequently threatens to occlude the coronary artery (87). Generally, the formation of atheromatous plaques begins in adolescence with accumulating cholesterol in the walls of vessels. Low density lipoprotein cholesterol oxidates, and macrophages activate starting inflammatory processes. These processes eventually weaken the plaque which makes it prone to rupture (88). Risk factors for IHD can be divided into modifiable and non-modifiable. Modifiable risk factors include e.g., smoking, diabetes, hypertension, abdominal obesity, dyslipidemia, and psychosocial stress. Non-modifiable risk factors are old age, male sex, rheumatic diseases, and genetically inherited factors (74). A type 2 myocardial infarction is defined as ischemic injury caused by an imbalance in the oxygen supply in the coronary circulation due to an acute stressor such as sustained tachyarrhythmias. Females present with a higher rate of myocardial infarction type 2 (86).

Treatment strategies for IHD aim to reduce symptoms, prevent complications, and decrease the risk of future events.

Age, disease and heart rate variability

HRV normally decreases with age (89). In normal aging an impact on the ANS reduces the regulations of the PNS and SNS systems, which manifests as changes in HRV. Obesity and pregnancy can also affect the reactivity of the ANS (90, 91). Previously published studies have shown decreased amplitude of the heart rate oscillations at all frequencies in older people (92, 93, 94). A meta-analysis also found lower mean RR-interval times and less total power in females (94).

HRV is affected by e.g., different conditions such as, in cardiovascular, neurological, rheumatic, and respiratory diseases, as well as in conditions resulting in chronic pain, psychological disorders, drug treatment and intoxications (95, 96, 97). Autonomic nerve damage can be caused by a primary disease of the nerve cell or by a disease that causes a damaging process in the peripheral or central autonomic nerves, such as multiple sclerosis. Autonomic neuropathy can manifest as arrhythmias, bladder disorders, postural hypotension, gastrointestinal disorders and sexual

dysfunction (97). Of the peripheral neuropathies, diabetes mellitus and ATTRv-amyloidosis are the most common conditions investigated with HRV. Spectral analysis of HRV in patients with ATTRv-amyloidosis and diabetes mellitus has shown that both SNS and PNS function is gradually reduced with disease progression (19, 98). When the ANS is affected, a dysfunction is obtained which leads to a decrease in HRV. This decrease can be in a singular component of the HRV spectrum or an overall general reduction (19).

Figure 8 shows a short-term HRV recording in a subject with reduced HRV. Note the absent beat to beat variation which reflects reduced parasympathetic activity. The heart rate is markedly increased after tilt, which indicates a functioning sympathetic activity. In cases with severe autonomic dysfunction, heart rate is nearly constant and does not change during autonomic provocations.

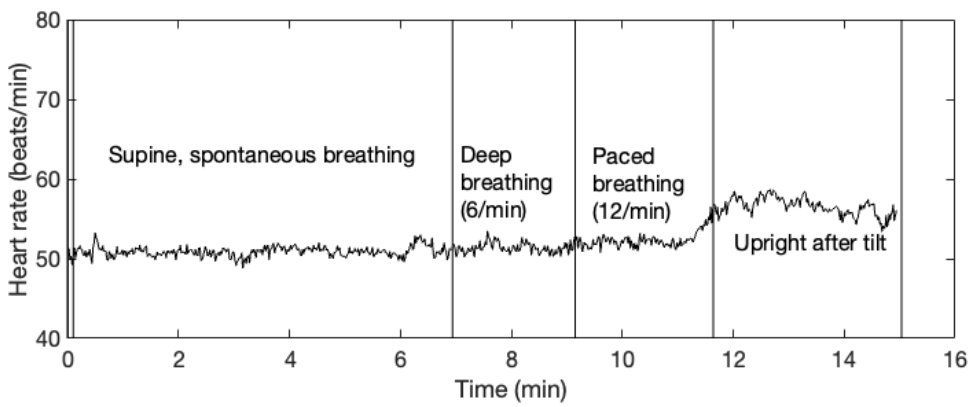

Figure 8. Illustration of fluctuations in heart rate during a short-term recording of HRV in one of the females with IHD that presented with reduced HRV. The four sections divided by vertical lines correspond to different parts of the investigation procedure.

The ANS is a regulatory system which plays and important part in maintaining homeostasis. Signs of autonomic dysfunction have been found in patients with RA (99). Existence of ANS dysfunction may be associated with autoantibodies pointed against ANS structures (100). Some, but not all, previously published data have shown that changes in ANS were associated with levels of inflammatory markers ERS, CRP and DAS28 (101). Cardiovascular involvement in RA can lead to changes in the hearts structure and function, which may affect HRV (102).

RA is associated with chronic systemic inflammation, and inflammation plays a significant role in the development and progression of IHD. Inflammation affects the ANS, leading to an imbalance between SNS and PNS and this imbalance can lead to a decreased HRV in several ways (103, 104). In response to infections, injuries, or autoimmune reactions, the immune system releases cytokines such as Interlukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha). These cytokines can influence the activity of autonomic centers in the brain, leading to alterations in autonomic balance (105). Additionally, chronic, or excessive SNS activation can disrupt the balance between SNS and PNS activity, including possible suppression of the PNS. The inflammatory responses, particularly during chronic illnesses, can directly lead to reduced activity of the PNS as well. Suppression of PNS can lead to decreased HRV and reduced ability to recover from stress (106).

Low HRV has been seen in patients with RA indicating cardiac autonomic dysfunction (107). Previous studies have shown that lower HRV is associated with development of hypertension and diabetes in this patient group, where some even arguing that the autonomic dysfunction leads the development of RA (108). Furthermore, psychological factors such as chronic pain, fatigue, and reduced quality of life in RA patients can lead to psychological stress (109). Individuals with IHD may aswell experience significant stress and anxiety which, in turn, can reduce HRV and negatively impact HRV by influencing the activity in ANS and cardiac health.

Over the past decade HRV has been extensively studied in patients surviving an acute myocardial infarction. In patients with a history of myocardial infarction, HRV is used in risk stratification and earlier studies have shown an increased risk of mortality, within few years after a myocardial infarction, in patients with decreased HRV. An abnormal HRV can predict sudden as well as non-sudden cardiac death after myocardial infarction. Reduced HRV is an independent risk factor for cardiac death, with the same predictive value as reduced left ventricular function and arrhythmias (19, 110, 111). Spectral analysis also shows reduced HRV, particularly decreased HF, in patients with CAD without previous myocardial infarction or heart failure. Clinically it results in reduced vagal tone and increased sympathetic output (19).

HRV is a means to understand the stability of the SNS and PNS in relation to autonomic nerve function. The alteration between each heartbeat is high in PNS activation and low in SNS activation. HRV gives a good prognosis in terms of arrhythmias and a low HRV can indicate cardiovascular diseases (95, 112). By understanding how HRV changes in different diseases and, in turn, which components, i.e., which branches of the ANS are affected, diagnostics and treatment could be facilitated.

Research rationale

Clinical physiological examinations are valuable for investigation of symptoms which correlate with heart and/or lung function. In our days, we become older and older, so it is imperative to understand and identify normal cardiovascular changes which occur with aging. It is important to correctly assess the ability of diagnostic methods to distinguish between changes due to a suspected heart and/or lung disorder and changes due to normal aging. The use of reference materials is prerequisite to be able to interpret investigations within clinical physiology. Additionally, reference materials help ensure that results from examinations are of high quality and that the methods provide reliable and consistent results.

Therefore, a special focus in this thesis is on reference values, they are a basis for reliable diagnoses. Since the dimensions, weight and size of the heart and lungs are not comparable between females and males, and not consistent throughout life, sex- and age-specific reference materials are essential. Given that multicultural communities become more common, and that we live longer than ever, it is of great importance that reference materials also evolve.

Another focus in this thesis has been on developing the knowledge about the relatively new and complex examination of HRV and how it is expressed in healthy subjects and in patients with two different diseases: RA and IHD. As health care moves towards personalized medicine, early sex-specific knowledge about changes due to disease becomes even more important. Tailoring treatments and interventions based on individuals' sex, age and physiological profile, in combination with early screening, can hopefully lead to better health outcomes in the future.

Objectives

Overall Aim

The purpose of this thesis was to further describe the properties of commonly applied clinical physiological investigations, in regard to age and sex, when assessing the cardiovascular and pulmonary systems of healthy individuals. Additionally, this thesis aimed to assess the relationship between HRV and two different disease states, cross sectionally and longitudinally.

The specific aims were:

Paper I: To assess age and sex differences in 12-lead ECG at rest and 24-hour ECG in subjectively healthy subjects. In addition, to conduct a 20-year follow-up study to assess whether ECG deviations observed at baseline were related to development of CVD.

Paper II: To compare observed lung function, based on dynamic spirometry measurements, in a cohort of subjects from Northern Sweden to two discordant reference materials of lung function.

Paper III: To evaluate the longitudinal change of cardiac autonomic function by analysis of HRV in a cohort of patients with RA. Furthermore, to investigate potential associations between HRV and RA disease activity, development of hypertension and the long-term development of CVD in patients with RA.

Paper IV: To assess overall long-term survival in females with IHD compared to healthy controls. Furthermore, to assess the significance of cardiac autonomic function on prognosis in females with IHD.

Materials and Methods

General Overview

Table 1. Overview of included methods and materials of the papers.

Paper	I	II	III	IV
Methods				
Analysis of ECG	X			
Analysis of spirometry		X		
Analysis of HRV			X	X
Assessment of cardiovascular diseases	X		X	X
Age-dependency	X	X		
Sex-dependency	X	X		
Longitudinal	X		X	X
New mathematical modelling		X		
Materials				
UGPHS subjects	X	X	X	X
HRV reference material			X	X
RA patients			X	
The women's heart trial				X

ECG=Electrocardiography, HRV=Heart rate variability, UGPHS=Umeå general population heart study, RA=Rheumatoid arthritis.

Study populations

Umeå General Population Heart Study

Paper I, II, III and IV

In all four studies subjects from the Umeå General Population Heart study (UGPHS) were included. UGPHS was developed as a reference material for echocardiographic examinations in the late nineties. A randomized sample of 1000 individuals was taken from the National Swedish Tax Agency's birth records, including 500 females and 500 males. All living in the county of Västerbotten. These individuals were divided into five-year age intervals, depending on the year of birth (1905,1910,1915,1920 up to 1975). Totally 15 age groups resulted from this stratification. They

received a letter regarding the study's content as well as a request to participate. The inclusion criteria were absence of any known cardiovascular or systemic disease and no use of medications that could possibly affect heart and lung function. This was examined via a telephone interview by a specialized research nurse. Subjects with diabetes, hypertension, hyperlipidemia, stroke, transient ischemic attack, previous rheumatic fever, and intermittent claudication were excluded. Hypertension was defined as blood pressure >160/90 mmHg (in paper I) due to the wide age range. If an individual was willing to participate and met all the study criteria, a specially designed questionnaire was sent before the investigations began.

Different examinations were performed at different time points between 1998-2000, resulting in diverse numbers of subjects in the different examinations. In paper I, a total of 219 subjects were included. Of those, 196 subjects were examined with 12-lead ECG at rest and 112 with long-term ECG, 89 subjects performed both examinations. Paper II included spirometry's from 284 subjects. HRV was recorded in 126 subjects and two subsets were included as age and sex matched controls in paper III and IV respectively. In paper III we used 43 females and 13 males as controls and in paper IV 36 females were used as controls. Figure 9 presents study enrolment from UGPHS population.

