Cardiac arrhythmias and heart rate variability in familial amyloidotic polyneuropathy

A clinical study before and after liver transplantation

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Old age is not so bad
when you consider the alternatives

Maurice Chevalier
Abstract

Familial amyloidotic polyneuropathy (FAP), found in the northernmost counties in Sweden, is a rare, lethal and inherited amyloidosis. The disease is caused by mutated transthyretin (TTR). The mutation is characterized by an exchange of valine for methionine at position 30 (ATTRVal30Met). FAP is characterised by progressive polyneuropathy affecting both the peripheral and autonomic nervous system (ANS). Cardiac arrhythmia and autonomic disturbances are common as well as gastrointestinal symptoms: such as constipation and diarrhoea.

Today, orthotopic liver transplantation (LTx) is the only treatment to stop the progression of FAP. The rationale for this is because 95% of TTR is synthesized by the liver, a liver transplantation should abolish the production of new mutated amyloidogenic TTR. The first liver transplantation for FAP was performed in Sweden 1990.

Heart complications and autonomic disturbances are common in FAP patients both before and after liver transplantation. The aim of the present study was three-fold: to determine whether liver transplantation affects the natural course of cardiac arrhythmias and cardiac autonomic function; to predict the risk of ventricular arrhythmias; and to elucidate heart rate variability (HRV) patterns by power spectrum analysis and Poincaré plots.

In total, ninety-seven Swedish FAP patients were included in the studies. The patients underwent 24-hours electrocardiography (Holter) recordings, and/or signal averaged electrocardiography (SAECG) and heart rate variability.
The study showed that many patients developed cardiac arrhythmias and conduction disturbances after LTx. Approximately 25 percent of patients were pacemaker treated after LTx. The SAECG recordings disclosed that many FAP patients had ventricular late potentials (LP) compared with healthy subjects, and that LP were associated with nonsustained ventricular arrhythmia. Analyses of heart rate variability (HRV) showed reduced autonomic function in the majority of patients. Some patients had high HRV with broadband power spectra and Poincaré graphs with a fan or complex pattern. These novel findings could be an indicator of ECG abnormalities (subtle atrial arrhythmia) in FAP patients instead of reflecting normal cardiac autonomic modulation. The HRV studies also showed that LTx preserves cardiac autonomic function in FAP.

In conclusion, cardiac arrhythmias, late potentials and reduced heart rate variability were common in Swedish patients with FAP, whether they underwent liver transplantation or not. The absence of LP may indicate a low risk for ventricular tachycardia in FAP patients.
Original papers

This thesis is based on the following papers*, which are referred to by their Roman numerals in the text.


III. Urban Wiklund, Rolf Hörnsten, Marcus Karlsson, Ole B Suhr, Steen M Jensen. Abnormal heart rate variability and subtle atrial arrhythmia in patients with familial amyloidotic polyneuropathy.* Annals of Noninvasive Electrocardiology (accepted for publication)

IV. Rolf Hörnsten, Ole B Suhr, Steen M Jensen, Urban Wiklund. Heart rate variability and ventricular late potentials after liver transplantation for familial amyloidotic polyneuropathy. In manuscript.

V. Rolf Hörnsten, Ole B Suhr, Bert-Ove Olofsson, Urban Wiklund. Liver transplantation preserves cardiac autonomic function in familial amyloidotic polyneuropathy: a long-term study of heart rate variability. In manuscript.

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### List of abbreviations

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<tr>
<th>Abbreviation</th>
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<tr>
<td>AHA</td>
<td>American Heart Association</td>
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<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
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<td>ARG</td>
<td>Arginine</td>
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<td>ARVD</td>
<td>Arrhythmogenic right ventricular dysplasia</td>
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<td>ATTR</td>
<td>Amyloidotic transthyretin</td>
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<td>CMV</td>
<td>Cytomegalovirus</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>ECG</td>
<td>Electrocardiography</td>
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<td>ES</td>
<td>Extra systolie</td>
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<tr>
<td>FAP</td>
<td>Familial amyloidotic polyneuropathy</td>
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<tr>
<td>FAPWTR</td>
<td>Familial Amyloidotic Polyneuropathy World Transplant Register</td>
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<tr>
<td>FIR</td>
<td>Finite impulse response</td>
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<tr>
<td>FQRS</td>
<td>Filtered QRS-complex</td>
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<tr>
<td>HF</td>
<td>High frequency</td>
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<tr>
<td>HIS</td>
<td>Histidine</td>
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<tr>
<td>HRV</td>
<td>Heart rate variability</td>
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<tr>
<td>ICD</td>
<td>Implantable cardioverter defibrillator</td>
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<tr>
<td>ILE</td>
<td>Isoleucine</td>
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<tr>
<td>LAS</td>
<td>Low amplitude signal</td>
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<tr>
<td>LEU</td>
<td>Leucine</td>
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<tr>
<td>LF</td>
<td>Low frequency</td>
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<tr>
<td>LP</td>
<td>Late potentials</td>
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<tr>
<td>mBMI</td>
<td>Modified body mass index</td>
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<tr>
<td>MET</td>
<td>Methionine</td>
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<td>LTx</td>
<td>Orthotopic liver transplantation</td>
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<td>PHE</td>
<td>Phenylalanine</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PND</td>
<td>Polyneuropathy disability scoring system</td>
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<tr>
<td>PSD</td>
<td>Power spectral density</td>
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<tr>
<td>RMS</td>
<td>Root mean square</td>
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<tr>
<td>SAECG</td>
<td>Signal averaged electrocardiography</td>
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<td>SAP</td>
<td>Serum amyloid protein</td>
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<td>SSA</td>
<td>Senil systemic amyloidosis</td>
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<tr>
<td>SVT</td>
<td>Supraventricular tachycardia</td>
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<tr>
<td>TTR</td>
<td>Transthyretin</td>
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<tr>
<td>VAL</td>
<td>Valine</td>
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<tr>
<td>VLF</td>
<td>Very low frequency</td>
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<td>VT</td>
<td>Ventricular tachycardia</td>
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Introduction

In the mid of the 1980s my interest for cardiac arrhythmias started when some physicians at our department introduced me into the fields of Holter-ECG. At the same period short-term recordings of heart rate variability (HRV) were introduced at our laboratory as a method to non-invasively study autonomic function, foremost in patients with familial amyloidotic polyneuropathy (FAP).

In the beginning of the 1990s we started a study where HRV and late potentials (LP) from signal averaged electrocardiography (SAECG) were analysed as a risk evaluation for ventricular tachycardia (VT) in patients who have had a myocardial infarction during the last week. These studies linked up my interest to full-blown status for non-invasive arrhythmia diagnostic methods. Previous studies in FAP patients have foremost shown conduction disturbances and amyloid infiltration in the heart from analyses of ECG or echocardiography. My first hypothesis was that there could be a connection between arrhythmogenic right ventricular dysplasia (ARVD) and FAP. Both these conditions have a form of infiltration in the heart muscle. The knowledge about ARVD shows that these patients have increased risk for ventricular arrhythmia and positive LPs.

