End-stage heart failure and left ventricular mechanical support.

Clinical and fundamental aspects.

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ISBN 90-393-3100-6

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Clinical and fundamental aspects.

Eindstadium hartfalen en mechanische ondersteuning van de linker ventrikel.
Klinische en fundamentele aspecten.

(Met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de Rector Magnificus, Prof. Dr. W.H. Gispen,
ingevolge het besluit van het College voor Promoties
in het openbaar te verdedigen
op dinsdag 24 september 2002 des namiddags te 2.30 uur

door

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geboren op 6 augustus 1957, te Driebergen-Rijsenburg

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Financial support by the Netherlands Heart Foundation and the Prof.R.L.J. van Ruyven Foundation for the publication of this thesis is gratefully acknowledged. Additional financial support, provided by Novartis Pharma BV, Roche Nederland BV and Merck, Sharp & Dohme BV is much appreciated.

Voor Maria
Lisa, Simon en Ireen

List of abbreviations

ACE	angiotensin converting enzyme
AT	anaerobic threshold
ATP	adenosin triphosphate
CI	confidence interval
DAB	diaminobenzidine
DCM	dilated cardiomyopathy
DNA	deoxyribonucleic acid
FasL	Fas-ligand
FLICE	caspase-8
FLIP	FLICE inhibitory protein
HRP	horseradish peroxidase
HTx	heart transplantation
IABP	intra-aortic balloon pump
IHD	ischemic heart disease
IVS	interventricular septum
LV	left ventricle
LVAD	left ventricular assist device
LVEF	left ventricular ejection fraction
MAP	mean arterial pressure
MHC	myosin heavy chain
NF-$_\kappa$B	nuclear factor kappa beta
NO	nitric oxide
NSAID	non-steroidal anti-inflammatory drug
NYHA	new york heart association
PAR	poly-ADP-ribose
PARP	poly-ADP-ribose polymerase
PCWP	pulmonary capillary wedge pressure
PVR	pulmonary vascular resistance
RAP	right atrial pressure
REMATCH	randomized evaluation of mechanical assistance for the treatment of congestive heart failure
RNA	ribonucleic acid
RQ	respiratory exchange ratio
RV	right ventricle
TACE	tnf$_\alpha$ converting enzyme
TGF$_\beta$	transforming growth factor beta
TNF$_\alpha$	tumor necrosis factor alpha
TNFR1	tnf receptor 1
TNFR2	tnf receptor 2
TPG	transpulmonary gradient
TUNEL	terminal dUTP nick end labeling
VE	minute ventilation
VCO_2	carbon dioxide production
VO_2	oxygen consumption

Contents

Chapter 1

General Introduction

Heart failure

Heart failure is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood (1). Coronary artery disease is the underlying cause of heart failure in approximately two thirds of patients with left ventricular systolic dysfunction (2). Other important causes of heart failure include hypertension, valvular disorders and cardiomyopathies (3). Defining heart failure is not simple, and many definitions have been used throughout the years. A pragmatic definition of heart failure would be: a clinical syndrome caused by an abnormality of the heart and identified by a characteristic pattern of hemodynamic, renal, neural and hormonal responses (4). After the initial cardiac injury, patients may remain asymptomatic for a long time (Fig.1).

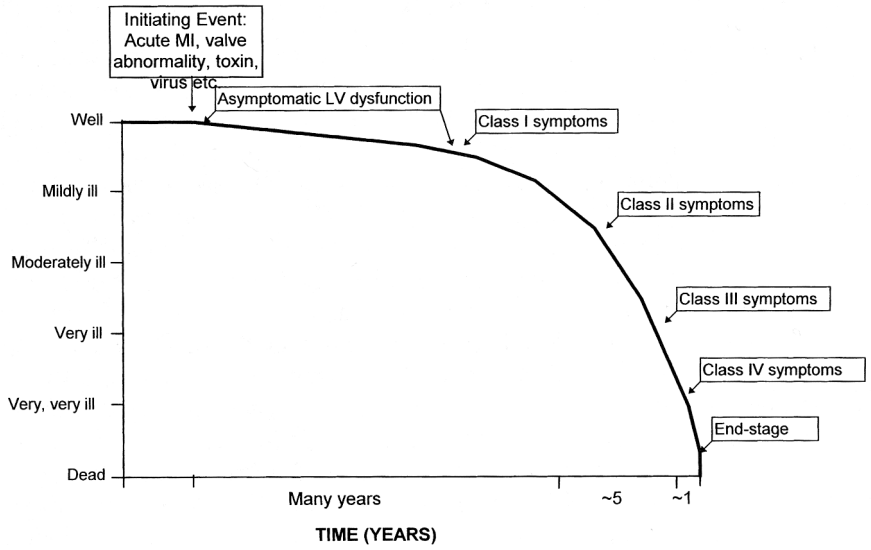

Figure 1. Heart failure is a progressive, lethal syndrome characterized by accelerating deterioration, following a period of asymptomatic left ventricular dysfunction. (Reproduced with permission from AM Katz. Heart Failure. Pathophysiology, molecular biology, and clinical management. Lippincott Williams and Wilkins, Philadelphia, 2000)

The cardinal manifestations of heart failure are dyspnea and fatigue, which may limit exercise tolerance, and fluid retention, which may lead to pulmonary

and peripheral edema (1). There is no simple pathophysiologic explanation for the genesis of these symptoms. Complaints of dyspnea are not related to the level of pulmonary artery pressure (5), and the limiting symptom during progressive exercise testing can be altered from fatigue with slow exercise protocols to dyspnea with faster exercise protocols (6). Furthermore, in heart failure there is no good correlation between the severity of cardiac dysfunction and the degree of functional impairment (7,8). It is now thought that abnormalities in peripheral blood flow and in skeletal muscle are important for the origin of symptoms and may also play a role in the progression of the syndrome (9).

The scope of the problem of heart failure

Heart failure is one of the fastest growing problems in cardiovascular medicine. This increase can be contributed to aging of the population and improved survival after myocardial infarction, as well as better treatment of heart failure patients (10), since treatment does not cure the disease but improves survival of the patients. The incidence of heart failure in the general population rises very steeply with age, from 0.02 cases per 1000 population per year in those aged 25-34 years, to 11.6 in those aged 85 years and over (11,12). A higher incidence of heart failure was found in the Framingham cohort, increasing steeply to an incidence of 27 cases per 1000 population per year in men 80 to 89 years old (13). The incidence of heart failure is about twice as high in males than in females (11). Coronary artery disease and hypertension are the most frequent causes of heart failure (11). It is estimated that the prevalence of heart failure and left ventricular dysfunction in a Western European population is about 1 % of the population (14). Heart failure is now the most common discharge diagnosis in patients older than 65 years. Recently a new approach to the classification of heart failure has been suggested by the American College of Cardiology and the American Heart Association, that emphasizes both the evolution and the progression of the disease and can be used to complement the New York Heart Association functional classification(1). In this classification four stages of heart failure are identified: Stage A identifies the patient who is at high risk for developing heart failure but has no structural disorder of the heart; Stage B refers to a patient with a structural disorder of the heart but who has never developed symptoms of heart failure; Stage C denotes the patient with past or current symptoms of heart failure associated with underlying structural heart disease; and Stage D designates the patient with end-stage disease who requires specialized treatment strategies such as mechanical circulatory

support, continuous inotropic infusion, or heart transplantation (1). In the evaluation of patients with heart failure a complete echocardiographic examination is the single most useful diagnostic test (1) but will not be discussed in this text. The prognosis of heart failure is grim and as poor as many malignancies. In the general population the mortality of patients with heart failure is twice that of persons without heart failure showing an 1, 2 and 5 years' survival of 89%, 79% and 59%, respectively with a fivefold increase in sudden death rate (15). In patients with NYHA class III or IV symptoms, the one-year mortality was 35% and the combined rate of mortality and/or readmission in hospital 81 % (16). Patients with end-stage heart failure have an even poorer prognosis: 75 % mortality after one-year (17). Mortality is either sudden or due to progressive pump failure. Sudden death, mostly caused by ventricular fibrillation or ventricular tachycardia, accounts for nearly half of the cardiovascular deaths (18). Progressive pump failure accounts for the other 30-50% and becomes even more important in end-stage heart failure. A small number of patients die due to electromechanical dissociation or bradycardia.

Treatment

The management of patients with heart failure has changed considerably during the last decades. Current therapy has two important goals: improvement of symptoms and improvement of prognosis. A challenge for the near future will be the prevention of left ventricular dysfunction by aggressively treating ischemic heart disease, and preventing the transition of asymptomatic cardiac dysfunction to symptomatic heart failure.

Treatment of every heart failure patient has to be individualized and begins with defining the cause of the syndrome. In some patients this directly indicates the optimal therapy, as for instance in the case of patients with primary valvular pathology. In ischemic heart disease, the diagnosis of hibernating myocardium can also influence the therapy substantially. Other potentially reversible causes, like hyperthyroidism, anemia, or alcohol abuse, have to be looked for.

General measures are often overlooked, but may influence the therapeutic response considerably. Weight reduction in the obese, sodium and fluid restriction, reduction of alcohol intake and cessation of smoking have to be stressed. The patient has to be educated about the importance of adequate drug dosing and the early detection of fluid retention by regular weighing. Drugs like NSAID's should be avoided because of their potential adverse effects like fluid retention and renal failure. Regular exercise in stable patients should be encouraged, because this can improve exercise capacity, even in patients with

severe heart failure (19).
Pharmacological therapy in heart failure comprises an increasing number of drugs but will not be discussed here, because it is outside the scope of this thesis. In the treatment of patients with end-stage heart failure the pharmacological options are limited and therefore, heart transplantation is often considered.

Heart transplantation

The first human heart transplantation was performed in 1967. It was followed by a period of hectic activity in heart transplantation worldwide. The initial results, however, were very disappointing, resulting in the discontinuation of heart transplantations in most centers. The majority of patients died because of acute rejection or infection. The development of a biopsy forceps by Philip Caves in Stanford enabled the monitoring of acute rejection, further supported by a histologic grading system introduced by Billingham (20). In 1980 cyclosporin was introduced as immunosuppressive treatment after transplantation. This resulted in a considerable improvement in 1-year survival in comparison with the "classic" immunosuppression with corticosteroids and azathioprine. From that time a dramatic expansion of heart transplantation occurred. To date, more than 57.000 heart transplants have been performed worldwide (21). However, from 1996 the annual number of transplants is declining, and now approaches 3000, despite the fact that all transplant centers accept increasingly older donors, with a maximum age of 55-60 years old. Age distribution of patients receiving heart transplants clusters between the ages of 40-60 years. One-year survival is 80-85 % with a further constant mortality rate of 4% per year. The patient half-life (time to 50% survival) is 9.1 years, and in those surviving the first year, the patient half-life is 11.6 years (21). Functional improvement is impressive, enabling patients to live a near normal life and often to resume work. Due to the shortage of donor hearts, heart transplantation can only be offered to a highly selected group of patients. It requires lifelong immunosuppressive medication and close follow-up. Major problems comprise acute rejections, infections, cardiac allograft vasculopathy, hypertension, renal failure and malignancies.

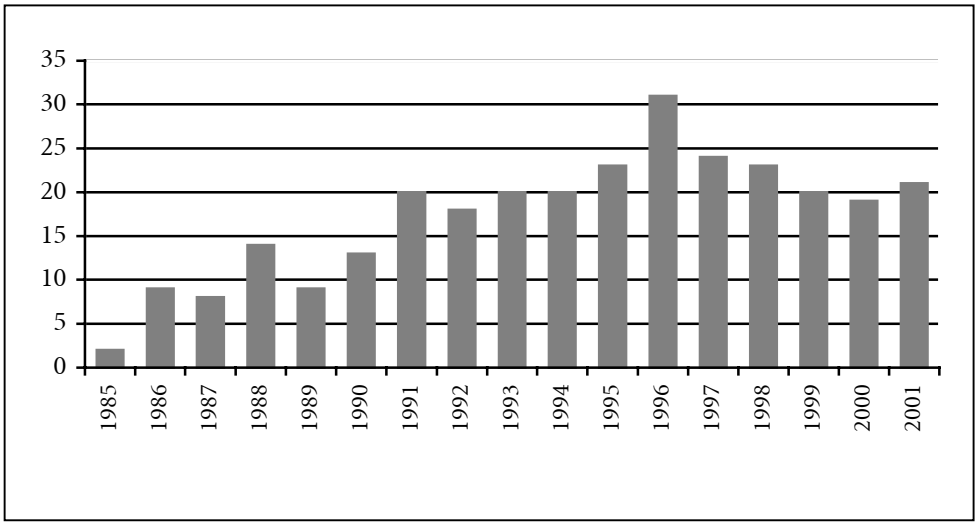

Figure 2. The number of heart transplantations performed per year in the University Medical Center Utrecht

In the Netherlands two centers are performing heart transplantations, Rotterdam and Utrecht. Due to the lack of donor-organs, both centers together transplant only about 40 patients each year, despite the acceptation of more and more marginal and older donors.

In the University Medical Center Utrecht, from the beginning of heart transplantation in 1985, 300 transplants have been performed. Figure 2 shows the yearly number of transplants. Actuarial survival is shown in figure 3, demonstrating a 5-year survival of 75 % and a 10-year survival of 64 %.

The recently introduced law on organ donation has not resulted in an increase of donor-organs, but in contrast, seems only to has made things worse. The number of available donor organs is also influenced by the increased traffic safety precautions and by the improved treatment options to prevent brain death.

It is not to be expected that the number of available donor-hearts will increase substantially during the coming years. Therefore, it is necessary to explore other therapeutic options for patients with end-stage heart failure, such as mechanical circulatory support.

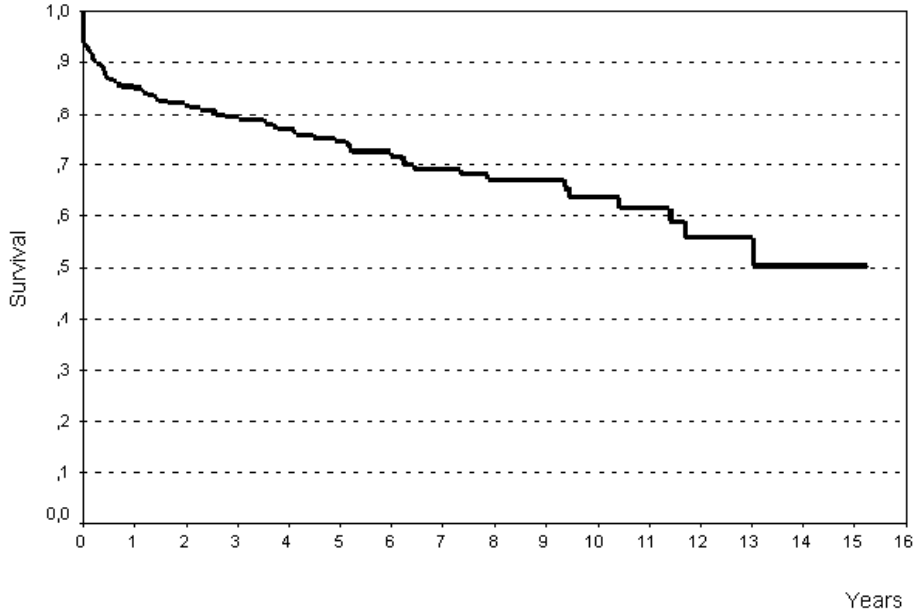

Figure 3. Actuarial survival after heart transplantation in the UMC Utrecht

Mechanical circulatory support

Mechanical circulatory support was first used in 1954 with the implementation of the cardiopulmonary bypass by Gibbon. This not only led to the breakthrough of cardiac surgery, but also stimulated research into techniques for supporting the circulation in severe heart failure. In 1969, only two years after the first human heart transplantation, a pneumatic total artificial heart was used for the first time as a bridge to transplantation (22). Subsequently, a variety of extracorporeal and implantable assist devices was developed. These devices can be arbitrarily divided in devices meant for short-term support (up to $\pm$ 2 weeks), or long-term support. Short-term devices include the intra-aortic balloon pump (IABP) and external centrifugal and pneumatic devices, like the Abiomed BVS 5000.

The IABP was first used in 1967 and since that time in millions of patients. Its main indications are postcardiotomy low cardiac output and pump failure after acute myocardial infarction. The increased waiting period for donor hearts limits its use as a bridge to transplantation. The IABP results in a modest increase in cardiac output up to 20%. The main shortcoming of the IABP is that it does not produce flow on its own; it merely augments heart function

modestly.
On the other hand, external centrifugal and pneumatic devices can support the circulation even in the absence of native heart function. The duration of support with these devices, however, is limited due to bleeding, thromboembolism, infection and renal dysfunction (23). The main indication is hemodynamic support in postcardiotomy shock.
Some examples of long-term assist devices are the Thoratec (Thoratec, Pleasanton, Calif), the Novacor (WorldHeart, Ottawa) and the HeartMate (Thoratec, Pleasanton, Calif).
The Thoratec ventricular assist device can be used for univentricular, as well as for biventricular support. It is a paracorporeal, pneumatically powered system, using a large driving console, although a portable driver recently has become available. The pumps are placed externally on the anterior surface of the abdomen, limiting mobilization of the patient. Furthermore, intensive anticoagulation is required (24).
The Novacor is an electrically driven, implantable pump, limited to left ventricular support. It has a smooth blood-contacting surface that requires anticoagulation and nevertheless has a higher thromboembolic rate than the other devices (25).
The HeartMate is also an implantable pump, which supports only the left ventricle (Fig. 4). Two models are available; a pneumatically driven version (IP), with an external driving console and an electrically driven version (VE). The pump consists of a titanium housing with a flexible Biomer polyurethane diaphragm bonded to a rigid pusher plate. The diaphragm divides the pump into two halves: a blood chamber and an air chamber in case of the IP system or an electrical motor chamber in case of the VE system. The air chamber of the IP system is connected to an external console by a transcutaneous driveline (Fig. 5). In contrast to the Novacor its textured polyurethane and sintered titanium lining makes anticoagulation unnecessary, except for low-dose aspirin. It has a low thromboembolic rate (3-6%), but a higher rate of reported device-related problems compared to the other devices (25-27). In the REMATCH Study Group the probability of device failure was 35% at 24 months (17). The device is implanted in an intra-abdominal position. The device is connected by cannulas, containing porcine valves, between the apex of the left ventricle and the base of the aorta (23) (Fig. 6). Maximum stroke volume of the blood chamber is 83 ml. The flow range is approximately 2-10 liters/min. It can be operated in a “fixed rate mode” and an “auto rate mode”. In the auto mode the HeartMate functions in a ‘fill-to-empty’ manner which permits the device to vary its flow based on the patient’s physiological need, dependent on left

ventricular filling.

Figure 4. The HeartMate VE left ventricular assist device.

The HeartMate is presently only approved for the use as a bridge to heart transplantation. More than 2700 patients around the world have been treated with this device (26), representing an overall experience of over 500 patient-years of support. Survival to transplantation in several reports varied from 65 to 76% (26-28). Furthermore, outcome after heart transplantation in patients supported with an internal LVAD is as good as that of patients transplanted after support on inotropic medical therapy (29). Obviously the use of LVAD's as a bridge to transplantation does not result in more heart transplantations; but the LVAD can help the sickest patient, who would die on medical therapy, survive to transplantation. Recently the suitability of the HeartMate LVAD as a long-term myocardial-replacement therapy was evaluated in patients ineligible for heart transplantation (REMATCH) (17). This study showed a survival rate at one year of 52% in the device group and 25% in the medical therapy group ($p = 0.002$). The rates at two years were 23 % and 8 % ($p = 0.09$), respectively. These findings establish the LVAD, not only as a bridge, but also as an alternative to heart transplantation in selected patients with end-stage heart failure (17), although longevity with the current device is limited by a high incidence of infections and device malfunctions.

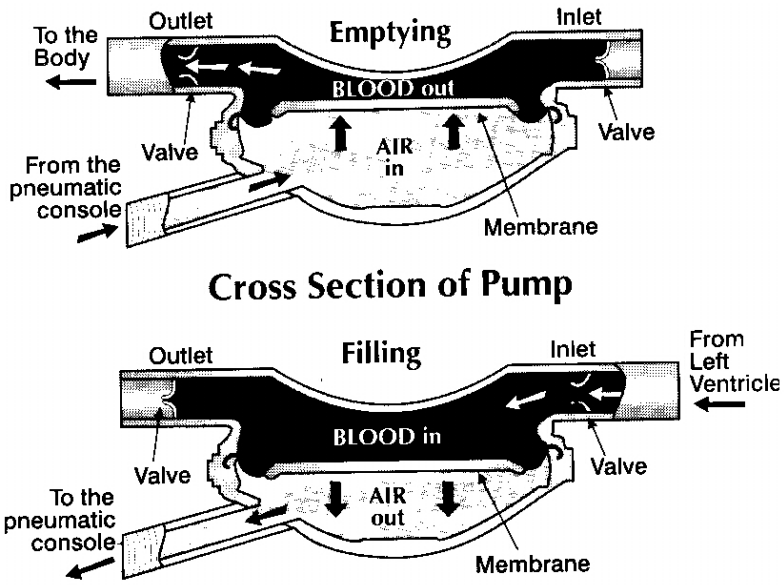

Figure 5. Cross section of the HeartMate IP LVAD showing a blood chamber and an air chamber divided by a pusher plate. Pulses of air, delivered by the pneumatic console, result in displacement of the pusher plate, propelling the blood out of the pump. Two porcine xenograft valves provide unidirectional flow.

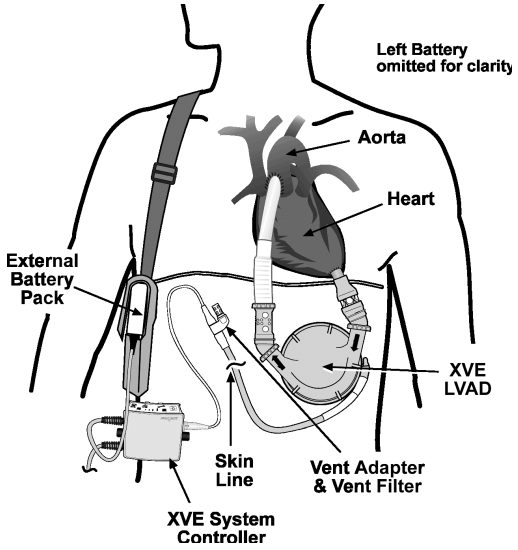

Figure 6. Schematic diagram of the HeartMate VE left ventricular assist device. The inflow canula is implanted in the left ventricular apex. The outflow graft is connected end to side to the ascending aorta.

Besides the use of LVAD's as a bridge to transplantation or as an alternative to heart transplantation it is suggested that they can be used as a bridge to recovery of cardiac function (30,31). Prolonged mechanical circulatory support effectively unloads the left ventricle, resulting in a decrease of cardiac dimensions (32-34) and cardiomyocyte size (35,36), improvement of myocyte contractile function (37,38) and decrease of neurohormonal activation (39,40). Few centers have limited experience with weaning patients from the device (41-43), whereas others question the feasibility of this policy (44,45). At this moment it is not yet clear if reversed remodeling of the left ventricle after mechanical support will be sufficient to allow for device removal; also, it remains to be seen how long this effect will last.

Newer developments in mechanical support.

Several newer devices will become available within a couple of years. These devices will offer greater durability and longevity, enabling their use as a realistic alternative to transplantation. Some examples are the HeartMate II (Fig 7) and the Jarvik 2000 (30), high-speed rotary axial flow pumps, which are fully implantable and much smaller than the present HeartMate. In the future, they will be powered by a transcutaneous energy transfer system.

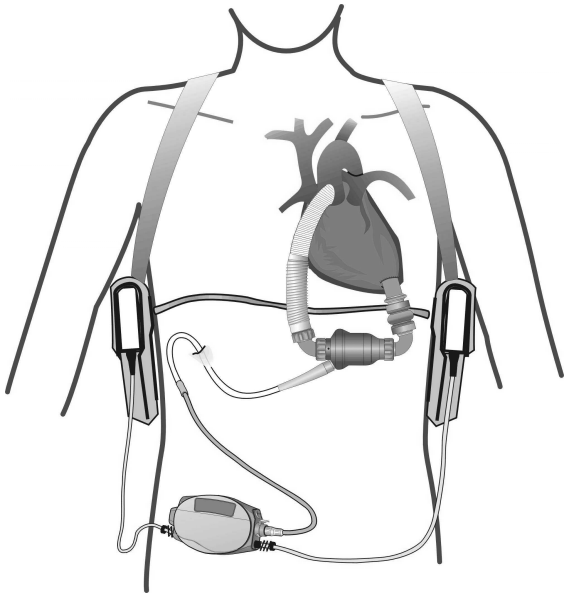

Figure 7. The HeartMate II axial flow pump

The HeartMate III is a centrifugal pump of which the rotor is magnetically suspended, which will extend the lifespan of this system greatly. A completely new total artificial heart (Abiocor) will also be available in the near future. In contrast to the previous devices, the Abiocor will replace the native heart. Indications and contra-indications for these newer devices will be an area of research for the coming years.

LVAD's as a means to study fundamental aspects of end-stage heart failure.

Implantation of a LVAD requires the removal of a part of the apex of the left ventricle to allow for insertion of the inflow canula. Therefore, the use of a LVAD as a bridge to transplantation offers the unique opportunity to obtain human myocardial tissue from patients with end-stage heart failure at a time when they are in a critical hemodynamic condition. Later in the course of therapy, when the patient has recovered thanks to the LVAD support, heart transplantation offers a second opportunity to obtain human myocardial tissue. In this way, human myocardial tissue of patients with end-stage heart failure, at two points in time, can be compared. This allows the study of more fundamental aspects of end-stage heart failure, like the evaluation of the morphology of the contractile filaments, and the role of apoptosis and its regulating mechanisms in end-stage heart failure.

The ultrastructure of the heart

The heart is composed of a syncytium of longitudinally arranged myocytes, interspersed with connective tissue elements and blood vessels (46). Each myocyte is surrounded by a cell membrane, the sarcolemma, and is filled with rodlike bundles of myofibrils and many mitochondria (47) (Fig. 8). These myofibrils are the contractile elements, consisting of serially connected contractile units known as sarcomeres. The sarcomere is composed of contractile, regulatory, and structural proteins arranged into thick and thin filaments (Fig.9) (46). The sarcomere is limited on either side by the Z line. In the Z lines the thin contractile filaments are anchored, consisting mainly of two strands of actin intertwined with tropomyosin. At regular intervals along this structure the troponin complexes are situated (Fig. 10). Troponin T binds the troponin complex to tropomyosin; troponin C is a calcium binding protein, necessary for the excitation-contraction coupling, and troponin I inhibits the interaction between actin and myosin (3). The thick contractile filaments mainly consist of myosin, which are anchored at the M line, in the middle of

the sarcomere. Titin is a large elastic molecule that supports myosin and binds it to the Z line (47). Sarcomere shortening in the contracting heart occurs when the thin filaments are pulled toward the center of the A-band by a rowing motion of the myosin cross-bridges (3). The energy required for these contractions is derived from ATP hydrolysis by myosin.