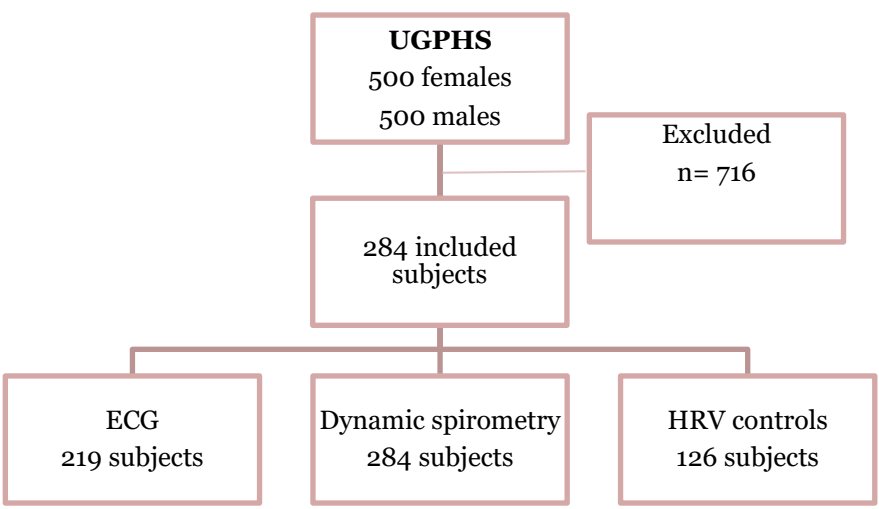

Figure 9. Study enrolment from the UGPHS population.

Clinical Physiology HRV controls

Paper III and IV

In paper III and IV, HRV data were obtained both from UGPHS and from the reference material used at the department of Clinical Physiology, Umeå University hospital. This data consisted of 90 subjects (n=45 females) in the age range 20-80 years and, was recorded between 1994-1996. The subjects comprising this reference material were randomly selected from the population register (subjects > 50 years of age) or recruited mainly among hospital staff (60, 113). The subjects did not have any diagnosis of cardiac arrhythmias or neurological disorder, were not treated with medications affecting blood pressure or heart function and all were normotensive (≤140/90 mmHg).

Cardiovascular morbidity in patients with RA

Paper III

Paper III originates from the project "Cardiovascular morbidity in patients with RA" (114, 115, 116) which was a prospective case-controlled study on an inception cohort of patients with newly diagnosed RA conducted at the Rheumatology department, University Hospital of Umeå, Sweden, during the years 2000-2004. The study included 50 patients, all of which were younger than 60 years of age and living in the county of Västerbotten, Sweden. One hundred subjects (76 females and 24 males) were used as controls. Two age-matched controls per patient were selected. The controls for this study originated from UGPHS and from the reference material used in HRV examinations at the department of Clinical Physiology, Umeå University Hospital, Sweden, A total of 33 females and 11 males were used from the HRV reference material, and 43 females and 13 males from UGPHS.

The Women´s heart study

Paper IV

The Women´s heart study was a randomized controlled trial conducted during the years 1997-1999. The study included 197 females with IHD, all of which were younger than 80 years of age and living in the county of

Västerbotten, Sweden. The inclusion criteria included that all subjects, from 1996 onwards, should have had either; their first or recurrent incident of acute myocardial infarction; or had been subjected to coronary angioplasty or coronary artery by-pass graft surgery; or, had angina pectoris with CAD confirmed by angiography and treated non-invasively. Of the 197 females that were included, 93 underwent HRV examinations. A total of 141 female subjects were included as controls, and 70 of those underwent HRV examinations. The controls were derived from both UGPHS (36 females) and the HRV reference material (34 females) used at the department of Clinical Physiology, Umeå University Hospital, as described above (Figure 10).

Summary of included subjects

Table 2 presents age and sex distribution of all included subjects in all four studies. Figure 10 shows the enrolment of HRV controls in Papers III and IV.

Table 2. Age and sex distributions of the subjects in all four papers.

	Age (years) Mean (SD)	**Age (years)** Min-max
Paper I		
Females (n=100)	60 (18.9)	25-90
Males (n=119)	59 (19.6)	23-90
Paper II		
Females (n=136)	56 (18)	25-90
Males (n=148)	56 (18.7)	23-90
Paper III patients		
Females (n=38)	44(11.1)	18-60
Males (n=12)	54 (7.5)	35-60
Paper III controls		
Females (n=76)	45 (12.6)	22-65
Males (n=24)	53 (7.6)	35-61
Paper IV patients		
Females (n=197)	61 (8.4)	35-77
Paper IV controls		
Females (n=141)	58 (13.4)	35-80

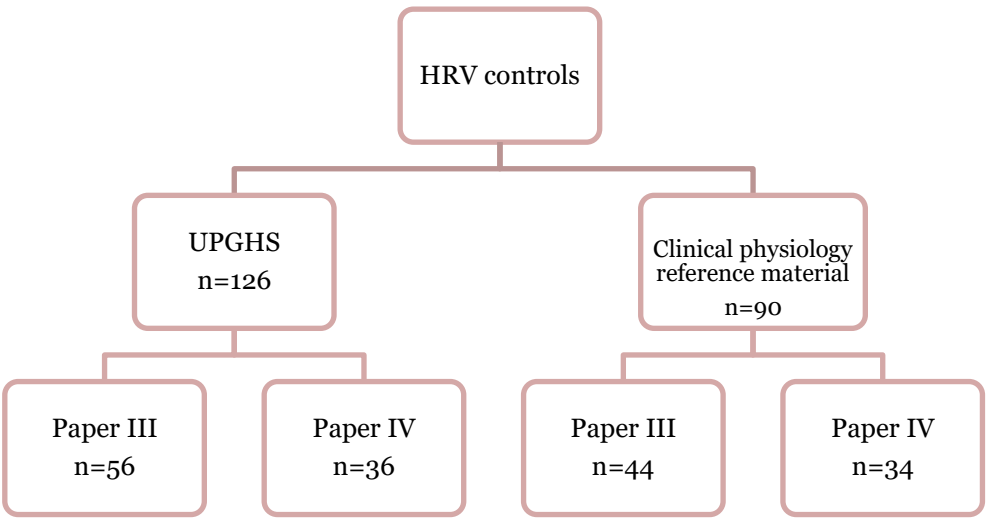

Figure 10. HRV controls enrolment in Paper III and IV

Ethical considerations

All studies were approved by the regional ethics review board in Umeå, Paper I, Dnr: 2017-537-32 M, addition to 98-129. Paper II, Dnr: 98-129, Paper III, Dnr:05-068 M. Paper IV, Dnr: 2021-02720, addition to 97-75. All participants provided written informed consent to participate in the studies. All studies were performed in accordance with the declaration of Helsinki.

Diagnostic methods

The studies in this thesis include data from several clinical and laboratory examinations. These are described in the following section.

12-lead ECG at rest (Paper I)

Twelve-lead ECG at rest examinations were performed with the subjects in supine position. The precordial electrodes (V1-V6) were placed in anatomical positions at the subject's chest as follows: V1: intercostal space 4 dexter parasternal, V2: intercostal space 4 sinister parasternal, V3: between V2 and V4, V4: intercostal space 5 sinister medio clavicular line, V5: anterior axillary line, V6: middle axillary line. The extremity

electrodes Yellow: left wrist, Green: left foot, Red: right wrist, Black (earth): right foot. A paper speed of 50 mm/s and gain setting of 10 mm range, and a test signal of 1 mV was used. The leads were presented in the Cabrera format (117). The interpretation was made according to the Minnesota Code Manual for ECG at rest (118). The analysis was manually performed by one investigator (SE) based on the following definitions and criterias as presented in Table 3.

Table 3: ECG parameters and criteria for classification.

Parameters	Criteria
Frequency	Normal frequency: 50-100 beats/min, tachycardia > 100 beats/min, bradycardia < 50 beats/min.
Rhythm	Regular, sinus rhythm/irregular, AF/flutter.
Focus	Sinus rhythm, ectopic rhythm, extra systoles.
P wave	Positive in I, II, avF and V3-V6. Form: soft and monophasic, Duration: 0.12 s, Amplitude: <2.5 mm.
PQ duration	Short <0.12 s, normal 0.12-0.22 s, extended >0.22 s.
QRS complex	Amplitude: sum of S-wave in V1 or V2 and the R-wave in V5 <3.5 mV, Duration: 0.06-0.10 s, Morphology: RBBB, duration >0.12 s and with an appearance like an M in V1-V2 and a wide S-wave in V5. LBBB duration >0.12 s and with an appearance like a deep and wide V (S-wave) in V1-V2 and a M-shaped complex in V5-V6.
Axis deviation	Normal: 0-90 degrees, left: < 0 degrees, right: >90 degrees
ST section	Measured in the J-point in leads V1, V5 and V6. Pathological if the form was elevated or reduced ≥ 0.1mV
Q wave	Normal duration: < 0.03 s.
R wave	Progression
T wave	Positive in leads I, II and V3-V6
QT duration	Normal: Females: 390-460 ms, males: 390-450 ms. Corrected according to Bazzets formula: QTc females abnormal > 460 ms and >450 ms for males.
U-wave	Absent, present

AF=Atrial fibrillation, RBBB=Right bundle branch block, LBBB=Left bundle branch block.

24-hour long term ECG (Paper I)

Twenty-four-hour long-term ECG was performed with three channels recording, using five color coded electrodes on the subject's chest. Exploring lead V2 (brown) was placed in the fourth intercostal space adjacent to sternum on the right side, the other exploring lead V5 (red) was placed in the fifth intercostal space in the anterior axillary line on the left side. The reference electrodes were placed beneath the right (white) and left (black) clavicle, and the green electrode used as grounding lead

was placed at V5R position. Testing was conducted using a Braemer DL 700 digital ECG recorder (Braemer Inc., Burnsville, MN USA). Subjects were instructed to follow their daily routines and note in a diary any 'heart related' symptoms, as well as time and duration of physical activities and wake up and sleep time.

The analysis was made automatically by the Aspect Holter system software (GE Healthcare Sverige AB, Danderyd, Sweden) and then edited and examined by one investigator (SE). The interpretation was made according to Bjerregard (28, 119) and the long-term ECGs were evaluated as presented in Table 4.

Table 4: Classification of normal and abnormal findings according to Bjerregard(119).

Normal	**Abnormal**
Sinus rhythm	AF, Flutter, Nodal
Lowest heart rate<50 beats/min (based on 1 min)	Lowest heart rate <40 beats/min (based on 1 min)
Pause ≤ 2 s	Pause> 2 s, AV-block II, III SA-block
PAC	20–40 y:> 10 PAC/24 h 40–60 y: >100 PAC/24 h 60–80 y: >1000 PAC/24 h
2 different PAC morphologies	>2 different PAC morphologies
>50 years; paroxysmal SVT	≤ 50 y: paroxysmal SVT >50 y: >2 SVT episodes or SVT longer than 10 beats
PVC	≤ 50 y:> 100 PVC/24 h >50 y: > 200 PVC/24 h
2 different PVC morphologies	>2 different PVC morphologies
>50 years: single paired PVC	≤50 y: single paired PVC PVC in bigeminy R on T PVC VT

PAC=Premature atrial contraction, SVT=Supraventricular tachycardia, PVC=Premature ventricular contraction, VT=Ventricular tachycardia. Definitions: SVT; 2 or more ectopic beats and heart rate > 100 beats/min. VT; > 3 PVC consecutive and heart rate > 100 beats/min.

Dynamic spirometry (Paper II)

Prior to spirometry, the subject's sex, age, height, weight, and ethnicity were noted. Dynamic spirometry was performed with the subject in a sitting position with the feet firmly on the floor and the neck slightly

extended. A nose clip was put on the nose and the subject was required to close his/her lips tightly around the mouthpiece. The procedure followed the ATS/ERS recommendations (36).