In the late 1990s, when the index case presented below was examined, the idea for my thesis work elucidated, and I started to collect data for further analysis in patients with FAP. In particular, I had the impression that these three non-invasive techniques – Holter-ECG, HRV and SAECG – have a great potential for risk evaluation in patients for arrhythmic death.
The disease Familial amyloidotic polyneuropathy

Amyloid and Amyloidosis

The word amyloid originates from the Latin word amylum and the Greek word amylon, which means starch or cellulose. In 1854, Wirchow stated the name (1). However, in 1859 it was proven that amyloid consisted of proteins, not cellulose (2). The deposits of fibrillar proteins, amyloid, progressively destroy normal tissue structures.

In 1922, a specific test was introduced to determine amyloid – the staining reaction with Congo red (3). The diagnosis of amyloidosis is still based on positive Congo red staining. Amyloidosis compromises a heterogeneous group of disorders, some systemic (4, 5) and some localised, as in Alzheimer’s disease (4, 5). Common features to all these conditions are extra cellular deposits of fibrillar proteins of different organs and tissues (6, 7), leading to organ dysfunction and, ultimately death (8).

Transthyretin

Familial amyloidotic polyneuropathy (FAP) is caused by mutated transthyretin (TTR) – a 127-amino acid that is primarily synthesized in the liver. TTR is a secreted protein present in the blood and cerebrospinal fluid and is a carrier of thyroxine and retinol-binding protein-retinon (vitamin A) complex(9, 10). TTR was discovered in 1942 by electrophoresis of cerebrospinal fluid(11). Initially, TTR was named prealbumin because it
migrates in front of albumin during electrophoresis (12). TTR synthesis occurs mainly in the liver (13), but it is also synthesised in choroid plexuses of the brain (14, 15), and in the pigment epithelium of the eye (16).

Both natural TTR and mutated variants of TTR are involved in the amyloid disease, but a mutation in TTR accelerates the process of amyloid fibrillogenesis and is the most important risk for TTR amyloidosis (9, 10). Today there are more than hundred TTR single site variants that have been associated with TTR amyloidosis (17), and the majority of mutations are associated with amyloid disease (18). The most frequent mutation associated with polyneuropathy is the ATTR Val30Met, whereas the most common mutation is the ATTR Val122Ile. The latter is associated with a late-onset cardiomyopathy (19), and is carried by approximately 4% of the African American population (20).

**Familial amyloidotic polyneuropathy**

FAP is a form of TTR amyloidosis, a lethal autosomal inherited disorder induced by accumulation of insoluble fibrillar proteins (amyloid) in the tissues in amounts sufficient to impair normal function. It was first describe by Andrade 1952 (21) and in 1968, the first reports come from Sweden and Japan (22, 23). Deposition of TTR amyloid is associated with a variety of human diseases (24-26) – the tranthyretin-associated hereditary amyloidoses (ATTR). When the peripheral nerves are affected prominently the disease is termed FAP. FAP is caused by mutated transthyretin in which valine is exchanged for methionine (27-31). Val30Met (substitution of valine by methionine at the 30 position on the light amino acid chain) is the most common mutation associated with FAP families worldwide (32-35). The main feature of FAP,
regardless of genetic mutation, ethnic background or geographic location, is progressive sensimotor and autonomic neuropathy (8, 28, 35, 36).

The amyloid polyneuropathy tends to involve small, unmyelinated fibers, disproportionately affecting the autonomic nervous system in sensation of pain and temperature. Typically, sensory neuropathy starts in the lower extremities followed by motor neuropathy within a few years. The initial signs of sensory neuropathy are paresthesia and hypoaesthesia of the feet. Temperature and pain sensation are impaired earlier than vibration and position sensation. At full-blown stages of the disease, sensory loss, muscle atrophy and weakness of extremities shows a glove and stocking distribution. Footdrop, wristdrop and disability of the hands and fingers are frequent symptoms of motor neuropathy. Autonomic neuropathy quite often accompanies the sensory and motor deficits (37). The symptoms includes orthostatic hypotension, constipation alternating with diarrhoea, attacks of nausea and vomiting, delayed gastric emptying (26), sexual impotence (38), and urinary retention or incontinence (39).

Ocular symptom may be present secondary to infiltration of the vitreous leading to vitreous opacities and elevated intraocular pressure (24). FAP patients may have renal amyloid with significant protein loss and subsequently renal insufficiency (25). Cardiomyopathy may be seen in the advanced stages of the disease (40). Cardiac arrhythmias and conduction disturbance are common in the disease (41-43), and often leads to pacemaker treatment (44).

The disease usually begins in the third or fourth decade, but the onset of symptoms may be later. For Japanese patients with the ValMet 30 mutation
the mean age of onset is 35 years (35), for Portuguese patients the mean age is 33 years (45). The mean age of onset in Val30Met of Swedish ancestry is later than in patients of Japanese or Portuguese ancestry (46). It has been speculated that the gene was transmitted from Portugal to Sweden and Japan during close commercial contacts between the countries in the 15th century. In a recent genetic study (unpublished data) on 14 Swedish, 18 Portuguese and 13 Brazilians families, the authors concluded that the origin of FAP depends on two different independent origins that occurred approximately 400 and 700 years ago, respectively. This finding makes it clear that the earlier hypothesis about an association between the Swedish population and the Portuguese population was wrong.

Although penetrance varies greatly among the geographic and ethnic foci, the outcome of FAP is invariably progressive and fatal (36). In Sweden, FAP has been diagnosed mainly in the two northernmost counties. Since the first case in 1965 (47), about 2500 carriers with positive genetic testing for TTR mutation have been recognized, and about 10% have developed the FAP disease. The life expectancy of patients with FAP is severely shortened and Swedish patients usually die after mean interval of 13 years from the first symptoms (26, 48, 49), from progressive worsening of neuropathy, secondary infections, cachexia or sudden death (50).

The main heart complications in FAP ATTR Val30Met are severe cardiac arrhythmias and conduction disturbances. The natural course of the disease often leads to permanent pacemaker treatment (51). Note that heart failure is a rare finding in FAP Val30Met patients (52).
Liver transplantation

The only treatment to halt the progression of FAP is liver transplantation (LTx). TTR is mainly produced in the liver and a liver transplantation should stop the production of new mutated amyloidogenic TTR. In 1990 in Sweden, the first FAP patient underwent orthotopic LTx for FAP (53). Since then, more than 100 FAP patients have been liver transplanted in Sweden. LTx has become an established world-wide treatment of the disease (54). Approximately 100 FAP patients are transplanted every year (Familial Amyloidotic Polyneuropathy World Transplant Register, accessible at: www.fapwtr.org). Most of the transplantations are performed on patients with FAP (ATTR Val30Met), but patients with other mutations have also undergone LTx (54).

After LTx, FAP patients have very low levels of mutant TTR in serum (55). Although the status of the patients seems unchanged after transplantation concerning amyloid deposits (54, 56-59), ocular manifestations appear after LTx (60), caused by a local production of TTR in the eye. Moreover, a progression of cardiomyopathy was found in FAP patients with the ATTR Val30Met mutation when they were examined 4 to 6 years after LTx, noted as increased left ventricular wall thickness and left atrial dimension (61). Other studies also have shown of a rapidly progressing cardiomyopathy after liver transplantation for certain TTR mutations (62-64). Thus, LTx does not seem to alter the natural course of the amyloid cardiomyopathy.
After the introduction of liver transplantation as a treatment of the disease, increased attention has been directed toward heart complications, which were the main cause of postoperative death (39%) according to World Transplant Register (36). The modified body mass index (mBMI), duration of disease, type of mutation and degree of autonomic involvement are important prognostic factors for the survival after of LTx (65).