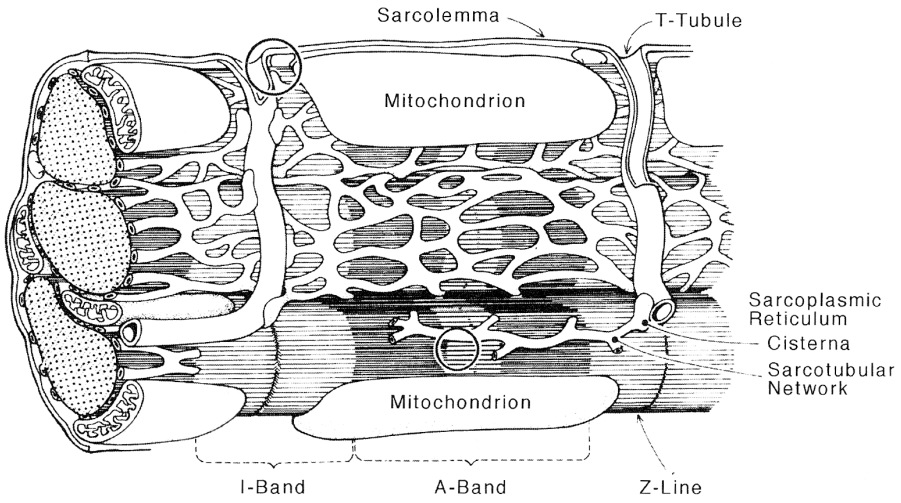

Figure 8. Ultrastructure of the cardiomocyte. Each sarcomere is bounded by two Z-lines in which the thin contractile filaments are anchored. The thin contractile filaments on either side of the Z-line represent the I-band. The A-band represents the region of the sarcomere occupied by the thick filaments into which the thin filaments extend from either side. (Reproduced with permission from AM Katz. Heart Failure. Pathophysiology, molecular biology, and clinical management. Lippincott Williams and Wilkins, Philadelphia, 2000)

Two isoforms of myosin heavy chain (MHC) are known in the heart: α-MHC and β-MHC. α-MHC contains high ATPase activity, which results in fast muscle contraction and high myocardial contractility. Human atrium contains mainly the α-MHC isoform. On the other hand β-MHC has low ATPase activity, resulting in a slower, weaker, but more efficient contraction. Human ventricular myocytes contain 90 % β-MHC isoform (3). Heart failure is associated with a shift in MHC isoform from the fast (α-MHC) to the slow type (β-MHC), especially in atrial myocytes, but also in ventricular myocytes (48). This re-expression of immature fetal cardiac genes like fetal contractile proteins and

natriuretic peptides is a feature of cardiac hypertrophy (49). It can be adaptive and promote a more favorable myoenergetic economy, but the functional implications of many of these changes are still unclear (50). Mutations in many cardiac proteins can be responsible for familial cardiomyopathies. Mutations in myofibrillar proteins are thought to result mainly in hypertrophic cardiomyopathy whereas mutations in cytoskeletal proteins mostly result in dilated cardiomyopathy (49). This dichotomy probably is a too simple pathophysiologic model, because mutations in β-myosin heavy chain and troponin T have recently been associated with dilated cardiomyopathy as well (51,52). Therefore, divergent signaling pathways have to be present, remodeling the heart in ways that result in either a dilated or a hypertrophic phenotype. Maybe in the future, these pathways may be used to direct therapeutic interventions to prevent or to abrogate disease progression in cardiomyopathy.

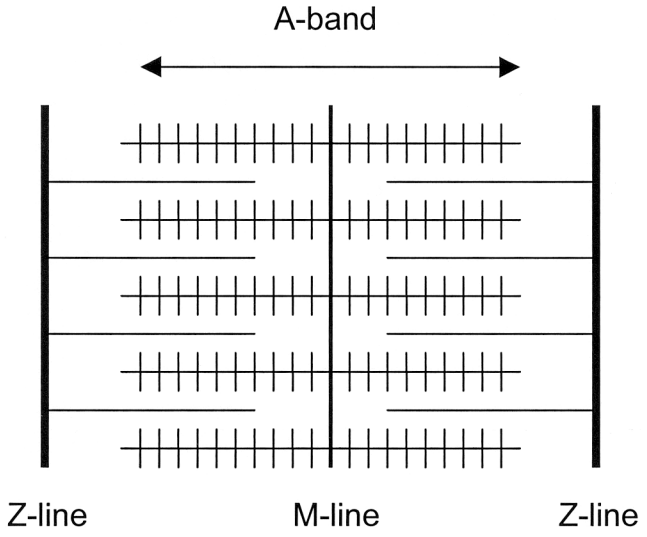

Figure 9. The sarcomere is limited on either side by a Z-line, in which the thin contractile proteins (actin/tropomyosin complex) are anchored. The myosin filaments are anchored in the M-line, in the middle of the sarcomere. The myosin heads, attached to the thick filaments, interact with the thin filaments. As a result the actin filaments move inward towards the center of the sarcomere, drawing the Z-lines closer together.

Mechanisms of heart failure

Heart failure may be viewed as a progressive disorder that is initiated after an

index event resulting in either loss of cardiomyocytes or deterioration of myocyte function, producing a decline in pumping capacity of the heart. Regardless of the nature of the initial event, a variety of compensatory mechanisms become activated (53). These compensatory mechanisms include the adrenergic nervous system (54), the renin-angiotensin system (55) and cytokine systems (56, 57).

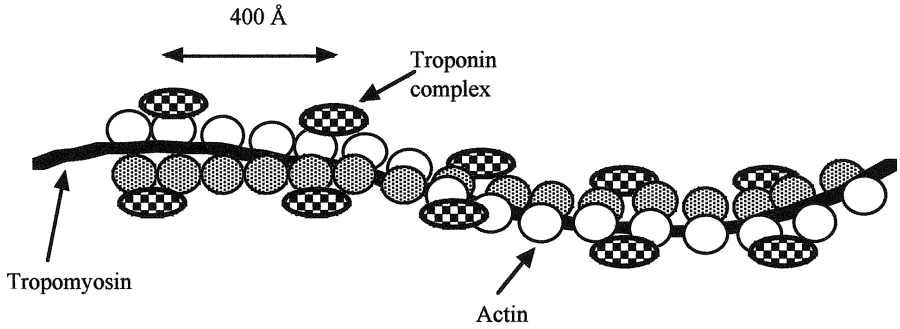

Figure 10. The thin contractile filament consisting of two strands of actin intertwined with tropomyosin. At regular intervals the troponin complexes are situated, consisting of troponin T, C and I.

This neurohormonal activation in heart failure is similar to the response to hypovolemia, hemorrhage, and exercise. It is an old evolutionary mechanism aimed at maintaining blood pressure, perfusing the brain, and exercising muscles so as to sustain physical activity (4). In the short-term these systems modulate cardiovascular function, so that the functional capacity of the patient is initially preserved. Why and when the transition to symptomatic heart failure occurs is unknown, but it is accompanied by further activation of neurohormonal and cytokine systems as well as adaptive changes within the myocardium, collectively referred to as remodeling (53).

Remodeling

Cardiac remodeling may be defined as genome expression, molecular, cellular and interstitial changes that are manifested clinically as changes in size, shape and function of the heart after cardiac injury (58). The central feature of myocardial remodeling occurring secondary to hemodynamic overload is an increase in myocardial mass associated with a change in the shape of the ventricles (59). Pressure overload results in increased myosin heavy chain

synthesis in the heart in vivo, within hours (50). The increase in systolic wall stress results in parallel addition of sarcomeres, increasing the width of individual cardiomyocytes and therefore causing concentric hypertrophy at the gross morphologic level. On the other hand, pure volume overload results in the addition of sarcomeres in series and lengthening of cardiomyocytes, causing eccentric hypertrophy at the gross morphological level (59). A number of factors have been identified as potential causes of myocyte hypertrophy, as indicated in table 1 (60). Initially, this hypertrophy is compensatory to the increased workload of the ventricle. Later, the process becomes maladaptive leading to ventricular failure. In this phase several events take place, like necrosis, apoptosis, fibrosis, increased fibrillar collagen deposition, and fibroblast proliferation. Structural changes reported in cardiomyocytes in this phase are loss of myofilaments and alterations in cytoskeletal proteins (61). In addition, vacuoles, myelin figures, increased amounts of lipofuscin and lipid droplets, all morphological consequences of cellular degeneration, are reported (62).

norepinephrine
angiotensin II
endothelin 1
fibroblast growth factor (FGF)
transforming growth factor-ß$_1$ (TGF ß$_1$)
tumor necrosis factor-α (TNFα)
interleukin-1β (IL-1β)
G protein 130-signaling cytokines

Table 1. Mediators of hypertrophy in heart failure

Evidence that neurohormonal activation itself can contribute to the progression of heart failure is for example based on a study in rats, showing that angiotensin II infusion was associated with altered sarcolemmal permeability and myocytolysis, with subsequent fibroblast proliferation and scar formation (63). This effect could be successfully blocked by captopril. Angiotensin II is also thought to release TGF-ß, which is contributory to phenotypic transformation of recruited interstitial fibroblasts into myofibroblasts (64). These myofibroblasts are responsible for an increased production of collagen I and III and also demonstrate contractile behavior, which governs matrix remodeling, including scar thinning (64).

Another finding supporting the potential adverse effects of neurohormonal activation is that adrenergic stimulation in isolated cardiomyocytes resulted in cyclic AMP-mediated calcium overload with a resultant decrease in RNA and protein synthesis, as well as decreased cell viability (65).
Ongoing myocyte cell death is thought to be an important mechanism leading to progressive ventricular dysfunction (66). The importance of myocyte cell death in the genesis of heart failure can simply be deduced from experience after myocardial infarction. The extent of the necrotic area determines the hemodynamic consequences and prognosis. Cardiogenic shock shortly after acute myocardial infarction will only be seen when approximately 50 % of the left ventricular myocardium is necrotic, whereas patients with smaller infarctions will rarely develop cardiogenic shock (67). Animal studies also stress the importance of progressive cell death in heart failure. For example, inhibition of myocyte cell death by insulin-like growth factor-1 in models of ischemic cardiomyopathy resulted in attenuation of ventricular dilatation, reactive hypertrophy, and diastolic wall stress (68,69). In this particular study necrosis was the predominant form of myocyte cell death, but it underscores a role for cardiomyocyte cell death, either by necrosis or by apoptosis, in the origin of heart failure.
Fundamental in the hypothesis of the role of cadiomyocyte cell death in heart failure is the concept that adult cardiomyocytes are terminally differentiated and have no capacity for self-renewal. Recent literature, however, has challenged this dogma (70-72). This potential for myocyte proliferation might counteract some myocyte cell death, although we do not yet know how extensive this effect will be (73). Therefore, myocyte cell death is still thought to be an important factor in the pathogenesis of heart failure. Necrosis is the main form of cell death in myocardial infarction. Apart from necrosis, however, apoptosis may play a role in cardiomyocyte cell death although it is not yet known to what extent (74,75).

Apoptosis

There is no field of basic cell biology and cell pathology that is more confusing and more unintelligible than is the area of apoptosis versus necrosis (76). Cell death is a process that contains a point of no return long before any necrotic changes can be seen by light microscopy (77). Therefore, some reports recommend that the term necrosis should be reserved for those changes that occur after cell death regardless of the pathway by which the cells died (78). These necrotic changes include karyolysis, pyknosis, karyorhexis, condensation

and intense eosinophilia of the cytoplasm, loss of structure and fragmentation (77). In this view the two major forms of cell death are apoptosis and oncosis (Fig.11). Oncosis is cell death characterized by cellular swelling, organelle swelling, blebbing and increased membrane permeability. It is often caused by ischemia and toxic agents and leads to DNA breaks in a nonspecific fashion (77). Cell removal is by inflammation (79), eventually leading to fibrotic scarring.

Apoptosis, or programmed cell death, is an evolutionary conserved genetically programmed process by which multicellular organisms regulate their cell numbers. It is critical in embryonic development, eliminating unneeded cells and in tissue homeostasis, eliminating for example damaged, premalignant cells and immune-effector cells (80). First reported by Kerr et al. (81) in 1972, this energy dependent form of cell death does not evoke a fibrotic reaction, in contrast to oncotic cell death.

Apoptosis is characterized by cell shrinkage, nuclear chromatin condensation (pyknosis), preservation of mitochondrial and sarcolemmal integrity and the emission of cell organelles by pseudopodia called budding (77, 79). DNA is broken down into segments that are multiples of approximately 185 base-pairs, due to specific cleavage between nucleosomes. Apoptosis hardly induces an inflammatory response and cell removal is done by macrophages or neighboring cells. Apoptosis can be initiated by an internal clock, or by extracellular agents such as hormones, cytokines, killer cells, and a variety of chemical, physical and viral agents (77).

The suggested model of apoptotic cell death and oncotic cell death, both resulting in necrosis (77) is basically attractive but has not received much support in literature. Most reports oppose apoptosis to necrosis. For simplification, the latter model will be used further. Apoptosis and necrosis may share common mechanisms in the early stages of cell death (82) and intermediate types of cell death, called aponecrosis have been seen (83) as well as secondary necrosis in initially apoptotic cells (82). The activation of caspases and the presence of ATP are crucial for the determination between apoptotic and necrotic cell death (84).

The detection of apoptosis is based on different molecular methods including the typical ladder pattern of DNA in agarose gel electrophoresis, as a result of the DNA fragments that are multiples of 180-200 base-pairs. This is a very sensitive technique, but does not reveal which cell type is apoptotic in the tissue (79).

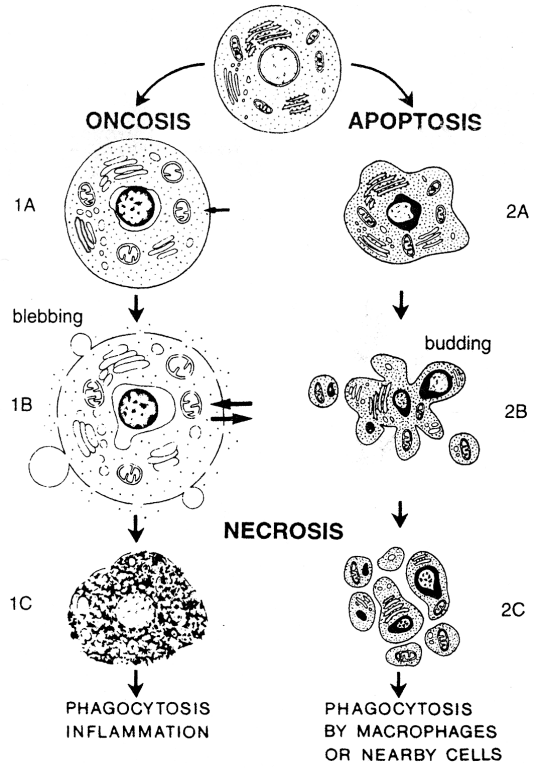

Figure 11. The two types of cell death. On the left side oncosis characterized by cellular swelling, organelle swelling, blebbing and increased membrane permeability. On the right side apoptosis is shown characterized by cell shrinkage, nuclear chromatin condensation (pyknosis), the preservation of mitochondrial and sarcolemmal integrity and the emission of cell organelles by pseudopodia called budding. (Reproduced with permission from Majno G and Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 1995; 146: 3-15).

The most widely used technique for the detection of apoptosis is the in situ end-labeling of fragmented DNA (TUNEL) method (85). This sensitive method enables the in situ visualization of the apoptotic process at the single cell level, but has several limitations (86). It is not yet known how many DNA strand breaks will result in a positive signal, and positivity may persist after complete DNA fragmentation has occurred. Furthermore, necrotic cardiomyocytes and cardiomyocytes showing postmortem autolysis may also be labeled (87) as well

as viable cardiomyocytes showing DNA repair (88) and RNA synthesis (89).
These limitations of the TUNEL method may partly explain the different percentages of apoptotic cardiomyocytes reported in end-stage heart failure patients. Narula et al. (90) reported an apoptotic index of 5-35%, Olivetti (91) 0.23% and Saraste (92) 0.12%. In patients with end-stage heart failure who received a left ventricular assist device, however, apoptosis of cardiomyocytes could not be demonstrated, but apoptosis of cardiac interstitial cells, such as fibroblasts and endothelial cells, was not uncommon (93).
Apoptosis is a very fast process, taking 6 to 24 hours at most (85, 86). Therefore, the initially reported levels of apoptosis of up to 35 % (90) are unrealistically high, because this would result in the demise of all cardiomyocytes within very short time. The widely varying values of incidence of apoptosis, reported in the literature, point out that the role of apoptosis in heart failure needs substantiation (86). Despite the large number of reports in literature dealing with apoptosis and heart failure, a causal role for apoptosis in human heart failure has not been proven, but there are many arguments that apoptosis of cardiomyocytes is a feature of both ischemic and non-ischemic heart failure (94). Myocardial stretch results in an increased incidence of apoptosis (95). The same holds for exposure to angiotensin II (96,97) and norepinephrine (98). Consistent with these findings, ACE-inhibitors (99) and beta-blockers (100,101) attenuate cardiomyocyte apoptosis. Furthermore, increased cytosolic calcium, oxygen free radicals and hypoxia can lead to apoptosis (66). Ischemia itself also increases cardiomyocyte apoptosis incidence (102-104). In acute myocardial infarction, apart from necrosis, apoptosis is reported (105), especially in the border zone (106). In hibernating myocardium, continuing myocyte loss by apoptosis may explain the lack of complete functional recovery after revascularization (107-109).

Regulation of cardiac apoptosis

The regulation of apoptosis is a very complicated process in which many factors are involved. In this overview, only the essential factors will be discussed. A key phenomenon of apoptotic cell death is the activation of a unique class of cysteine proteases, known as interleukin converting enzymes (ICE), more recently referred to as caspases (110,111). They exist as pro-enzymes that are activated by cleavage at specific protein sequences. Following activation these enzymes have proteolytic activity and cleave specific substrates. Caspase-3, 8 and 9 are important in cardiac myocyte apoptosis. Caspase-3 represents the final common pathway of the caspase cascade,

activating endonuclease, which degrades DNA (112).
Apoptosis can be schematically initiated in two ways: "death-receptor" mediated apoptosis and "mitochondrion" dependent apoptosis (113) (Fig. 12).
Death-receptor mediated apoptosis involves the binding of extracellular death signal cytokines, like TNFα and FasL, to their cognate cell surface receptors, TNF-R1 and Fas, respectively (113). This binding activates death domain proteins, which interact with caspase-8 (formerly named FLICE), further initiating the apoptosis cascade. The interaction between death-receptors and death domains can be inhibited by FLICE (caspase-8)-inhibitory proteins (FLIPs) (114), which are expressed at high levels in the heart (115).

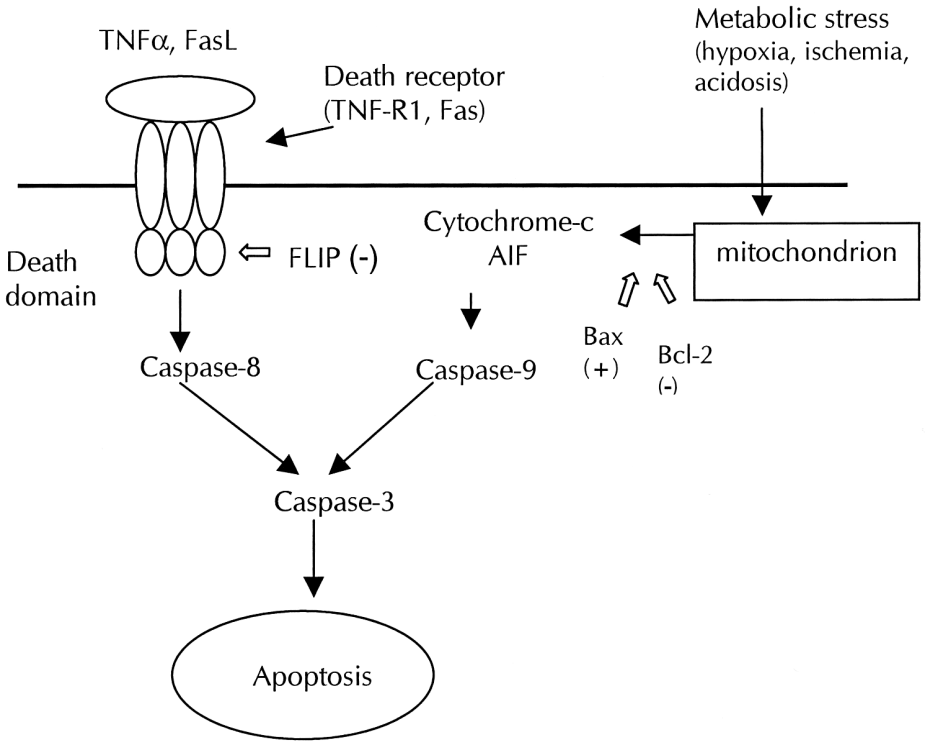

Figure 12. Schematic representation of the caspase cascade in apoptosis. On the left side death receptors are activated by TNFα or FasL resulting in caspase-8 and caspase-3 activation. Caspase-8 (FLICE) can be inhibited by FLICE inhibitory protein (FLIP). On the right side of the figure the mitochondrial pathway is shown, in which caspase-9 activation results in apoptosis by activating caspase-3. The balance between Bax and Bcl-2 regulates this process. AIF = apoptosis inducing factor. Binding of TNFα to TNF-R1 can also activate NF-κB which can stimulate production of nuclear survival proteins, blocking apoptosis (not shown).

Mitochondrion dependent apoptosis results from metabolic stress, like hypoxia, ischemia and acidosis, resulting in the mitochodrial release of cytochrome-c, or apoptosis-inducing factor (AIF), both activating caspase-9, that subsequently activates caspase-3 (112). The release of cytochrome-c can directly be blocked by Bcl-2, a member of a family of human apoptosis regulating proteins (116). Bcl-2 also binds to and inactivates the pro-apoptotic family member Bax (112). The ratio of Bcl-2 to Bax, the so-called "death switch" is often used as an indicator of apoptosis (66).
Another regulator of apoptosis is the transcription factor NF-κB. Binding of TNFα to TNF-R1 can result in apoptosis by activation of the death domain pathway, but it can also trigger NF-κB activation, which leads to gene products that block the apoptotic pathway (117). It was thought that TNFα can only result in apoptosis if NF-κB is blocked simultaneously (118). But to complicate things even more, it has been shown that NF-κB itself can promote apoptosis, depending on the cell type and the nature of the apoptosis-inducing stimulus (118). A factor also involved in the regulation of apoptosis is the tumor suppressor protein p53, which is believed to induce apoptosis in response to DNA damage (66) and other signals like stretch-mediated release of angiotensin II (97).

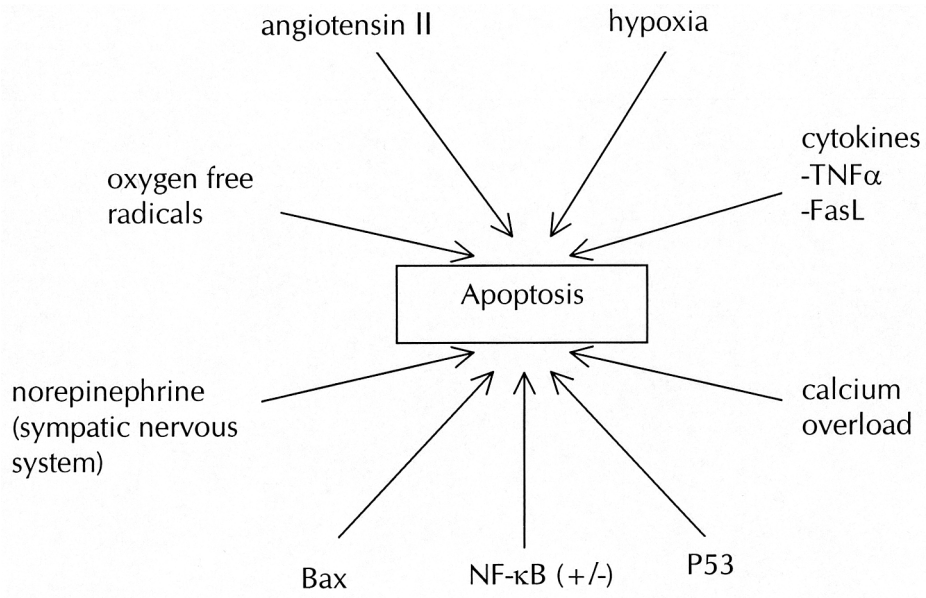

Figure 13. Some triggers of cardiomyocyte apoptosis in heart failure.

In heart failure many stimuli are known to result in apoptosis (Fig.13). The exact mechanisms by which these stimuli trigger apoptosis are often unknown.

In the transition between compensated hypertrophy and overt cardiac failure apoptosis may be a critical event. This has been suggested by a study in spontaneously hypertensive rats. Rats showing signs of heart failure had a five-fold increase in the number of apoptotic cardiomyocytes in comparison with rats without heart failure (119).

Another argument in favor of the importance of apoptosis in the transition of compensatory hypertrophy to heart failure comes from a study in transgenic mice that harbor a knockout of the gp 130 cytokine receptor. The gp130 cytokine receptor is a common subunit of the interleukin-6 family of cytokine receptors, that has been shown to promote cell survival and compensatory hypertrophy in the presence of an apoptotic stimulus in vitro (120). When gp130 knockout mice were exposed to acute pressure overload, they developed dilated cardiomyopathy accompanied by massive myocyte apoptosis, whereas the control mice exhibited compensatory hypertrophy (120). This suggests that gp 130 mediates a myocyte survival pathway that acts by blocking the onset of myocyte apoptosis during pressure overload. It also suggests that cardiac myocyte apoptosis and hypertrophy may be activated by common signaling pathways (120). In this way the balance between apoptotic and hypertrophic pathways determines whether chamber dilatation will occur (121).

Despite the large number of studies of apoptosis in heart failure, we do not yet know with certainty if myocyte apoptosis contributes to the progression of human heart failure, or if it is a homeostatic process that serves to dismantle dysfunctional myocytes in an orderly manner (50). The presence of cardiomyocyte apoptosis in heart failure however, has raised hopes that inhibition could prevent or slow down the loss of contractile cells (122). Prevention of apoptotic myocyte loss may be directed at several levels. Therapy directed at the adverse pathophysiologic condition of heart failure includes ACE-inhibitors (99) and β-blocking agents (101). Therapy inhibiting the intracellular regulatory mechanisms of apoptosis can be directed at the Bcl-2/Bax ratio or death domain proteins. Finally, therapy can be directed at the effector pathways of apoptosis like the caspases. Several important questions still need to be answered considering the use of anti-apoptotic therapy in heart failure. The beneficial effect of ACE-inhibitors and β-blocking agents has been repeatedly demonstrated. However, it has not been shown whether direct inhibition of apoptosis could delay or prevent the development of heart failure. Also, anti-apoptotic therapy for heart failure may not apply to all types of heart failure. It is also a question if the immediate benefits of rescuing myocytes from, for example, TNF-induced apoptotic cell death, may be mitigated by the

unaffected negative inotropic effect of TNF that seems to be independent from its pro-apoptotic activity (122). It is possible that inhibiting apoptosis may simply result in a shift towards necrosis, which may have more deleterious effects on neighbouring cells and ultimately a worse outcome (74). Importantly, the safety of direct anti-apoptotic therapy has not been tested. Given the role of apoptosis in the removal of premalignant cells and the downregulation of immune-effector cells, directly blocking apoptosis may be associated with significant deleterious consequences like lymphoma and autoimmune disorders (74). Therefore, a lot of research has to be performed in this area, before anti-apoptotic therapy can be introduced in patients with heart failure.