Dynamic spirometry started with a rapid and complete inhalation, followed by a rapid forced exhalation lasting at least six seconds. At least three, with a maximum eight, measurements were conducted, with a duplicability criteria of ≤ 5 % variation from the second highest value. The highest recorded FEV_1 value was used in the analysis. The maximum FVC value was used when the FEV_1/FVC ratio was calculated. Review of the reports was made by one investigator (SE) and those who had abnormal values were reviewed by a physician with expertise in lung function testing. The normal values were defined according to the ATS/ERS guidelines (36). The definition of airway obstruction was FEV_1/FVC < LLN, and the definition of small FVC was FVC< LLN. The LLN corresponds to the Z-score -1.64.

Model of the age-dependency in spirometry data

In Paper II, we determined a model for the observed lung function parameters in our cohort by rederiving the parameters in the LMS model (47) for FEV_1, FVC and FEV_1/FVC with different models for females and males, henceforward referred to as the rLMS model. We assumed that the data did not deviate from a normal distribution, i.e., used L=1. This hypothesis was verified by analyzing the residuals of the estimated model. The rLMS model was based on spirometry data from our cohort (UGPHS) and the output was compared with the published GLI reference values (47) and the OLIN model (49).

Heart rate variability (Paper III and IV)

Short-term recording of HRV

HRV for patients in Paper III was recorded at inclusion (baseline examination) and again after 5 years. In Paper IV, HRV was recorded at inclusion, approximately 4 months after their cardiac event.

All subjects were informed not to drink coffee or tea, eat a larger meal or smoke two hours prior HRV testing. Height and weight were noted, and systolic and diastolic blood pressure were collected at the time of each HRV recording. The definition for hypertension was blood pressure ≥140/90 mmHg, previous diagnose with hypertension and/or using antihypertensive medication at the time of measurement.

Prior to the examination, four ECG electrodes (single channel ECG) were placed on the right and left shoulders and the right and left hips. The examination began with the subject in the supine position. After six minutes with spontaneous breathing in supine position the subjects were asked to perform paced breathing, first with deep breathing at a rate at six breaths per minute for one minute corresponding to the breathing frequency 0.1 Hz, and then with normal spontaneous breathing at a rate of 12 breaths per minute for one minute. After the paced breathing procedures, a passive tilt of around 70 degrees towards the upright position was performed and sustained for four minutes with spontaneous breathing (Figure 11). Blood pressure was measured in the supine and upright positions with sphygmomanometer.

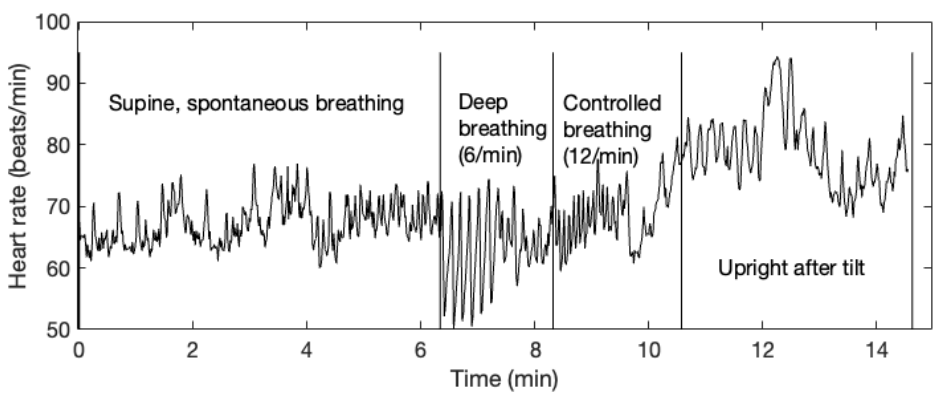

Figure 11. Short-term recording of HRV using the protocol utilized at the department of Clinical Physiology at Umeå University Hospital. This recording is from one of the females with IHD that presented with normal findings.

Recordings in the supine and upright position were analyzed as 2-minutes segments, and recordings with paced breathing were analyzed as 1-minute segments. All analyses used the spectral analysis method and were performed only on segments with normal sinus rhythm. Sequences with spurious arrhythmic beats or artefacts were manually replaced by linear interpolation. The irregularly sampled RR intervals were converted to equidistant samples by cubic spline interpolation followed by resampling at 2 Hz.

The power spectrum was estimated by autoregressive modelling (58). HRV was determined by assessing the total spectral power (0.005-0.50 Hz, PTOT), and the VLF, LF and HF components (Figure 7). The LF/HF ratio was measured as a marker of sympathovagal balance. Two-time domain indices were also used, SDNN and RMSSD. The analyze and interpretations were performed by specialist biomedical scientists and engineers at Umeå University Hospital.

Age and sex differences in HRV

The age dependency in HRV was estimated based on HRV recordings taken from the 201 subjects in the combined control data set (Figure 10).

First a linear regression model including age, sex and the interaction between age and sex was evaluated. However, no statistically significant interaction was found in any HRV parameter. Thus, no differences between males and females were found in the slope of the regression line.

Therefore, the final regression model included only age, where the estimated slope was used for age-correction of all HRV parameters.

Age-corrected HRV indices were calculated according to:

$$\hat{X}_{age,i} = X_i + \beta_1 \cdot (age_i - 50)$$

where age is expressed in years, and β_1 is the estimated slope of the regression line. Figure 12 shows the age-dependency in HRV in the supine position in controls. Solid lines show the estimated age-dependency and 95 % confidence intervals based on linear regression in all available controls in the age range 20-80 years (N=201, 103 males and 98 females).

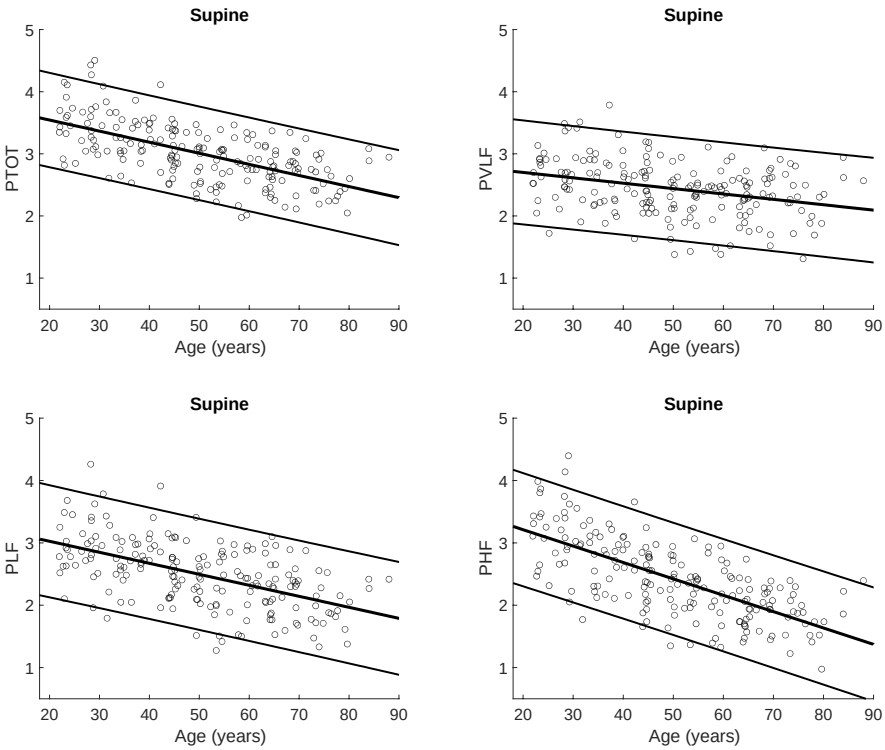

Figure 12. Age dependency in HRV in healthy controls in supine position. PTOT=Total power, PVLF=Power of the very low frequency component, PLF=Power of the low frequency component, PHF=Power of the high frequency component.

The corresponding slope for each HRV parameter and procedure is presented in Table 5.

Table 5. Data for calculation of age corrected HRV parameters. All HRV parameters were logarithmically transformed (base 10) before the slope of the linear regression line was determined.

	β_1 in different positions			
	Supine	**Deep breathing**	**Paced normal breathing**	**Upright**
PTOT (ms^2, log)	-0.018	-0.018	-0.022	-0.019
VLF (ms^2, log)	-0.009	-0.012	-0.012	-0.013
LF (ms^2, log)	-0.017	-0.019	-0.017	-0.023
HF (ms^2, log)	-0.027	--	-0.026	-0.021
LF/HF	0.010	--	0.009	0
SDNN (ms)	-0.009	-0.009	-0.010	-0.009
RMSSD (ms)	-0.013	--	-0.013	-0.010

$p<0.001$ for all test, except for LF/HF upright. PTOT=Total power, PVLF=Power of very low frequency, PLF=Power of low frequency, PHF=Power of high frequency, SDNN=Standard deviation of RR intervals, RMSSD=Root mean square of successive differences.

Figure 13 illustrates how HRV parameters in the supine position vary with age in females and males. Several HRV parameters showed a sex difference in the effect size, i.e., the intercept of the regression line. However, as seen in the figure the differences were very small.

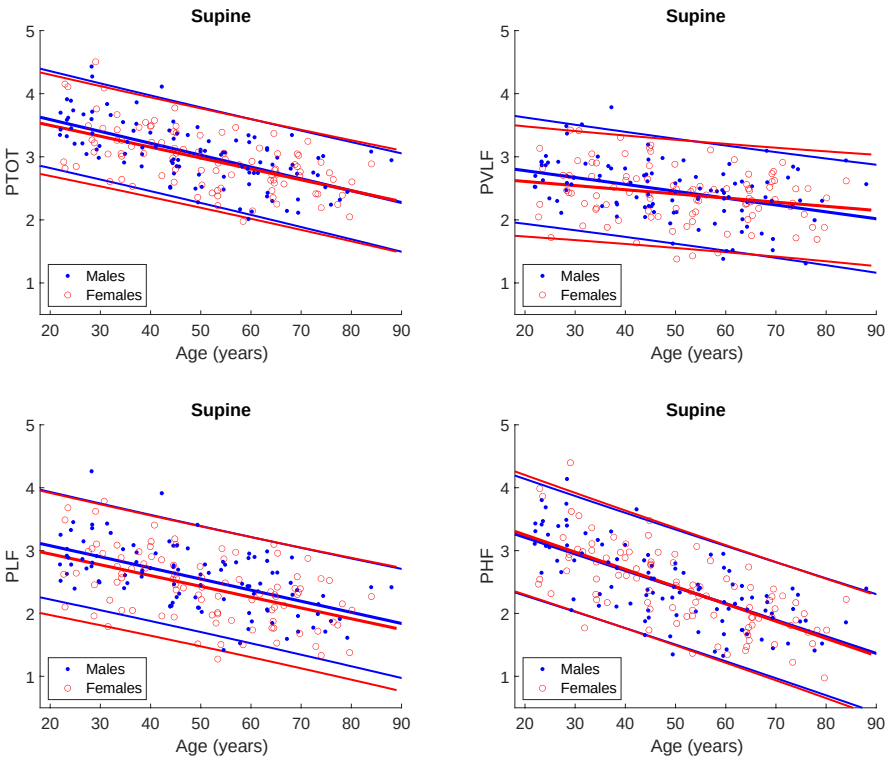

Figure 13. Linear regression with 95% confidence intervals of how HRV parameters relates to age and sex. PTOT=Total power, PVLF=Power of very low frequency, PLF=Power of low frequency, PHF=Power of high frequency.