**Worldwide transplanted FAP patients’ characteristics**

The following data were obtained from FAPWTR (www.fapwtr.org, accessed at 2006-11-30):

- Sex distribution of transplanted patients (%): Male 57
- Age at time of transplantation (years): Mean 40.7 +/-10.9, range 21-70.
- Duration of disease before transplantation (years): 4.2+/-2.9, range 0.3-30.
- Cardiac related deaths and sepsis account for at least 50% of deaths after transplantation.

**Indications for orthotopic liver transplantation**

The following clinical findings are all required before LTx is performed:

1. Biopsy proven amyloidosis
2. Genetic testing proving mutation
3. Symptoms such as paresthesia and hypothesia of the feet.

**Biopsy of amyloid**

Biopsy tissue (nerve, rectal, abdominal fat pad) is tested for amyloid using Congo red staining to confirm the presence of amyloid. Stained tissue will be viewed under polarized light used to demonstrate amyloid characteristic “apple-green” birefringence (66, 67).
Degree of the FAP disease

The polyneuropathy disability scoring system is used to assess the patients motor function: where 0 denotes patients without any impairment; I sensory disturbances, but preserved walking capability; II impaired walking, but able to walk without stick or crutches; IIIA walking with help of one stick or crutch; IIIB walking with the help of 2 sticks or crutches, and IV confined to a wheelchair or bedridden (39).

Another indicator for the degree of the disease is the modified body mass index (mBMI), which is used as indicator of malnutrition. BMI is a measure of body fat based on height and weight that applies to both men and women. The index mBMI is obtained by multiplying BMI (weight/height², in kg/m²) with the serum albumin (g/l) to compensate for oedema (26, 39). Patients with mBMI>600 have improved survival after liver transplantation (65).
Non-invasive ECG recordings

_Ambulatory electrocardiography (Holter-ECG)_

After the second World War Norman Jeffery Holter (68) started the development of 24-hours ambulatory ECG recording. He first tried to activate a frog muscle nerve from distance and later on measure brain activity from a rat. Using an amplifier, a radio transmitter, and an oscilloscope, he was able to broadcast EEG signals from the brain to a nearby radio receiver – at first only 2-4 feet away, later a block away. After that, he could investigate living systems with the greatest possible degree of freedom.

Later, N.J Holter transmitted ECG signals from a person who was moving around instead of just lying in a bed. It was easier to measure the heart signal compared with brain signal because it was about 10 times greater in magnitude. Initially, the received signals were displayed on an oscilloscope and photographed. In 1949, Holter developed the telemetry briefcase: by using transistors it became possible to record the ECG signals with a tape recorder.

Holter-ECG is the most widely used method to evaluate symptoms suggested of cardiac rhythm disturbances, such as syncope, pre syncope, dizziness, and palpitation (69, 70). However, other transient events are less commonly related to rhythm abnormalities: shortness of breath, chest discomfort, weakness or neurological symptoms such as a transient ischemic attack.

If arrhythmias are thought to be causative in patients with transient symptoms, an ECG has to be recorded at the same time that the patient has symptoms.
With such a recording, one can determine if the symptoms are related to an arrhythmia.

Four different outcomes are possible with Holter-ECG (71):

1. The patient may have symptoms and a simultaneously recorded cardiac arrhythmia capable of producing such symptoms. This outcome is the most useful for further treatment.
2. The patient may have symptoms although no arrhythmia is found in the Holter-ECG recording. This outcome is also useful because it shows that the symptoms are not related to rhythm disturbances.
3. The patient may remain asymptomatic during simultaneously recorded cardiac arrhythmias. This outcome has equivocal value because the recorded arrhythmia may or may not be relevant to the symptoms.
4. The patient may remain asymptomatic and no arrhythmias are found in the recording. This finding gives no useful information.

Holter-ECG is a useful tool to diagnose of all types of arrhythmias and to identify the underlying mechanisms (particularly for supraventricular arrhythmias), as for risk stratification and intervention (70).
Heart rate variability

In the eighteenth century, Albrecht von Haller noted that beat-to-beat fluctuations in a normal heart rate (HR) are not absolutely regular, and that these fluctuations are in synchrony with respiration. In the four last decades the clinical significance of these beat-to-beat fluctuations – normally referred to as heart rate variability (HRV) – has been mentioned in the literature. Measuring fetal HRV was the first major clinical application of HRV. Hon and Lee et al. reported in 1965 that the beat-to-beat changes are the first noted alteration before fetal distress occurs (72, 73). Numerous studies have demonstrated that HRV measurements can be a useful tool in assessing the function of the autonomic nervous system with regard to cardiac function (74, 75).

The autonomic nervous system (ANS) is a neural network that regulates the heart rate by the parasympathetic and sympathetic nervous system, two subsystems that often oppose each other. The parasympathetic system slows down the heart rate and the sympathetic system speeds it up. Cathecolamines, such as norepenephrine and acethylcoline are the major neurotransmitters in the sympathetic system and parasympathetic system, respectively (76).

HRV is considered to reflect the cardiac autonomic modulation. There are different methods to estimate the variability between heartbeat intervals (77-81). Data are collected from short-term recordings (one hour or shorter) as well as long-term recordings (24-hour recordings). HRV is calculated using both time domain and frequency domain indices, based on statistical, geometric and power spectra measures of the R-R intervals (77). Power
spectrum analysis of HRV has shown that the fluctuations occur in different spectral regions, where the different spectral components reflect autonomic activity related to respiration, regulation of blood pressure and thermoregulation (82).

Frequency domain analysis, i.e., how the power (variance) is distributed as a function of frequency, is used in this thesis. Power spectral analysis gives a quantitative measure of the variability, and the power spectrum is often divided into very low frequency (VLF; below 0.04 Hz), low frequency (LF; 0.04-0.15 Hz), and the high frequency (HF; 0.15-0.5 Hz) regions. The VLF component reflects slow variations in HR such as due to thermoregulation. The LF component reflects both parasympathetic and sympathetic activity, whereas the HF component represents parasympathetic activity related to respiration (80).