Cytokines

As mentioned above, cytokines are thought to play an important role in heart failure as mediators of cardiomyocyte hypertrophy and cardiomyocyte apoptotic cell death. Cytokines are soluble peptides, which mediate cell-to-cell interactions via specific cell surface receptors and regulate the activation, differentiation, growth, death or acquisition of effector functions of the immune system (123). In addition, they mediate effects of the cells of the immune system on other cells and tissues. They usually act in an autocrine (on the cell that produced them) or paracrine (on cells close by) manner. Well over 100 different human cytokines have now been identified (Table 2). They act by binding to specific receptors at the cell membrane, setting off a cascade that leads to induction, enhancement or inhibition of a number of cytokine-regulated genes in the nucleus (124).

Name	**abbreviation**	**examples**
interleukins	IL	IL-1β, IL-2, IL-6, IL-10
interferons	IFN	IFNα, IFNβ, IFNγ
tumor necrosis factors	TNF	TNFα, TNFβ, FasL
growth factors	GF	NGF, EGF
colony stimulating factors	CSF	M-CSF, G-CSF
chemokines	-	RANTES, MCP-1

Table 2. Some common cytokines

In this overview of the cytokines mainly TNFα and FasL will be discussed, because these cytokines are especially thought to have a pathogenic role in

heart failure.

The biologic effects of cytokines seem to be determined primarily by the local level in the body compartment in which they are produced and not by the serum levels. However, continuous infusion of TNFα in a study in rats, resulted in a time dependent, partially reversible depression of left ventricular function (125). Elevated circulating levels of TNFα in patients with severe heart failure have been repeatedly reported (56, 57), especially in cachectic patients (126). The interpretation of these results however, is hampered by the natural variability of circulating cytokine levels (127). Analysis of circulating levels of cytokines and their cognate receptors in 1200 patients with advanced heart failure, enrolled in the Vesnarinone trial, showed that increased levels of TNFα, interleukin-6, and the soluble TNF receptors were associated with increased mortality (128). TNFα mRNA and TNFα protein have also been demonstrated in explanted hearts from patients with dilating cardiomyopathy or ischemic heart disease undergoing heart transplantation (129). Transgenic mice with cardiomyocytes producing TNFα all died of heart failure, supporting a causal role for TNFα in the pathogenesis of human cardiac disease (130). Furthermore, in animal studies, chronic ß-adrenergic stimulation led to a significant increase in mRNA and protein expression of TNFα throughout the myocardium (131). A provisional study with a specific TNFα antagonist (Etanercept) in 47 heart failure patients resulted in a significant improvement in ejection fraction and a trend toward improvement in functional status, further underlining the possible role of TNFα in the progression of heart failure (132). However, a large study with this TNFα antagonist was recently discontinued because of lack of benefit.

TNFα is synthesized as a large inactive protein, which is activated by a tissue metalloproteinase called TNFα converting enzyme (TACE) (133). Like TNFα, TACE has been demonstrated in cardiomyocytes of patients with dilated cardiomyopathy (DCM) (134).

Two types of TNFα receptors have been identified: a 55-kDa (TNFR1) and a 75-kDa (TNFR2). Both receptors are widely expressed on human cells, including adult cardiomyocytes (135). In heart failure patients TNF receptor proteins were decreased in comparison to non-failing hearts, possibly correlating with increased levels of soluble TNF receptors in the serum as a consequence of receptor shedding (129). The soluble receptors are able to bind circulating TNFα. In this way the cytotoxic effects of TNFα may be neutralized. Binding of TNFα to cardiac TNFR1 results in a negative inotropic effect in cardiomyocytes (135). This myocardial depression can be the result of direct alterations in intracellular calcium homeostasis leading to decreased levels of peak intracellular calcium during the systolic contraction (136) or through enhanced

activity of nitric oxide (NO) synthase in the myocardium (137). In addition, TNFα effectively uncouples the beta-adrenergic receptors from adenyl cyclase via an effect on the G inhibitory protein (138). Induction of apoptotic cell death by TNFα is also predominantly mediated by TNFR1 (139). TNFR2 is thought to contribute to TNFα toxicity by recruiting TNFα for interaction with TNFR1. In this way a lower concentration of TNFα is needed for TNFR1 signal transduction (135).

Another molecule belonging to the TNF family is Fas-ligand (FasL). Binding to its receptor Fas, like TNFα, results in apoptosis of the Fas bearing cell (140). This is an important mechanism in the down-regulation of immune reactions as well as in T cell-mediated cytotoxicity (141). After myocardial ischemia this interaction is also thought to be directly involved in cell death (142). A study in rats showed constitutive expression of FasL and upregulation of Fas in relation to diastolic but not systolic wall stress. This did not result however, in enhanced cardiomyocyte apoptosis (143).
This suggests that other regulators of cardiomyocyte cell death have to be present. It is here that FLICE-inhibitory protein (FLIP) probably plays an important role because it can inhibit apoptosis induced by all known human death receptors and is highly expressed in the heart (115).

Aim of the thesis

The main purpose of this thesis was to study the role of left ventricular mechanical support in end-stage heart failure. In addition, the availability of myocardial tissue from patients with end-stage heart failure, at two points in time, during the implantation of the LVAD, and after support, at the time of heart transplantation, offered the unique opportunity to investigate some fundamental aspects of end-stage heart failure in man.
As heart transplantation can only be used for a small number of heart failure patients, we were especially interested if a LVAD can be successfully used as an alternative to heart transplantation. Furthermore, we were interested to find out to what extent mechanical support with a LVAD might result in myocardial recovery.
In **chapter 2** the clinical results of the HeartMate LVAD as a bridge to heart

transplantation in our center are presented. **Chapter 3** deals with the exercise performance of patients while on a LVAD in comparison with the situation after heart transplantation, to examine if a LVAD can be an alternative to heart transplantation, regarding exercise performance. In **chapter 4** recovery of cardiac histology is reported during mechanical support with a LVAD in patients with end-stage heart failure. For this purpose the contractile myofilaments are analyzed using immunohistochemistry. **Chapter 5** deals with cardiomyocyte apoptotic cell death in clinical heart failure and some of the mediators and receptors involved, and the effect of LVAD support on it. In **chapter 6** the morphology of the contractile myofilaments of the left ventricle is compared with that of the right ventricle and the interventricular septum, using large transmural biopsies and small biopsies taken with a bioptome. This was done to assess if right ventricular endomyocardial biopsies may be used to monitor recovery of cardiac histology in the left ventricle.
Chapter 7 contains a general discussion.

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Chapter 2.

Left ventricular assist device as bridge to transplantation in patients with end-stage heart failure.

Eight year experience with the implantable HeartMate LVAS.

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Neth Heart J 2002; 10: 267-271

Abstract

OBJECTIVE- To evaluate the use of left ventricular assist devices (LVAD) as bridge to heart transplantation (HTx) in patients with end-stage heart failure.
METHOD- Between March 1993 and December 2001, 38 patients with refractory end-stage heart failure underwent HeartMate LVAD (Thoratec, Pleasanton Calif.) implantation.
RESULTS- 33 of the 38 patients (87%) survived the implantation and perioperative period. There were 5 perioperative deaths (13%), 2 of right ventricular failure, 2 as a result of bleeding and 1 probably due to septic shock at the time of LVAD implantation. Three patients (9%) died late in the postoperative period due to septic shock, mechanical failure of the device and a cerebral embolus resulting from LVAD endocarditis, initiated by an acute cholecystitis. Twelve patients (32%) had one or more infectious episodes during long-term assist, of which 1 patient died. Four patients are still on the device, waiting for heart transplantation. Twenty-six patients (76%) underwent HTx after 206 ± 129 days of support.
CONCLUSION- These results show the efficacy of LVAD support as a bridge to heart transplantation in patients with end-stage heart failure. Major long-term complications are infections and mechanical failure of the device.

Introduction

In the treatment of patients with end-stage heart failure, heart transplantation is still the only option that provides both a better life expectancy and a substantially better quality of life [1,2]. It mostly results in a dramatic improvement in general wellbeing and in exercise performance [3,4]. However, since the start of heart transplantations in the Netherlands in 1984 the number of procedures has been limited due to shortage of suitable donor hearts. Every year the heart transplant centers in Utrecht and Rotterdam together perform 40-50 heart transplants. This number has been fairly stable during the last decade, despite all measures to improve donation and despite the tendency to accept hearts from older donors.

The low number of heart transplantations is in sharp contrast to the growing number of patients with end-stage heart failure [5]. This discrepancy has resulted in long waiting times and a high (15-20%) mortality on the transplantation waiting list. Moreover, many potential transplant candidates not even make it to the waiting list, because of acute hemodynamic deterioration. To reduce this high mortality rate, mechanical circulatory support can play an important role as a bridge to transplantation. Implantable left ventricular assist devices like the HeartMate (Thoratec, Pleasanton Calif.) and the Novacor (WorldHeart, Ottawa) are the most suitable devices as bridge to transplant, because they can support the failing heart for months or even years [6-10].

The Heart Lung Center Utrecht (HLCU) started a bridge to transplantation program in 1993 using the implantable HeartMate pneumatic and later the vented electric left ventricular assist device.

In this article the results of 8 years experience with this device are presented.

Methods

Description of the HeartMate® device and mode of implantation.

The HeartMate® left ventricular assist system (LVAS) consists of an implantable pneumatic (IP) or a vented electric (VE) left ventricular assist device (LVAD) (fig 1). The pump consists of a titanium housing with a flexible Biomer polyurethane diaphragm bonded to a rigid pusher plate. The diaphragm divides the pump in two halves: a blood chamber and an air chamber in case of the IP system or an electrical motor chamber in case of the VE system. The air chamber of the IP system is connected to an external console by a transcutaneous driveline. By delivering programmed pulses of air the console provides the displacement of the diaphragm propelling the blood through an outflow graft into the arterial

circulation. Both the outflow and inflow graft contain porcine xenograft valves, providing uni-directional flow. Differences in air pressure between this closed system and the ambient air are equalized by manually venting the system on the console, which also has a display continuously showing information about stroke volume, filling status and pump rate.

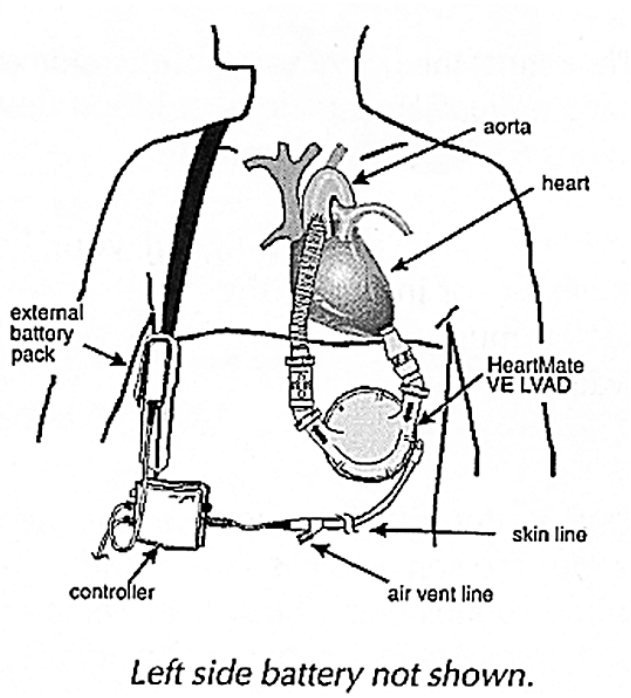

Figure 1: Schematic diagram of the HeartMate VE left ventricular assist device. The inflow canula is implanted in the left ventricular apex. The outflow graft is connected end to side to the ascending aorta. The transcutaneous skinline is connected to the controller.

In case of a VE system the pump is electrically driven and continuously vented through an almost identical driveline connected to a small controller and energized by rechargeable batteries. The LVAD has a maximal stroke volume of 83 ml and a maximal beat rate of 120 beats per minute. The pump can function in a fixed mode or in an automatic mode, allowing the device to vary its flow dependent on the left ventricular filling volume.
The unique feature of the HeartMate® blood pump is the blood-contacting surface. These textured biomaterials are to promote the formation of a thin, well-adhered pseudointimal lining on the inner side of the pump. This non-thrombogenic

neointimal layer reduces the need for anticoagulation, as are the porcine xenografts; only 80 mgr. of aspirin a day is required for antithrombotic prophylaxis.
Implantation of the device is accomplished through a median sternotomy and laparotomy. The pump is implanted in the left upper quadrant of the abdomen, intra-or extraperitoneally. The inlet canula connects the apex of the left ventricle to the pump, while the outlet canula is connected end to side to the ascending aorta using a Dacron® vascular graft. (Fig 1). Both canulae pass the diaphragm.
Since 1997 the driving console has been replaced by a smaller, portable one (HeartPak) (fig 2) which is charged by exchangeable batteries and offers the patients a better and more extended range of mobility.

Patients.
From March 1993 to December 2001, 38 patients, (34 males, 4 females, mean age 38 ± 13 years, range 16-62 years) received the HeartMate® LVAD as a bridge to heart transplantation. Indication for implantation was cardiogenic shock refractory to drug treatment in all cases (table 1). Dilating cardiomyopathy was the underlying cause in 23 patients (60%), ischemic heart disease in 15 (40%).
Pre LVAD all patients were on high-dose intravenous inotropic medication (dopamine, dobutamine and milrinon) and 16 patients were also supported by an intra-aortic balloon pump. An external ventricular assist device had been implanted in 3 cases (1 Hemopump and 2 Abiomed BVS 5000). The pneumatic HeartMate IP was used in 32 patients, the electrical HeartMate VE in 6.

Statistical analysis
Data are presented as the mean ± SD. Statistical analysis was performed with two-tailed paired Student t test. A p-value < 0.005 was considered significant.

Results.

In this group of 38 patients, 33 survived the implantation and early postoperative period (87%). Five patients died in the first 30 days post implant (peri-operative mortality 13%), 3 patients (9%) died late in the postoperative period (Table 2).

Table 1. Characteristics of LVAD patients at the time of implantation (n = 38)

Male / Female	34 / 4
Age (yr.)	38 ± 13
DCM / IHD	23 / 15
LVEF (%)	14 ± 5
Cardiac Index (L/min/m^2)	2.0 ± 0.7
MAP (mm Hg)	62 ± 11
RAP (mm Hg)	13 ± 7
PCWP (mm Hg)	24 ± 7
PVR (dyne sec cm^{-5})	196 ± 94
TPG (mm Hg)	9 ± 4.2
IABP / other support	16 / 3
Mean duration support (days)	172 ± 140

Figure 2: The three models of the HeartMate Left Ventricular Assist System. The Implantable Pneumatic system with the original console and with the portable console (HeartPak), and the Vented Electric device.

Table 2. Patient mortality and cause of death (n = 8)

Patient	Sex	Age	Survival/days	Cause of death
# 3	M	42	133	Mechanical failure
# 5	M	20	3	RVF
# 6	F	52	8	RVF
# 10	M	62	0	Bleeding
# 18	M	38	435	Cerebral embolus
# 23	M	49	35	Septic shock
# 25	M	25	6	Bleeding
# 30	M	24	0	Septic shock at time of implantation

RVF: right ventricular failure

Mean duration of support for the 38 patients was 172 ± 140 days with a longest duration of 557 days and a cumulative experience of 6522 days (nearly 18 years).
Successful implantation of the HeartMate® LVAS resulted in an immediate improvement of the hemodynamic situation in all patients. Cardiac index increased from 2,0 ± 0,7 l/min/m^2 pre implantation to 3,0 ± 0,5 l/min/m^2 24 hours post transplantation ($p < 0.0001$).
Renal and hepatic function normalized within 6 weeks (Table 3). Use of the mechanical pump device did not cause hemolysis (normal serum Hb and haptoglobin levels), or thrombocytopenia (platelet count 211 ± 81.10^9 /L pre-implantation versus 289 ± 81.10^9 /L, 6 weeks post implantation) in any of the patients.
Four patients are still on the device. Of the remaining 34 patients, 26 (76%) underwent heart transplantation, after an average of 206 ± 129 days on the device. At the time of transplantation all patients were in NYHA functional class 1 and were fully mobilized. After an intensive training and instruction program seven patients have been discharged from the hospital awaiting heart transplantation at home.

Table 3. Hepatic and renal function pre and post implant HeartMate (n = 31)

Variable	mean ± SD pre implant	Mean ± SD 6 weeks post implant	p-value
Creatinin (μmol/l)	159 ± 79	74 ± 27	P < 0.0003
Tot.bilirubin (mmol/l)	27 ± 18	14 ± 10	p < 0.01
ASAT (U/l)	103 ± 129	25 ± 9	p < 0.03
ALAT (U/l)	99 ± 114	23 ± 13	p < 0.01

COMPLICATIONS

Right ventricular failure (RVF)
RVF early after implantation occurred in 12 patients (32%). All but 3 patients were successfully treated with positive inotropic agents, vasoactive agents and optimal oxygenation. In three patients temporary support of the failing right ventricle was necessary using an external device (Abiomed®). Weaning of this device was only successful in one single case, 2 patients died in the postoperative course due to complications related to right ventricular failure (air embolism and a hypotensive cerebral infarction).

Bleeding.
Severe postoperative bleeding requiring blood transfusion and reoperation occurred in 5 patients. Two of these died. One patient died due to multi-organ failure after a complicated surgical procedure with long duration of extra-corporeal circulation. The other patient died due to an irreparable disrupture of the left ventricular apex-inlet canula connection, 7 days after implantation.

Thrombo-embolic complications.
Thrombo-embolic events occurred in only three patients, but resulted in death in two. One patient suffered a massive cerebral infarction resulting from LVAD endocarditis 418 days post implant. The LVAD endocarditis probably originated from an acute cholecystitis. Another fatal thrombo-embolic event occurred due to mechanical failure of the control unit. The third patient suffered episodes of amaurosis fugax and recurring abdominal pain suggestive of embolic renal disease, for which oral anticoagulation was successfully installed. The overall thrombo-embolic complication rate was 7.9 % or 0.014 events per patient month,

despite the use of only low dose aspirin as anti-thrombotic prophylaxis in all cases. If the patients with the device malfunction and the LVAD endocarditis are excluded, the thrombo-embolic complication rate was 2.6 % or 0.005 events per patient month.

Device related infections

Twelve patients (32%) had driveline and pocket infections, primarily staphylococcus aureus. Nine of these patients had positive blood cultures. Treatment in all patients consisted of intravenous antibiotics in combination with local treatment. One patient died as a result of septic shock caused by coagulase negative Staphylococcal infection 45 days after LVAD implantation. In 5 patients surgical treatment was necessary consisting of pocket exploration and transposition of an abdominal rectus muscle flap. One patient was kept on long term antibiotic treatment under suspicion of endocarditis of the porcine xenografts in the device. Explantation of the device, at the time of heart transplantation, however, did not reveal signs of endocarditis.

Mechanical complications

Few minor mechanical defects such as driveline electrical wire fractures, display dysfunction, and driver sensor dysfunction occurred, not causing pump function to cease. Major dysfunction of the device occurred in 2 patients. In one case pump function ceased because the pneumatic driving console got jammed, resulting in the nursing staff having to drive it manually for a short period. The console had to be replaced and the patient did not suffer any adverse effects. Mechanical dysfunction of a pneumatic device in a second patient however happened to be fatal (patient # 3 in table 2). A combination of a failing sensor and the vent valve not closing properly after a routine venting procedure at 19 weeks' post implantation, resulted in a low stroke volume, while the patient was asleep and the devices low flow alarm not going off, due to the failing sensor. This low flow state caused the patient to suffer a fatal stroke due to cerebral embolism originating from an intraventricular thrombus.

Surgical complications during heart transplantation

The presence of a LVAD resulted in a more complex heart transplant procedure. In five patients (19%) this lead to increased bleeding requiring re-operation. In one patient a hepatic laceration due to adhesions had to be oversewn. The abdominal wall could be closed primarily in all patients; the diaphragm had to be reconstructed in some.

Discussion.

The use of implantable left ventricular assist devices as bridge to transplantation for heart transplant candidates, who deteriorate while waiting for a donor heart, is now widely accepted [11-13]. The results are very encouraging, especially considering the poor condition of the patients at the time of LVAD implantation, who were facing imminent death. This treatment not only leads to increased survival, but also to complete restoration of renal and liver function and impressive improvement of functional class, comparable to the situation after heart transplantation, as we have reported previously [4].

Our preference for the HeartMate LVAD over other implantable devices was based on its blood-contacting inner surfaces, promoting the formation of a biological lining, not necessitating the use of anticoagulants and diminishing the risk of thrombo-embolic complications[14]. This study and studies by others confirm the low risk for thrombo-embolism with this device [7, 12, 14, 15].

The overall patient survival until transplantation of 79 % in this study is promising, considering the long mean duration of support (172 ± 140 days). The latter is the reflection of the long waiting time for heart transplantation in our transplant program.

Given this, our policy in the last 2 years has been to discharge patients from the hospital while on the device after they had been fully recovered and after they had been extensively trained to use the device[16]. This requires good cooperation of the patient and intensive follow-up and support facilities of the hospital.

Considering survival in this study one has to bear in mind the young mean age of the patients, which is younger than in other published studies [6-8].

Right ventricular failure has been reported a serious problem after LVAD implantation with risk of air-embolism during the implantation procedure and inadequate filling of the device resulting in low output thereafter. This problem is inherent to the fact that only the left ventricle is supported and it did occur in almost one third of the patients in this study, as has been reported by others [12, 17, 18]. Predictors of right ventricular failure are high right atrial pressure, high transpulmonary gradient and an acute decrease in pulmonary artery pressure with LVAD implantation[17]. Growing experience and better patient selection is probably the explanation that no fatalities due to RVF occurred in the second half of the study.

Device-related infections, partly due to the presence of transcutaneous drivelines are reported to be another major problem [19-22]. The rate of 32 % device-related infections in our patients corresponds to reports in literature. In two patients an infectious episode turned out to be fatal. Therefore, the infection problem needs

careful attention. Totally implantable devices with transcutaneous energy transmission, which are currently being clinically investigated, may decrease the risk of driveline related infections.

There were various mechanical problems, especially of the pneumatic driving consoles, but except for one case, these were not fatal. The need for continuous support by an experienced technical department, however, proves to be increasingly mandatory.

In conclusion, this study shows promising results using the HeartMate IP and VE LVAD as a bridge to transplantation in patients with end-stage heart failure. The main drawbacks are the mechanical complications and the high risk for infections, partly related to the transcutaneous driveline. Future devices may diminish these problems, allowing longer periods of event-free support. Based on the present experience and future technical improvements, LVAD's may not only be used as a bridge to transplantation, but also as an alternative to transplantation. The recently reported results of the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) Study Group, showing a significant improval of survival one year after LVAD implantation vs. the medical-therapy group, support this idea [23].

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Chapter 3.

Exercise performance in patients with end-stage heart failure after implantation of a left ventricular assist device and after heart transplantation; an outlook for permanent assisting?

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J Am Coll Cardiol 2001; 37: 1794-1799

Abstract

OBJECTIVES- We sought to study exercise capacity at different points in time after left ventricular assist device (LVAD) implantation and subsequent heart transplantation (HTx).

BACKGROUND- The lack of donor organs warrants alternatives for transplantation.

METHODS- Repeat treadmill testing with respiratory gas analysis was performed in 15 men with a LVAD. Four groups of data are presented. In group A (n = 10) the exercise capacities at 8 weeks and 12 weeks after LVAD implantation were compared. In group B (n = 15), the data at 12 weeks are presented in more detail. In group C (n = 9), sequential analysis of exercise capacity was performed 12 weeks after LVAD implantation and at 12 weeks and one year after HTx. In group D, exercise performance one year after HTx in patients with (n = 10) and without (n = 20) a previous assist device was compared.

RESULTS- In group A, peak oxygen consumption (VO_2) increased from 21.3 ± 3.8 to 24.2 ± 4.8 ml/kg/min ($p < 0.003$), accompanied by a decrease in peak minute ventilation/carbon dioxide production (VE/VCO_2) (39.4 ± 10.1 to 36.3 ± 8.2; $p < 0.03$). In group B Peak VO_2 12 weeks after LVAD implantation was 23.0 ± 4.4 ml/kg/min.

In group C, levels of peak VO_2 12 weeks after LVAD implantation and 12 weeks and one year after HTx were comparable (22.8 ± 5.3, 24.6 ± 3.3 and 26.2 ± 3.8 ml/kg/min, respectively; p = NS). In group D, there appeared to be no difference in percent predicted peak VO_2 in patients with or without previous LVAD (68 ± 13 % vs. 74 ± 15 %; $p < 0,37$), although, because of the small numbers, the power of this comparison is limited (0,45 to detect a difference of 10%).

CONCLUSION- Exercise capacity in patients with a LVAD increases over time; 12 weeks after LVAD implantation, VO_2 is comparable to that at 12 weeks and one year after HTx. Previous LVAD implantation does not seem to adversely affect exercise capacity after HTx.

Introduction

Aging of the population and prevention of premature death from ischemic heart disease by improved medical and surgical management have led to a growing incidence of heart failure. Improved medical therapy for chronic heart failure has further reduced mortality, thereby expanding the number of patients with heart failure (1). Heart transplantation (HTx) is the ultimate treatment option for end-stage heart failure. However, due to a shortage of donor hearts, this therapy can be offered to only a very small number of patients. Therefore, mechanical circulatory support of the failing heart has been suggested as an alternative to HTx. To date, two types of internal left ventricular assist devices (LVADs) are available: the TCI HeartMate (Thermo Cardiosystems Inc., Woburn, Masschusetts) and the Novacor (World Heart Corporation, Ottawa, Canada), both mainly used as a bridge to transplantation. In selected patients the results are excellent with approximately 75 % successful bridging to transplantation and LVAD support time lasting up to three to four years (2,3,4). Based on the growing experience with these devices, it seems realistic to use the LVAD as an alternative to HTx or long-term bridging (in terms of years) to transplantation in youngsters.

One of the prerequisites for permanent or semi-permanent assisting is that exercise performance, although not normal, should be adequate for daily life activities, as is the case after HTx. Until now, few studies are available evaluating exercise capacity after LVAD implantation (5-10). Most studies are based on small numbers of patients from different institutions, with divergent postoperative care, rehabilitation programs and exercise testing protocols, which may limit direct comparison of the results. Only one study compares exercise performance in patients after LVAD implantation and following HTx (6). Data on the ventilatory response to exercise and the progress of exercise performance after long-term LVAD implantation have not yet been reported. Therefore, we performed repeated exercise testing with respiratory gas analysis in a cohort of consecutive patients with end-stage heart failure with an assist device and after HTx. To find an effect of LVAD implantation on exercise performance after HTx, a comparison was made between a group of post-transplant patients with and without a previous LVAD.

Methods

Patients with a LVAD.