Measurement of disease activity in patients with RA (Paper III)

Patients with RA donated blood at baseline and at five-year follow-up. All blood samples were separated into plasma, serum, and buffy coat cells by centrifugation at 4400xg for 15 minutes and stored at -80°C. After thawing the levels of IL-6 (ng/L) were measured in serum using ELISA (R&D Systems, Abingdon UK). Soluble CRP levels (mg/L) were measured according to routine methods at Umeå University Hospital, and ESR (mm/h) was measured according to the Westergren method. Calculation of DAS28 included ESR, number of tender and swollen joints and the visual analogue scale for global health (68). Pain was assessed using the visual analogue pain scale score (0-10)(120).

Medical record review (Paper I, III and IV)

In Paper I, a 20-year follow-up was performed. The follow-up was carried out by reviewing the subjects' medical records (Evry NCS care portal, version 5.11.8.1, Evry Sweden AB, Eskilstuna, Sweden), specifically assessing CVD and ECG abnormalities from date of inclusion to autumn 2019. Data from inpatients notes, visits to outpatients' clinics and health centers, as well as diagnostic codes were reviewed. Cardiovascular disease was defined as myocardial infarction, heart failure, angina pectoris, stroke, transient ischemic attack, intermittent claudication and, or valvular heart disease. Electrocardiographic abnormalities included conduction disorders, morphological deviations, supraventricular and/or ventricular arrhythmias and if they had received pacemaker or ICD therapy. Also, if the subject had a diagnosis of hypertension and/or diabetes, as well as vital status were noted.

In Paper III, eleven years after inclusion, a review of the patient´s medical record (Evry NCS care portal, version 5.11.8.1, Evry Sweden AB, Eskilstuna, Sweden) was performed. Cardiovascular events including myocardial infarction, stroke, transient ischemic attack, pulmonary embolism, deep vein thrombosis and if the patient had undergone heart valve surgery or coronary artery bypass graft were assessed. The review was performed as described by earlier publications (116, 121). All events were verified by experienced clinicians.

In Paper IV, a 20-year follow-up was performed by reviewing the subjects' local medical record system (Evry NCS care portal, version 5.11.8.1, Evry Sweden AB, Eskilstuna, Sweden) and recording the vital status. This follow up was performed in December 2022.

The controls vital status was assessed through both the medical record system and the Swedish Population Register. Only all-cause mortality and date of death were reported in this thesis. These follow-ups were performed on 2019-09-09 and 2023-09-01.

Statistics

In all manuscripts, continuous variables were presented as means and standard deviation (SD). For categorical variables, numbers (n) and percentages (%) were used. In all four studies $p \leq 0.05$ was considered as statistically significant.

Paper I

Chi-square tests were used for comparisons between baseline and follow-up findings as well as ages and numbers of arrhythmic events. For comparison of sex and age differences ANOVA for repeated measurement was used. Statistical analyses and data processing was performed in Microsoft Office Excel 2016 (Microsoft corporation., Redmond, WA, USA), Matlab (MathWorks Inc., Natick, MA, USA) and SPSS version 27 (IBM, Armonk, NY, USA).

Paper II

Association between age and height were assessed using linear regression. The evaluation of different models of spirometry data based on age was performed by predicting the mean and LLN at different ages. For each subject the corresponding predicted values and residuals were calculated based on the subjects age, height, and sex for all included models. The Kolmogorov-Smirnov test was used for testing the normality of the residuals. The one-sample t-test was used for testing the deviation from the mean. A two-sample Kolmogorov-Smirnov and ANOVA test were used for investigating the differences in the distribution of residuals. Z-scores were calculated for all variables. Density plots were used for testing the distribution of Z-scores. Statistical analyses and data processing were

performed in Microsoft Office Excel 2016 (Microsoft corporation, Redmond, WA, USA) and Matlab (Mathworks Inc, Natick, MA, USA). Computational analyses were performed using R version 4.1.3 and the GAMLSS package version 5.4-1.

Paper III

Comparisons between controls versus patients and patients versus patients at and between baseline and follow-up were performed using independent and paired t-tests respectively. Nonparametric Spearman correlation analysis was used when associating CRP, ESR, IL-6 and DAS28 with HRV-parameters. HRV values were logarithmically transformed using base 10 and age adjusted based on the controls age dependency. The results of the HRV analysis were presented as Z-scores and modelled by linear regression. Data processing and statistical analyses were performed in Matlab (Mathworks Inc, Natick, Ma, USA) and SPSS version 28 (IBM, Armonk NY, USA).

Paper IV

HRV data were age adjusted, based on the age-dependency results from the controls, and logarithmically transformed using base 10. Continuous data was presented as mean and SD. All-cause mortality was analysed by Kaplan-Meier survival curves, and the log-rank test was used for testing the differences between groups. Chi-square tests were used for comparing the number of subjects that were alive or not at the various timepoints (10, 15, 20 years). For comparison between the different groups, independent t-tests were used. Linear regression analysis was performed for testing the relationship between survival and HRV since there was no censored data regarding vital status (all included cases were followed during the whole study period of 20 years). The HRV indices were used as dependent variables and heart rate and vital status as independent variables. Data processing and statistical analyses were performed in Matlab (Mathworks Inc., Natick, Ma, USA) and SPSS version 28 (IBM, Armonk NY, USA).

Results and discussion

Paper I

Main results

Age-dependent changes were seen in certain ECG parameters (heart rate, PQ-duration, QTc and P-wave duration). Sex-dependent differences were also noticed, males showed increased QRS, PQ and P-wave durations and larger R+S amplitude compared to females as presented in Table 6.

Table 6. Mean (SD) of variables from standard 12-lead ECG at rest in three different age groups for females and males.

12-leadECG at rest	**Age < 50 y (23-50)**		**Age 50-65 y**		**Age > 65 y (65-90)**	
	Females (n=19)	**Males (n=20)**	**Females (n=19)**	**Males (n=31)**	**Females (n=26)**	**Males (n=27)**
Heart rate (beats/min)	64 (11)	60 (12)	67 (10)	64 (7)	**68 (11)** *	**68 (13)** *
PQ duration (ms)	**153 (26)** †	**169 (20)** †	***174 (36)*** *†	***178 (21)*** *†	***170 (22)*** *†	***176 (25)*** *†
QRS duration (ms)	**87 (10)** †	**92 (8)** †	**87 (11)** †	**86 (16)** †	**78 (13)** †	**91 (10)** †
QT duration (ms)	383 (26)	380 (30)	388 (48)	378 (30)	389 (28)	391 (39)
QTc (ms)	392 (29)	378 (30)	***408(41)*** *	***391 (32)*** *	***412 (26)*** *	***412 (32)*** *
P-wave duration (ms)	**84 (20)** †	**91 (16)** †	**93 (15)** *†	**100 (13)** *†	**92 (15)** *†	**102 (23)** *†
P-wave amplitude (mV)	0.19 (0.07)	0.15 (0.04)	0.16 (0.05)	0.16 (0.05)	0.16 (0.04)	0.16 (0.05)
R+S (mV)	**2.2(0.5)** †	**2.5 (0.6)** †	**2.3(0.6)** †	**2.6(0.5)** †	**2.4(0.6)** †	**2.6(0.5)** †

Bold values indicate statistical significance * Indicates statistical significance compared to <50 y. † Indicates statistical significance compared to females.

Of the 219 subjects that performed ECG examinations (12-lead at rest, long-term ECG, or both) 17 % of the females and 20 % of the males presented with pathological findings at baseline. A summary of the

proportion of all pathological ECG divided into age groups is presented in Table 7. Pathological findings at the ECG examinations are summarized in Table 8 and 9. The most common finding when assessing 12-lead ECG at rest was left ventricular hypertrophy. The main finding when assessing long-term ECG was PACs and furthermore, eight subjects presented with ventricular tachycardia (VT).

Table 7. Summary of pathological ECG recordings according to sex and age at baseline examinations.

	Age <50 years		Age 50-65 years		Age >65 years	
Pathological ECG (%)	**Females (n=23)**	**Males (n=30)**	**Females (n=22)**	**Males (n=36)**	**Females (n=42)**	**Males (n=43)**
Overall	31	29	21	36	53	47
Standard	17	33	14	14	38	37
Long-term	33	6	31	36	50	50

ECG=Electrocardiogram

Table 8. Summary of ECG findings in 12 lead ECG at rest in females and males.

Abnormal findings at 12-lead ECG at rest	**Females/Males**		
	< 50 years (n=23/30)	**50-65 years (n=22/36)**	**>65 years (n= 42/43)**
Atrial fibrillation	0/0	0/0	0/1
Bradycardia	2/4	1/0	1/2
Nodal rhythm	0/1	0/0	0/0
AV-block I	0/0	1/1	3/4
Short PQ-duration	1/0	0/0	0/1
Prolonged QT-duration	1/0	1/0	1/1
Left axis deviation	0/0	0/0	2/3
Left or right AFB	0/0	1/2	1/2
RBBB/LBBB	0/0	0/1	2/2
ST-T changes	0/3	0/0	5/1
Abnormal T-wave	0/0	0/0	6/2
LVH	0/4	1/1	4/6

ECG=Electrocardiogram, RBBB=Right bundle branch block, LBBB=Left bundle branch block, AFB=Anterior fascicular block, LVH=Left ventricular hypertrophy.

Table 9. Summary of findings in 24-hour ECG in females and males

	n
Abnormal findings at 24-hour ECG	**(Females/Males)**
< 40 years10 PAC/24 h	1/0
40-59 years 100 PAC/24 h	0/2
>60 years 1000 PAC/24 h	3/3
<50 years PSVT	1/0
>50 years >2 episodes of SVT	7/8
< 50 years >100 PVC/24h	2/0
>50 years >200 PVC/24h	6/7
<50 years PVC in pair	3/1
PVC in bigeminy	5/2
R on T PVC	1/0
VT	4/4
AV-block II type 1	4/0

ECG=Electrocardiogram, PAC=Premature atrial contraction, PSVT=Paroxysmal supraventricular tachycardia, PVC=Premature ventricular contraction, VT=Ventricular tachycardia, AV-block=Atria-ventricular block.

At the follow-up 30 % of the females and 36 % of the males had developed CVD, and hypertension was seen in 45 % of the females and 58 % of the males as presented in Table 9. Males had significantly more ECG abnormalities than females. There were also an increasing proportion of subjects with ECG abnormalities, CVD, and hypertension with increasing age.

Table 10. Summary of findings at follow-up according to sex and age at baseline examination.

	Number of individuals							
Follow-up findings	**Age <50 years**		**Age 50-65 years**		**Age >65 years**		**All**	
	Females	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**
Number of follow-ups	n= 27	n= 29	n= 25	n= 38	n= 52	n= 35	n= 104	n=102
Alive (%)	100	97	80	79	23	23	57	65
Cardiovascular disease (%)	0	3	28	32	46	69	30	36
ECG abnormalities (%)	4	7	20	34	31	83	21**	43
Hypertension (%)	15	41	60	47	54	83	45	58
Diabetes (%)	7	7	4	3	6	20	6	10

ECG=Electrocardiogram, ** Indicates p <0.01 (females vs males).

Results discussion

This study aimed to assess the properties of the ECG related to age and sex in a healthy population. With increasing age, the central and peripheral cardiovascular systems are affected. The main reason for this is changes in collagen isoforms which results in tissues becoming stiffer (122). A contaminant reduction of the elastic filaments provides increased fibrosis, and reduced compliance of both the cardiac and vascular walls. This results in the loss of the Windkessel effect and vasculature becomes ridged, which consequently leads to the development of hypertension or heart failure, which creates a positive feedback effect resulting in additional cardiac and vascular dysfunction (123, 124, 125, 126).