Finally, it is important to note that HRV may encompass two aspects: one reflecting normal cardiac autonomic control (normal sinus rhythm) and one reflecting random variations due to underlying abnormalities in cardiac control or function (erratic rhythm) that may be associated with worse outcomes (83). The most common method for analysing HRV is by using R-R intervals as a substitute for variations in PP-intervals in the electrocardiogram (ECG). Thus, in all cases where the ventricle contractions is not the direct result of a signal from the sinus node, differences between R-R intervals will not reflect the autonomic input on the sinus node (84). A phenomenon of particular interest in this thesis, related to this problem, is subtle multifocal atrial rhythm.
**Signal averaged electrocardiography**

Signal averaging techniques were first used in neurological science (85). In the end of 1960, Sherlag et al. developed a catheter technique to measure His bundle activity in an animal and later in man and this leads subsequently to recordings of various potentials from the heart (86). In 1969, Han recorded electrical signals in the canine heart, and noted that during ventricular pacing there was activation that persisted beyond the QRS complex, which were referred to as delayed ventricular potentials (87). In the early 1970s, El-Sheriff et al demonstrated fragmentation, delay and continuous electrical activity in reentrant ventricular ectopic beats and ventricular tachycardia in the canine model. Thus, resulted that late potentials was established as a marker of damaged or diseased myocardium; the substrate for reentrant ventricular tachycardia (88). Subsequently, Fontaine and co-workers reported the use of signal averaging technique. In 1973 and 1974, Nancy Flowers reported the first surface recordings from His bundle activity with signal averaging technique and noise reduction (89, 90). In late 1970s, non-invasive recordings of His bundle activity took a new direction and the recordings of late potentials became more interesting.

In 1982, Simson made important technical contributions for noise reduction which included bidirectional filtering and the combination of X, Y, Z leads into its vector magnitude. In the mid and late 1980s, Breithard and Gomes et al studied the prognostic significance of late potentials in the post-infarction patients (91, 92). In 1987 the studies by Kuchar and Gomes et al established the predictive value of late potentials in risk stratification of post-infarction patients (93, 94). One major limitation is that patients with bundle branch block normally are excluded from analyses of late potentials. In 1991, a Task
Force Committee reported on the standards for analysis of late potentials and its potential benefits (95)

Signal averaging is the most common computerised method for improving the signal-to-noise ratio of the ECG. The process of signal averaging is performed to detect low-amplitude cardiac signals, which are not identifiable with standard ECG techniques (95, 96). Signal averaging is based on the assumption that the interfering noise is random and that the waveform of interest repeats with every heartbeat included in the average. SAECG recordings are normally analysed off-line, i.e., data are stored on the computer while the patient is connected to the system, but the recordings are analysed later. Reduction of the noise is the most important procedure in the SAECG examination (97). Noise in the SAECG has three principal origins: interference signals, electronic sources and physiological sources.
Do FAP patients have potentially life-threatening ventricular arrhythmias?

Many of the investigations performed in this thesis are inspired by the finding of 17 minutes episode of sustained ventricular tachycardia (VT) in a Holter recording performed in a liver transplanted FAP patient. Because of the impact this patient has had on this thesis work, the patient is described as an index case below.

A middle-aged patient was referred to our hospital with increasing pain in both his feet. The symptoms progressed in the following years, and peripheral biopsies and genetic analyses confirmed the diagnosis of FAP (ATTR Val30Met mutation). After five years of disease, the patient underwent liver transplantation. A pre-transplant 24-hours Holter-ECG investigation showed frequent ventricular premature beats and short episodes of non-sustained ventricular tachycardia (VT).

After nine years, the patient received Ciproxin treatment for an infection that turned out to be caused by cytomegalovirus (CMV). During hospital stay, one episode of sustained VT was detected, which was successfully treated with Sotalol. A Holter-ECG performed a few weeks later showed same findings as the recording before LTx.

Later the same year two investigations with SAECG were performed, both showing abnormal late potentials (LP) (96). Invasive programmed electrical stimulation, however, failed to induce sustained VT. The patient, nevertheless, continued to suffer from episodes of dizziness and cardiac palpations. During
telemetry and Holter-ECG recording after a suspect grand mal seizure, several episodes of sustained VT lasting up to seventeen minutes were recorded (Figure 1). Consequently, a transvenous cardiac defibrillator (ICD) was implanted.

One year later, SAECG re-examination showed continuing abnormal LP. Echocardiographic examinations from the pre-LTx evaluation to the latest post-transplant follow-ups showed an increasing left ventricular septum thickness (from 10 to 14 mm). Furthermore, post-transplant Holter-ECG recordings have disclosed sino-atrial and second-degree atrio-ventricular blocks with pre-syncope.

Figure 1. ECG obtained from the patient showing a sustained ventricular tachycardia (modified from Fig 1, Paper II).
To our knowledge, this is the first report of a FAP (ATTR Val30Met) patient with documented sustained VT several years after liver transplantation. It is possible, that the initial CMV infection and treatment may have facilitated the development of the VT. However, the repeatedly abnormal LP at subsequent follow-ups, the increasing hypertrophy of the ventricular septum and the development of conduction disturbances, as well as the time interval from infection to the sustained VT give support to the suspicion of an amyloid related arrhythmia.

Further support for an amyloid related arrhythmogenesis is found in a study of patients with primary amyloidosis, in which the presence of LP was associated with a higher risk of sudden death in patients with infiltration of amyloid fibrils in the myocardium (98).
Aims of the study

The principal aim of the present study was to examine the presence of cardiac arrhythmia and cardiac autonomic dysfunction in Swedish patients with familial amyloidotic polyneuropathy, examined before and after liver transplantation. The specific aims were:

1. to determine whether liver transplantation affects the natural course of cardiac arrhythmias and conduction disturbances.

2. to predict the risk of development of ventricular arrhythmia.

3. to determine if 24-hours HRV power spectra would verify previous findings from short-term recordings that FAP patients have a reduced HRV.

4. to investigate whether liver transplantation affects the cardiac autonomic function and cardiac arrhythmias by analysis of 24-hours HRV and SAECG recordings before and after liver transplantation.

5. to determine whether early or late follow-up examinations after liver transplantation shows progress or regress of cardiac autonomic dysfunction by analysis of short-term HRV.
Summary of papers

Paper I: Liver transplantation does not prevent the development of life-threatening arrhythmia in familial amyloidotic polyneuropathy, Portuguese type (ATTRVal30Met) patients

In paper I, the aim was to investigate the occurrence and development of cardiac conduction and rhythm disturbances in Swedish FAP patients who underwent liver transplantation. The study included 17 men and 13 women.

The number of patients with ECG abnormalities increased after LTx. The proportion of patients with a Holter registration classified as normal was 47 % before LTx and decreased to 29 % after LTx. Twenty-four percent had a pacemaker device implanted 5 to 8 years after LTx because they had bradyarrhythmia and symptoms such as, dizziness and syncope/presyncope.

The study showed that the development of cardiac conduction disturbances and arrhythmia appear to continue after liver transplantation. Therefore, regular Holter-ECG examinations should be performed after LTx, because of the potential risk that FAP patients receiving transplants may develop fatal arrhythmia also several years after LTx.
**Paper II: Ventricular late potentials in familial amyloidotic polyneuropathy**

In paper II, we investigated the occurrence of ventricular late potentials (LP) in patients with FAP. We hypothesized an increased prevalence of LPs and possible association between the occurrence of LPs with ventricular arrhythmia on Holter ECG and echocardiographic data. The study included 29 men and 26 women.

The study showed that LPs were common findings in older FAP patients (46%), compared with a healthy control group (15%). Moreover, the occurrence of LP in patients older than 60 years was associated with nonsustained ventricular arrhythmia and left ventricular thickness. Long-term follow studies are required to find the prognostic significance of these new findings, although absence of LP may indicate a low risk for ventricular tachycardia.