Fifteen consecutive male patients (age 37 ± 12 years [mean $\pm$ SD]) with refractory end-stage heart failure treated in our center with a pneumatic LVAD (TCI HeartMate) were included in the study (Table 1). All patients were in critical hemodynamic condition, with a mean ejection fraction of 13 ± 5 %, cardiac output 3.2 ± 0.5 l/min and a mean arterial pressure of 62 ± 9 mm Hg, despite high doses of inotropic support and sometimes intra-aortic balloon counterpulsation. All patients were eligible as transplant candidates or were already on the waiting list. After LVAD implantation, patients could be mobilized at an early stage and participated in an intensive rehabilitation program supervised by a physical therapist, aimed at dynamic exercise, strength and endurance (see Appendix).

Table 1. Characteristics of patients with a left ventricular assist device

Male	15
Age (y)	37 ± 12
Length (cm)	179 ± 9
Weight (kg)	71 ± 8
Duration (mo)	4.7 ± 5.8
DCM	8
IHD	7
LVEF (%)	13 ± 5
CO (l/min)	3.2 ± 0.5
MAP (mmHg)	62 ± 9
PVR (d.s.cm^{-5})	162 ± 54
Assist time (days)	181 ± 125

Duration = time between onset of heart failure symptoms and implantation of LVAD (months);

Heart transplant patients.

A cohort of 20 consecutive male heart transplant recipients (mean age 52 ± 10 years) without a previous HeartMate was used for comparison. All patients were in NYHA functional class I.

Study design.

Maximal exercise capacity 8 weeks after LVAD implantation was compared with that 12 weeks after implantation (Group A, $n = 10$). All patients performed

an exercise test 12 weeks after implantation (Group B, n = 15).To support the hypothesis that exercise performance, with an assist device, is sufficient and comparable to that after HTx, we performed a sequential analysis of exercise capacity 12 weeks after LVAD implantation, as well as 12 weeks and one year after HTx in the same patients (Group C, n = 9). Finally, exercise capacity one year after HTx in the post-LVAD group was compared to that in a cohort of transplant patients without a previous assist device (Group D).

Exercise studies.

All patients underwent treadmill exercise testing using a 2-min staged modified Naughton protocol or a modified Bruce protocol. Continuous breath-by-breath respiratory gas analysis (Oxycon, Jaeger inc, Breda, Netherlands) was performed. Measurements included heart rate, blood pressure, oxygen consumption (VO_2), carbon dioxide production (VCO_2), minute ventilation (VE) and respiratory exchange ratio ($RQ = VCO_2/VO_2$). Peak VO_2 was defined as the average VO_2 during the last minute of exercise and is expressed as ml/kg/min as well as ml/min. In addition, to correct for differences in age among the study groups, the percentage of the predicted values was calculated according to Jones (11,12). Oxygen consumption at the anaerobic threshold (AT) was identified as the oxygen uptake before the systematic increase in the ventilatory equivalent for oxygen (VE/VO_2), without a concomitant increase in the ventilatory equivalent for carbon dioxide (VE/VCO_2), together with the V-slope method (13,14). The ventilatory response to exercise was defined as VE/VCO_2 at peak exercise (15). All tests for patients with a LVAD were performed in the automatic mode of the device, allowing LVAD output to follow an increase of venous return.

Statistical analysis.

Data are presented as the mean ± SD. Statistical analysis was performed with two-tailed paired and unpaired Student t tests, as appropriate. For the sequential analysis of the three exercise tests in the same patients, repeated measures analysis of variance (ANOVA) was used. If relevant, 95% confidence intervals (CIs) of differences were calculated. A p-value < 0.05 was considered statistically significant. All analysis were performed using SPSS version 8.0 for Windows.

Results

All 15 patients with a LVAD completed exercise testing 12 weeks after implantation. In 10 of these patients, a test at eight weeks after implantation was available. There were two late deaths: one due to device failure 133 days after implantation and the other due to repeated cerebral embolism 432 days after implantation. Thirteen patients (87%) were successfully bridged to HTx after a mean duration of support of 181 ± 125 days (range 71to 455 days). Early after HTx, three patients died: one patient died from peri-operative bleeding and two died from intractable infections. One patient was not fit to perform an exercise test 12 weeks after HTx due to pneumonia at that time. Thus, 9 patients completed exercise testing 12 weeks after LVAD implantation, as well as 12 weeks and one year after HTx (Fig.1).

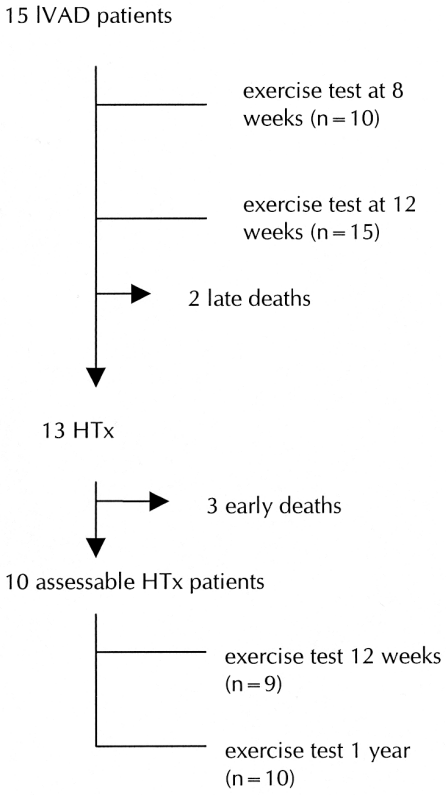

Figure 1. Flow diagram of patients with a left ventricular assist device (LVAD) and exercise tests.

Before LVAD implantation, all 15 patients were in NYHA functional class IV. After LVAD implantation, all patients demonstrated an impressive improvement in general well-being and complete recovery of organ function. Eventually, all cardiac medication could be stopped, even when previous right ventricular failure in the LVAD group was present. Only aspirin, 80 mg/day, was given to prevent thromboembolic complications.

Table 2. Comparison of exercise performance 8 and 12 weeks after LVAD implantation in the same patient (n = 10).

	8 weeks	**12 weeks**
peak VO_2(ml/kg/min)	21.3 ± 3.8	24.2 ± 4.8*
% predicted	53 ± 9	60 ± 10*
peak VO_2 (ml/min)	1438 ± 286	1671 ± 317*
% predicted	47 ± 7	54 ± 6*
AT (ml/kg/min)	14.8 ± 2.2	15.8 ± 4.0
VE/VCO_2	39.4 ± 10.1	36.3 ± 8.2 †
RQ	1.2 ± 0.1	1.2 ± 0.1
Pumprate (min^{-1})	108 ± 19	115 ± 18
Pumpflow (l/min)	7.2 ± 1.1	7.6 ± 1.1

* $p \leq 0.003$ 12 weeks post LVAD versus 8 weeks post LVAD.

† $p < 0.03$ 12 weeks post LVAD versus 8 weeks post LVAD.

Group A: Comparison of exercise capacity 8 and 12 weeks after LVAD implantation (Table 2). Peak VO_2 increased from 21.3 ± 3.8 at 8 weeks to 24.2 ± 4.8 ml/kg/min at 12 weeks after implantation ($p < 0.003$; 95% CI of the difference: 4.7 to 1.3). Furthermore, the ventilatory response to exercise (VE/VCO_2) decreased from 39.4 ± 10.1 at 8 weeks to 36.3 ± 8.2 at 12 weeks after implantation ($p < 0.03$; 95% CI of difference 0.4-5.8).

Group B:Exercise performance 12 weeks after LVAD implantation (Table 3). Twelve weeks after LVAD implantation, all patients were in NYHA functional class I (apart from the limitations imposed by the drive lines and the console of the device). Peak VO_2 averaged 23.0 ± 4.4 ml/kg/min. (range 17.2 to 32.1), or 58 ± 9% of the predicted value (range 43 to 80%), sufficient for normal activities of daily life (14). There was no difference in peak VO_2 between patients with ischemic heart disease (n = 7) and those with dilated cardiomyopathy (n = 8) (21.6 ± 2.6 vs. 24.2 ± 5.4 ml/kg/min).

Table 3. Exercise performance 12 weeks after LVAD implantation (n = 15)

age (y)	37 ± 12
peak VO_2 (ml/kg/min)	23.0 ± 4.4
% predicted	58 ± 9
peak VO_2 (ml/min)	1620 ± 301
% predicted	54 ± 8
AT (ml/kg/min)	15.4 ± 3.7
RQ	1.2 ± 0.1

Group C: Sequential analysis of exercise performance in 9 patients 12 weeks after LVAD implantation and then at 12 weeks and one year after HTx (Table 4, Fig. 2). In this group, three exercise tests were compared by repeated measures ANOVA. Peak VO_2 demonstrated some improvement, although statistically insignificant: 22.8 ± 5.3, 24.6 ± 3.3 and 26.2 ± 3.8 ml/kg/min, respectively (p = 0.26; 95% CI of difference between 12 weeks after LVAD and 12 weeks after HTx: -1.91to 5.47). Expressed as percentage of the predicted value, these were 58 ± 12%, 63 ± 10% and 69 ± 13%, respectively (p = NS). The patients' AT increased from 14.4 ± 4.0 ml/kg/min with a LVAD to 15.9 ± 3.3 and 18.7 ± 2.5 ml/kg/min 12 weeks and one year after HTx, respectively (p = NS). The ventilatory responses (VE/VCO_2 at peak exercise) 12 weeks after LVAD implantation and 12 weeks and one year after HTx were not different (37.2 ± 7.8, 33.0 ± 4.4 and 33.7 ± 4.7, respectively).

Group D: Exercise capacity one year after HTx with and without a previous LVAD. Because of the large difference in the mean age between both groups, these data were only analyzed as percentage of predicted peak VO_2. Without a previous assist device (n = 20), peak VO_2 one year after HTx was 74 ± 15% versus 68 ± 13% of the predicted value in patients with a previous LVAD (n = 10; p = 0,37 [NS]; 45% power to detect a difference of 10% with a two-sided significance of 0,05).

Table 4. Sequential analysis of exercise performance in nine patients at 12 weeks after LVAD implantation and then at 12 weeks and one year after heart transplantation

	12 weeks after LVAD	**12 weeks after HTx**	**1 year after HTx**
peak VO_2 (ml/kg/min)	22.8 ± 5.3	24.6 ± 3.3	26.2 ± 3.8
% predicted	58 ± 12	63 ± 10	69 ± 13
peak VO_2 (ml/min)	1524 ± 326	1722 ± 357	1988 ± 513
% predicted	51 ± 7	57 ± 10	67 ± 13
AT (ml/kg/min)	14.4 ± 4.0	15.9 ± 3.3	18.7 ± 2.5
VE/VCO_2	37.2 ± 7.8	33.0 ± 4.4	33.7 ± 4.7
RQ	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.0

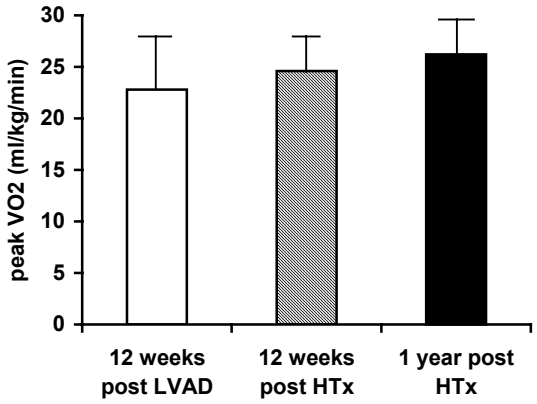

Figure 2. Peak VO_2 (ml/kg/min) in nine patients 12 weeks after LVAD implantation and then at 12 weeks and one year after HTx (p = NS by repeated measures analysis of variance).

Discussion

The results of this study suggest that exercise performance after implantation of a TCI HeartMate LVAD in a group of patients with end-stage heart failure, who underwent intensive postoperative rehabilitation, is sufficient for activities of normal daily life. An increase in maximal exercise performance from 8 to 12 weeks could be demonstrated (Table 2), most likely due to postoperative

convalescence and systematic strenuous training. One may speculate that longer assist times will result in an even better exercise performance. This improvement in exercise performance over time was accompanied by a significant decrease in the ventilatory response to exercise (VE/VCO_2). This variable is often increased in patients with severe heart failure and is considered an independent and even better prognostic marker than reduced peak VO_2 (15,16). Twelve weeks after implantation, peak VO_2 averages 23.0 ml/kg/min, compatible with Weber class A(17). Sequential analysis of exercise performance in nine patients at 12 weeks after LVAD implantation and then at 12 weeks and one year after HTx demonstrated similar peak VO_2, AT and VE/VCO_2 values. Therefore, apart from the limitations imposed by the operating console and the drive line of the HeartMate, activities with placement of a LVAD are expected to be comparable to those after HTx.

Furthermore, exercise performance of patients one year after HTx with a previous LVAD approximates that of post-transplant patients without a previous LVAD. This indicates that the impact of pre-transplant LVAD, per se, implying a thoracotomy and laparotomy with a diaphragmatic incision through which the inlet conduit of the HeartMate is passed, together with the potential detrimental effects of cardiopulmonary bypass, does not appear to lead to late consequences in terms of diminished exercise capacity. However, the presence of a LVAD certainly complicates the transplant procedure and may lead to excessive bleeding. This may have contributed to the peri-operative death of one of the patients. In contrast, the excellent general condition before HTx, in all probability, is an advantage for post-transplant rehabilitation, although this could not be translated in terms of a shorter hospital stay after HTx.

Comparison with previous studies. Our results seem to diverge from those of other studies on this subject. In the Experience with left Ventricular Assist Device with Exercise (EVADE) trial (6), peak VO_2 one to three months after LVAD implantation was reported to be 14.5 ± 3.9 ml/kg/min, compared with 17.5 ± 5.0 ml/kg/min after HTx. Mancini et al.(7)compared exercise performance in 20 patients with a LVAD with that of heart failure patients without mechanical support. Peak VO_2 in the LVAD group was 16.0 ± 3.8 ml/kg/min.

Several factors may account for these differences. The EVADE study is a multi-center study comprising 18 patients. Because it was a multi-center study, postoperative care, rehabilitation and exercise testing may not have been identical. Exercise testing was performed earlier after implantation (51.9 ± 20.4

days), when peak VO_2 was not yet optimal (Table 2). Furthermore, the mean age and weight of our patients were considerably lower than those in EVADE (37 ± 12 vs. 48 ± 12 years, and 71 ± 8 kg versus 76 ± 12 kg, respectively), resulting in a higher predicted peak VO_2 (39.6 vs. 33.6 ml/min/kg) and a higher normalized VO_2 per kilogram. Most of the aforementioned differences from the EVADE study also apply to the study of Mancini et al.(7). Furthermore, in that study, exercise testing was by way of a bicycle ergometer instead of a treadmill, as used in our study, which may render 10% to25% lower values of peak VO_2 (18). However, several small-scale studies in which exercise performance was measured by way of a treadmill demonstrated much lower peak VO_2 values (12-17 ml/kg/min) than those found in our study (8-10). Despite these differences in study design and patient characteristics, we believe that part of the excellent exercise performance of our patients with a LVAD is due to the intensive post-implantation training program.

Effect of training in heart failure. The influence of training on exercise capacity may be inferred from evidence showing that impaired exercise performance in patients with chronic heart failure is related not only to an impaired hemodynamic condition, but also to skeletal muscle abnormalities (19-21) like atrophy (22), as well as alterations in muscle histology and biochemistry (23). Sullivan et al. demonstrated, for the first time, that training could improve exercise capacity in patients with severe heart failure (24). Later, many other studies confirmed this, as reviewed by Piepoli et al. (25). Several studies using muscle biopsies or nuclear magnetic resonance spectroscopy confirmed the improvement in muscle metabolism after training, independent of central hemodynamic changes (26-29). These data demonstrate that in patients with chronic heart failure, despite a limited cardiac output response, an intensive training program can improve exercise capacity significantly.

After HeartMate implantation, total cardiac output will increase significantly, but pump flow is limited to approximately 10 liters/min. At rest, there is usually no contribution of the native heart; however, during exercise opening of the aortic valve has been observed, contributing to increased total blood flow. Based on a flow of 10 liters/min, a male patient should be able to reach a peak VO_2 of approximately 1400 ml/min, if he has a normal hemoglobin and oxygen extraction. Because this value is similar to that found in our study, we assume that exercise performance was optimal for the given blood flow. From the onset of our LVAD program, our policy was aimed at the reversal of skeletal muscle abnormalities by implementation of an intensive training program guided by a

physical therapist. The combination of an increase in cardiac output due to LVAD implantation and intensive post-implantation training likely resulted in optimal exercise capacity.

Study limitations. Due to an inherent, considerable mortality and morbidity during the course of LVAD implantation and after successive HTx, only a limited number of patients was available for a complete longitudinal study of exercise performance (n = 9, Group C). Therefore, additional analyses were performed on the larger data sets of patients at different time points after LVAD implantation (Group A, n = 10) and one year after HTx with and without a previous LVAD (Group D, n = 10 and n = 20, respectively). The relatively young age of the patients with a LVAD limits the value of direct comparison of VO_2 values with those of post-HTx patients without previous LVAD implantation. This can be partly overcome by using a percentage of individually predicted values. Furthermore, as in all small studies, an insignificant difference between two groups will have limited power. The patients in this study were relatively young, very motivated and male, which may have skewed the results and limited generalization to the whole population of patients with end-stage heart failure.

Conclusion. This study demonstrates that exercise performance in patients with severe heart failure treated with a TCI HeartMate LVAD, combined with an intensive rehabilitation program, increases over time and, at 12 weeks, is fully compatible with activities of normal daily life. Twelve weeks after implantation, exercise capacity is comparable to that at three months and one year after HTx. One year after HTx, there appears to be no difference in exercise performance between patients with and those without a previous LVAD as a bridge to transplantation. Therefore, with regard to exercise performance, a permanent LVAD holds promise as a potential alternative for HTx.

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Appendix

During the first two weeks after implantation of the HeartMate, the aim of training is to get the patient accustomed to exercise. Thereafter, an interval training program is started, with regular adjustment of the duration and intensity of the workout. Initially, the training regimen consists of 2-6 min of low-level activities, alternated with 1-2 min of rest. Training includes sessions on the bicycle and treadmill, as well as the rowing machine. Coordination is improved by several games, like badminton, tennis and volleyball. Intensity is adjusted according to the level of perceived exertion (2-4 corresponding to "light" to "somewhat hard") on the Borg scale of 0 to 10, with exertional dyspnea not exceeding 2 on a dyspnea scale of 0 to 4. Duration of exercise is gradually increased to 20-40 minutes/day three to five times a week. In addition, strength and endurance training of local muscle groups, according to the 5BX plan of the Royal Canadian Air Force (30) is performed.

Chapter 4.

Left ventricular assist device in end-stage heart failure: persistence of structural myocyte damage after unloading.

An immunohistochemical analysis of the contractile myofilaments

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J Am Coll Cardiol 2002; 39: 963-969

Abstract

OBJECTIVES – We sought to evaluate the contractile proteins in cardiomyocytes of patients with end-stage heart failure (HF) before and after mechanical support with a left ventricular assist device (LVAD).

BACKGROUND - Improvement of myocyte dysfunction has been suggested after LVAD support.

METHODS - Fourteen patients' myocardial biopsies taken at the time of LVAD implantation and after explantation, at the time of heart transplantation, were processed for routine hematoxylin-eosin staining and immunohistochemistry using monoclonal antibodies against actin, myosin, tropomyosin, troponin C and T and titin. A grading scale from 1 (abnormal staining of all myocytes, no cross-striation) to 5 (normal fiber anatomy and striation) was used. The cross-sectional area of cardiomyocytes was also measured.

RESULTS- The cardiomyocytes' cross-sectional area decreased after support, from 519 ± 94 μm^2 to 319 ± 53 μm^2 ($p < 0.001$). Actin, tropomyosin, troponin C, troponin T and titin at the time of LVAD implantation showed widespread distortion of architecture; their grades were: 1.4 ± 0.6, 2.3 ± 1.0, 2.1 ± 0.9, 2.1 ± 1.2, 2.0 ± 0.6, respectively. In contrast, myosin morphology was preserved (4.6 ± 0.7). After LVAD support ,actin, tropomyosin, troponin C, troponin T and titin showed improvement (grades 2.7 ± 1.3 [$p = 0.004$], 3.2 ± 1.2 [$p = 0.021$], 3.3 ± 0.9 [$p = 0.004$], 3.0 ± 1.1 [$p = 0.048$] and 3.1 ± 0.9 [$p = 0.001$], respectively), but no normalization. The myosin pattern deteriorated slightly (3.6 ± 1.6 [$p = 0.058$]).

CONCLUSIONS – After LVAD support, during a period of 213 ± 135 days in patients with end-stage HF, despite a decrease in the size of the cardiomyocytes, severe structural myocyte damage persisted. This does not support complete recovery of myocyte histologic features.

Introduction

Heart failure (HF) is a growing problem in cardiovascular medicine. Aging of the population, prevention of premature death by improved medical and surgical management as well as better therapy available for the treatment of chronic HF, results in an increasing number of patients with this syndrome. Heart failure may be considered a progressive disorder, initiated after an index event that results in a decline in pumping capacity of the heart. It is accompanied by activation of neurohormonal and cytokine systems, as well as adaptive changes in the myocardium called "remodeling" (1). This process of remodeling includes alterations in left ventricular (LV) geometry, alterations in extracellular matrix and alterations in the cardiomyocytes' size, function and number. Some of the changes reported in failing human cardiac myocytes are loss of myofilaments and alterations in cytoskeletal proteins(2). One canine study suggests reversibility of myocyte dysfunction, associated with an increase in the number of contractile elements, after treatment with beta-blockade (3). Also, in humans, reversibility of myocyte dysfunction has been suggested after mechanical support with a left ventricular assist device (LVAD) (4-8). This raises the option to use LVAD support as a bridge to recovery of cardiac function in patients with end-stage HF (9-11). The number of patients who have been weaned from the LVAD, however, is limited, and long-term results are not available. Unloading the heart with an LVAD will lead to a decrease in cardiac dimensions and neurohormonal activation (5,12,13). Whether this results in a significant and sustained reversal of myocyte dysfunction, however, is questionable (14,15). Few histologic studies on this subject are available (4-6,16,17). No microscopic studies of the contractile proteins in myocytes have been performed. The aim of this study was to evaluate the contractile proteins in cardiomyocytes by means of routine staining and immunohistochemical analysis of myocardial biopsies taken at the time of LVAD implantation and to compare these with biopsies taken at the time of heart transplantation (HTx). Furthermore the cardiomyocytes' cross-sectional area before and after LVAD support was compared.

Methods

Patient group

Fourteen consecutive patients (2 women and 12 men) with refractory end-stage HF treated with a pneumatic LVAD (Heartmate, Thoratec, Pleasanton,

California) as a bridge to transplantation were included in this study (Table 1). Eight patients had dilated cardiomyopathy (DCM) and six had ischemic heart disease (IHD).Thirteen patients were successfully transplanted. One patient died of a recurrent cerebral embolism. The mean duration of LV unloading was 213 ± 135 days (range 71-455 days). Before HTx, all patients were in New York Heart Association functional class I and demonstrated a very good exercise performance, as reported previously (18). All cardiac medication had been stopped. Written, informed consent was obtained from all patients.

Table 1. Characteristics of patients with an implanted LVAD

Male (n)	12
Female (n)	2
Age (years)	32 ± 12
Duration of HF* (months)	5.6 ± 6.1
DCM / IHD (n)	8 / 6
LVEF (%)	13 ± 5
CO (l/min)	3.4 ± 1.2
MAP (mmHg)	64 ± 13
IABP/other support (n)	7
Duration of LVAD support (days)	213 ± 135

* Time between onset of heart failure symptoms and implantation of LVAD (months).

Immunohistochemical analysis

Myocardial biopsy at LVAD implantation consisted of the LV apical core removed during Heartmate implantation. These biopsies were compared with LV tissue specimens of the explanted heart after HTx, from the apical half of the LV, outside the suture area of the inflow canula. In the one patient who died before transplantation, tissue specimen of the heart at autopsy were used. All biopsies were directly fixed in buffered formalin, embedded in paraffin and routinely processed for hematoxylin-eosin staining on 5-μm sections. Standard immuno-histochemical analysis was performed using a three-step avidin- biotin peroxidase reaction. In brief, the sections were deparaffinized and rehydrated, and endogenous peroxidase was blocked using a solution of methanol and hydrogen peroxide. Primary antibodies were applied to the sections for 1 hr and subsequently demonstrated using a biotinylated horse immunoglobulin G (IgG) anti-mouse IgG and horseradish peroxidase-labeled streptavidin. Immunovisualization was performed using 3,3 diamino benzidine tetra hydrochloride reagents and counterstaining with Mayer's hematoxylin. The sections were dehydrated, cleared and embedded in Pertex mounting medium

(Histolab, Göteborg, Sweden). Primary monoclonal antibodies used in this study were anti-sarcomere actin (Sigma, Zwijndrecht, the Netherlands), myosin slow (Sigma), tropomyosin (Sigma), troponin T (Sigma), troponin C (Novocastra) and titin (Novocastra, Newcastle upon Tyne, United Kingdom). Antigen retrieval, by boiling sections in a citrate-buffered solution for 15 min, was performed on the sections for all antibodies except myosin. Sections for myosin incubation were proteolytic-digested with pepsin for 15 min.

All slides contained large transmural biopsies and were examined completely by two to four investigators who aimed for consensus. In these biopsies, all longitudinal arranged cardiomyocytes were analyzed. In the case of IHD, only surviving myocytes were studied; the necrotic area was not taken into consideration. Besides providing a description of the histological findings, a grading system was also used for the immunohistochemical data, according to the following scale:

1. Almost all myocytes show a distorted architecture with absence of cross-striation
2. Some normal myocytes with cross-striation are present; most of the myocytes are abnormal, however
3. There are normal myocytes with cross-striation, along with myocytes without cross-striation, or myocytes without antigen staining over the full length of the contractile fiber
4. There are some myocytes with a distorted architecture; most of the myocytes are normal, however
5. Almost all myocytes show a normal architecture and cross-striation

To test for inter- and intra-observer variabilities, grading was repeated in half of the biopsies in a blinded manner, and the kappa value of reliability was determined. The cardiomyocytes' cross-sectional area was measured on 3 μm slides stained with modified azan, making use of a Videoplan morphometric program (Zeiss Kontron, Eching, Germany). Thirty cells were measured perpendicular to the long axis; oblique sections were excluded.

Control tissue

Before starting this study in patients with a LVAD, we optimized our technique of tissue handling and immunohistochemistry on all kinds of human myocardial biopsies. This was mostly done to rule out the effects of globally warm ischemia on the biopsies. As normal human LV myocardial tissue is not

available, atrial auricles obtained at open heart surgery from patients without HF were used for comparison.

Statistical analysis

Grading of the immunhistochemical data was compared by using the paired Student-t test. Inter- and intra-observer variabilities were tested using kappa analysis. A comparison between DCM and IHD was made by using the unpaired Student-t test. All data were calculated with SPSS version 8.0 for windows. A p value < 0.05 was considered significant.

Results

Hematoxylin-eosin staining.