Age and sex difference were found in both the P-wave duration and PQ-duration, with females exhibiting shorter durations for each variable. Surprisingly, for both males and females the longest PQ-duration was seen in age group 50-65 years of age. The PQ-duration reflects the hearts electrical system, measuring the conduction time from the atria to the ventricles. There are several reasons for prolonged PQ-duration, one of those is fibrosis due to collagen degeneration. Therefore, it might have been expected that the age group > 65 years of age would have had the longest conduction times. However, most cases of AV-block I was seen in the oldest subjects (>65 years of age).

Males had longer QRS duration in the age-groups < 50 and over 65 years of age compared to females. Healthy adolescent males have larger left ventricular mass compared to females resulting in longer QRS duration (127). Clinically, it would be reasonable that the reference values should differ between females and males, since our study, as well as other studies, indicate that females have shorter QRS duration (128, 129, 130). However, currently the reference values for both sexes are the same which can lead to consequences for females, e.g., in electrophysiology, where cardiac resynchronization therapy devices are implanted, one criterion is prolonged QRS-duration. Thus, females may receive medical care and interventions at a later stage when the same ECG reference values are used for both sexes.

An age-dependency was found in QTc duration. The QTc duration was longer in the oldest age groups (> 65 years of age) in both males and females compared with the other age-groups (< 50 and 50-65 years of

age). In the age group < 50 years and 50-65 years of age, females had longer QTc than males, while in the age group > 65 years of age, durations were equal. Earlier studies have found that QTc duration is significantly longer in females, due to female hormones (26, 131, 132). Prolonged QTc duration can cause aberrant ventricular repolarization that may lead to ventricular arrhythmias. Torsades de point is a lethal ventricular arrhythmia that is more prevalent in females. Today there are different reference material for females and males regarding QTc duration, however there are no references for different ages.

In 1992 Furberg et al presented findings that illustrated that major ECG abnormalities are common in the elderly population regardless of earlier heart disease. The authors showed that the prevalence of ECG deviations in those who were normotensive and had no history of coronary heart disease, increased with age (133). This was observed in our results as well. With increasing age, a greater proportion of ECG abnormalities, CVD and hypertension were seen in both sexes.

Eight subjects (4.1 %) had pathological T-wave changes and they were seen in both females and males >65 years of age. T-wave changes are seen in several disease states e.g., myocardial ischemia, however minor alterations in morphology can also be a part of the normal aging process and cause T-wave changes. Moller et al concluded that T-wave abnormalities should be given a special consideration (134), and T-wave changes should be interpreted in the broader clinical context, taking into consideration the subject's medical history and symptoms along with physical examination.

ST-T changes were seen in 9 (4.6 %) subjects, and 5 (2.5 %) subjects had pathological Q-waves. ST-T changes can range from normal to severe and are often a sign of myocardial damage or mild myocardial ischemia, so it is important to know the differential diagnoses, to determine the cause of these ECG changes. The same goes for pathological Q-waves; they may occasionally be seen in subject without cardiac disease, but most often as the cause of a serious cardiac event, such as secondary to a myocardial infarction or septal hypertrophy.

Since the inclusion criteria in this study included the absence of any known cardiovascular or systemic disease, it is surprising that these

findings occurred. However, non-specific T-wave changes can be seen in incipient hypertrophy and pathological T-waves can be seen in lateral leads when left ventricular hypertrophy is present (but undiagnosed). This was found in all age groups examined in our study and may therefore be a reason for those abnormalities. In our study, only one definition was used for pathological Q-waves. Therefore, it is difficult to draw any major conclusions about their occurrence. In order to investigate the actual clinical relevance, more criteria should be applied.

The most common finding in long-term ECG recordings were PACs which occurred both sporadically and in abnormal amounts. Ninety-eight percent of the males and 89 % of the females presented with PACs (one or more). Sporadic PACs are normal, although an abnormal amount of PACs may be a sign of underlying heart disease (135). Premature atrial contractions can initiate and also predict risk of developing AF (136). Only one subject in our study presented with AF on their long-term ECG recording at baseline. Episodes of AF or atrial flutter have been associated with increased mortality and have an estimated incidence between 3 to 15 % in diverse populations (137). Atrial fibrillation is the most common type of SVT, but AF is uncommon in healthy subjects although the risk is increased in males, with advancing age, and with conditions such as hypertension, sleep apnea syndrome and alcohol consumption (138). Previous studies had presented that the risk of developing AF increased almost 50 %, from 6-7 % to 13-17 %, with aging between 65-74 up to 75-90 years of age (137, 139, 140, 141).

Regarding PVC, 86 % of females and 84 % of males presented with PVCs (one or more). A recent study revealed that atrial or ventricular premature contractions that occur > 1000/day irrespective of origin are associated with increased risk of heart failure, AF, stroke, and mortality in individuals with and without a history of CVD (142). The Framingham study reported that more than 30 PVC/hour was associated with a 2.3-relative risk for death in men without any known coronary disease (143). Bjerregard also conveyed an association between PVCs and developing IHD (28). In our study 11 % of the subjects had > 1000 premature contractions/day, regardless of type. Sixty-three percent of those were deceased at the 20-year follow up. However, since we have not followed up on cause of death, it is difficult to speculate whether this has had any impact or association with cardiac events or mortality.

Paper II

Main results

Two hundred and eighty-five subjectively healthy adults (137 females, 48 %) between 20-90 years of age were included and assessed with dynamic spirometry. The rederived rLMS model was based on the 269 subjects with normal spirometry results. Figure 14 shows a comparison of the rLMS model with the original GLI and OLIN models.

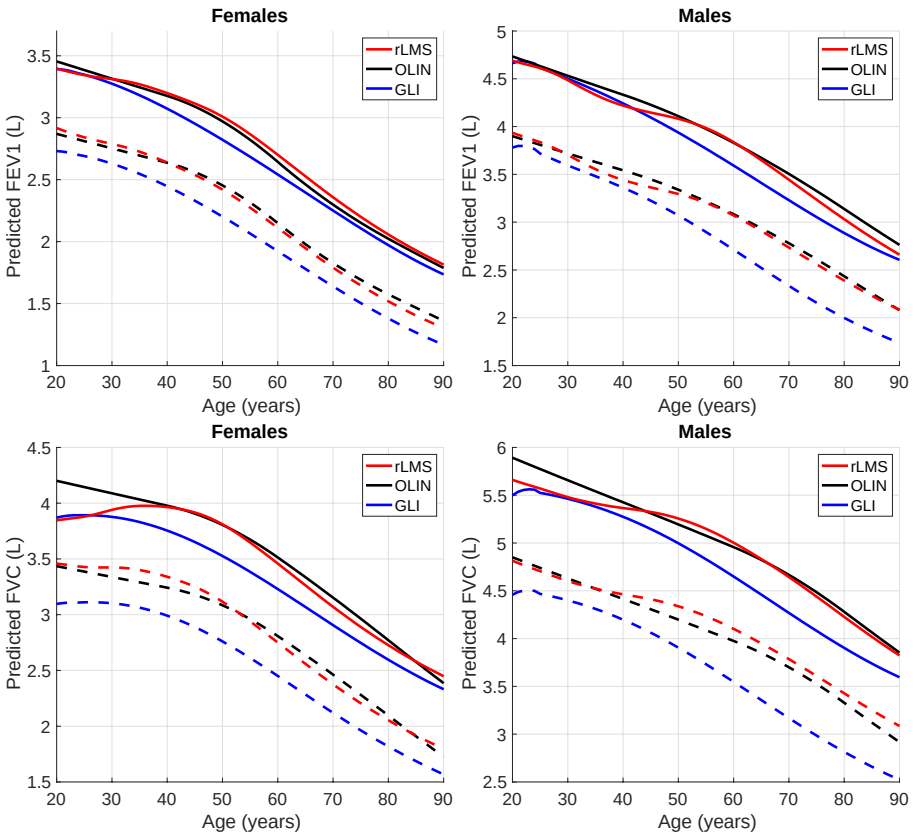

Figure 14. Comparison between the new model (rLMS) and the two other models of sex and age dependent changes in lung function. Predicted reference values of mean (solid lines) and LLN (dotted lines) are plotted against age for a female of height 164 cm, and a male of height 178 cm.

In this material of subjectively healthy subjects, 16 presented with pathological results according to ATS/ERS guidelines. The number of subjects below LLN were determined for all three models and is presented in Table 11. For rLMS the expected number of subjects was around 5% due to the definition of LLN. Fewer subjects had pathological findings when the GLI reference values were used compared to the OLIN reference values.

Table 11. Proportion of the included 131 females and 138 males that presented with Z-scores < LLN for the different models of the sex- and age-dependency in lung function.

	Females			**Males**		
	rLMS	**OLIN**	**GLI**	**rLMS**	**OLIN**	**GLI**
FEV_1 (%)	5.3	6.9	1.5	5.8	7.2	1.4
FVC (%)	3.8	3.8	0.8	5.1	2.2	0.0
FEV_1/FVC (%)	6.9	2.3	3.8	6.5	11.6	3.6

FVC=Forced expiratory vital capacity; FEV_1=Forced expiratory volume in one second.

Figure 15 illustrates how predicted lung function based on the rLMS model differs between sexes for two different body heights. The estimated FEV_1 in a female of height 164 cm is on average 0.49 liters lower than for a male of the same height. The difference at height 178 cm is 0.65 liters. The corresponding differences for FVC are 0.70 liter at 164 cm and 0.89 at 178 cm, respectively.

Figure 16 shows the relation between height and spirometry variables in our cohort. Figure 17 shows the estimated annual decrease in height, where females exhibited a decrease of 0.19 cm/years (CI 0.13-0.24 r^2 = 0.27, F= 48.8, p< 0.001) and males 0.14 cm/years (0.08-0.19, r^2 = 0.125, F = 26.0, p< 0.001).

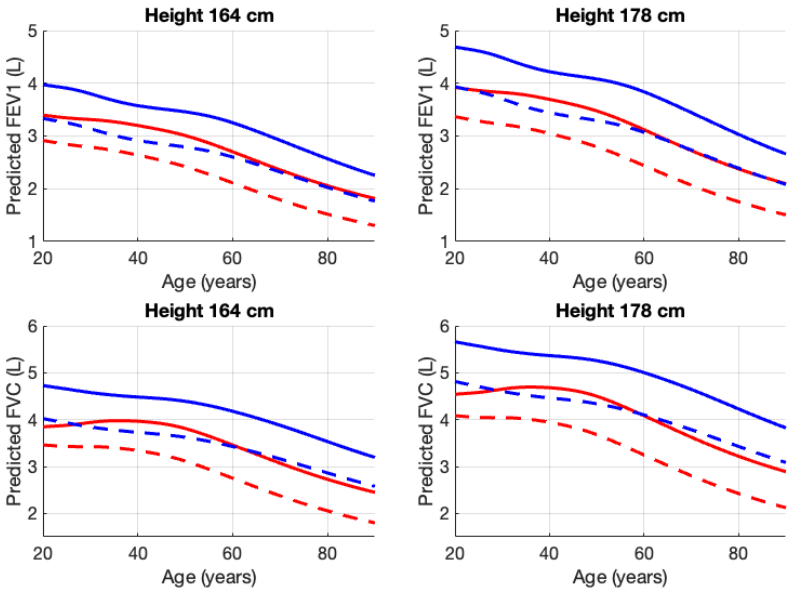

Figure 15. Predicted dynamic spirometry variables according to the rLMS model in females (red) and males (blue) for different heights and ages.