**Paper III: Abnormal heart rate variability and subtle atrial arrhythmia in patients with familial amyloidotic polyneuropathy**

In paper III, which was a study before LTx, the aim was to analyse HRV patterns in 24-hours ECG recordings. Findings from power spectrum analysis of HRV were compared with those of Poincaré plots. The study included 28 men and 23 women.
The study showed that most of the FAP patients had reduced HRV, but some cases had high HRV indicating rhythm disturbances instead of reflecting normal cardiac autonomic modulation. These novel findings were substantiated by a broadband spectral pattern and by Poincaré plots with a fan or complex pattern.

**Paper IV: Heart rate variability and ventricular late potentials after liver transplantation for familial amyloidotic polyneuropathy**

In paper IV, we investigated if there was a regressive or progressive development of ventricular late potentials and HRV after LTx. Therefore, the study compared recordings of SAECG and 24-hours HRV obtained before LTx with those obtained 1-2 years after LTx. The study included 10 men and 11 women.

The study showed that reduced HRV and ventricular late potentials were common before LTx, but no significant changes were observed after LTx. Marked increased HRV was found in three patients after LTx, but this was caused by the development of intermittent atrial arrhythmia, and did not reflect an improvement in cardiac autonomic control. The study did not show any significant improved cardiac autonomic control after LTx.
In paper V, we performed a long-term follow-up study of heart rate variability to determine the impact of liver transplantation on cardiac autonomic function in FAP patients. Data were recorded in 57 FAP patients (29 men and 28 women) before and between 2-14 years after LTx: as early (less than 30 months) and as late (more than 42 months) follow-up recordings. HRV was analysed by power spectral analysis and Poincaré plots.

The study showed reduced sympathetic cardiac autonomic modulation at the early follow-up after LTx, compared with before LTx. The cardiac autonomic dysfunction was relatively unchanged between the early and late follow-ups. Liver transplantation preserves the cardiac autonomic function in FAP, but not the development of other disturbances in cardiac function – such as arrhythmia and conduction disturbances.
Material and methods

Patients

All patients (54 men and 43 women) included in the present series of studies were adult Swedish FAP patients, between 24 and 80 years of age at first examination (mean 53.5 years). The examinations were performed as part of their clinical evaluation before eventual liver transplantation, or performed as follow-up investigations after transplantation. All were examined at the University Hospital in Umeå, Sweden from 1990 to October 2006. The liver transplantations (paper I, IV and V) were performed at Huddinge University Hospital, Huddinge, or Sahlgrenska University Hospital, Göteborg both in Sweden.

The diagnosis of TTR-amyloidosis was in all patients confirmed by findings of amyloid deposits in the skin or intestinal biopsy specimens, and by positive genetic testing for TTR-mutation: all patients carried the ATTR Val30Met trait except two who carried His88Arg or Phe33Leu, respectively.

Mean duration of symptomatic disease to first examination was 3.5 years (range 0.5-12.0 years). The PND score disclosed that 12 patients had score 0, 48 patients score 1, 19 patients score 2, 13 patients score 3, and finally 3 patients score 4. Overall for the FAP patients, mean mBMI at a mean age of 53.5 years was 950±197, range 420-1534. Compared with healthy normal subjects mean value in same mean age group (968) the patients had slightly reduced mBMI.
All patients gave informed consent and were treated according to the Declaration of Helsinki. The studies were approved by the Ethical Committee of Umeå University. All studies were performed retrospectively, except paper II that was a prospective study. Figure 2 shows an overview of the FAP patients included in the studies.

Figure 2. FAP patients included in the studies.
Controls

In paper II, FAP patients were compared with 94 healthy subjects, 44 men and 50 women, with a mean age of 54 years (age range 33-80 years). In paper V, FAP patients were compared with 90 healthy subjects (45 men and 45 women), mean age 50 years (age range 20-80 years), respectively. The subjects in the control series were randomly selected from the population register or recruited from the hospital staff. They did not have any neurological disorder or cardiac arrhythmia, were not treated with drugs known to affect heart rate variability, and had normal blood pressure.

Twenty-fours hours ambulatory ECG recordings (I, II, III,IV)

Examination

The presence of cardiac conduction and rhythm disturbances was determined based on standard 24-hours Holter-ECG monitoring. The recordings are non-invasive and relatively simple to perform. After selecting the sites for electrode application, four (paper I) or five (paper II-IV) colour coded silver-silver chloride electrodes were attached to the subjects chest. Placements with five electrodes were as follows: exploring leads (V2 brown and V5 red) were located in the fourth right intercostal space adjacent to the sternum and in the left anterior axillary line over the fifth intercostal space, respectively; reference electrodes (white and black) were placed underneath the right clavícula; and earths (green) at V5R position. The leads were positioned so as to achieve optimal P-waves and QRS-complexes as well as to minimize disturbances. After attaching the electrodes the patients were connected to a portable recording unit (Tracker II, Reynolds Medical Ltd, UK, or Braemer
DL 700, Braemer inc. Burnsville, MN, USA). The recorder was attached to a belt worn around the waist and did not interfere with strenuous activity or even vigorous exercise.

Subjects followed their normal daily routine and recorded a diary of symptoms and activity levels, which included a wide variety of activities such as symptoms from the heart (palpitations, dizziness), other examinations at hospital, athletic activity, dinner, onset of sleep and moment of waking. All the recordings started in the afternoon and the subjects came back approximately at the same time the day after.

The ECG recordings were automatically analysed by a PC-based Holter system (Aspect Holter System, GE Healthcare, Borlänge, Sweden) and then examined and edited by one investigator.

Our specific definitions of rhythm and conduction disturbances in paper I- IV were as follows:

- Supraventricular tachycardia (SVT) was defined as the presence of series of more than five consecutive supraventricular beats in series at a heart rate more than 100 beats/minute.
- Ventricular rhythm was defined as series of more than two consecutive ventricular extra systoles with a heart rate below 100 beats/minute.
- Ventricular tachycardia (VT) with a heart rate more than 100 beats/minute (paper I, II, III and IV)
- Ventricular arrhythmia was defined as more than 10 ventricular beats/hour (paper II, III and IV) (99).
Methodological considerations

The following additional arrhythmic events were defined by the software:

- Ventricular beats in series: More than one ventricular beat in series.
- Ventricular beats in bigeminia: More than two ventricular beats alternating with normal sinus beats.
- Early ventricular beats: Ventricular beats which took place within the QT-interval before the previous QRS-complex, according to Bazetts formula. \( QTc = \frac{QT}{\sqrt{RR}} \) in sec and heart rate more than 60 beats/minute.\(^{(100)}\)
- Supraventricular beats in series: More than one supraventricular beat in series.
- Supraventricular beats in bigeminia: More than two supraventricular beats alternating with normal sinus beats.
- Tachycardia: a heart rate more than 100 beats/minute and at least 20% over mean heart rate the last minute. "Sudden tachycardia".
- Bradycardia: a heart rate below 50 beats/minute and at least 20% below mean heart rate the last minute. "Sudden bradycardia".
- Episodes with tachycardia or bradycardia are estimated at 4 heart beats.
- Asystoli: a R-R interval more than 3 seconds.