At the time of LVAD implantation, all myocardial biopsies showed widespread hypertrophy of the myocytes, striking vacuolization and absence of normal cross-striation. Increased amounts of lipofuscin and interstitial fibrosis were seen (Fig 1a). After LVAD support, at the time of HTx, the biopsies showed less hypertrophy and vacuolization and some improvement in cross-striation (Fig 1b). None of the patients, however, showed complete normalization. Interstitial fibrosis appeared to increase in most patients.

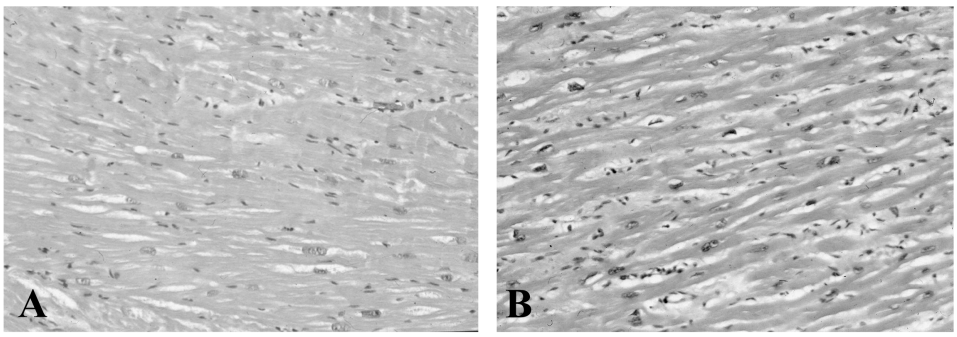

Figure 1: Hematoxylin-eosin staining in left ventricular biopsies (original magnification x50) before and after LVAD support. (A) Before LVAD support, there were hypertrophic myocytes with varying nucleair sizes, with vacuolization and interstitial fibrosis. (B) After LVAD support, the myocytes showed less hypertrophy and vacuolization, together with a decrease in myocyte size and an increase in interstitial fibrosis.

To rule out the effect of the implantation of the inflow cannula on this fibrosis,

we also examined longitudinal sections of the complete LV. These biopsies showed that the interstitial fibrosis was diffusely present throughout the whole LV. The inflow cannula only caused a small local ring of dense fibrous tissue directly around it. The widespread variation in myocyte histologic features in both series of biopsies was remarkable, both at LVAD implantation and HTx. Areas with almost normal structure were neighbored by areas showing severe morphological disorganization.

Actin

The myocardial biopsies at the time of LVAD implantation showed severe distortion of actin-staining pattern in nearly all myocytes, with barely any cross-striation visible (Fig 2a). Within some vacuoles, granular material staining for actin was discerned.

At the time of HTx, some improvement in actin pattern was observed, with partly restoration of cross-striation (Fig 2b). Large areas, however, still demonstrated an abnormal architecture.

Myosin

Remarkably, biopsies taken at the time of LVAD implantation showed an almost normal myosin architecture (Fig 2c). In the vacuoles, no myosin-positive material was discerned. In contrast to the partial improvement in actin staining, myosin staining demonstrated a slight deterioration at the time of HTx (Fig 2d).

Tropomyosin, roponin C and T and Titin

The staining pattern for these antibodies was comparable to that of actin staining, but less pronounced. At the time of LVAD implantation, some cross-striation was seen, but there were also large areas with disorganization and a lack of contractile material. Within individual cardiomyocytes, a wide variability of the staining pattern was occasionally observed. At the time of HTx, biopsies showed some improvement of cross-striation, but areas with disorganization persisted. Within each cardiomyocyte, the staining pattern appeared to be more homogeneous after LVAD support than before it. Between cardiomyocytes, however, the variability in the staining pattern increased. This frequently resulted in alternation of structurally normal myocytes with apparently abnormal myocytes in the same contractile fiber - a peculiar phenomenon not mentioned before in the published data (Fig 3).

The titin-staining pattern was comparable to that of the other thin contractile filaments.

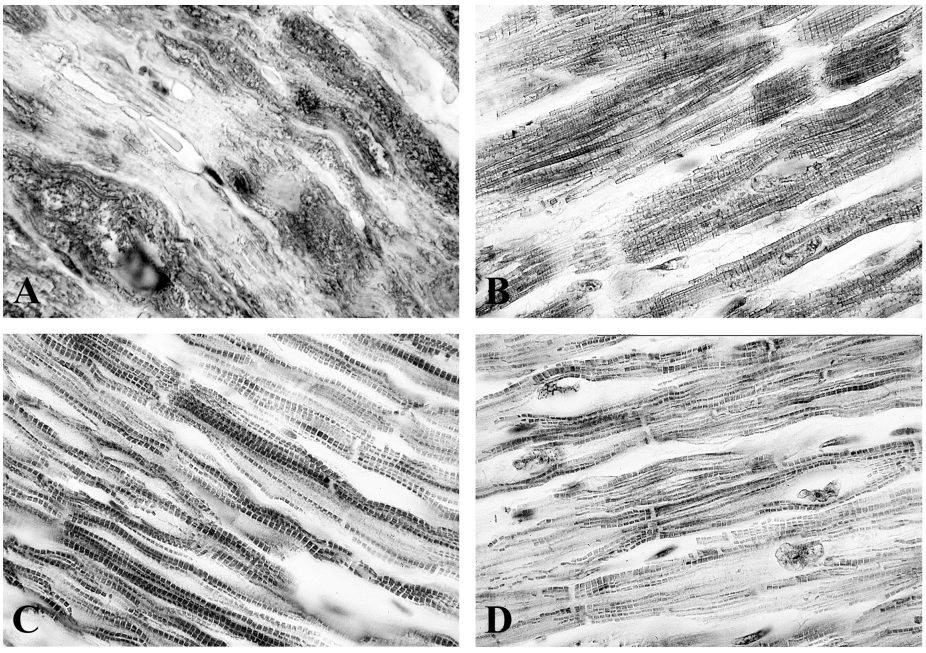

Figure 2: A: Actin staining before LVAD support shows a severe distortion of the architecture and absence of cross-striation in nearly all myocytes (grade 1). B: Actin after LVAD support demonstrates some improvement in the staining pattern, with partial restoration of cross-striation (grade 3). C: Myosin before LAVD support displays a normal architecture (grade 5). D: After LVAD support, some deterioration is observed (grade 3). (original magnification x250).

Grading of the contractile proteins

A clear pattern can be delineated (Table 2, Figure 4); the thin, contractile filaments and titin demonstrated widespread disorganization at the time of LVAD implantation and showed improvement after support ($p = 0.004$ for actin pre-vs. post-support). In contrast, myosin, the main constituent of the thick contractile filament, displayed an almost normal morphology, demonstrating a slight deterioration after LVAD support ,although not significant ($p = 0.058$).

The inter- and intra-observer kappa values were 0.55 and 0.63, respectively, indicating fair agreement. Ischemic heart disease showed a worse staining pattern of the thin contractile filaments at the time of LVAD implantation than DCM ($p < 0.05$ for actin, troponin C and titin). After LVAD support, this

difference disappeared.

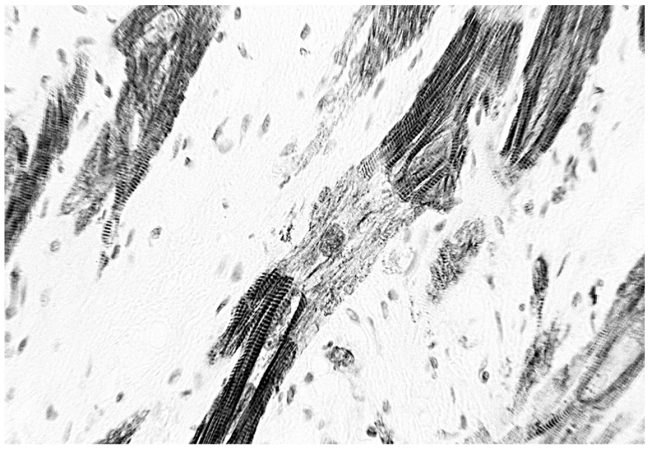

Figure 3: Tropomyosin staining after LVAD support show the alternation of histochemical normal myocytes with abnormal ones in the same contractile fiber (original magnification x150).

There was no correlation between the grading of the contractile elements in individual patients and the duration of HF symptoms, the duration of LVAD support, the dose and duration of catecholamine support before LVAD implantation or the use of an intra-aortic balloon pump before support.

Cross-sectional area of the cardiomyocytes

The cardiomyocytes' cross-sectional area decreased significantly, from 519 ± 94 μm^2 before LVAD support to 319 ± 53 μm^2 after support ($p < 0.001$), a reduction of $36 \pm 19\%$. Normal values in five control-patients showed a cross-sectional area of 226 ± 67 μm^2.

Normal atrial auricle tissue

The atrial cardiomyocytes of patients undergoing coronary artery bypass graft surgery showed a normal staining pattern of the contractile proteins (Fig. 5).

Table 2. Grading scale of the contractile proteins

	LVAD implantation	Heart transplantation
Actin		
DCM	1.6 ± 0.7	2.6 ± 1.4
Ischemic	1.0 ± 0.0*	2.8 ± 1.3
All	1.4 ± 0.6	2.7 ± 1.3 (p = 0.004)
Myosin		
DCM	4.8 ± 0.5	3.4 ± 1.6
Ischemic	4.3 ± 0.8	3.8 ± 1.6
All	4.6 ± 0.7	3.6 ± 1.6 (p = 0.058)
Tropomyosin		
DCM	2.6 ± 0.9	3.3 ± 1.3
Ischemic	1.8 ± 1.0	3.2 ± 1.2
All	2.3 ± 1.0	3.2 ± 1.2 (p = 0.021)
Troponin C		
DCM	2.5 ± 0.8	3.0 ± 0.9
Ischemic	1.5 ± 0.8*	3.7 ± 0.8
All	2.1 ± 0.9	3.3 ± 0.9 (p = 0.004)
Troponin T		
DCM	2.0 ± 0.9	2.6 ± 1.2
Ischemic	2.2 ± 1.6	3.5 ± 0.8
All	2.1 ± 1.2	3.0 ± 1.1 (p = 0.048)
Titin		
DCM	2.3 ± 0.5	3.3 ± 1.0
Ischemic	1.7 ± 0.5*	3.0 ± 0.6
All	2.0 ± 0.6	3.1 ± 0.9 (p = 0.001)

Data are presented as the mean value ± SD. * $p < 0.05$ for IHD versus DCM, at the time of LVAD implantation.

Discussion

In the present study, for the first time, to the best of our knowledge, immunohistochemical data on contractile proteins in cardiomyocytes are reported in patients with end-stage HF treated with an LVAD. This study showed that the myosin structure was preserved in patients with end-stage HF before LVAD implantation. In contrast, the thin, contractile filaments and titin

demonstrated widespread distortion of the normal staining pattern, with actin displaying the worst degradation. After LVAD support, a partial improvement in morphology of the thin contractile filaments and titin had occurred. In contrast, myosin demonstrated a slight deterioration in histologic features. Furthermore, after LVAD support, the staining pattern within each cardiomyocyte appeared to be more homogeneous. Between different cardiomyocytes, however, the variability in the staining pattern persisted, leading sometimes to the alternation of apparently abnormal myocytes with normal ones in the same contractile fiber. This may affect cardiac contractility in a negative way and may also affect the spread of the electrical impulse, creating a condition for re-entry.
Finally, a significant decrease in the cardiomyocytes' cross-sectional area after LVAD support was demonstrated, although normalization was not complete.

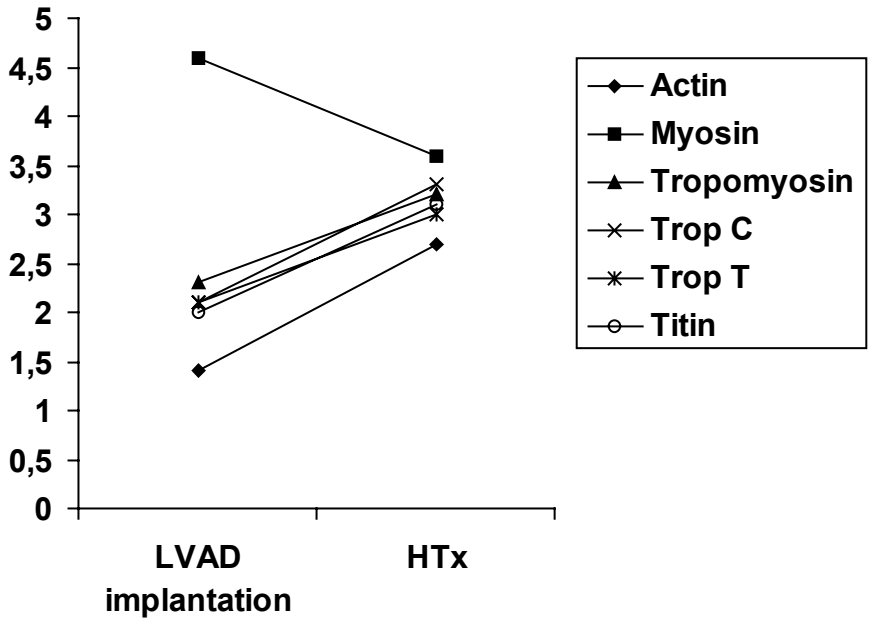

Figure 4: Grading of the contractile proteins in myocytes before and after LVAD support. Myosin staining showed some deterioration (grade 4.6 before LVAD implantation vs. 3.6 after LVAD support; $p = 0.058$). In contrast, the contractile proteins of the thin filaments and titin showed improvement (grade 1.4 for actin before LVAD implantation vs. 2.7 after LVAD support; $p = 0.004$). Severe structural myocyte damage persisted, despite a period of LVAD support of 213 ± 135 days.

The role of immunohistochemistry in HF

Immunohistochemic studies on myocardial tissue in chronic HF are scarce and comprise mainly hearts with DCM (2,19,20). With respect to the thin contractile filaments and titin, our results correspond to these studies. In contrast to the study reported by Hein et al. (19) the myosin pattern in our patients with end-stage HF was remarkably preserved. This relative sparing of myosin, however, has been demonstrated before in a study of dogs in whom regional ischemia was induced by ligation of the left anterior descending coronary artery (21).

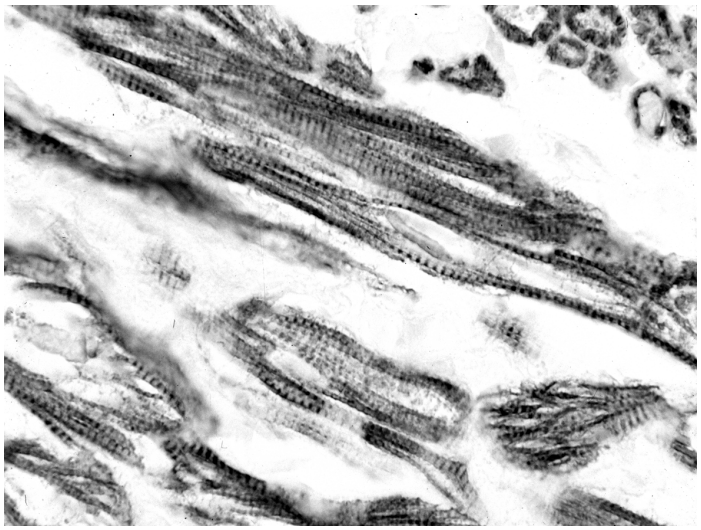

Figure 5: Human atrial cardiomyocytes show normal cross-striation (actin staining, grade 5; original magnification x250).

Recovery of myocyte function

Despite persistence of widespread areas with a distorted myocyte architecture, as shown in this study, LVAD implantation leads to an impressive improvement in the hemodynamic condition in our patients (18), as well as in those reported in other studies. The decrease in cardiomyocyte size is in accordance with other studies (17,22, 23). Furthermore, LVAD support results in a decrease in cardiac dimensions (4-6,17,24), an improvement of myocyte-(7,25,26) and mitochondrial function (27, 28), an improvement of neurohormonal activation (12,13,17), an increase in beta- receptor density (29) and disappearance of antibodies against beta- receptors (9) as well as a decrease in serum interleukin

(IL)-6 and IL-8 (30). Recently, a decreased susceptibility to apoptosis (31) and a reduction in tumor necrosis factor-alpha activity in nonischemic cardiomyocytes after support were shown (32). Histologic studies after LVAD support showed a reduction of wavy fibers, contraction band necrosis and myocytolysis, accompanied by an increase in myocardial fibrosis (4-6,8,9,16,17), although recently, a reduction in the collagen content was also reported after LVAD support (23).

These alterations, however, probably resulted from unloading of the heart with consequent circulatory recovery and may not be specific for LVAD support at all. Medical therapy (e.g. angiotensin-converting enzyme inhibitors and beta-adrenergic blockers) also improves the circulatory status and slows deterioration of the failing heart, a benefit that is partly due to inhibition of remodeling (14, 33). Furthermore, spontaneous improvement in LV function is well known in myocarditis and in peripartum cardiomyopathy and was also reported in 27 % of patients with recent-onset DCM (34). This may partly explain the number of patients being weaned from LVAD support in some studies. It is evident that in these situations, an LVAD can play an important role in keeping a patient alive and gaining time for cardiac improvement. At present, however, there is no evidence available that LVAD support can be a permanent cure for patients with HF (15). The extent of myocardial recovery after LVAD support is highly variable (11,35,36), and there are conflicting data on the amount of fibrosis after support. The increased fibrosis demonstrated in our study and by others (4,6,37), will have a negative impact on systolic and diastolic cardiac function, limiting the chance for complete recovery. Furthermore, the inadequate presence of titin after LVAD support, as seen in our study, can hamper the formation of new sarcomeres, because titin is necessary as a template for the organization of newly synthesized myosin and actin filaments (19).

A new and intriguing strategy that combines mechanical and intensive pharmacological therapy to promote reverse remodeling and uses clenbuterol to induce physiologic hypertrophy has been suggested - the Harefield approach (38, 39). This requires further studies to determine the long-term efficacy and to elucidate the concepts of reverse remodeling (39).

Study limitations

In principle, abnormalities observed in cardiac biopsies could partly be caused by global warm ischemia of cardiac tissue after obtaining the biopsies. Diseased human hearts are extremely susceptible to the effects of ischemia,

particularly the contractile proteins (20). Therefore, we optimized our tissue handling so that 90 % of the biopsies were fixed within 10 minutes. The normal architecture of the myosin pattern in the patients with HF argues against global ischemia as a potential mechanism for our findings, because in the study of Hein et al.(20), it was shown that all contractile proteins are equally sensitive to the effect of global ischemia. Furthermore, our control biopsies were also exposed to the potential effect of global ischemia, but showed a normal staining pattern of the contractile proteins. Most importantly, in this study, every patient is its own control. The improvement in the staining pattern of all thin contractile proteins and titin in the biopsies after LVAD support, which are handled in the same way as before LVAD placement, is a strong argument against the effects of global warm ischemia.
Another problem is that biopsies may not be representative of the whole heart. This problem is overcome, in the greater part in this study, by the quantity of available tissue (1-3 cm. biopsies).
All our patients had end-stage HF. It cannot be ruled out that patients with less severe forms of HF may actually show more recovery of myocyte histologic features after LVAD support.
The rather long duration of LVAD support in this study may mean that the optimal period of recovery has been exceeded, because some clinical and animal studies suggest that recovery is complete within a couple of weeks (11, 26, 40).
In our patients with LVAD support, all cardiac medications were stopped. It is not known to what extent this could have influenced recovery; this will be an area for future research.

Conclusion

Despite impressive hemodynamic recovery, a decrease in the cardiomyocytes' cross-sectional area and an improvement in staining pattern of the thin contractile elements and titin, our findings endorse the persistence of severe structural myocyte damage after a long duration of LVAD support (213 ± 135 days). Our findings do not support complete recovery of myocyte histologic features after a period of unloading of the heart by LVAD support.

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Chapter 5.

Cardiomyocyte cell death in patients with end-stage heart failure before and after support with a left ventricular assist device: low incidence of apoptosis despite ubiquitous mediators.

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Submitted

Abstract

BACKGROUND- Left ventricular assist device (LVAD) implantation in patients with end-stage heart failure results in impressive hemodynamic improvement. The effects on myocardial apoptosis and its mediators are unknown.

METHODS- Myocardial biopsies from 17 patients at the time of LVAD implantation and after explantation, at the time of heart transplantation (HTx) were examined by TUNEL reaction and with antibodies against Fas Ligand (FasL), Fas, TNFα receptor 1 (TNFR1), TNFα receptor 2 (TNFR2), TNFα, TNFα converting enzyme (TACE), Poly (ADP-ribose) polymerase (PARP), Poly ADP-ribose (PAR), Caspase 3 and FLICE inhibitory protein (FLIP).

RESULTS- Apoptosis incidence was low: 0.8 % of cardiomyocytes (range 0-3) before support, and 0.1 % (range 0-0.6) positive cardiomyocyte nuclei after support ($p < 0.01$). This was accompanied by low expression of caspase 3 and high expression of the DNA repair enzyme PARP. Its product PAR increased after support. Mediators and receptors inducing apoptosis, and FLIP were widely present before and after support.

CONCLUSION- Despite the abundant presence of mediators and receptors inducing apoptosis, the incidence of apoptosis itself was low before and after mechanical support. The abundant expression of FLIP may suggest an important role for this protein in the inhibition of cardiomyocyte death.

Introduction

Heart failure is a progressive disorder accompanied by activation of neurohormonal and cytokine systems and adaptive changes in the myocardium called remodeling.[1] Myocytes and fibroblasts are the major cardiac cells involved in this remodeling process. Ongoing myocyte cell death is thought to be one of the mechanisms leading to progressive ventricular dysfunction.[2] There is accumulating evidence that this cell death occurs partly through apoptosis, although it is not yet known to what extent.[3] Different degrees of apoptosis have been documented in end-stage heart failure.[4-6] Only few studies have dealt with the occurrence of apoptosis in patients with end-stage heart failure treated with a left ventricular assist device (LVAD).[7,8] No systematic study has been performed on apoptosis mediators and receptors in patients with end-stage heart failure treated with a LVAD.

The aims of this study were, firstly, to evaluate the apoptotic process and its related mediators in patients with acutely deteriorating end-stage heart failure, and secondly, to investigate the effect of LVAD support on these processes. For this study we performed immunohistochemical analysis of myocardial biopsies taken at the time of LVAD implantation and compared these with biopsies taken at the time of heart transplantation (HTx).

Methods

Patient population

This study included 17 patients (15 males, 2 females; mean age 34 ± 13 years, range 18-53) with refractory end-stage heart failure treated with a LVAD (HeartMate, Thoratec, Pleasanton, California) as a bridge to transplantation. Ten patients had dilated cardiomyopathy (DCM) and 7 ischemic heart disease (IHD). All patients were in NYHA class IV and on intravenous inotropic medication. Seven patients were also treated with an intra-aortic balloon pump. Mean LV ejection fraction was 15 ± 5 %. Mean cardiac output was 3.7 ± 1.4 L/min; mean arterial pressure 64 ± 12 mm Hg. All patients were successfully transplanted after a mean duration of left ventricular support of 211 ± 115 days (range 71-455). While on the LVAD all patients were in NYHA functional class I and had a very good exercise performance.[9] All cardiac medication had been stopped, except for low dose aspirin. Written informed consent was obtained from all patients.

Immunohistochemical analysis

Myocardial biopsy at LVAD implantation consisted of the left ventricular apical core removed during HeartMate implantation. These biopsies were compared to large left ventricular free wall tissue specimen of the explanted heart after HTx. All slides contained large transmural biopsies and were examined completely by three investigators. All biopsies were directly fixed in buffered formalin and embedded in paraffin. Primary antibodies were used against Fas ligand (FasL), Fas, TNFα- receptor 1 and 2 (TNFR1, TNFR2) (all Sanvertech, Santa Cruz, California USA), TNFα (Hycult Biotechnology, Uden, the Netherlands), TNFα-converting enzyme (TACE, a gift from Immunex Corporation, Seattle, USA), Poly-ADP-ribose polymerase (PARP, Sanvertech), Poly-ADP-ribose (PAR, Kordia Life Sciences, Leiden, the Netherlands), Caspase 3 (CCP32, Dakopatts, Denmark) and FLICE Inhibitory Protein (FLIP long and short, Sanvertech).

Detection of these primary antibodies was performed using either a combination of horseradish peroxidase (HRP) labelled second and third antibodies, or using biotinylated second antibody with HRP labelled streptavidin. HRP of all immuno reactions was visualised using diaminobenzidine (DAB) development with H_2O_2 as substrate. After counterstaining with Mayer's Haematoxilin, sections were dehydrated in ethanol, cleared in xylol and embedded in Pertex mounting medium (Histolab, Göteborg, Sweden).

For FLIP (L + S) visualisation, all incubations were performed on aceton fixed frozen sections, sections were incubated overnight at 4 ^{0}C using an isotype mouse IgG1 as control. The demonstration of FLIP was performed using Powervison system (Klinipath, the Netherlands).

Apoptosis was demonstrated using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) according to the method of Wijsman et al.[10] As positive control biopsies we used cardiac samples of heart-transplant patients with severe acute rejection, as discussed previously.[11]

Quantitative FLIP mRNA expression

RNA was isolated using TRIzol™ Reagent (GibcoBRL, Rockville, USA). Three µg of RNA was used for cDNA synthesis using oligoDT and random primers. Primers and probes were designed for use at the ABI Prism 7700 sequence Detection System (Applied Biosystems, New Jersey, USA). FLIP L/S forward and reverse primers: 5'-CCA-CTG-GAA-AGG-ATT-CTG-AAA-GAA-3' and 5'-AAG-CTC-ACA-AGG-GTC-TTG-CAG-TA-3', FLIP L/S probe: 5'-FAM-CAG-CTC-

CGG-GCC-AGT-CAA-CAG-AA-TAMRA-3′ (Biosource, Foster City, USA). Porphobilinogen deaminase (PBGD) was chosen as a reference gene, using PBGD forward and reverse primers: 5′-GGC-AAT-GCG-GCT-GCA-A-3′ and 5′-GGG-TAC-CCA-CGC-GAA-TCA-C-3′, PBGD probe: 5′-VIC-CAT-CTT-TGG-GCT-GTT-TTC-TTC-CGC-C-TAMRA-3′ (Applied Biosystems).
For quantitative RT-PCR, the Taqman™-PCR core reagent kit was used (Applied Biosystems). PCR reactions were carried out in a final volume of 50 µl containing: 1x Taqman™ buffer (final concentration), 40 mmol dNTP's and 1.25 Units Ampltitaq Gold. In case of FLIP 2.5 pmol of both primers, 7.5 pmol probe and 150 mmol $MgCl_2$ were added. The reaction mixture for PBGD contained 15 pmol of both primers, 10 pmol probe and 250 mmol $MgCl_2$. The following thermocycle profile was used: 95 °C for 10 min followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. Each sample was run in duplo. The following formula was used to quantify our samples: relative quantity = $2^{-\Delta\Delta Ct}$, $\Delta\Delta Ct = \Delta Ct$ (sample) - ΔCt (calibrator) and ΔCt = Ct (target) – Ct (reference). The calibrator sample, cDNA of PHA stimulated blood mononuclear cells of a healthy volunteer, was run each time and therefore, it was possible to make an interrun comparison. For normal comparison FLIP m-RNA was also quantified in 7 myocardial biopsies peroperatively taken from donor hearts and from patients undergoing coronary bypass operation.