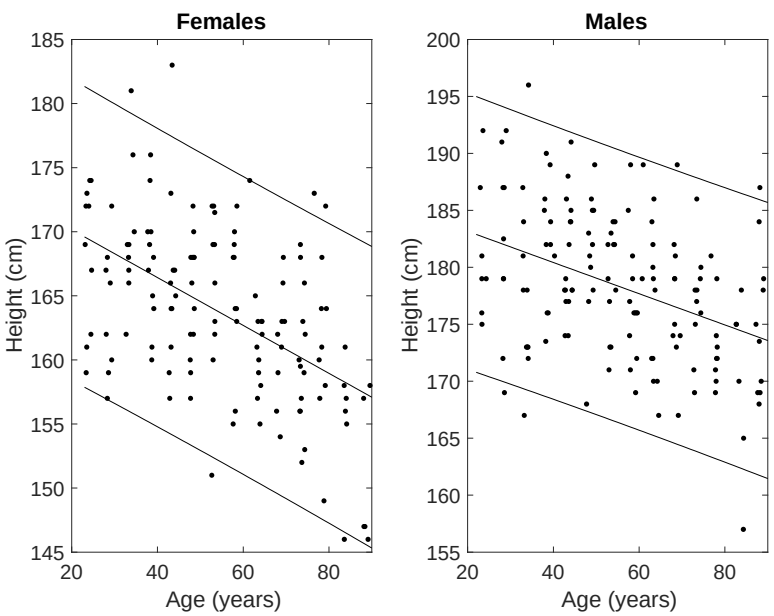

Figure 16. Age at vs height examination for all included males and females in Paper II. Lines show estimated linear regression lines and 95 % CI for observations.

Results discussion

A new model for lung function was derived (rLMS) and compared to the present GLI and OLIN reference values using a cohort of subjects from Northern Sweden. The estimated rLMS-model in our study correlated well with the previously published model from the OLIN study (49).

As figure 14 shows, there were small differences between the predicted values in our rLMS model compared to the OLIN model, but larger differences compared to the GLI model. For age range 40-90 years the GLI model predicts a lower mean than our rLMS model and the OLIN model. The GLI model also shows a distinctly lower LLN for the variables FEV_1 and FVC for all ages. Since FEV_1 is used routinely in the clinical setting to grade respiratory diseases, and both our and the OLIN study have shown that the GLI model underestimates this parameter, using this reference material may lead to incorrect diagnoses when used in its current form in the Swedish population.

Another reason for this discrepancy could be that the LMS model type could be unsuitable for this type of modelling. On the contrary, it has been shown to be very good for constructing age-related models and it has been used to build growth curves in America, England, and the Netherlands (144, 145, 146). In our study we showed that the LMS model can be updated with coefficients suitable for a particular population, enabling the production of clinically applicable sex and age specific reference materials adjusted for a population, if there are large enough reference populations to base the models on. We have also shown that the LMS model suits the Swedish population if the model parameters are updated, in the same way as shown for the Finnish population (147). One reason why the current GLI reference values for Caucasians are not suitable for our population may be that we, in the Nordic countries, have on average taller individuals compared to southern Europe.

Reliable and continuously updated reference materials are required when interpreting lung function examinations in both a clinical and research setting. A patient must receive the same treatment based on a lung function examination regardless of where in the world they are examined. It is clear that this is not the case in the populations of Sweden and Finland since both of our results, and earlier studies, have revealed that the internationally recommended reference equations (GLI) are discordant

with results from these national studies (48, 52, 147). Differences in lung function between other ethnic groups have also been found; a large cohort study (PURE) revealed that Asian and African Americans had a lower FEV_1 compared to European and white North American populations, even after adjustment for height, sex, and age (148). Ethnicity is thus an important factor to consider when interpreting spirometry data (149).

A decrease in all examined spirometry variables with age was seen in in both sexes. This was expected since in childhood, and when growing up, lung capacity should increase, while later in life a loss of elastic recoil in the lung tissue occurs decreasing lung function (46).

The difference in average height between sexes indicates a need for separate reference equations for females and males. Males tend to be taller than females and it is known that height influences lung function since it is a function of chest size (46). Different populations also have different average heights. In our study group, the sex specific average height was 164 cm for females and 178 cm for males, whereas in a study investigating reference equations for a population from southeast Asia, the average height for females was 151.5 cm and for males 163.9 cm (150).

Although we get shorter with increasing age, we have successively become taller in general as a population over the years according to Statistics Sweden. The Central Bureau of Statistics has recorded height data since 1980 and this shows that Swedes have become, on average, 2 cm taller since then (scb.se). Since body characteristics of different populations changes over time, especially in developing countries, there will always be a need for updated reference materials over time (8). Thus, this is another indication for the need of diverse equations for different populations, which are easy to update as the population evolves.

Performing a lung function examination is physically demanding and it is necessary to be able to understand the instructions. It is likely that compliance to these instructions is lower in children and the elderly. A previous study stated that physical fitness was associated with lung function and that is something that must be consider when interpreting spirometry tests in the elderly (151).

In summary, our results emphasize that the GLI model underestimates the pulmonary function in the adult Swedish population, but also that the model can be improved. Using incorrect reference equations when interpreting spirometry examinations can lead to incorrect diagnosis, which can lead to great suffering for the individual and unnecessary costs for the healthcare system.

Paper III

Main results

Fifty patients (38 females, 76 %), all with newly diagnosed RA, were included in this study and compared to 100 sex- and age- matched controls (76 females, 76 %). For comparison of age-corrected HRV in RA patients (12 males and 38 females) and controls, 100 age- and sex-matched controls (24 males and 76 females) were selected from the control data set. This selection was performed since the number of male RA patients was much lower than the number of male controls.

Patients with RA presented with findings suggestive of impaired cardiac autonomic function, both in the early stage of the disease and at the five-year follow-up. As presented in Figure 18, the supine position during normal breathing shows no differences between the patients with RA and controls, however there was a tendency towards a lower HRV in the patient group. During deep breathing (6 breaths/min, Figure 19) patients with RA had lower HRV in PTOT, LF and SDNN compared to controls, and after five years these differences were more pronounced. During deep breathing, HF, HF/LF and RMSSD were not analyzed since HF displays overlap with the respiratory rate because the respiratory curve is not completely sinus shaped.

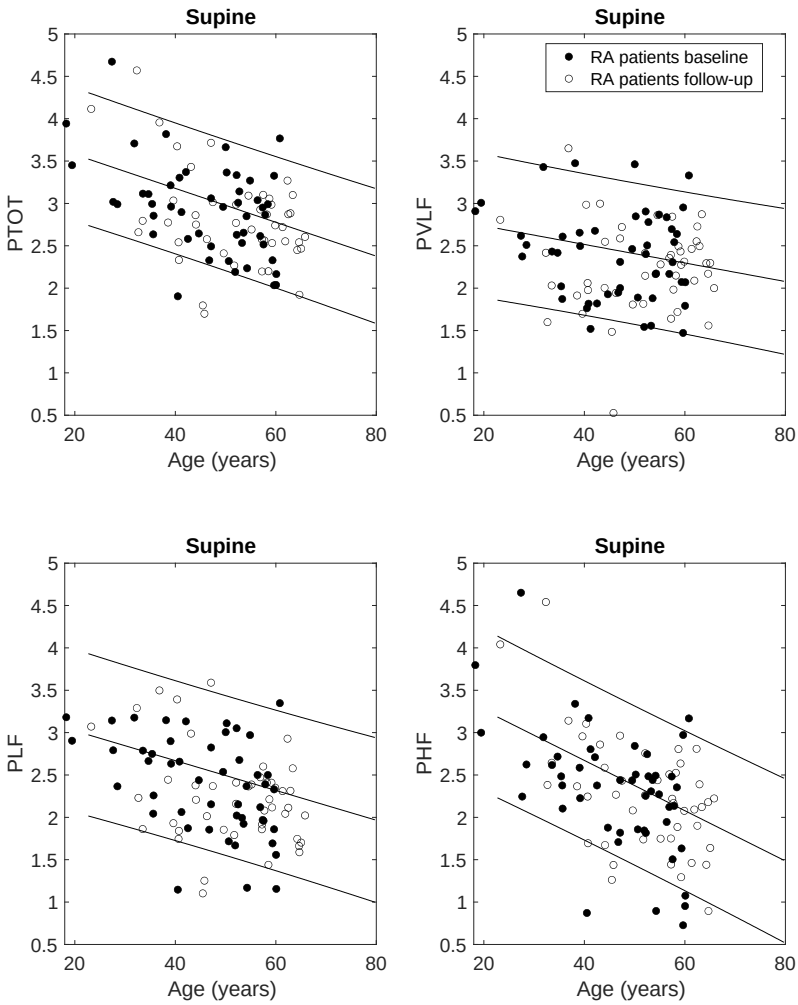

Figure 18. HRV in patients with RA in the supine position. Lines show estimated linear regression lines compared to 95% confidence interval for controls. PTOT=Total power, PVLF=Power of the very low frequency component, PLF=Power of the low frequency component, PHF=Power of the high frequency component.

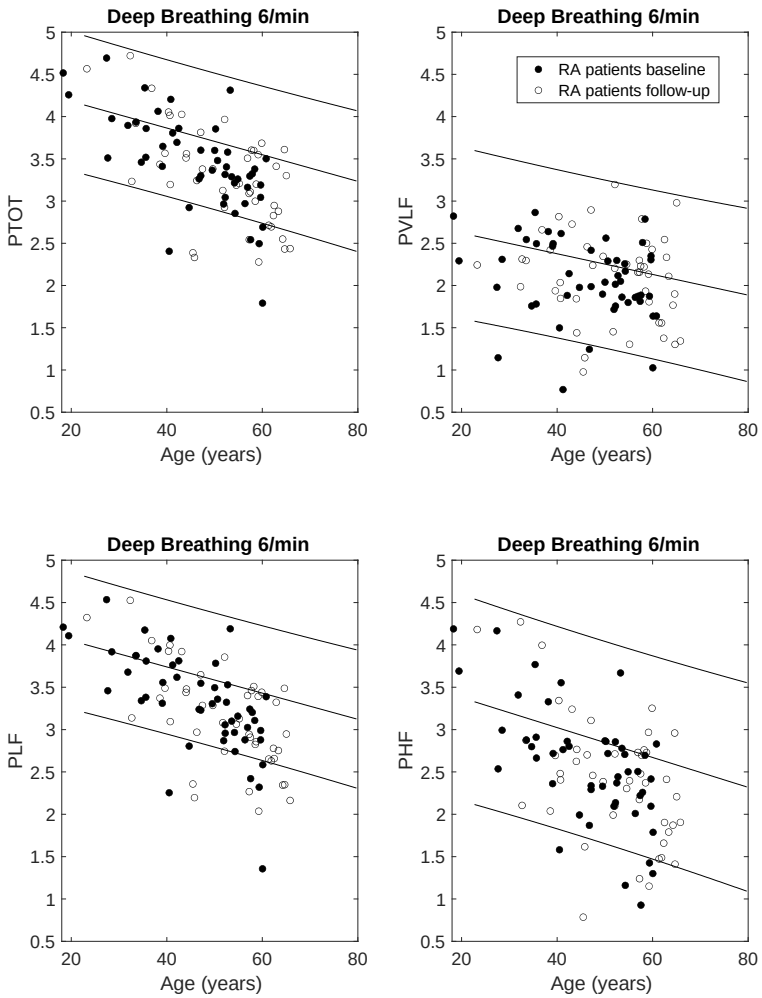

Figure 19. HRV in patients with RA during deep breathing (6 breaths/min), compared to 95 % CI for controls. PTOT=Total power, PVLF=Power of the very low frequency component, PLF=Power of the low frequency component, PHF=Power of the high frequency component.

Table 12. HRV parameters reflecting parasympathetic modulation in patients with and without CVD.