Signal average electrocardiography (II, IV)

All recordings (paper II, and IV) where performed with an ECG device with high-resolution ECG option (Siemens-Elema MegaCart, Siemens-Elema AB, Stockholm, Sweden). Orthogonal bipolar X, Y and Z leads were recorded until 300 heart cycles were recorded. The sampling rate was 1,000 samples/second and the correlation limit 98%. The recorded signal was
digitised, and the resulting data underwent signal averaging and filtering using Fir4 (finite impulse filter) band pass filter (95) with range of 40-250 Hz. A high-pass cut-off frequency of 40 Hz was used for filtering because time-domain results analysed at 40 Hz-filtering showed the highest sensitivity and specificity for predicting arrhythmic events in previous studies (91, 101).

The vector magnitude (VM) includes all three leads X, Y and Z, and is given by:

$$VM = \sqrt{x^2 + y^2 + z^2}$$

Figure 3 shows an example of a SAECG recording in a subject without LPs.

The following quantitative SAECG variables were calculated from the vector magnitude and visually inspected and adjusted:

- **FQRS-duration:** filtered QRS-complex duration, which includes the duration of possible late potentials
- **RMS40:** root mean square voltage of the terminal 40 ms of the filtered QRS-complex.
- **LAS40:** duration of the low amplitude signals (<40µV) of the terminal filtered complex at 40 Hz filtering, i.e. the duration of the last part of the filtered QRS-complex which is maximum 40µV above baseline.

SAECG by time domain analysis was considered to be positive if two or more of the following criteria were fulfilled (95):

1. Filtered (F)QRS-complex duration >114ms
2. RMS40<20 µV; and
3. LAS40>38 ms at 40 Hz filtering
Figure 3. Typical SAECG recording in a subject without late potentials. Left: unfiltered complexes; middle: filtered complexes; right: corresponding vector magnitude.

Methodological considerations
Before the recordings begin, the subjects should be given an opportunity to relax for a few minutes. A calm, warm, relaxed subject, lying on a comfortable, sufficiently long and wide couch in a well-heated examination room generates a minimum of muscular interference. Turn off all unnecessary electrical devices in the room, group the electrode- and power cables to minimize electromagnetic disturbances, and never use 50 Hz filter. An enabled 50 Hz filter causes very small distortions in the latter part of the QRS complex, i.e. "ringing". The reason for that is that late potentials have
amplitudes of 5-40µV, so a 50 Hz filter could produce ringing of the same magnitude and in the same part of the QRS complex.

Good electrode quality and correct, careful electrode attachment is of vital importance to results in the recording of high-resolution ECG. Before attaching the electrodes (Ag/AgCl), clean the electrode sites carefully with alcohol or similar, lightly abrade the skin at the electrodes sites with sandpaper or similar. The electrode placement (paper 2, and 4) were of bipolar electrode configuration and the electrode cable designations according to AHA (American Heart Association) and are placed as following (Figure 4):

- **RL** Right arm
- **LA** Left arm
- **RL** Right leg
- **LL** Left leg
- **V1** On the right midaxillary line on a level with the fifth intercostal space
- **V2** On the left midaxillary line on a level with the fifth intercostal space.
- **V3** At top left of sternum
- **V4** On the left iliac crest
- **V5** On the back on a level with fourth intercostal space and left sternal border
- **V6** In the fourth intercostal space on the left sternal border

**Important settings for SAECG recordings**

Before data acquisition is started three important settings must be done. Firstly, the correlation limit: This value designates the morphological similarity between heart beats, e.g., 0.98=98%. Beats found to have a lower value in the data acquisition will be excluded from further analysis. Secondly, to choose target noise level or target number of beats level: Acquisition can be
terminated when one of these levels are reached – a) the noise level is less than 0.3μV, and/or b) the number of heart cycles reached 300 beats. Thirdly, choose filter type “FIR4 or FIR6” (FIR= Finite Impulse response).

Noise in the SAECG recording has three principal origins:

- Interference noise: usually appears as a periodic disturbance superimposed on the ECG waveform.
- Electronic noise: random fluctuations, generated by electronic devices and has similar power at all frequencies.
- Physiological noise: the most important noise source, contributing 75-95% of noise in the resting ECG. Physiological noise originates from muscle activity and occupies a wide frequency bandwidth over short periods of time.

**Figure 4.** Simplified picture of an orthogonal bipolar electrode configuration. Note that one lead is placed on the back of the patient. From these lead placements, the 3 orthogonal components, X, Y and Z are derived.
Heart rate variability

Short-term recording of HRV (paper V)
After attaching ECG electrodes, short-term recordings of HRV were performed as following (Figure 5): after about 5 minutes supine rest, the blood pressure was measured and continuous recording of ECG and respiration (impedance measure) was started. Free spontaneous breathing was continued for 6 minutes, the subjects were than instructed to perform controlled breathing: first at a rate of 6 breaths per minute during one minute; then at a rate of 12 breaths per minute during one minute. After passive tilting to 70 degrees head-up position, the recording was continued during 4 minutes. During the last minute the blood pressure was measured again.

Spectral analysis was performed on segments without artefacts and arrhythmic beats. Recordings in the supine and upright positions were analysed as 2-minutes segments, whereas no analysis was preformed on the 1-minute sequences with controlled breathing in this study.

The R-R interval data was transformed to an evenly sampled (2 Hz) time series by cubic spline interpolation, as illustrated in Figure 6. The power spectral density (PSD) was estimated by autoregressive modelling(77), after both the mean and linear trends had been removed.
Figure 5. Typical short-term HRV recording in a healthy subject. Top: recorded heart rate. Bottom: power spectra for HRV (solid line) and respiration (dashed) in the supine position (left figure), and after passive tilt (right figure), respectively.
HRV was quantified by calculating the mean heart rate, the total spectral power (0.003-0.5 Hz) and the power of three different spectral components: 1) very low-frequency (VLF) component (below 0.04 Hz), reflecting several physiological variables, such as thermoregulation and the renin-angiotensin system; 2) low-frequency (LF) component (0.04-0.15 Hz), which is attributed to baroreceptor-mediated blood pressure control, reflecting both sympathetic and parasympathetic activity(102); 3) high-frequency (HF) component (0.15-0.50 Hz), which is related to the respiratory rate at normal breathing and used as an estimate of parasympathetic activity(77). The LF/HF ratio was calculated as an indicator of sympathovagal balance. The recording and analysis software was developed at our research department.
24-hours recordings of HRV (paper III and IV)

Before power spectral analysis, a careful arrhythmia analysis was performed using the same software package and the same procedure was performed as used for analysis of regular Holter recordings. Heartbeats were classified as: normal beats, supraventricular extrasystolic beats, ventricular extrasystolic beats, or beats of uncertain origin. The classification of heartbeats was carefully examined and confirmed by one investigator. After editing, R-R intervals and the classification of heartbeats were exported from the software package for further analyses using Matlab (Mathworks Inc., Natick, Mass., USA). Before HRV was analysed, undetected ectopic beats were removed by additional filtering, where RR intervals were removed if they differed more than 30% from the mean of the preceding and following RR intervals (103, 104).