Statistical analysis

Results are given as mean ± SD. Student's paired or unpaired t test was used for statistical comparison, as appropriate. All data were calculated with SPSS 8.0 for windows. A p value < 0.05 was considered significant.

Results

Apoptosis

The number of TUNEL positive nuclei was counted per 150 cardiomyocyte nuclei at magnification 400 x and expressed as percentage. Only dark brown stained nuclei were counted as positive; ambiguously stained nuclei, which were especially present in the biopsies before LVAD implantation, were scored negative.
A low incidence of TUNEL positive myocytes was seen both at the time of LVAD implantation and after support, at the time of HTx. In 15 patients this was 0.8% (range 0-3%) of cardiomyocyte nuclei positive before, and 0.1%

(range 0-0.6 %) after LVAD support ($p < 0.01$) (Table 1).

Table 1 Histochemical data of apoptosis before and after LVAD support

	TUNEL	Caspase-3	PARP	PAR
before LVAD	0.8 % (0-3 %)	weakly present	3.6 ± 0.6	1.0 ± 0.0
after LVAD	0.2 ± 0.4* (0-0.6 %)	weakly present	3.2 ± 0.9	1.6 ± 0.8†

* = $p < 0.01$; † = $p < 0.007$
TUNEL: percentage of positive staining nuclei (range).PARP: Poly (ADP-ribose) polymerase; PAR: Poly ADP-ribose; grade1 = 0-25% of myocytes positive; 2 = 25-50% of myocytes positive; 3 = 50-75% of myocytes positive; 4 = 75-100% of myocytes positive.

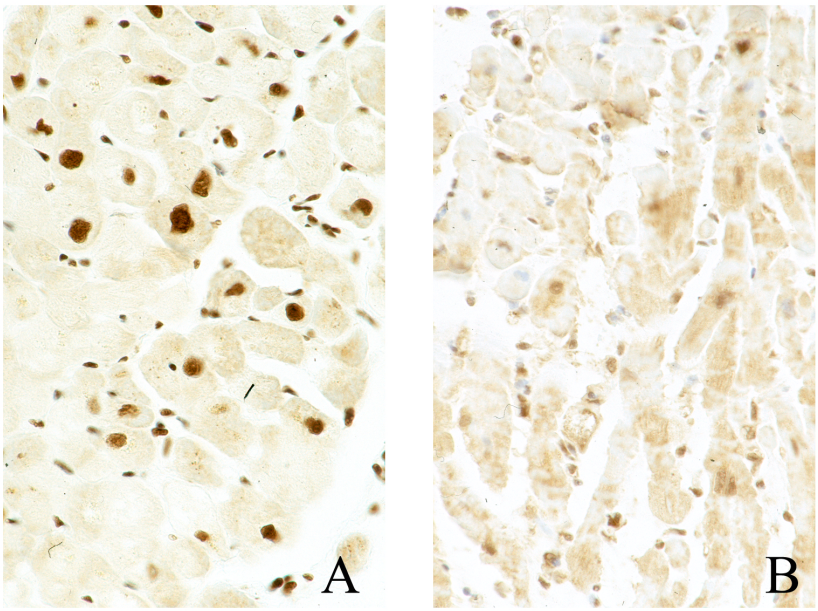

Figure 1. In this figure an example of high incidence TUNEL positivity is shown (A). This TUNEL positivity did not correlate with high caspase-3 activity (B) suggesting that the TUNEL staining in this patient was not indicative of apoptosis. (original magnification 100 x).

In two patients a remarkably high incidence of TUNEL positivity was observed before LVAD implantation: 26 % and 97 % positive cardiomyocyte nuclei, respectively (fig 1a). In these two patients TUNEL reaction was repeated on frozen sections, lacking the proteolytic digestion with pepsin which is required for the TUNEL reaction on paraffin.[11] These two slides showed complete absence of TUNEL positive cardiomyocyte nuclei, suggesting that the TUNEL positivity on the paraffin slides was not indicative of apoptosis. No frozen tissue was available of all patients, however. After LVAD support the incidence in these two patients decreased to low numbers, in accordance with the other patients.

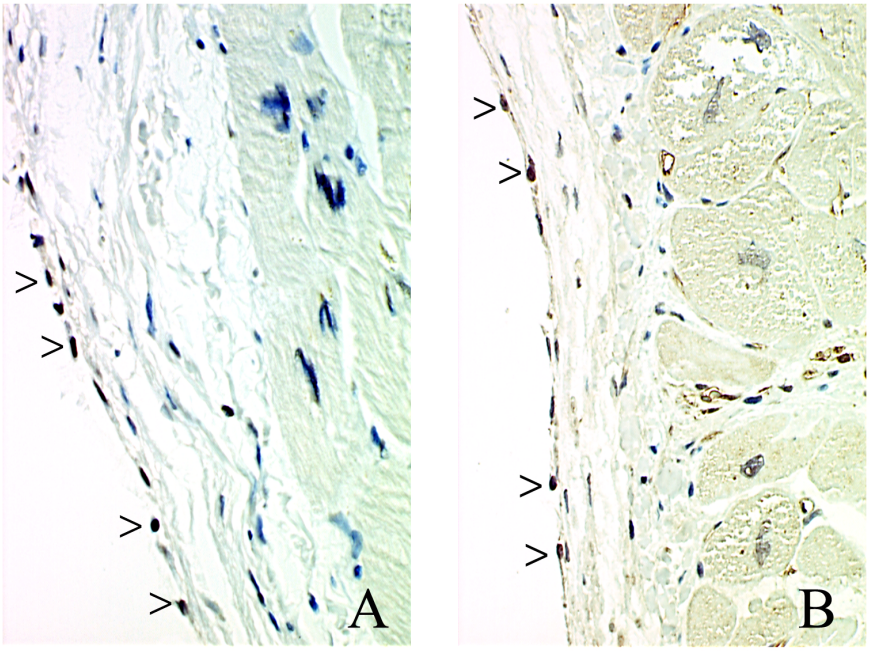

Figure 2. This shows the normal situation of positive TUNEL staining in endothelial cells (endocard) (A) corresponding with caspase 3 activity (arrowheads) (B). (original magnification 100 x).

In all patients a higher incidence of TUNEL positivity was seen in infiltrating cells, endothelial and endocardial cells (fig 2a) and in fibrous tissue surrounding infarcted areas. In general, the subendocardial area showed more

TUNEL positive cardiomyocytes than the deeper layers. Regarding cardiomyocyte apoptosis, hearts of patients with DCM showed lower numbers than hearts of patients with IHD. Especially patients with recent infarctions showed higher numbers of apoptotic cardiomyocytes. The number of TUNEL positive myocytes related neither with the dose and duration of inotropic support nor with the hemodynamic data before LVAD implantation in this patient group.

Caspase 3

Most myocytes showed a weak cytoplasmic staining of caspase 3 (Table1). However, nuclear expression was scarce. After LVAD support the picture was similar. Endothelial and endocardial cell nuclei were often positive before and after LVAD support (fig 2b). There was no difference between IHD and DCM. TUNEL positive nuclei were often caspase 3 positive. In the two patients that showed a very high percentage of TUNEL positivity, nuclear expression of caspase 3 was much lower (fig 1b), suggesting that the TUNEL staining in these patients was not indicative of apoptosis, as was also suggested by the TUNEL reaction on frozen sections.

Poly (ADP-ribose) polymerase (PARP)

This DNA repair enzyme was widely present in the nuclei of cardiomyocytes before and after LVAD support (fig 3a). We quantified the activity by using the following grading: 1 = 0-25% of myocytes positive; 2 = 25-50%; 3 = 50-75%; 4 = 75-100%. For the whole group the grading decreased from 3.6 ± 0.6 to 3.2 ± 0.9 ($p < 0.05$) (Table 1).

There was no difference between DCM and IHD patients.

Poly ADP-ribose (PAR)

In contrast to PARP expression, the nuclear expression of its product, Poly ADP-ribose (PAR) was only very weak. We used the same scoring system as with PARP, resulting in 1.0 ± 0.0 before support and 1.6 ± 0.8 after support ($p < 0.007$) (Table 1).

FasL

Before and after LVAD support almost all myocytes showed a weak cytoplasmic staining for FasL in DCM hearts as well in ischemic hearts. Expression at the cellular membrane was only sporadically observed.

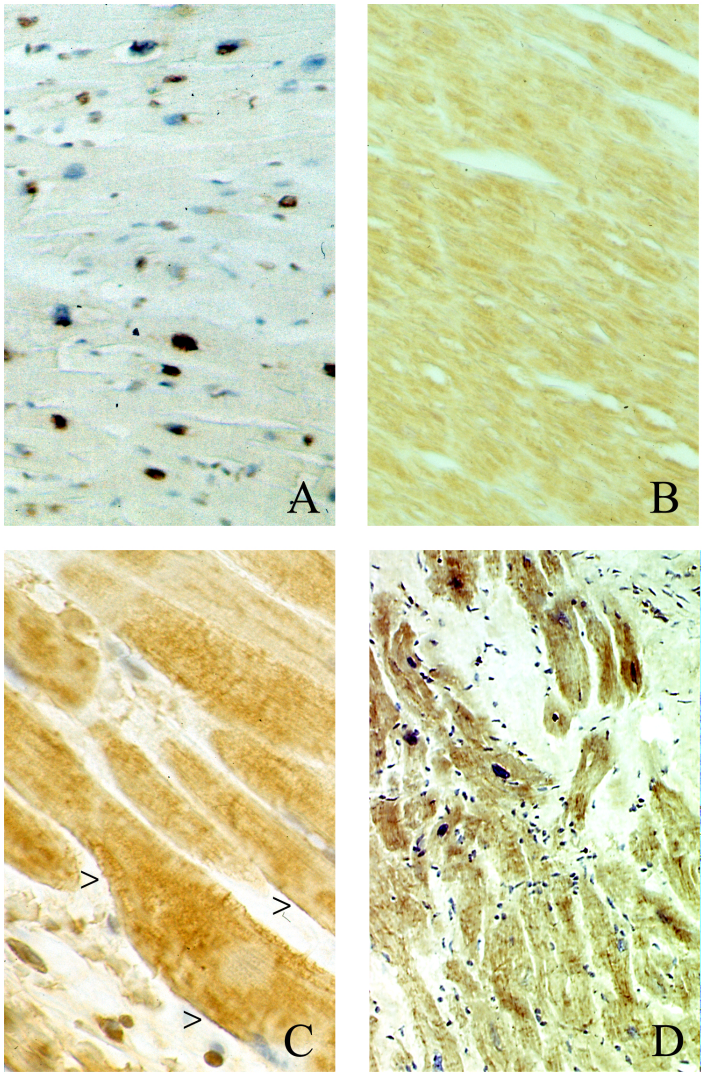

Figure 3. Regulators of apoptosis in patients with end-stage heart failure, before LVAD support. **A**, PARP expression showing almost all cardiomyocyte nuclei positive (original magnification 50 x); **B**, Fas staining, demonstrating diffuse weak cytoplasmic expression (original magnification 50 x); **C**, Diffuse cytoplasmic presence of TNF receptor 1, with some expression on the sarcolemma (arrowheads) (original magnification 150 x); **D**, cytoplasmic expression of FLIP (original magnification 100x).

Fas

Most of the myocytes demonstrated a weak cytoplasmic expression of Fas activity (fig 3b) and sometimes at the cell surface. After LVAD support there was no noticeable change in staining pattern. Again, no difference between DCM and IHD patients was noted. Some intercalated discs showed positive staining.

TNFα

Both DCM and IHD patients showed positive staining for TNFα in part of the myocytes. The activity sometimes had a patchy localization with more positive staining on the endocardial side of the myocardium and in the papillary muscles and less staining on the epicardial side. After LVAD support most myocytes still showed TNFα expression, although it was slightly diminished in some patients.

TNFα Converting Enzyme (TACE)

TACE staining before and after support followed that of TNFα in most patients but, on the whole, demonstrated a slightly weaker expression. Most activity was detected in the cytoplasm and not , as might have been expected, at the sarcolemmal site.

TNFα Receptor 1 (TNFR1)

All myocytes showed cytoplasmic staining for TNFR1 as well as expression at the cell membrane (fig 3c). After LVAD support some patients showed no surface expression anymore, both in the DCM and the IHD group, but in the majority of patients expression was unchanged.

TNFα Receptor 2 (TNFR2)

Before LVAD support almost all DCM patients showed cytoplasmic and sarcolemmal expression of TNFR2. After support this expression decreased slightly. In some patients it was no longer detected. In patients with IHD the expression of TNFR2 before and after LVAD support appeared to be a little less pronounced than in the DCM group.

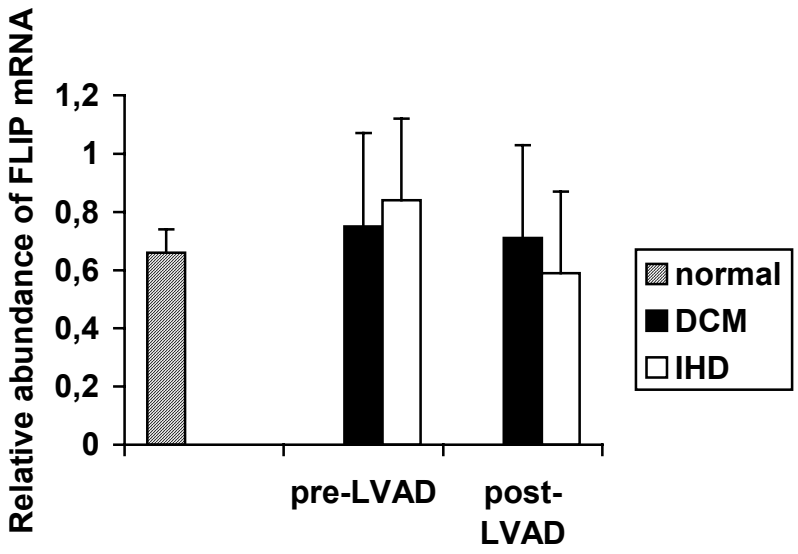

Figure 4. FLIP m-RNA in normal cardiac tissue and in DCM and IHD patients with end-stage heart failure. The relative abundance of FLIP m-RNA, measured by quantitative PCR, is comparable in normal cardiac tissue and in heart failure patients before and after LVAD support (p = NS).

FLIP

FLIP m-RNA was demonstrated in cardiac tissue, in the same amount before LVAD implantation as after LVAD support (0.79 ± 0.31 relative units before, 0.65 ± 0.33 after support; p = NS) . It did not differ from the amount in hearts without heart failure (0.66 ± 0.07, fig 4). Immunohistochemistry confirmed the presence of FLIP mainly in the cytoplasm of cardiomyocytes (fig 3d). No difference was observed before and after LVAD support. Isotype controls were negative.

Discussion

In the present study, comprising a group of relatively young patients with severe heart failure, a remarkably low incidence of apoptotic cardiomyocytes was demonstrated (0.8%) together with a low caspase 3 activity. In contrast, expression of the DNA repair enzyme PARP was quite high. Its product PAR, on the other hand, was mostly absent, but increased after LVAD support, along

with a further reduction in TUNEL positive cardiomyocytes. This suggests decreased apoptosis and increased DNA-repair after LVAD implantation, probably due to a reduction in myocardial stretch and decreased neurohormonal activation.

Apoptosis inducing ligands (TNFα and FasL) were present in most of the cardiomyocytes and decreased only slightly or not at all after LVAD support. TACE expression coincided with that of TNFα, although on a lower level of activity. Transmembrane receptors belonging to the TNF receptor superfamily (TNFR1, TNFR2, Fas) were present both in the cytoplasm and on the membrane of most cardiomyocytes in end stage heart failure, although Fas surface expression was only week. After LVAD support the expression of TNFR2 in DCM patients decreased slightly. FLIP was widely distributed in the cardiomyocytes before and after mechanical support, comparable to the level in normal hearts.

Apoptosis in heart failure

Progressive loss of cardiomyocytes by apoptosis is thought to be an important pathogenic mechanism of heart failure.[3] Initially, high levels of apoptosis were reported (5-35%), [5] but in subsequent studies apoptosis rates of less than 1 % were presented,[4,6] corresponding with the results in our study. Maybe end-stage heart failure represents a "burnt-out" state, characterized by minimal ongoing apoptosis.[3] Some caution has to be taken because of the sometimes localized nature of the apoptotic process, with a preference for the subendocardial regions. An additional problem in the determination of the role of apoptosis in heart failure is the limited specificity of the TUNEL method, because necrotic cardiomyocytes and postmortem autolysis as well as vital cardiomyocytes showing DNA repair and RNA synthesis have been reported to lead to TUNEL positivity as well.[12-14] This may be the explanation for the high incidence of TUNEL positive cardiomyocytes in two patients in this study and in some previous reports on apoptosis in heart failure. The absence of caspase 3 staining in these two patients and the absence of TUNEL positivity on frozen sections made us believe that the extensive positive TUNEL staining on the paraffin slides in these patients is not caused by apoptosis. In these patients the combination of proteolytic pretreatment required on paraffin sections for the TUNEL reaction and a particular status of nucleus pre-LVAD may have caused the TUNEL positivity. This may also be the reason for the ambiguously stained nuclei by the TUNEL reaction in most patients. Also this staining was mainly observed pre-LVAD.

The low expression of caspase 3 in this study and in others[15,16] is in accordance

with the low incidence of apoptosis because caspase-3 represents the final common pathway of the caspase cascade resulting in DNA cleavage.[17] The high expression of the DNA repair enzyme PARP that we found suggests that the cardiomyocytes were relatively resistant to apoptosis.[18] Caspase 3 inactivates PARP early in the apoptotic process,[19] but the antibody used in this study recognizes both the full-length molecule and the 85-kDa cleaved fragment. Other studies, however, have already shown negative immunostaining for 24-kDa PARP fragments in end-stage heart failure.[15] In contrast to cardiomyocytes, endothelial and endocardial cells, exhibited considerable presence of apoptosis as was also reported by others.[8] This may suggest that myocardial apoptosis is promoted by the diffusion of soluble pro-apoptotic mediators, like TNFα, FasL or free radicals, from the blood or from endothelial cells.[20,21]

Apoptosis in patients treated with a LVAD

Only few other studies on apoptosis in LVAD patients are available.[7,8,16] In the study of Bartling et al.[7] a decrease of apoptosis after LVAD support was demonstrated by DNA fragment laddering. In contrast to our study these authors did not compare TUNEL stained biopsies before and after LVAD treatment. Therefore, it can not be excluded that the attenuation of DNA fragmentation after LVAD support may have been the result of a reduction in apoptotic interstitial cells. These may make a major contribution to the apoptotic ladder patterns, as is demonstrated for instance in acute rejection after heart transplantation[11] and in transgenic mice with cardiac-specific overexpression of TNFα.[22] The study of Francis et al.[8] showed very little evidence for cardiomyocyte apoptosis in patients with end-stage heart failure, but found overexpression of Bcl-2 and PCNA (proliferating cell nuclear antigen). Birks et al.[16] demonstrated elevated myocardial caspase-9 expression in patients with end-stage heart failure at the time of LVAD implantation, but caspase-3 was not elevated, in accordance with our study. They did not perform TUNEL reactions in those biopsies, however.

Apoptosis related mediators in heart failure and after LVAD

TNFα is synthesized as a large inactive protein, which is activated by a tissue metalloproteinase called TNFα converting enzyme (TACE).[23] Two types of TNFα receptors have been identified: a 55-kDa (TNFR1) and a 75-kDa (TNFR2). Both receptors are expressed on adult human cardiomyocytes.[24] Induction of apoptotic cell death by TNFα is predominantly mediated by

TNFR1.[25]

TNFα mRNA and protein and TACE have been demonstrated before in explanted hearts from patients with end-stage heart failure.[26,27] After LVAD support decrease of immunohistochemic TNFα expression in a study with 8 patients has been reported.[28] The largest reduction however, was in the 4 patients who could be weaned from the assist device, suggesting that the reduction of TNFα was not only caused by unloading, but maybe also by the natural history of the underlying disease. In our study, reduction of TNFα expression and TACE after LVAD support was only very modest.

Another mechanism for the induction of apoptotic cell death is the cross-linking of Fas and Fas-L.[29] This is an important mechanism in the down-regulation of immune responses and effector activities, such as in T cell-mediated cytotoxicity [30] and ischemic myocardial cell death.[31] A study in rats, however, showed no enhanced cardiomyocyte apoptosis despite the presence of FasL and Fas. [32]

It is assumed that FLIP may contribute to the blockade of the death signaling pathways in cardiomyocytes.[31,33] This protein is abundantly present in the heart, as was also demonstrated in our study. In this way most cardiomyocytes are protected from apoptotic cell death, in contrast to other cardiac cells, like endothelial cells. Other inhibitors of apoptosis, like NFκB and anti-apoptotic factor A1, a member of Bcl-2 family proteins are also potential regulating factors of cardiomyocyte cell death in end-stage heart failure, but were not investigated in this study.

Conclusion

In the conundrum of end-stage heart failure we still do not know if progressive cardiac dilatation occurs because of progressive deterioration of myocyte function, or because of a reduction in myocyte number.[34] In the present study, a low incidence of apoptotic cardiomyocytes was observed, despite the abundant presence of apoptosis-inducing mediators and their specific receptors. To us, this would suggest that deterioration of myocyte function is more important than the reduction in myocyte number. This is in agreement with our previous study in which we demonstrated severe structural damage of the contractile proteins in end-stage heart failure, which did not completely resolve after LVAD support, [35] while the number of apoptotic cardiomyocytes in this study decreased significantly after LVAD support. The abundant expression of FLIP may suggest an important role for this protein in the inhibition of cardiomyocyte death.

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Chapter 6.

Similar left and right ventricular cardiomyocyte morphology after support with a left ventricular assist device; utility of right ventricular biopsies to monitor left ventricular reverse remodeling.

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Submitted

Abstract

OBJECTIVES- To evaluate if the morphology of the contractile proteins in cardiomyocytes of patients with end-stage heart failure, treated with a left ventricular assist device (LVAD), is identical in the left- and right ventricle (LV, RV) and in the interventricular septum (IVS) and can be monitored by biopsies taken with a bioptome.

BACKGROUND- Application of a LVAD as a bridge to recovery of cardiac function requires monitoring of myocyte recovery. The use of right ventricular biopsies for this could be feasible, if morphologic findings in the RV coincide with those in the LV.

METHODS- At the time of heart transplantation myocardial biopsies of LV, RV and IVS from 13 patients after LVAD support were compared using immunohistochemistry with monoclonal antibodies against the contractile proteins. Additionally, in 5 patients, small biopsies obtained with a diagnostic bioptome were compared with large transmural biopsies. Hemodynamic monitoring was performed when the patients were fully recovered from the implantation, to rule out persistent RV failure.

RESULTS- The staining pattern of actin, myosin, tropomyosin, troponin T and C was identical in the biopsies of LV, RV and IVS. Small biopsies taken with a bioptome appeared to be representative for the larger biopsies. Hemodynamic monitoring showed absence of RV failure in our study group.

CONCLUSION- In the absence of RV failure, morphology of the contractile myofilaments after LVAD support for 215 ± 143 days, is identical in LV, RV and IVS, allowing for monitoring of the possible occurrence of LV reverse remodeling by RV biopsies.

Introduction

The growing number of heart failure patients and the shortage of donor hearts warrant exploration of other therapeutic options for patients with end-stage heart failure. Left ventricular assist devices (LVAD's) can be used successfully as a bridge to transplantation (1-4). In selected patients, LVAD's may even offer an alternative to transplantation, with significant survival and quality of life benefits one year after implantation, compared to patients treated pharmacologically (5). This includes a favorable exercise capacity while on the device, which is comparable with that after heart transplantation (6).

Some reports suggest the use of LVAD's as a bridge to recovery of cardiac function in selected patients (7-9), although long-term results are scarce. Others question the use of a LVAD as a bridge to recovery (10,11). Alternative treatment protocols, combining mechanical support with intensive pharmacological therapy, or other modalities such as growth hormone, gene therapy and myocyte or stem cell transplantation, may hold the key to better long-term results (12,13). The appropriate monitoring of recovery, however, is a critical step in all these therapeutic options.

Most studies of reversal of myocyte dysfunction have only dealt with left ventricular tissue. Recently one study, looking at LV and RV myocyte size, SERCA 2a content and force-frequency relations of isolated superfused trabecula, suggested differential left and right ventricular reverse remodeling after LVAD support. This might suggest that reduction of mechanical load is a primary factor underlying reverse remodeling (14).

We previously reported the persistence of severe structural damage of the contractile proteins in left ventricular myocytes after a long duration of LVAD support, using immunohistochemistry (15). We wondered whether this technique might be used to monitor recovery in LVAD patients on a prospective base, by inspecting right ventricular biopsies obtained via the usual jugular vein approach. Obviously, this would only be feasible if the findings in the right ventricular biopsies would coincide with those in the left ventricular biopsies.

Therefore, the aim of this study has been to compare the state of the contractile myofilaments in cardiomyocytes using immunohistochemical analysis of myocardial biopsies taken from the left ventricular free wall (LV), the interventricular septum (IVS) and the right ventricular free wall (RV) after LVAD support, at the time of heart transplantation (HTx). In addition, small biopsies taken with a diagnostic bioptome were compared with large transmural

biopsies.

Methods

Patient population

This study included 13 patients (12 males, 1 female) with refractory end-stage heart failure treated with a pneumatic LVAD (HeartMate, Thoratec, Pleasanton, California) as a bridge to transplantation (Table 1). Six patients had dilated cardiomyopathy (DCM) and 7 ischemic heart disease (IHD). One patient died due to recurrent cerebral embolism, the other 12 patients were successfully transplanted. Mean duration of LVAD support was 215 ± 143 days (range 71-455). While on the LVAD all patients were in NYHA functional class I and showed good exercise performance as reported before (6). After the initial postoperative period, all cardiac medication had been stopped, except for low-dose aspirin. Written informed consent was obtained from all patients.

Table 1. Characteristics of LVAD patients

Male	12
Female	1
Age (y)	34 ± 13 (range 18-55)
DCM / IHD	6 / 7
LVEF (%)	14 ± 5
MAP (mmHg)	64 ± 13
IABP/other support	6
Duration of LVAD support (days)	215 ± 143

Immunohistochemical analysis

Large transmural myocardial biopsies after LVAD support, at the time of heart explantation were taken from the LV, IVS and RV. All biopsies were directly fixed in buffered formalin, embedded in paraffin and routinely processed on 5 μm sections. Standard immuno-histochemical analysis was performed using a three step avidin- biotin peroxidase reaction. In brief, sections were deparaffinised , rehydrated and endogenous peroxidase was blocked using a solution of methanol and hydrogenperoxide. Primary antibodies were applied to the sections for 1 hr and subsequently demonstrated using a biotinylated horse IgG anti mouse IgG and HRP labeled streptavidin. Immunovisualisation was performed using DAB reagent and counterstaining with Mayer hematoxilin. Sections were dehydrated, cleared and embedded in Pertex. Primary monoclonal antibodies used in this study were: anti sarcomere actin

(Sigma, Zwijndrecht, the Netherlands), myosin slow (Sigma), tropomyosin (Sigma), troponin T (Sigma) and troponin C (Novocastra, Newcastle upon Tyne, UK). Antigen retrieval by boiling sections in citrate buffered solution for 15 min was performed to the sections for all antibodies except myosin. Sections for myosin incubation were proteolytically digested with pepsin for 15 min.