	Patients with CVD (n=9)	Patients without CVD (n=40)	*p*-value
Supine, deep breathing (6/min)			
PTOT (log, ms²)	3.25 (0.61)	3.42 (0.41)	0.16
LF (log, ms²)	3.10 (0.69)	3.29 (0.42)	0.15
Upright, normal breathing			
PTOT (log, ms²)	2.70 (0.32)	2.76(0.51)	0.38
LF (log, ms²)	2.21 (0.37)	2.31 (0.52)	0.30
HF (log, ms²)	1.54 (0.41)	1.90 (0.70)	0.07

Data is given as mean (standard deviation). HRV parameters are log-transformed and age-corrected. HRV=Heart rate variability), CVD=Cardiovascular disease, PTOT=Total power, LF=Low frequency component, HF=High frequency component.

A negative correlation was found between baseline HRV (all parameters) and SBP in patients with RA compared to controls.

At time of inclusion (disease onset), patients with RA had a positive correlation between heart rate and the inflammatory markers IL-6 and CRP.

At the eleven-year follow-up 20 % (8 patients) of the patients had experienced a cardiovascular event, and one patient had died due to myocardial infarction.

Results discussion

RA is a systemic disease which is associated with several co-morbidities. Cardiovascular disease has been found to be the most common cause of premature death in patients with RA. A combination of risk factors such as hypertension, dyslipidemia, and chronic inflammation, is believed to contribute to this increased risk (65).

Patients with RA presented with a lower HRV with different provocations, as well as due to postural changes, which agrees with previously published data (152). The longitudinal results revealed that patients with RA still had less variability during the deep breathing maneuver, five years after the initial investigation. These results indicated that parasympathetic tonus was reduced. However, this imbalance was not detected in the supine position during spontaneous breathing which is the most common test

carried out in these patients. The relationship between HRV and RA is complex and multifactorial (104) and the mechanisms for reduced HRV in patients with RA is unclear.

The relationship between hypertension and RA were investigated. Earlier studies have reported a higher prevalence (35-65 %) of hypertension in patients with RA compared to the general population (78, 153, 154, 155). Several factors can contribute to this increased prevalence including, chronic inflammation which can lead to endothelial dysfunction, and medications such as corticosteroids which can lead to hypertension as a side effect, as well as stress resulting from living with a chronic disease. In our study 18 % (9 patients) had hypertension at baseline and 22 % (11 patients) had developed hypertension until the five-year follow-up. These numbers are relatively low compared to other studies.

Negative correlations between HRV and SBP were found when the subjects were in the supine position with normal, and deep breathing. In addition, a tendency to lower HRV was observed at baseline during deep breathing in patient who developed hypertension compared to those who did not. In the 22 % (11 patients) of the subjects who developed CVD during the study period of eleven years, we found lower baseline HRV parameters compared to those who did not develop CVD, such as HF in upright position, and LF during deep breathing. Although the latter results were not statistically significant. HRV could be a method to, at an early stage, screen for the risk of developing hypertension in patients with RA. This could allow treatment to be started in a timely manner, consequently leading to reduced risk of future CVD.

Another explanation for hypertension in these patients could be related to reduced baroreflex sensitivity, which has been reported in patients with RA previously (104). The baroreceptor reflex contributes to maintaining homeostasis, by controlling blood pressure fluctuations. One of the key ways in how this is done is by moderating adequate outflow of sympathetic signals to the heart and resistance vessels (22). The regulation of blood pressure in different body positions is a very important function of the ANS, with an upright position, in particularly, putting great demands on blood pressure regulation. Central neural arrangements such as behavioral and humoral are involved in the regulation of the cardiovascular system including the baroreflex. In states of normal blood

pressure (normotension) the baroreceptors are persistently functioning and inhibiting sympathetic efferent action. A reduced baroreflex sensitivity results in decreased inhibitory activity and an imbalance in the sympathovagal outflow to the heart, causing chronic adrenergic stimulation (104). A specific test of the baroreflex was not performed, but the result showed a lower PNS activity in patients with RA, who theoretically have an increased sympathetic response, which may contribute to the above theory.

Previous studies have shown that developing hypertension at a younger age increases the risk of CVD and all-cause mortality (156, 157). Few studies have examined the age of onset for hypertension (157), however a multicenter cohort study found that subjects with hypertension onset at 40 to 49 years of age had higher CVD risk than patients with hypertension onset at 60 to 65 years of age (158). In our cohort including patients with RA, the mean age at baseline was 46.6 years, and at the five-year follow-up the mean age was 51.7, this means that almost all patients in our cohort had developed hypertension with an early onset, which may be an explanation for CVD development in patients with RA.

At the 11-year follow-up, 9 patients presented with various cardiovascular events, and one had died due to a myocardial infarction. All these patients presented with somewhat lower HRV parameters when parasympathetic modulation was assessed, i.e., HF in the upright position and LF during deep breathing, compared to those who did not have a cardiovascular event. The differences were however not significant (Table 11). Reduced parasympathetic modulation has been associated with an increased risk of cardiovascular events such as myocardial infarction, arrhythmias, and sudden cardiac death (159). An explanation for this association may be that a lower HRV is linked to a reduced ability for the cardiovascular center to adapt, making it more exposed to disturbances and stressors.

Earlier publications (160, 161, 162, 163, 164) have shown that an increased heart rate is an independent predictor of mortality in conditions such as myocardial infarction, hypertension and diabetes mellitus, but also in the general population (160). Patients with RA presented with higher heart rate in the supine position, both at baseline and at five-year follow-up, compared to controls in the present study. The elevated heart rate seen in patients with RA could be due to decreased activity in the PNS and

increased activity in the SNS which, in turn, can be explained by the chronic inflammatory process (104). Positive correlations between the inflammatory markers IL-6 and CRP and heart rate at baseline examinations were found. This may explain why patients with RA had elevated heart rate during baseline, and not at the five-year follow-up; their inflammation has decreased due to adequate treatment and management. The other aspect that may explain why patients with RA still had higher heart rate than the controls after five years, may be related to low physical activity, medications, depression and/or anxiety (96, 104, 165, 166, 167).

An international cross-sectional study from 2008 found that a low proportion of patients with RA exercise regularly. Three centers in Sweden participated in the study, and 61 % of the patients reported no regular physical activity (165). At the five-year follow-up there was a positive correlation between DAS28 and heart rate during normal and deep breathing in the supine position. A study from 2015 revealed that DAS 28-values are associated with physical activity (167). The present study was unable to prove causality between DAS28, and heart rate/physical activity thus further investigations are required.

It is important for healthcare providers to be aware of different factors that can contribute to CVD when caring for patients with RA. This is essential to monitor and treat conditions, such as blood pressure, to actively decrease CVD mortality and morbidity in this patient group. Managing hypertension in these patients may involve lifestyle modifications, changes in medications and addressing the underlying systemic inflammation to reduce the risk of cardiovascular complications associated with both rheumatism and hypertension.

Paper IV

Main results

Long-term survival and cardiac autonomic function were assessed in 197 females with, and 141 without IHD. Females with IHD presented with lower HRV in one or multiple HRV parameters, during all procedures, compared to controls. Differences in survival were seen after 20 years,

with females with IHD having a lower proportion of survival. After 15 years, or less, no differences were seen (Table 13).

Table 13. Proportion of survival in all included subjects after 10, 15 and 20 years.

Survival	Females with IHD (n=197)	Females without IHD (n=141)	*p*-value
10-year (%)	90.3	91.4	0.72
15-year (%)	80.2	80.9	0.88
20- year (%)	59.4	72.3	**0.01**

IHD = Ischemic heart disease. Bold values indicate statistical significance.

When dividing the patients and controls into age groups, <60 years of age and >60 years of age, there were differences in survival, compared to controls, after 10 years. This was only seen in the younger age group (<60 years of age). In the older age group (>60 years of age), there were no significant differences (Figure 20).

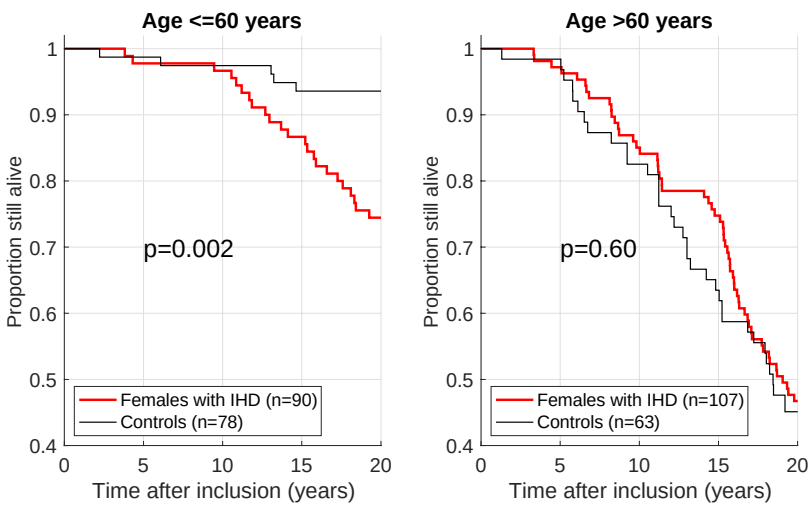

Figure 20. Survival curves derived from Kaplan-Meier analysis where subjects are divided into <60 years and subjects >60 years.

Females with IHD presented with lower HRV in multiple HRV parameters, during all procedures, compared to controls as presented in Table 14 (only significant differences are shown).

Table 14. Comparison of HRV parameters in females with IHD and controls.

	Females with IHD (n=93)	Controls (n=70)	*p*-value
Supine, normal breathing			
LF (ms^2, log)	2.24 (0.42)	2.41 (0.45)	**0.01**
LF/HF	-0.10 (0.39)	0.01 (0.34)	**0.044**
Supine, deep breathing (6/min)			
PTOT (ms^2, log)	3.28 (0.49)	3.71 (0.34)	**<0.001**
LF (ms^2, log)	3.12 (0.53)	3.60 (0.34)	**<0.001**
Supine, paced normal breathing (12/min)			
PTOT (ms^2, log)	2.89 (0.41)	3.06 (0.41)	**0.009**
LF (ms^2, log)	2.10 (0.38)	2.33 (0.42)	**<0.001**
HF (ms^2, log)	2.64 (0.51)	2.83 (0.46)	**0.02**
Upright, spontaneous normal breathing			
LF (ms^2, log)	2.15 (0.48)	2.53 (0.41)	**<0.001**
HF (ms^2, log)	1.74 (0.49)	2.06 (0.44)	**<0.001**

Data is given as mean (standard deviation). HRV parameters were logarithmically transformed. IHD=ischemic heart disease; HRV=Heart rate variability; PTOT=Total power; HF=Power of high frequency component; LF=Power of low frequency component. Bold values indicate statistical significance.

Females with IHD had higher BMI and SBP compared to the controls (Table 15).

Table 15. Clinical characteristics of all included subjects.

	Females with IHD (n=197)	**Females without IHD (n =141)**	***p*-value**
Age (years)	60.9 (8.4)	58.1 (13.4)	**0.03**
BMI (kg/m²)	27.3 (8.3)	24.7 (3.3)	**<0.001**
SBP (mm Hg)	138 (18)	133 (24)	**0.03**
DBP (mm Hg)	79 (11)	77 (9)	0.16

Data is given as mean (standard deviation), IHD=Ischemic heart disease, BMI=Body mass index, SBP=Systolic blood pressure, DBP=Diastolic blood pressure. Bold values indicate statistical significance.

Results discussion

Twenty years after the patients first cardiac event, 59.5 % of the patients were still alive. Such a long follow-up time is uncommon. According to Statistics Sweden, Central Bureau of Statistics, mortality after a myocardial infarction has been decreasing in Sweden for several decades. The Central Bureau of Statistics reported that, of the subjects who suffered a myocardial infarction in 2021, 15 % died the same day, 22 % died within 28 days and within a year 30 % had died.