Power spectral analysis was performed only on R-R intervals related to normal beats by means of fast Fourier transformation. All data from the 24-hour recording were divided in segments with a length of 300 seconds, and averaged spectra were calculated according to Welch method(105). Ectopic beats and episodes with poor signal quality were replaced by interpolation. However, segments with more than 4% interpolated data were discarded. The percentage of segments used for HRV analysis was used as an indicator of the quality of the recording and the presence of arrhythmia. HRV was only analysed in recordings with more than 70% used time.

The total power (in the frequency region 0.003-0.50 Hz), and the power of the VLF, LF and HF components were calculated as average data over 24 hours recordings. In addition, The LF/HF ratio was calculated as an indicator of sympathovagal balance. The average R-R interval value was calculated from
accepted beats (mean RR). Finally, power spectra were determined for each hour of the recording.

**Poincaré plot analysis**

Poincaré plots were constructed as scatterplots of all pairs of R-R intervals were two successive heart cycles were classified as normal (106). Poincaré plots based on data from complete 24-hour recordings often have one of the following patterns (Figure 7) (106): a) a comet, with an increasing variability with increasing R-R interval, corresponding to the “normal” HRV pattern; b) a torpedo, with the same dispersion along the diagonal irrespectively of mean R-R interval, a pattern found in subjects with low HRV; c) a fan-shaped, a triangular or V-shaped dispersion along the diagonal; d) a complex pattern, with groupings of points off the diagonal. Undetected ectopic beats or other heartbeats reflecting cardiac conduction disturbances often results in a fan-shaped pattern or in a complex pattern. Although only beats classified as normal sinus beats were used to generate the Poincaré plots, all sinus beats immediately after the ectopic beats were also removed.

The geometric appearance of the Poincaré plot was described by rotating the coordinate axis 45° counter clockwise to the normal axis. Then, the standard deviation along new y-axis (SD1) and the standard deviation along the new x-axis (SD2) were calculated. SD1 describes the fast beat-to-beat (short-term) variability of the HR, whereas SD2 describes the long-term variability (107). The ratio SD1/SD2 was used as an indicator of undetected arrhythmic beats and artefacts (83). Figure 8 shows and example of the different methods for analysis of 24-hour HRV data.
Figure 7. Poincaré plots for three different 24-hour recordings. Left: patient with normal HRV with a comet shaped Poincaré plot, where the variability increases with increasing R-R interval; Middle: Patient with reduced HRV with a torpedo shaped Poincaré plot, showing a low variability without any dependence of mean R-R interval; Right: patient with a fan shaped Poincaré plot, a typical finding in patients with subtle atrial arrhythmia.

Figure 8. Three different methods to analyse 24-HRV recordings, illustrated for a healthy subject. Left: average 24-hour PSD, showing both a LF and HF peak. Middle: Hourly spectra, showing a marked day-night variation in HF power. Right: Comet shaped Poincaré plot, with SD1 och SD2 indicated.
Results and discussion

Cardiac arrhythmias and conduction disturbances in FAP patients
Paper I, II, III, IV and V.

All papers studied cardiac arrhythmias and conduction disturbances in FAP patients before LTx (paper I, II and III), and both before and after LTx (paper I, IV and V).

Paper I was a long-term follow-up study of Holter-ECG recordings, including recordings more than 42 months after LTx. The study included 17 men and 13 women and showed an increased severity of cardiac arrhythmias and conduction disturbances when recordings before LTx were compared with those after LTx. The proportion of patients with a Holter-ECG classified as normal was 47 % before LTx, 35% at the short-term follow-up recording, and decreased to 29 % at the long-term recording. In addition, 24 % had a pacemaker device implanted 5 to 8 years after LTx because they had bradyarrhythmia and symptoms such as dizziness and syncope/presyncope.

In paper II, we focused on ventricular arrhythmia before LTx and hypothesized an association between ventricular arrhythmias and late potentials. The study included 29 men and 26 women, and they were compared to 94 healthy controls. Ventricular arrhythmia was defined as following: presence of 3 or more ventricular beats in a series, or more than 10 ventricular extrasystoles per hour. Eighteen patients (38%) had episodes with nonsustained ventricular arrhythmia (an episode of three or more ventricular beats in series and/or more than 10 VES/hour). In addition, 25 patients (53%)
had episodes of rhythm or conduction disturbances in their Holter-ECG recordings.

In paper III, the aim was to determine if 24-hours HRV power spectra would verify previous findings from short-term recordings that FAP patients have a reduced HRV. The study included 28 men and 23 women. Using methods for HRV analysis – power spectral analysis and Poincaré plots – we found signs of subtle atrial arrhythmia in 18 (45%) FAP patients, an arrhythmia not identified during regular Holter analysis. In total, paroxysmal or intermittent atrial arrhythmia was found in 38% of the patients.

In paper IV, which was a short-term follow-up study of 24-hour HRV recordings, performed up to 28 months (mean 21.7 months) after LTx. The study compared data from ambulatory Holter recordings before LTx with data collected after transplantation. The study included 10 men and 11 women. The proportion of patients who had a Holter-ECG recording classified as abnormal was 62% before LTx and increased to 71% after LTx. One patient was pacemaker treated shortly after the Holter-ECG examination, as prevention before LTx.

In paper V, the aim was to investigate the impact of liver transplantation on the autonomic nervous system by spectral analysis of HRV and Poincaré plots, where 21 patients performed follow-up recordings more than 10 years after LTx. The study included 29 men and 28 women. The study showed that the patients continued to develop cardiac arrhythmia after LTx, and that approximately 25% needed pacemaker treatment after LTx.

In all studies (I, II, III, IV and V) we disclosed that cardiac arrhythmias were common in FAP patients, and that rhythm and conduction disturbances had a
progressive development after transplantation. Summing findings in study I and IV, only 43% had normal Holter recordings before LTx, which decreased to 32% after LTx. The most common finding was supraventricular rhythm disturbances.

Approximately 25% needed pacemaker treatment after transplantation and this is a sign of more severe conduction disturbances. These signs of rhythm and conduction disturbances are in accordance with earlier studies (41, 51, 108). The most common symptoms in pacemaker treated patient were dizziness and syncope/presyncope. Two Japanese histopathologic studies showed that wild-type TTR can assemble into amyloid in the heart after LTx (109, 110). The cause of pacemaker treatment of our liver transplanted FAP patients could be continued deposition of amyloid within the heart after LTx, especially within the conductive system. All FAP patients were on immunosuppression therapy (tacrolimus or cyclosporine) after LTx, but to our knowledge no study has reported that this treatment might cause or contribute the development of cardiac arrhythmias and conduction disturbances. In the present studies (I, IV and V), there were no obvious differences between the two different drugs.

The findings in paper I, IV and V show that LTx does not seem to halt the development of heart complications in FAP. Although FAP and other transthyretin amyloidosis are rare diseases the physician handling FAP patients should be aware of possible heart related complications also after LTx (44, 61, 64). Typical complications include heart block and/or restrictive heart failure and also VT.
Late potentials in FAP patients

Paper II and IV.

These two papers studied late potentials from SAECG recordings in FAP patients before LTx (paper II), and both before and after LTx (paper IV). Paper II, which was a study before LTx, showed that late potentials were common findings in older FAP patients (46%), compared with a healthy control group (15%). Among the younger patients (less than 60 years old) the difference between FAP patients and controls were, 30% and 22% respectively. In this study, nonsustained ventricular arrhythmia (VT and >10 VES/hour) was found in 58% of FAP patients.