All slides contained large transmural biopsies and were examined in a blinded fashion by three investigators. In these biopsies all longitudinally arranged cardiomyocytes were analyzed. In case of ischemic heart disease only surviving myocytes were studied; the necrotic area was not taken into consideration. A grading system was used, as reported before (15):

1. Almost all myocytes show a distorted architecture with absence of cross-striation.
2. Some normal myocytes with cross-striation are present; most of the myocytes, however, are abnormal.
3. Normal myocytes with cross-striation along with myocytes without cross-striation or myocytes without antigen staining over the full length of the contractile filament.
4. Some myocytes with distorted architecture; most of the myocytes however are normal.
5. Almost all myocytes show normal architecture and cross-striation.

To test for inter- and intra-observer variability, kappa value of reliability was determined.
Furthermore, we investigated the potential damage of a biopsy forceps on the immunohistochemical morphology of the contractile proteins, and if the histologic findings of the myofilaments in these small biopsies were representative for larger biopsies. For this purpose, in 5 patients additional biopsies of the right ventricular side of the IVS were taken with a Caves bioptome directly after explantation of the heart and compared to the corresponding large IVS biopsies. The immunohistochemical protocol for these biopsies was the same as for the larger biopsies.

Hemodynamic measurements

To be informed about the left and right ventricular filling pressures, we performed Swan-Ganz catheterisation in all patients right before LVAD implantation and 6-8 weeks after implantation, when organ function had recovered and the patients were fully rehabilitated. The measurements after

LVAD implantation were performed with the device operated in the "auto" mode.

Statistical analysis

All data are presented as the mean ± SD. The grading of the immunohistochemical data on the contractile proteins in the 3 different biopsies were compared by two-tailed paired Student-t test and Friedman rank test. Comparison between DCM and IHD was made by an unpaired Student-t test. Inter-and intra-observer variability was tested using Kappa analysis. All data were calculated with SPSS 8.0 for windows. A p value < 0.05 was considered significant.

Results

Grading of the contractile proteins

The gradings of the contractile proteins for LV, IVS and RV, are shown in figure 1. For actin they amounted to: 2.4 ± 0.9, 2.0 ± 1.1 and 3.0 ± 1.0, respectively, and for myosin: 4.2 ± 1.2, 4.0 ± 1.3 and 4.3 ± 1.0. Tropomyosin showed the following gradings: 3.5 ± 0.9, 3.0 ± 0.9 and 3.7 ± 1.0. For troponin T they were 3.2 ± 1.0, 3.1 ± 1.0 and 3.6 ± 0.9, respectively, and for troponin C: 3.2 ± 1.0, 2.9 ± 0.9 and 3.5 ± 0.9. There were no significant differences between the gradings of the LV, IVS and RV.

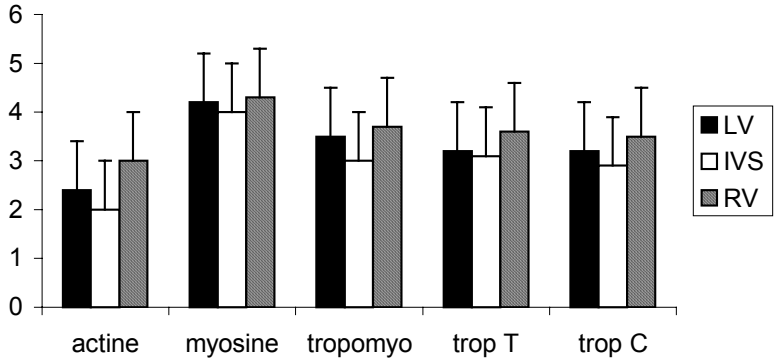

Figure 1: Grading of the contractile proteins in myocytes of LV, RV and IVS after LVAD support showing identical findings throughout the heart. For explanation of the grading system see text.

Typical examples for actin and myosin are shown in figure 2 and 3. Comparison between patients with DCM and IHD showed that the grading of the contractile proteins did not differ between these groups (Fig.4). The inter- and intra-observer kappa values were 0.57 and 0.63, respectively, indicating fair agreement.

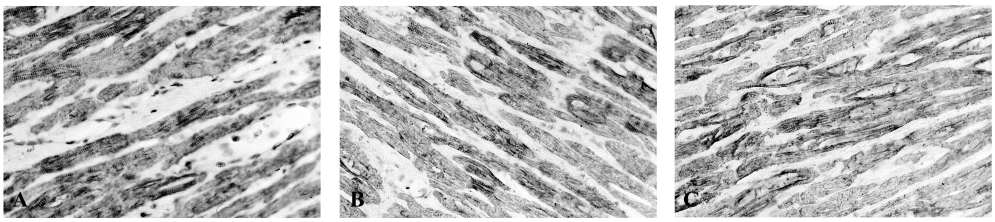

Figure 2: An example of actin staining in the LV (A), RV (B), and IVS (C) (all grade 2) (original magnification 100x).

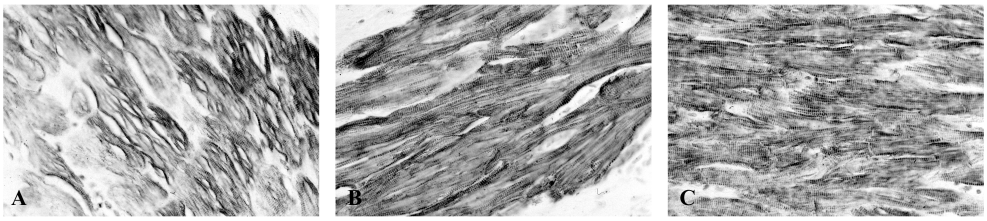

Figure 3: An example of myosin staining in the LV (A) (grade 4), RV (B) (grade 5) and IVS (C) (grade 4) (original magnification 100x).

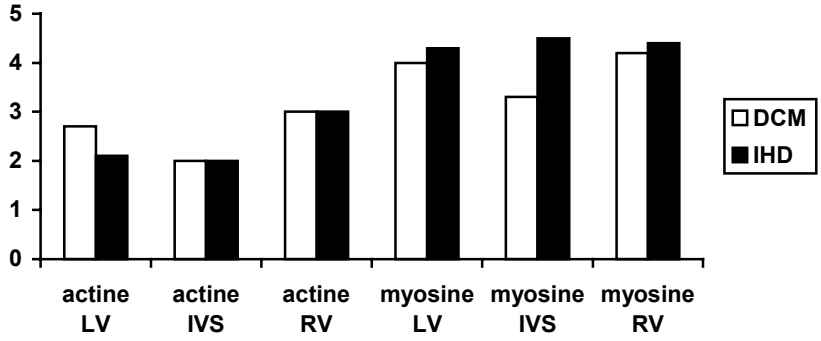

Figure 4: Comparison of the grading of the contractile proteins between patients with DCM and IHD showing no difference.

Analysis of the 5 small biopsies from the IVS, taken with the Caves bioptome, showed that also in these biopsies the contractile myofilaments could be well assessed. No mechanical damage of the myofilaments, by the bioptome, was visible. Comparison with the corresponding large
biopsies showed that the morphology of the contractile proteins was identical in the small and the large biopsies (Fig. 5).

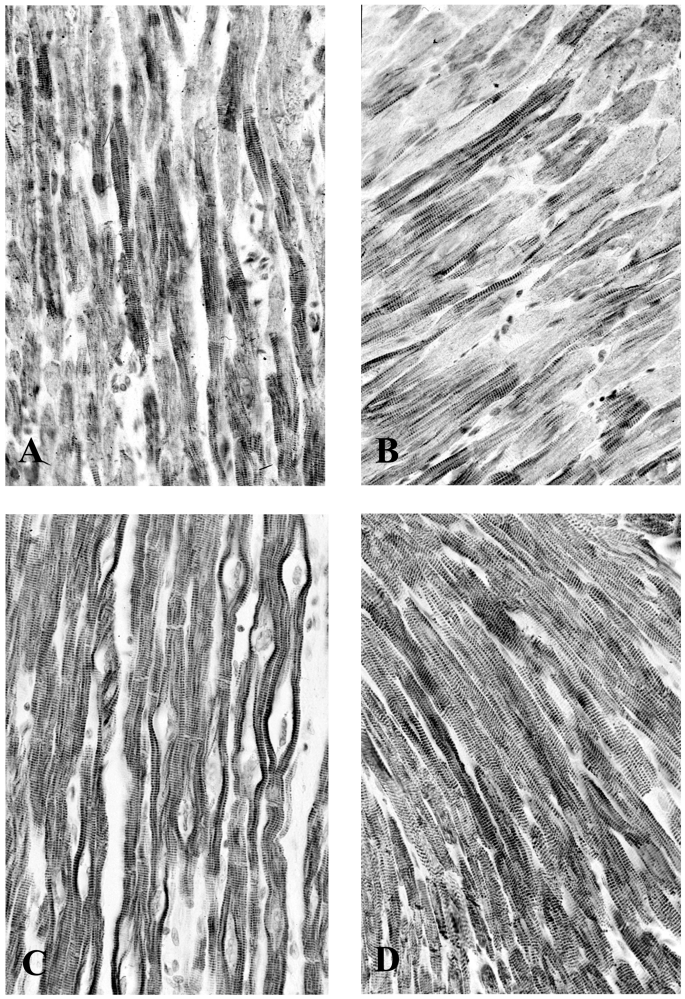

Figure 5: Comparison of small biopsies taken with a diagnostic bioptome (A and C) and large transmural biopsies (B and D) from the same heart. A and B actine staining (grade 2); C and D myosin staining (grade 5). (original magnification 100 x)

Hemodynamic data

Before LVAD implantation all patients demonstrated severely compromised hemodynamics (Fig. 6). Mean pulmonary capillary wedge pressure was 25 ± 6 mm Hg, mean arterial pressure 64 ± 13 mm Hg with a mean cardiac output of 3.5 ± 1.1 L/min. Right atrial pressure was also increased: 11 ± 6 mm Hg (range 1-23), with normal pulmonary vascular resistance, 170 ± 90 dynes.sec.cm $^{-5}$. In this patient group 8 out of the 13 patients had right atrial pressures higher than 10 mm Hg at the time of LVAD implantation, suggesting RV failure. Directly after LVAD implantation, 5 out of 13 patients had symptomatic RV failure resulting in inadequate filling and low output of the device, for which treatment with positive inotropic and vasoactive agents was instituted successfully. There appeared to be no relation between the grading of the contractile proteins after LVAD support in individual patients and the left and right ventricular filling pressures before LVAD implantation, nor with clinical symptomatic right ventricular failure shortly after LVAD implantation.

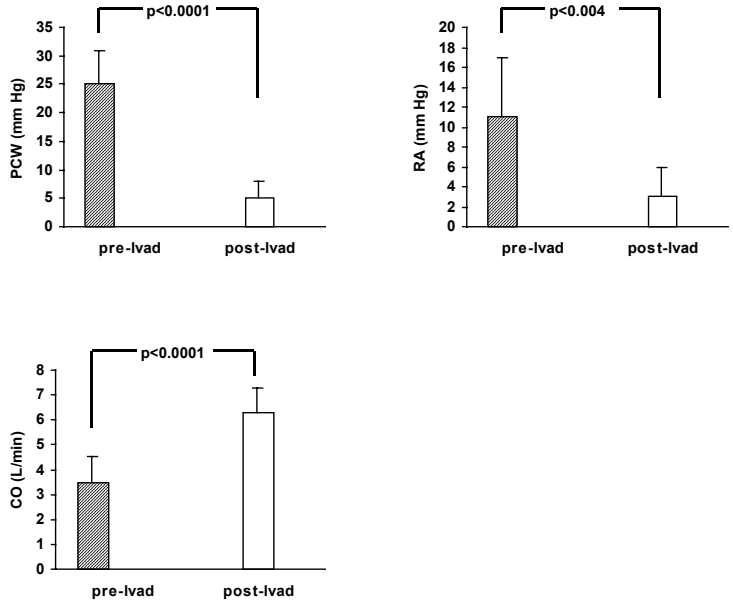

Figure 6: Hemodynamic data at the time of LVAD implantation and after implantation when the patients were fully rehabilitated. Results are presented as mean ± SD. PCW = pulmonary capillary wedge pressure, RA = right atrial pressure, CO = cardiac output.

After the direct postoperative period, when all patients were fully mobilized, no clinical RV failure was apparent. Hemodynamic measurements at that time showed low filling pressures of both right and left ventricles (Fig. 6). Right atrial pressure was 3 ± 3 mm Hg ($p < 0.004$ vs. pre-LVAD), pulmonary capillary wedge pressure 5 ± 3 mm Hg ($p < 0.0001$ vs. pre-LVAD), pulmonary artery pressure 19/8, mean 12 mm Hg. Cardiac output was 6.3 ± 1.1 L/min ($p < 0.0001$ vs. pre-LVAD). This means that the LVAD was functioning adequately and emptied the LV completely. Furthermore, RV function was completely restored.

Discussion

The main finding in this study of relatively young patients supported with a LVAD for 215 ± 143 days is the identical morphology of the contractile proteins in LV, IVS and RV, despite the fact that only the LV is supported. Furthermore, biopsies taken with a diagnostic bioptome show a good quality of histologic findings, similar to the much larger transmural biopsies. The staining pattern of the contractile proteins in this study is in agreement with our previous study (15): an intermediate grading for the thin contractile proteins, with actin showing the lowest grading and a higher score for the thick contractile protein myosin. In this patient group we could not demonstrate a relationship between increased RV filling pressures before LVAD implantation, or symptomatic RV failure directly after implantation, and the grading of the contractile proteins of the RV and IVS after long-term assist. This could be explained by the long duration of LVAD support in this patient group (215 ± 143 days). The similar morphologic structure of the contractile myofilaments of LV and RV in this study suggests that cardiac recovery after LVAD support, if it occurs, appears to be the same, throughout the whole heart. Our findings seem to be in contrast with those of Barbone et al (14), who demonstrate differential left and right ventricular reverse remodeling after LVAD implantation, suggesting that reduction of mechanical load is a primary factor underlying this process. In that study reverse remodeling was studied by looking at LV and RV myocyte size, SERCA 2a content and force-frequency relations of isolated superfused trabecula. An important difference with our study is that in the study of Barbone et al. (14) the LVAD support time was considerably shorter (77 ± 71 vs. 215 ± 143 days) and right atrial pressure during LVAD support much higher (11 ± 6 vs. 3 ± 3 mm Hg) than in our study, suggesting that right ventricular function had not completely been recovered. In our patient group 8 out of 13 patients had signs of right ventricular failure before LVAD implantation. Five

out of the 13 patients had symptomatic right ventricular failure during and directly after LVAD implantation. Right ventricular failure is one of the main complications in LVAD implantation, occurring in about one third of the patients (3). The hemodynamic data in our study, after LVAD implantation, suggest that RV function had recovered sufficiently. It is in this situation that we demonstrate identical morphology of the contractile proteins in LV, IVS and RV. Regarding the data of Barbone et al. (14) the differences of the results between RV and LV in that study were small. Furthermore, in the expression of SERCA 2a and the force-frequency relation, they used normalized ratio's to demonstrate a difference between RV and LV results, ignoring the pre-existing absolute difference between RV and LV. Therefore, we think there is still no definite answer to the question of whether the process of reverse remodeling is mainly caused by hemodynamic unloading or by the normalized biochemical milieu. LVAD support results in improvement of neurohormonal activation (16-18) which might be responsible for reverse remodeling throughout the whole heart. On the other hand hemodynamic unloading of the LV may also influence RV function. Both beneficial and detrimental effects have been reported (19). The decreased LV volume during LVAD support may alter the septal and LV contributions to RV contraction (20). The main effect of LVAD support, however, is improvement of RV function, by decreasing RV afterload (19,21).
The observation of identical morphology of the contractile proteins in LV, IVS and RV in this study was done in patients who were not weaned from the device; all were transplanted, except for one patient who died before transplantation. Therefore, from this study we can not definitely conclude that the results would have been the same in patients demonstrating enough reverse remodeling to allow for LVAD removal. We merely demonstrated identical morphology of the contractile filaments in LV and RV after a rather long period of LVAD support, in the absence of persistent RV failure. In the present study, we have not examined if the same holds true in case of persistent RV failure or after a shorter period of LVAD support; therefore, this remains a matter of speculation.

Conclusion

This study demonstrates that the morphology of the contractile myofilaments after support with a LVAD, in the absence of RV failure, is identical in LV, IVS and RV. Furthermore, small biopsies taken with a diagnostic bioptome are representative for the histologic findings of the myofilaments in larger biopsies and do not show damage by the bioptome. Therefore, with regard to the

morphology of the contractile myofilaments, prospective biopsies of the RV may be a way of monitoring reverse remodeling of the LV during LVAD support.

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Chapter 7.

General discussion

The aim of this thesis was to study the role of left ventricular mechanical support in end-stage heart failure, particularly if this device can be used as an alternative to heart transplantation, or as a treatment option leading to recovery of cardiac function.

The impact of heart failure in medicine is expanding. The annual increase in prevalence suggests a growing epidemic, mainly caused by aging of the population (1). On the other hand, this growing epidemic, seen in the perspective of the greatly improved management of virtually all forms of heart disease that lead to heart failure, seems to be a paradox (2). It has to be remembered, though, that many forms of heart disease that can now be successfully treated, are not really cured. For example, premature death from hypertension and acute myocardial infarction are prevented, allowing for chronic adaptive changes, eventually leading to heart failure (2).

The pathophysiology of heart failure is very complex and no single conceptual paradigm has withstood the test of time (3). Initially the "cardiorenal model" was applied in which heart failure was viewed as a problem of excessive salt and water retention that was caused by abnormalities of renal blood flow (4). Later, the "hemodynamic model" developed, centering around reduced cardiac output and excessive peripheral vasoconstriction (4). Subsequently, the "neurohormonal model" was introduced in which heart failure progresses as a result of the overexpression of biologically active molecules that are capable of exerting toxic effects on the heart and the circulation, contributing to disease progression independently of the hemodynamic status of the patient (3). All these models laid the foundation of important therapeutic strategies, but especially the neurohormonal model greatly improved the conceptual thinking of heart failure. Furthermore, it contributed to the introduction of ACE-inhibitors and later ß-blockers leading to a major improvement of prognosis in heart failure (5-11). Later, also inflammatory cytokines, endothelin and oxidative stress were introduced in this model (12). All these pathways, as well as increased wall stress, can give rise to apoptotic cell death, progressive remodeling, and altered gene expression, resulting in progression of myocardial dysfunction (13).

But even this complex neurohormonal model fails to completely explain disease progression in heart failure (3). This can be inferred from the Kaplan-Meier survival curves of ACE-inhibitor and ß-blocker trials. The curves from treated and non-treated patients clearly begin to diverge at 6 months, to become parallel again between 18 and 48 months. This suggests that there may

be an attenuation or loss of effectiveness of neurohormonal antagonism as heart failure progresses. This may be explained by incomplete inhibition of the renin-angiotensin or the adrenergic systems in heart failure, or by alternative metabolic pathways for neurohormones that are not antagonized by the current medications (3). However, the addition of an angiotensin receptor blocker to an ACE inhibitor to block remaining angiotensin II levels completely, as was done in the Val-HeFT study (14), surprisingly, did not result in a further decrease in mortality. Patients on a combination of ACE-inhibitor and ß-blocker who received additional angiotensin receptor blocker, did even worse on the end points mortality and morbidity (14). Other investigations on endothelin receptor antagonists and TNFα binding proteins have not been very encouraging either (15). Therefore, the assumption that the addition of other antagonists will be effective cannot be taken for granted and the recent results of the above mentioned trials may suggest that the string of successes with neurohormonal antagonists may have run its course (15). Research on the optimal dose and combination of neurohormonal antagonists has to continue but one has to bear in mind that it will be increasingly difficult to attain additional survival benefit on top of that already achieved with the present antagonists.

Fundamental aspects of end-stage heart failure

The treatment of patients with end-stage heart failure with a LVAD entails the unique opportunity to obtain myocardial biopsies at two points in time: at the time of LVAD implantation, when the hemodynamic situation is severely compromised, and after a period of unloading, at the time of heart transplantation. In that same period blood samples can be taken to monitor "peripheral" effects of neurohormonal activation during unloading of the left ventricle. Maybe this model can help contribute to the understanding of the complex pathophysiology of heart failure in man. Chapter 4 and 5 show some results. Chapter 4 focuses on the morphology of the contractile proteins in cardiomyocytes. For this study we used immunohistochemistry, which enables the examination of large biopsies, in contrast to electron microscopy in which only very small biopsies can be studied. Considering the widespread variation in myocyte histology which we observed, with areas showing an almost normal structure adjacent to areas with severe morphological disorganization, the use of electron microscopy may underestimate the severity of the morphological alterations. In the biopsies of the patients with severe heart failure, at the time of LVAD implantation, we found an impressive disturbance of the normal

staining pattern of titin, actin and its related proteins tropomyosin, troponin C and T. Myosin in contrast, showed normal morphology. The disturbance of the staining pattern of the thin contractile proteins was more impressive than would be expected from the routine HE staining. Potentially, this technique could be used to make myocardial biopsies in heart failure patients more informative.
The abnormal staining pattern of the thin contractile filaments with specific monoclonal antibodies suggests that the expression of the contractile proteins is disturbed in the failing human heart (16), and that heart failure will result in loss of contractile elements (17). This has been confirmed using electron microscopy, which shows large cellular areas containing only mitochondria, ribosomes and glycogen (17). It seems obvious that these changes of the contractile proteins result in a decrease of myocyte contractile function. The regulators of these contractile protein alterations are not known but are probably numerous. Cleavage of membrane-associated actin has been reported in human neutrophils during apoptosis (18). Therefore, it can be speculated that activation of the apoptotic cascade in heart failure may directly result in damage to actin and its related contractile proteins in the cardiomyocytes. Apoptotic cardiomyocyte death, on the other hand is limited by the presence of FLICE Inhibitory Protein (FLIP) which inhibits death domain related apoptosis. This protein is abundantly present in the heart, as is shown in chapter 5. After LVAD support the apoptotic process is less activated, probably through decreased neurohormonal stimulation, and by a reduction in myocardial stretch as a result of left ventricular unloading. This may result in partial restoration of thin contractile protein morphology. Pharmacological intervention to improve this process appears an interesting area of future research.
Chapter 5 deals with apoptotic cell death and its regulators. Apoptosis is thought to play a role in ventricular remodeling (19), although controversy exists about its importance (20). Despite many studies performed on this subject it is still not clear if progressive myocyte loss precedes and causes progressive cardiac dilatation, or whether, alternatively, progressively abnormal myocyte function leads to end-stage cardiac dilatation, which then triggers cell loss (21). Progressive myocyte loss may either constitute an adaptation to preserve function of less-affected cells, or it may be a maladaptation resulting in further dilatation (21). The discussion about the importance of apoptosis in the progression of heart failure is further complicated by the limitations of the techniques used for detecting apoptosis. The frequently used TUNEL method is a very sensitive but not a very specific technique, since oncotic and autolytic cells, as well as living cells undergoing DNA repair or RNA synthesis may also be labeled (20). In our study we tried to substantiate the incidence of apoptosis

by also evaluating the caspase-3 expression, which constitutes the final common step in the apoptotic cascade. Our observations indicate that the very high percentage of TUNEL positive cardiomyocytes which we saw in two patients, was not caused by apoptosis, because it was not accompanied by high caspase-3 expression.

In patients with end-stage heart failure we noted a low incidence of apoptotic cardiomyocytes (0.8 %) combined with low caspase-3 expression. Mediators of apoptosis and their receptors were all present. Considering the ample presence of conditions for apoptosis, the low incidence of cardiomyocyte cell death suggests that to a certain extent these cells are protected from apoptotic cell death. In cardiomyocytes the apoptosis-inhibitor FLIP was abundantly present, and comparable to the level in non-failing hearts. In endocardial and endothelial cells, showing a high incidence of TUNEL expression, FLIP expression was much lower. Therefore, we think that FLIP is an important inhibitor of cardiomyocyte apoptotic cell death in heart failure. Other inhibitors of apoptosis, like NF-κB may also influence the delicate balance between cell death and survival in heart failure, because in animal studies it has been suggested that the cytotoxic effect of TNFα is dependent on the blocking of NF-κB (22). This transcription factor has been recently investigated in patients undergoing LVAD implantation showing a significant decrease in the number of NF-κB positive cardiomyocyte nuclei after LVAD support (23).

To return to the conundrum of end-stage heart failure (21), the results in the studies of the LVAD patients show widespread alterations in morphology of the thin contractile proteins and titin (chapter 4) and only a low incidence of cardiomyocyte apoptosis, despite ubiquitous mediators (chapter 5). These findings may suggest that deterioration of myocyte function contributes more to the progression of heart failure than reduction in myocyte number. The predominance of apoptotic cardiomyocytes and TNFα in the subendocardial region of the heart, a preferred site for ischemia in the myocardium, may suggest that secondary ischemia, caused by increased wall tension as a consequence of dilatation of the heart, may be central in cardiomyocyte cell death. This ischemia activates death receptor and mitochondrial pathways of apoptosis, as well as regulatory proteins like FLIP and NF-κB. In the end the balance in the pro-and anti-apoptotic factors determines the extent of apoptosis. Progressive cardiomyocyte cell death in itself contributes to the decline of the contractile function and subsequent cardiac dilatation. This adds to the vicious circle.

Therapeutic options in end-stage heart failure

Complete antagonism of presently recognized neurohormonal activation does apparently not result in complete abrogation of disease progression. Therefore, other options for end-stage HF are warranted. Heart transplantation is the only treatment option that provides both a significant survival advantage and a substantial improvement in functional class (24). Therefore, it still deserves an important place in the treatment of patients with end-stage heart failure, although the number of donor hearts decreases year by year (25). In the Netherlands it is thought that this may be partly attributed to insufficient donor recognition and logistical problems in the donor hospitals. Considering the enormous efforts and the financial investments put into it, it appears very unlikely that this should be the only cause. Most discussions about the potential number of donors available in the Netherlands are confused by the entanglement of potential organ and tissue donors. In contrast to cornea and skin donation, heart donation is only possible in patients with severe neurological damage eventually resulting in brain-death. The treatment of these patients has been optimized over the years, aimed at preventing brain death. Furthermore, impressive safety precautions have been introduced in the car industry, like safety belts and airbags, combined with speed-limits, evidently reducing the number of casualties. Therefore, it is likely that the reduction in the number of donor hearts allocated is the result of a real reduction of available donor organs. It is evident that every effort has to be made to increase the number of the available donor organs and to optimize their usage, but it is realistic to assume that in this way only a minority of heart failure patients can be treated, like a drop in the ocean. That is why alternative treatment options have to be explored.