When all females with IHD were compared to controls, no differences in survival were seen until after approximately 15 years. When the females with IHD were divided into two ages groups, under and over 60 years of age, those younger than 60 years of age had a higher mortality already after 10 years while the older age group had a slightly better survival rate compared to the controls. The former finding can probably be explained by a strong genetic component and severe risk factors. Previous studies have also reported that younger females affected by acute myocardial infarction have a worse prognosis for survival (168, 169). The latter was somewhat surprising but perhaps it is an effect of the medical care and follow-ups following the cardiac event.

A reduced HRV has been shown to be a predictor of long-term cardiac mortality in females with CAD (170). Moreover, reduced HRV after myocardial infarction is a risk factor for ventricular arrhythmias and sudden cardiac death (171). The result shown that females with IHD had reduced HRV compared to controls in different procedures, most pronounced differences were seen during breathing provocations indicating impaired parasympathetic activity. The HF component reflects parasympathetic activity, and the vagal tone expressed from parasympathetic activity supports the dynamic autonomic regulation which is important for cardiovascular health i.e., defective vagal inhibition is associated with increased morbidity (172). The HF component were lower in females with IHD compared to controls.

When comparing HRV in supine position among females with IHD who were alive or deceased after 15 years, no differences were found. However, after correction for the effect of heart rate on HRV, a higher PTOT and VLF in upright position were found in those who were deceased after 10, 15 and 20 years. This indicates a high sympathetic activity in these patients. An overactive sympathetic nervous system can lead to a persistent stress response, raised heart rate, elevated blood pressure and vasoconstriction (173, 174). Moreover, sympathetic overdrive attenuates the parasympathetic activity resulting in decreased vagal influence in the SA-node. Additionally parasympathetic activity is believed to be protective against ventricular tachyarrhythmias while a sympathetic overdrive predisposes to ventricular fibrillation (102). Therefore, we can hypothesize that it might be the sympathetic dominance contributing to the exacerbation of CVD, and that this sympathetic dominance could explain the difference seen in HRV between those who were alive and those who were deceased.

Thus, there are various possible explanations for how low HRV increases the risk of death, but the exact pathophysiology remains unclear. Nevertheless, an autonomic dysfunction with increased sympathetic activity and reduced parasympathetic activity increases the risk of fatal arrhythmias and may therefore be the reason for increased risk of mortality. However, since we only investigated all-cause mortality in Paper IV it is difficult to discuss this further. Therefore, follow-up studies are needed in this cohort of females with IHD for evaluation on how the impaired HRV found in this study is associated with cardiac death.

Methodological considerations

In this thesis, methodological aspects due to age and sex have been in focus. In the coming chapter, methodological considerations regarding the clinical physiological methods studied, and limitations with each study, will be discussed.

ECG recordings

ECG examinations are used to assess the electrical activity of the heart and can be useful in detecting heart disorders. Sensitivity and specificity may vary depending on the specific purpose of the examination. For example, an ECG has better sensitivity to detect heart rhythm abnormalities than to identify blockage in coronary arteries.

It is important in ECG examinations at rest that patients are relaxed and calm. They should feel safe and well taken care of. The examination room should be warm, so artefacts are not produced due to a shivering patient. A 12-lead ECG is an examination that is quick to perform and requires little effort from the patient, however, a long-term ECG, even though the size and weight of the unit has dramatically changed since its inception, may be a burden to the patient during the registration period. Before placing the ECG electrodes, you should shave the skin if there is a lot of body hair, then exfoliate the skin lightly with sandpaper and wipe with alcohol. This skin preparation ensures adequate adhesion and contact of the ECG electrodes, both essential for optimal trace recordings. Electronic devices that could interfere with the investigation should also be avoided.

Spirometry

Performing dynamic spirometry can be challenging for both the patient and the operator. The forced exhalation must be done with the least possible delay after the maximum inhalation, otherwise there is a risk that the expiratory flow will be too low. Additionally, the exhalation portion must be explosive and fast, and requires the patient to be able to continue to exhale for at least six seconds. Other challenges complicating accurate performance of dynamic spirometry include patients with a serious illness, with a language other than Swedish or, with intellectual

disabilities. There is a limitation in how many attempts can be performed (maximum eight), and a competent operator who can encourage the patient to perform the maneuvers, whilst clearly explaining the instructions is also necessary.

Heart rate variability

Many HRV studies are usually based on examination in resting conditions, in Paper III as well as IV we showed that autonomous provocations were needed to be able to see differences compared to healthy controls. Thus, setting up measurement protocols that are able to catch all aspects of the autonomous system in different disease states is important to consider.

Consideration should be given when measuring HRV in different patient groups regarding breathing frequency. The efferent pathways in the SNS and PNS are affected by respiration. The respiratory effect can modify LF and mislead the interpretation of the autonomic balance using LF/HF ratio. Respiratory sinus arrhythmia generally becomes less pronounced at a high respiratory rate, depending on the dampening properties of the pacemaker cells and ANS (175). Low HF can also be misinterpreted as reduced vagal activity if the breathing frequency is below 0.15 Hz. Therefore, the HF component is not of interest during deep breathing where the vagal response is observed in the LF component. A method to ensure that the vagal activity related to breathing is in the HF band, is to perform controlled breathing with 12 breaths/min, corresponding to 0.2 Hz (176).

When interpreting HRV, it is of the utmost importance that the trace is free from arrhythmias, as even occasional extra systolic beats can hinder the possibility of assessing cardiac autonomic function, especially in short recordings (< 2 min) (60). An example is, AF, which usually causes very high HRV spectrum where no clear peaks are seen. HRV measurements are sensitive to artifacts and require high quality in data collection.

Limitations

Paper I

A limitation of paper I was that the upper blood pressure limit was 160/90 mm Hg, which is, by todays standard, well above the limit for hypertension and therefore some of the pathological findings at inclusion may be due to hypertension. Further limitations were that not all subjects underwent both 12 lead ECG at rest and Holter ECG. Moreover, only the local medical journals were reviewed, which resulted in not all subjects being followed up.

Paper II

In paper II, there were smokers or former smokers included in the population. Furthermore, there were a relatively small number of studied subjects and therefore the rederived LMS model cannot be used in clinical settings until it has been applied to a larger material.

Paper III

In paper III, HRV was not performed at 11-year follow-up in the patients with RA. Thus, the relationship between cardiovascular events and HRV at that timepoint could not be investigated. Another limitation was that the controls were only investigated with HRV once, thus the estimated age-dependency in the controls might be slightly biased.

Paper IV

In paper IV, risk factors such as BMI, hereditary predispositions and smoking habits were not considered during analysis. Cause of death was also not investigated, and we were therefore not able to evaluate how different HRV parameters are related to cardiac death.

Conclusions

This thesis had some overarching objectives; it aimed to deepen the methodological knowledge of different clinical physiological examinations, specifically to evaluate how sex and age affects the validity of these examinations. Furthermore, it aimed to assess how the autonomic nervous system was affected in two different disease states by studying HRV, cross sectionally and longitudinally.

We found that there are sex and age differences in several ECG parameters that may affect data interpretation and consequently clinical management. We were also able to verify that, when interpreting lung function, the LMS model used by GLI can be updated to better suit the population to be investigated. Furthermore, we found that diseases such as RA and IHD may lead to impaired cardiac autonomic function. We saw that autonomic functional changes in patients with RA was most evident in respiratory provocations, which is suggestive of a parasympathetic branch impairment. On the other hand, in females with IHD it was the provocation with posture change that identified the patients with higher mortality—consisting of those with highest sympathetic response in HRV.

Paper I

- In our population of 219 subjectively healthy persons of varying age and sex, there were sex and age differences in 12-lead ECG at rest parameters. This confirms previous findings and supports the need for the establishment of age- and sex-specific reference values for these parameters in the future.
- No associations between baseline ECG and CVD were found.

Paper II

- Our study showed that the reference values developed by GLI are not suitable for Caucasian population of Sweden. Based on larger cohorts it is possible to modify the LMS model and use updated coefficients for more accurate and reliable interpretations of spirometry examinations in this population.

Paper III

- Despite anti-rheumatic medications, patients with RA had autonomic imbalances both at an early state of the disease and at the five-year follow-up examination.
- No associations were found between HRV and RA disease activity.
- A negative correlation between reduced HRV and increased systolic blood pressure was found.

Paper IV

- After sympathetic stimulation, higher PTOT and VLF were associated with higher risk of mortality in females with IHD.
- Females with IHD < 60 years of age presented with a higher risk of mortality compared to controls, however females >60 years of age did not show the same pattern.
- Females with IHD had lower HRV compared to controls.

Future perspective

The development of ECG has been impressive, and what the future holds remains to be seen. I can imagine that the application of methods within artificial intelligence (AI) and machine learning will develop the investigation further. Still, I think caution is needed before AI applications are allowed to interpret the ECGs on their own, to make sure that the algorithms are as versatile and adaptive as the human brain. However, algorithms and advanced techniques can most likely be efficient in detecting deviations at an early stage and guiding clinicians on where to pay extra attention. I believe the future of ECG will involve increased accessibility, precision, and usability.

To achieve consistency in the interpretation of dynamic spirometry data, in the same way that exist for the performance of the examination, I believe that the work that GLI began in creating global reference materials is the right way to go. I think that it is very promising that the LMS model can be applied to dynamic spirometry data from different parts of the world, as this is a prerequisite to finalizing a global reference material.

HRV has been used in research for many years in various fields, including cardiovascular risk assessment and assessment of autonomic dysfunction or neuropathy. New areas of application are now being explored. For example; psychological studies have shown that stress causes reduced HRV which is associated with an increased risk of adverse health outcomes, including hypertension, and heart disease (177). In sports, HRV is used as a monitoring index to optimize training (178), and biofeedback, which is a therapeutic technique that can help regulate stress by carrying out relaxion techniques and controlled breathing, which has been shown to increase HRV (179).

As research in the HRV field continues to advance, HRV has the potential to play a larger role in clinical practice, particularly in health monitoring and preventive care, e.g., for identifying risk factors in various diseases, such as cardiovascular risk for patients with rheumatism, and for predicting the risk of cardiovascular events and sudden cardiac death in patients with IHD. To be able to use HRV to a greater extent in clinical routine, I think that the method must become more user-friendly. Further

standardization of the HRV measurement and analyzes is needed to facilitate comparisons and interpretations. In addition, larger and more representative databases are needed to be able to develop accurate reference values. HRV measurements must also be integrated into clinical decisions and the care process.

Performing examinations to identify risks can be questioned, and one problem that need to be addressed is how the result should be communicated to the patient. However, today risk assessments are used for a variety of diseases, such as the CHA_2DS_2VASc score that calculates the risk of stroke in patients with AF (180) or the Framingham risk score for CVD (181). Considering that research has shown that HRV can be improved by biofeedback (182, 183) and physical exercise(184), there may be a benefit in using HRV in individual risk assessment to measure effects after lifestyle changes.

In today´s society, I think we are more involved, interested and concerned in our own personal health than ever before. We have various "smart gadgets" that can measure different physical functions such as heart rate, HRV, blood pressure, and sleep patterns etc. This can be beneficial as well as detrimental to our health. It can provide inspiration and knowledge about our own bodies and well-being, and help follow the development of, for example, physical fitness. On the other hand, abnormal values measured by the 'smart gadgets' can create anxiety that could potentially have negative consequences for ourselves and the healthcare system due to increased care visits as well. Hence, if in the future we should be able to take advantage of the upsides of these new techniques, I think we need not only easily accessible, standardized and up to date, but most likely also individual, reference materials for each parameter. This will be a prerequisite to create an understanding of what we are measuring and when to seek care.

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“Most people do not realize what risk they run when they submit to an electrocardiogram” (Marriott 1960)”

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