Summing the occurrence of ventricular late potentials in paper II and IV, approximately 35% of FAP patients had positive late potentials, compared with 20% in a healthy group, respectively. Paper IV compared pre- and post LTx recordings but no differences in the occurrence of LP was found, i.e., the substrate for electrical re-entry in the myocardium is unchanged after LTx.

Paper II showed that LPs were associated with non-sustained ventricular arrhythmia and an increased size of the left ventricle in FAP patients older than 60 years. These findings of LP could have been caused by amyloid fibril infiltration in the myocardium. This is supported by findings in patients with AL (primary) amyloidosis, where the presence of LP was associated with a higher risk of sudden death (111). In those patients, amyloid fibril infiltration of the myocardium is a serious prognostic factor. Moreover, the main cause of death in TTR amyloidosis was related to cardiac complications (36), which emphasises the clinical relevance of the findings in paper II. Thus, the increase in LP may be linked to an increased risk for ventricular arrhythmia and sudden death in FAP patients. However, because of the relatively high
occurrence of LP in healthy individuals, SAECG may be difficult to use as a screening tool for the presence of ventricular arrhythmia in TTR amyloidosis. On the other hand, patients without LP probably have a low risk for ventricular arrhythmia.

**Autonomic function measured by both long-term and short-term recordings of heart rate variability**

Paper III, IV and V.

These three papers studied the autonomic function before LTx (paper III), and both before and after LTx (paper IV and V). All the recordings in paper III and IV were long-term HRV recordings measured by 24-hours Holter-ECG recordings. In paper V, which was a late follow-up, in some patients more than 10 years after LTx, the HRV recordings were performed in a controlled laboratory environment. Figure 9 shows typical short-term HRV recordings in FAP patients with moderate and severe autonomic dysfunction.

Paper III, which was a study before LTx, showed that most of the FAP patients had reduced HRV, but some cases had high HRV that indicated subtle atrial arrhythmia instead of normal cardiac autonomic modulation. These novel findings were substantiated by a broadband spectral pattern and by Poincaré plots with a fan or complex pattern. The study included 28 men and 23 women.

In paper IV, we analysed if there was a regressive or progressive development of cardiac autonomic function after LTx. The outcome showed conflicting results with both reduced and increased HF power after LTx. Although the increased HRV could reflect an improvement in cardiac autonomic control, we found that this reflected development of subtle atrial arrhythmia. The study included 10 men and 11 women.
In paper V, the aim was to evaluate the long-term impact of liver transplantation on the cardiac autonomic function in Swedish FAP patients. The study included 29 men and 28 women. HRV was analysed by analysis of heart rate variability and Poincaré plots in patients without arrhythmia. The study showed reduced sympathetic cardiac autonomic modulation at the early follow-up after LTx, compared with before LTx. This reduction could possibly reflect a deterioration in cardiac autonomic function during the time span between the pre-transplant HRV examination and LTx (mean 7.9 months), or be due to immunosuppressive medication. Furthermore, HRV showed a small increase between the early and late follow-up recordings. We hypothesise that this increase is caused by subtle atrial arrhythmia – a phenomenon that previously had been shown to contaminate HRV recordings in FAP patients. Although the autonomic dysfunction seemed unchanged after LTx, this study, as well as previous data from 24-hour recordings, showed
that patients continue to develop cardiac arrhythmia after LTx, and that approximately 25% needs pacemaker treatment.

All three studies showed that the majority of the FAP patients had reduced heart rate variability both before and after LTx, indicating a severe dysfunction in cardiac autonomic control. This is in agreement with many other studies in patients with FAP (37, 112-114). In particular, the reduced power of the HF component indicates a reduced vagal modulation of the sinus node, which could be due to a parasympathetic dysfunction.

A novel finding was that high HRV, in particular in the HF region, may be an indicator of subtle atrial arrhythmia. Therefore, the conflicting results with increased HRV in some patients and decreased HRV in other patients after LTx could partly be explained by this finding. This study showed that several patients increased their HRV after LTx because they developed subtle atrial arrhythmia, and did not improve their cardiac autonomic function. However, it cannot be ruled out that improvement in cardiac autonomic function may occur in individual patients, but those patients were not found in this study. Moreover, today the patients are transplanted earlier in the course of the disease. Further research is needed to study the occurrence and development of cardiac arrhythmias in these patients. Will these patients also develop cardiac arrhythmias after liver transplantation? Will they have less severe autonomic disturbances?

Short-term HRV is an important clinical evaluation for assessment of cardiac autonomic function before patients undergo liver transplantation. A previous study showed that FAP patients with severe cardiac autonomic dysfunction
have increased risk for intraoperative circulatory instability during transplantation (115).

**Why cardiac arrhythmia and autonomic dysfunction?**

The cause of cardiac arrhythmia and cardiac autonomic dysfunction in FAP patients is not yet fully investigated and there are probably many factors that contribute to the disturbances. The main factor could be the impact of amyloid fibrils in different organs, such as the conduction system in the heart and the autonomic nervous system. This is supported by the findings of heavy amyloid infiltration in the atrium (116) and the vagus nerve (117).

**Rolf’s reflections**

FAP is a very rare disease. Cardiac arrhythmias and conduction disturbances are life threatening and could lead to circulation collapse. On the other hand, with adequate treatment a satisfied general health can be reached. A liver transplantation is very expansive and it is important to determine the correct point of time for transplantation, which depends on how damaged or diseased the heart and the autonomic nervous system are.

Whether FAP patients underwent liver transplantation or not it is important that patients with potentially increased risk for sudden death perform regular examinations with Holter-ECG. Temporary disturbances in the cardiovascular regulation may lead to intensive care treatment, and can give serious neurological damages which results in reduced quality of life.
Conclusions

In this thesis, the Holter-ECG recordings indicate that rhythm and conduction disturbances are common both before and after LTx, and that the natural course of the cardiac involvement after LTx includes a progressive development of cardiac arrhythmias.

The occurrence of ventricular late potentials in FAP patients older than 60 years is associated with nonsustained ventricular arrhythmia and an increased size of the left ventricle. Absence of LP may indicate a low risk for ventricular arrhythmia.

Reduced heart rate variability is a common finding both before and after LTx, as shown by analyses of both short-term and 24-hour HRV recordings. A novel finding was that high HRV could be an indicator of ECG abnormalities – in particular subtle atrial arrhythmia – instead of reflecting normal cardiac autonomic modulation. Furthermore, liver transplantation seems to preserve cardiac autonomic function in FAP.

This thesis has shown that cardiac arrhythmia, ventricular late potentials and reduced heart rate variability are important complications of the FAP disease, and that the complications of this disease are not solved by liver transplantation.

Since the development of cardiac arrhythmias and conduction disturbances continue after liver transplantation, it is important to perform regular follow-up recordings of Holter-ECG. Analysis of short-term HRV is an important
pre-transplant recording, because it can identify patients at high-risk for intraoperative circulatory instability, as shown in an earlier study.

The three non-invasive methods used in this study – Holter-ECG, HRV and SAECG – have led to improvements in the diagnosis of cardiac arrhythmia and autonomic function in patients with FAP.
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