Mechanical support in end-stage heart failure

Mechanical support of the failing heart is almost as old as heart transplantation itself (26), but, only recent improvements of the devices have turned this into a regular treatment modality (27, 28). Implantable left ventricular assist devices are now mainly used as a bridge to transplantation, but the growing experience with these devices suggests that they may also be used as an alternative to heart transplantation (29).

In 1993 we started the implantation of LVAD's in the Heart Lung Center Utrecht, as part of our heart transplantation program. The results of this bridge to transplantation program are presented in chapter 2. The results are very encouraging with a long-term survival of 79 %. This is especially promising considering the very poor condition of the patients at the time of LVAD

implantation. Infections do remain an important problem with this technology, which is partly attributable to the external driveline. Frequent mechanical problems limit the longevity of the device used in this study (HeartMate), as was illustrated by the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) study group, which showed a probability of device failure of 35 % at 24 months (30). Nevertheless, this study is the first to show that mechanical support with a LVAD in selected patients with advanced heart failure resulted in substantial improvement in survival and may be an acceptable alternative to transplantation (30).

It is also important to know the improvement in exercise capacity after implantation of a LVAD, as a parameter of the quality of life with mechanical support. Therefore, we performed exercise testing with respiratory gas analysis at several points in time after LVAD implantation. The results, shown in chapter 3, confirm that exercise capacity increases over time and, at 12 weeks after LVAD implantation, is comparable to that at 12 weeks and one year after heart transplantation. With regard to exercise capacity a LVAD is fully compatible with activities of normal daily life, apart from the inconvenience of the console or the battery pack.

As mentioned above, mechanical problems with the HeartMate are an important item, limiting the large scale introduction of this device as an alternative to heart transplantation. It is hoped and expected that the newer devices using rotary axial flow pumps and magnetically suspended centrifugal pumps, like the HeartMate II and III and the Jarvik 2000, will offer greater durability with less wear and tear. A complicating factor of those devices is the lack of the textured blood-contacting surface of the HeartMate, which potentially increases the thrombo-embolic risk and requires anticoagulation. The same holds for the new totally implantable artificial heart (Abiocor), which was recently implanted in seven patients of whom already five have died after several weeks on the device. When the problems of durability, infections and thrombo-embolism with mechanical support in heart failure have been solved, we are well on the way to the use of mechanical support as a realistic alternative to heart transplantation. LVAD's may solve the problem of donor scarcity, and eliminate the adverse effects of immunosuppression. In addition, unlike transplantation, implantation of the device can be planned at an optimal time in the patient's course. What the effect of this policy will be on costs has to be analyzed and society has to decide if it can afford this kind of therapy (31).

Bridge to recovery

It has been suggested to use mechanical support as a bridge to recovery of cardiomyocyte function in severe heart failure, through a process called "reverse remodeling". This seems a plausible thought at first glance, considering the impressive hemodynamic improvement of the patients after implantation of a LVAD. Anecdotal reports of patients weaned from the assist device are available (32-34), although long term results are often lacking. Other reports question the use of a LVAD as a bridge to recovery (35,36), except for potential curable diseases like myocarditis and peripartum cardiomyopathy. Chapter 4 and 5 also deal with this subject, showing a persistence of cardiomyocyte hypertrophy after LVAD support, although cardiomyocyte cross sectional area decreased 36 %. The immunohistochemical staining pattern of the contractile filaments improved, but overall widespread areas of abnormal staining pattern persisted. After LVAD support the staining pattern within each cardiomyocyte appeared to be more homogeneous. Between different cardiomyocytes, however, the variability in staining pattern persisted. This resulted sometimes in the alternation of myocytes staining normally, with myocytes showing abnormal or absent staining, in the same contractile fiber. This suggests that recovery for the individual cardiomyocyt is an all-or-nothing phenomenon, exhibiting either complete recovery or complete disappearance of contractile filaments. Furthermore, after support most patients showed an increase of interstitial tissue, probably fibrosis.

An increase of fibrosis together with the deprivation of contractile proteins from myocytes will undoubtedly have a negative impact on overall cardiac contractility and electrical conduction, creating conditions for re-entry, which will increase the risk for lethal arrhythmias (37). Furthermore, also diastolic function may be affected adversely.

Besides the persistence of both cardiomyocyte hypertrophy and severe structural myocyte damage, the incidence of apoptotic cardiomyocytes in the patients with severe heart failure decreased after unloading (0.8 % of cardiomyocytes TUNEL positive before LVAD, 0.1 % positive after support; $p < 0.01$). As mentioned before, this may be attributed to the decrease of wall tension due to unloading of the left ventricle, and the decrease of neurohormonal activation after LVAD support. The apoptotic mediators and their receptors, however, all remained present, even if one assumes that the level of expression may have decreased a little. Immunohistochemistry may not be sensitive enough to detect minor differences. Therefore, the substrate for cardiomyocyte apoptotic cell death remains existent, despite the improved hemodynamics of the patients.

These data suggest that after LVAD support some recovery at the cellular level in the failing heart is taking place, but that we are still far from "treating" heart failure with an assist device. The idea that heart function can recover after unloading the left ventricle is based on the very old concept that rest is beneficial for patients with heart failure (38). Heart failure therapy, however, has changed dramatically over the years. The clinical picture of heart failure, for example, is dominated by impaired exercise performance, partly caused by skeletal muscle abnormalities (39). Many studies have demonstrated a beneficial effect of exercise training in heart failure patients (40) with improvement of skeletal muscle metabolism (41-44). This underscores that, in contrast to the previous mentioned old ideas, complete rest is not wholesome for disturbances of skeletal muscle metabolism in heart failure. Most probably, this applies to cardiac muscle as well: the mere unloading does not result in long-term improvement of cardiac function, except in those patients who received a LVAD for potentially curable diseases like myocarditis. Alternative treatment protocols, combining mechanical support with pharmacological therapy, growth hormone, gene therapy, or myocyte or stem cell transplantation, may hold the key to better long-term results (45,46). In these strategies monitoring of recovery is essential. Staining of the contractile myofilaments in myocardial biopsies by immunohistochemistry, as shown in chapter 4 may help to demonstrate sufficient recovery of cardiomyocyte histology, to allow for device removal. As most endomyocardial biopsies are taken from the right ventricle by a jugular access, using modified Caves bioptomes, it is essential to demonstrate that the histology of right ventricular biopsies is representative of the left ventricle as well.

In chapter 6, it is demonstrated that in patients showing only partial recovery, this is actually the case. Therefore, we postulate that in this patient group, which has gone through a long period of LVAD support, right ventricular biopsies can be used to monitor recovery in the left ventricle, provided there is no right ventricular failure. This will require sequential biopsies to monitor the process of recovery over time.

The concept, of using mechanical support of the failing left ventricle for temporary circulatory assistance, meanwhile taking the opportunity to provide intensive therapy aimed at reversal of remodeling, seems appealing. It will require elaborate research of the process of reverse remodeling and of the mediators amenable as targets for intervention. Furthermore, the optimal mode of monitoring recovery has to be determined and above all, long-term recovery has to be confirmed.

Mechanical support in end-stage heart failure has rapidly become very important. We are now at the brink of a new era in the treatment of patients with severe heart failure. Mechanical support has already established a place as bridge to heart transplantation; it is to be expected that very soon it can also be applied as an alternative to heart transplantation in selected patients. Hopefully, in the future it will be used as a bridge to recovery of cardiac function, in combination with other therapeutics aimed at reverse remodeling. Furthermore, mechanical support offers a unique opportunity to extend basic research on severe human heart failure, by the availability of myocardial tissue at several points in time, before and after unloading of the heart.

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Chapter 8

Summary

The aim of this thesis was to study the role of left ventricular mechanical support in end-stage heart failure, particularly if this device can be used as an alternative to heart transplantation, or as a treatment option leading to recovery of cardiac function.

Chapter 1 is a general introduction that provides an overview of some clinical aspects of heart failure with emphasis on the role of heart transplantation and mechanical circulatory support in end-stage heart failure. Furthermore, the complex pathophysiology of heart failure is discussed, focussing on the process of remodeling and the importance of apoptosis and cytokines, like tumor necrosis factor alpha.

Chapter 2 reports the results of the use of the HeartMate left ventricular assist device as a bridge to transplantation in the University Medical Center Utrecht. These results are very encouraging. Despite the very poor condition of the patients at the time of implantation of the left ventricular assist device (LVAD), 33 of the 38 patients (87%) who underwent LVAD implantation, survived the perioperative (30 day) period. In the early postoperative period right ventricular failure was an important problem, resulting in the death of two patients in this group. After this period the main problems are infections and mechanical problems, limiting the long-term use of this device. Twenty-six patients (76%) underwent heart transplantation after a duration of LVAD support of 206 ± 129 days.

Chapter 3 deals with the exercise performance of patients while on an LVAD. Exercise capacity increases over time, and at 12 weeks after LVAD implantation, peak VO_2 is comparable to that at 12 weeks and one year after heart transplantation (peak VO_2: 22.8 ± 5.3, 24.6 ± 3.3 and 26.2 ± 3.8 ml/kg/min, respectively). Therefore, with regard to exercise capacity an LVAD is fully compatible with activities of normal daily life, apart from the inconvenience of the console or the battery pack, and holds a promise as a potential alternative for heart transplantation.

Chapter 4 describes the histologic features of cardiomyocytes, with emphasis on the contractile proteins, in patients with end-stage heart failure undergoing LVAD implantation. In 14 patients myocardial biopsies taken at the time of LVAD implantation and after explantation, at the time of heart transplantation were compared using routine hematoxylin-eosin staining and immunohistochemistry with antibodies against the contractile proteins. At the time of LVAD implantation widespread distortion of the staining pattern of the thin contractile proteins and titin was seen. In contrast, myosin staining pattern was preserved. After LVAD support, during a period of 213 ± 135 days, the thin contractile proteins and titin showed improvement, but no normalization.

Cardiomyocyte cross-sectional area decreased 36% after LVAD support, but also did not normalize. The persistence of severe structural myocyte damage in this study group does not support complete recovery of myocyte histologic features after a period of unloading of the heart by LVAD support.

Chapter 5 focuses on cardiomyocyte cell death in patients with end-stage heart failure, before and after LVAD support. In 17 patients, myocardial biopsies at the time of LVAD implantation and after explantation, at the time of heart transplantation, were examined by TUNEL reaction to detect cardiomyocyte apoptosis. Furthermore, immunohistochemistry was used to be informed about regulators of apoptotic cell death. Before LVAD support apoptosis incidence was low (0.8% of cardiomyocytes), further decreasing after support (0.1% of cardiomyocytes). The apoptosis inducing mediators and their receptors were all present before and after LVAD support. The apoptosis inhibitory protein FLIP was widely expressed in cardiomyocytes before and after LVAD support, with m-RNA levels comparable to that in normal controls. This may suggest that FLIP is an important factor in the prevention of cardiomyocyte apoptosis.

Chapter 6 reports the comparison of the morphology of the contractile proteins in biopsies of the left- and right ventricle and the interventricular septum to investigate if right ventricular biopsies might be used to monitor reverse remodeling in the left ventricle. This was done because right ventricular biopsies can be easily obtained by a jugular vein approach. In 13 patients with end-stage heart failure, myocardial biopsies after LVAD support, at the time of heart transplantation were compared, using immunohistochemistry with antibodies against the contractile proteins. The staining pattern of these biopsies from the left- and right ventricle and from the right ventricular side of the interventricular septum was identical. Furthermore, small biopsies taken with a diagnostic bioptome were representative for the histologic findings of the myofilaments in larger biopsies. With regard to the morphology of the contractile proteins, prospective biopsies of the right ventricle may be a way of monitoring reverse remodeling of the left ventricle during LVAD support.

Chapter 7 is a general discussion of the results of the investigations included in this thesis. Mechanical circulatory support has already established an important place as bridge to transplantation. Patients with severe end-stage heart failure, facing imminent death, can be successfully kept alive with an LVAD, until a donor heart comes available. This does not solve, however, the shortage of donor organs. Probably, mechanical circulatory support can be used in the near future as an alternative to heart transplantation. In this way the

problem of the scarcity of donor organs can be overcome. The use of mechanical support as a bridge to recovery of cardiac function in end-stage heart failure is presently highly experimental and unpredictable. Only potentially curable diseases, like myocarditis may demonstrate enough recovery, allowing device removal. The concept, of using mechanical support of the failing left ventricle for temporary circulatory assistance, meanwhile taking the opportunity to provide intensive therapy aimed at reversal of remodeling, seems appealing. It will require elaborate research of the process of reverse remodeling and of the mediators amenable as targets for intervention. Furthermore, the optimal mode of monitoring recovery has to be determined and above all, long-term recovery has to be confirmed.

Chapter 9

Samenvatting

Dit proefschrift beschrijft de rol van mechanische ondersteuning van de linker ventrikel bij eindstadia van hartfalen, met name om te beoordelen of dit ook als alternatief voor harttransplantatie gebruikt kan worden, dan wel als behandelingsmogelijkheid om de hartfunctie langdurig te laten herstellen.

Hoofdstuk 1 is een algemene inleiding, waarin een overzicht wordt gegeven van een aantal klinische aspecten van hartfalen, toegespitst met name op harttransplantatie en mechanische ondersteuning van het hart bij eindstadia van hartfalen. Tevens wordt de complexe pathofysiologie van hartfalen besproken, in het bijzonder het proces van remodeleren en het belang van apoptose (geprogrammeerde celdood) en cytokines (regelaars van onder andere celgroei en celdood), met name tumor necrosis factor alpha.

Hoofdstuk 2 beschrijft de ervaring in het Universitair Medisch Centrum Utrecht met de HeartMate, een linker ventrikel steunhart, gebruikt als overbrugging naar harttransplantatie. De resultaten zijn zeer bemoedigend. Ondanks de zeer slechte algemene conditie waarin de patiënten zich bevonden op het moment van implantatie van het steunhart (Left Ventricular Assist Device, LVAD), overleefden 33 van de 38 patiënten (87%) de perioperatieve periode (30 dagen). In de vroeg postoperatieve periode is rechter ventrikel falen een groot probleem, hetgeen heeft geresulteerd in het overlijden van twee patiënten. Na deze periode zijn de belangrijkste complicaties infecties en mechanische problemen van het steunhart. Hierdoor wordt met name langdurig gebruik beperkt. Zesentwintig patiënten (76%) ondergingen een harttransplantatie na een periode van 206 ± 129 dagen aan het steunhart.

Hoofdstuk 3 gaat over het inspanningsvermogen van patiënten aan het steunhart. Het inspanningsvermogen neemt toe in de loop der tijd, waarbij de maximale zuurstofopname tijdens inspanning (piek VO_2), 12 weken na LVAD implantatie, overeenkomt met die van 12 weken en van een jaar na hart transplantatie (piek VO_2: 22.8 ± 5.3, 24.6 ± 3.3 and 26.2 ± 3.8 ml/kg/min, respectievelijk). Wat betreft het inspanningsvermogen is een steunhart geschikt om normale dagelijkse bezigheden te verrichten, hoewel de aandrijvingconsole of de draagbare accu's wel enig ongemak met zich mee kunnen brengen. Met betrekking tot het inspanningsvermogen kan mechanische ondersteuning dus als een bruikbaar alternatief voor harttransplantatie worden gezien.

Hoofdstuk 4 beschrijft de histologische kenmerken van de cardiomyocyten, in het bijzonder de contractiele eiwitten, bij patiënten met ernstig hartfalen, die een LVAD implantatie ondergingen. Bij 14 patiënten werden myocard biopten genomen ten tijde van de LVAD implantatie. Deze werden vergeleken met biopten genomen na explantatie, tijdens de

harttransplantatie. De biopten werden beoordeeld middels routine hematoxyline-eosine kleuring en met behulp van immunohistochemie, gebruik makend van antistoffen tegen de contractiele eiwitten. De biopten afgenomen tijdens de LVAD implantatie vertoonden uitgebreide afwijkingen van het kleuringpatroon van de dunne contractiele eiwitten en titin. Het beeld van myosine, het belangrijkste bestanddeel van de dikke contractiele eiwitten, daarentegen was vrijwel geheel normaal. Na LVAD ondersteuning, gedurende 213 ± 135 dagen toonden de dunne contractiele eiwitten en titin verbetering, echter geen normalisatie. De oppervlakte van de dwarsdoorsnede van de cardiomyocyten ten tijde van de LVAD implantatie was ruim twee maal zo groot dan bij normale cardiomyocyten. Na LVAD ondersteuning nam deze oppervlakte weliswaar 36% af, maar normaliseerde evenmin. Het persisteren van belangrijke structurele myocyt schade in deze patiënten groep pleit tegen een volledig herstel van de histologische eigenschappen van de cardiomyocyt, na een periode van LVAD ondersteuning

Hoofdstuk 5 houdt zich bezig met dood van cardiomyocyten in patiënten met een eindstadium hartfalen, voor en na LVAD ondersteuning. Bij 17 patiënten werden myocard biopten voor en na LVAD implantatie onderzocht op het voorkomen van apoptotische cardiomyocyten, middels de TUNEL reactie. Tevens werd met behulp van immunohistochemie geprobeerd een indruk te krijgen over de regulatoren van deze apoptose. De apoptose incidentie voor LVAD ondersteuning was laag (0.8% van de cardiomyocyten), en nam nog verder af na LVAD ondersteuning (0.1% van de cardiomyocyten). Apoptose inducerende mediatoren en hun specifieke receptoren waren zowel voor als na LVAD ondersteuning aanwezig. Het apoptose remmende eiwit FLIP was in ruime mate aanwezig in de cardiomyocyten, zowel voor als na LVAD ondersteuning, waarbij m-RNA niveaus vergelijkbaar waren met die van de normale controle groep. Dit suggereert dat FLIP een belangrijke rol speelt in het voorkomen van cardiomyocyt apoptose.

Hoofdstuk 6 vergelijkt de morfologie van de contractiele eiwitten in biopten van de linker- en rechter ventrikel en het interventriculaire septum, om te beoordelen of rechter ventrikel biopten gebruikt zouden kunnen worden voor het aantonen van herstel in de linker ventrikel tijdens ondersteuning met een LVAD. Rechter ventrikel biopten kunnen namelijk vrij makkelijk verkregen worden via het aanprikken van de vena jugularis interna in de hals. Bij 13 patiënten met ernstig hartfalen werden myocard biopten, afgenomen na LVAD ondersteuning, tijdens de harttransplantatie, vergeleken middels immunohistochemie met antistoffen tegen de contractiele eiwitten. Het

expressiepatroon van de contractiele eiwitten bleek identiek in de linker- en rechter ventrikel biopten evenals in de biopten afgenomen uit de rechter kant van het interventriculaire septum. Tevens werd gezien dat biopten, afgenomen met behulp van een diagnostisch bioptoom representatief waren voor de grotere biopten. Wat betreft de morfologie van de contractiele eiwitten kunnen rechter ventrikel biopten dus gebruikt worden om herstel van linker ventrikel functie aan te tonen tijdens LVAD ondersteuning.

Hoofdstuk 7 bevat een algemene discussie naar aanleiding van de resultaten van dit proefschrift. Mechanische ondersteuning van de circulatie heeft ondertussen al een belangrijke plaats verworven als overbrugging naar harttransplantatie. Patiënten met ernstig hartfalen die dreigen te overlijden kunnen op deze manier in leven worden gehouden, in afwachting van een donor hart. Deze behandeling lost echter het tekort aan donororganen niet op. Vermoedelijk zal echter binnen afzienbare tijd mechanische ondersteuning gebruikt kunnen worden als alternatief voor harttransplantatie. Hiermee kan het probleem van het tekort aan donor harten natuurlijk wel opgelost worden. Het gebruik van mechanische ondersteuning om de eigen hartfunctie langdurig te laten herstellen, moet momenteel als uiterst experimenteel en onvoorspelbaar worden beschouwd. Alleen potentieel herstelbare aandoeningen, zoals bijvoorbeeld myocarditis, kunnen zoveel herstel tonen dat verwijdering van het LVAD gerechtvaardigd is. Het idee om mechanische ondersteuning te gebruiken als tijdelijk hulpmiddel om in de tussentijd te proberen met uitgebreide aanvullende therapie langdurig herstel van de linker ventrikel functie te bewerkstelligen, is op zich aantrekkelijk. Daarvoor is het nodig uitgebreid onderzoek te doen naar dit herstelproces en de daarbij betrokken regulatoren. Tevens dient bepaald te worden hoe dit proces van herstel het best gemonitord kan worden en of dit ook leidt tot een blijvend herstel.

Dankwoord

Aan het einde van dit proefschrift wil ik graag iedereen bedanken die heeft bijgedragen aan de totstandkoming ervan. In het bijzonder wil ik noemen:

Mijn promotororen Prof. dr. E.O. Robles de Medina en Prof. dr. J.G. van den Tweel. Etienne ben ik zeer veel dank verschuldigd, omdat hij me de gelegenheid heeft geboden dit onderzoek te verrichten en mij altijd heeft gestimuleerd. Hij was altijd geïnteresseerd in de vorderingen en bereid de manuscripten van vele verbeteringen te voorzien. Het is tragisch dat hij, door zijn ziekte en voortijdig overlijden, niet meer de afronding heeft kunnen meemaken, maar ik weet zeker dat hij er trots op zou zijn geweest.
Beste Jan, hartelijk dank voor de mogelijkheid dit onderzoek grotendeels binnen jouw afdeling te laten plaatsvinden. Het onderschrijft de al jarenlang bestaande goede samenwerking tussen onze vakgroepen.

Mijn copromotoren Dr. R.A. de Weger en Dr. F.H.J. Gmelig-Meyling. Beste Roel, jouw steun bij dit proefschrift was onmisbaar. Zonder jouw hulp zou dit proefschrift er nooit gekomen zijn. Het is, denk ik, een goede zet geweest, om het al jarenlang lopende harttransplantatie onderzoek ook uit te breiden in de richting van het hartfalen. Dit proefschrift moet gezien worden als het eerste resultaat hiervan. Ik hoop dat er nog vele zullen volgen en verheug mij op voortzetting van de goede samenwerking.
Beste Frits, binnen de samenwerking speelde jij een belangrijke rol als back-bencher en wist op strategische momenten zinvolle ondersteuning te geven. Ook je tekstuele aanvullingen heb ik erg op prijs gesteld.

De leden van de beoordelingscommissie, Prof. dr. P.J. Slootweg, Prof. dr. A. Brutel de la Rivière, Prof. dr. C. van Echteld, Prof. dr. J. Klumperman en Prof. dr. D.J. van Veldhuisen dank ik voor hun bereidwilligheid dit proefschrift kritisch te beoordelen.

Een bijzonder woord van dank gaat uit naar Dick van Wichen die bij dit proefschrift een essentiële rol heeft gespeeld. Vol enthousiasme en met veel vakkundigheid heeft hij al het bioptmateriaal bewerkt voor immunohistochemie en heeft hij ook een belangrijke bijdrage geleverd bij de beoordeling ervan. Hij was ook onmisbaar bij het maken van de fotopagina's en de uiteindelijke uitvoering van het boekje. Beste Dick, je bent de "stille

kracht" waarop het hele lab Moleculaire en Immuun Pathologie leunt en voor mij de grote steunpilaar waardoor het mogelijk was om naast mijn klinische werkzaamheden dit onderzoek te doen.

Dr. J.R. Lahpor verdient natuurlijk ook mijn speciale dank. Beste Jaap, je speelt al vele jaren een belangrijke rol in het Utrechtse harttransplantatie programma. Zonder jou zou vermoedelijk de mechanische ondersteuning bij terminaal hartfalen in Utrecht niet zover zijn gekomen, als die nu is. Je flamboyante karakter draagt ruimschoots bij aan ons adagium: "never a dull moment".

Mijn directe collega's dr. J.H. Kirkels en drs. C. Klöpping. Beste Hans en Corinne, ik denk dat we er trots op mogen zijn dat de "pioniers-afdeling" van indertijd is uitgegroeid tot een volwaardige afdeling voor de behandeling van patiënten met ernstig hartfalen, met diverse behandelingsmogelijkheden, waaronder harttransplantatie en mechanische ondersteuning. Hier hebben jullie een belangrijke rol in gespeeld. Ik wil jullie bedanken voor alle hulp die jullie me hebben geboden bij het onderzoek en de extra tijd die ik heb kunnen gebruiken voor de afronding van dit proefschrift.

Onze secretaresses, Bea Vlasblom en Hanneke Schuurs dank ik voor de soepele manier waarop ze altijd de organisatie draaiende weten te houden en structuur weten aan te brengen in de chaos die bij hen op het bureau wordt gestort. Tevens een woord van dank aan onze verpleegkundige (NP) Albert Oosterom, de regelneef van het team, die voor bijna alles wel een oplossing heeft en overal kan bijspringen waar het nodig is. Albert, dit boekje toont waar al die "vrije" dagen goed voor waren. Natuurlijk mag ik ook onze transplantatie coördinator Patricia Batavier niet vergeten, al was het alleen maar voor de koffie die ze 's morgens vroeg voor iedereen inschenkt. Maar ook haar relativerende kijk op de wereld werkt vaak verhelderend.

De analisten van het "HTx-immunologie-pathologie" lab (waar zit dat eigenlijk?), met name Annette, Joyce en Yevan, verrichten veel werk, waardoor onderzoek als dit mogelijk is. Zij zorgen onder andere voor de opvang, bewerking en archivering van bloed en biopten van de patiënten en zijn nooit te beroerd om een speurtocht door het ziekenhuis te houden op zoek naar verdwenen materiaal. Ze zijn altijd zeer enthousiast en betrokken bij de klinische zaken. In het begin leek het wel eens dat wij twee verschillende talen spraken, maar geleidelijk aan zijn we elkaar steeds beter gaan begrijpen. Ook

mijn “collega-promovendus” Femke dank ik voor haar bijdrage aan het onderzoek en wens haar succes toe bij haar eigen promotie.

Mijn paranimfen, dr. Huub Meijburg en drs. Paul Westendorp, collega’s uit de opleidingstijd in het “oude” ziekenhuis aan de Catharijnesingel (those were the days) wil ik bedanken voor hun ondersteuning bij mijn promotie. Het succes van onze combi zeven jaar geleden sterkt mij enigszins in de gedachte dat het wel moet lukken.

Mijn collega’s van de maatschap Cardiologie van het Hart Long Centrum Utrecht wil ik bedanken voor hun steun bij de totstandkoming van dit proefschrift: dr. Carl Ascoop, drs. Egbert Bal, dr. Jurrien ten Berg, dr. Maarten-Jan Cramer, drs. Richard Derksen, drs. Frank Eefting, dr. Sjef Ernst, prof. dr. Richard Hauer, dr. Wybren Jaarsma, dr. Peter de Jaegere, dr. Hans Kirkels, drs. Corinne Klöpping, dr. Peter Loh, drs. Gijs Mast, dr. Thijs Plokker, dr. Benno Rensing, dr. Rienk Rienks, dr. Maarten-Jan Suttorp, drs. pieter Stella en dr. Erik Wever.

Tenslotte, Maria, Lisa, Simon en Ireen, mijn dierbaarsten. Eigenlijk mocht ik niets over jullie schrijven in dit boekje, maar ik neem toch maar de vrijheid om dat wel te doen. De afgelopen tijd is misschien wat hectisch geweest, maar jullie zorgden in elk geval voor een stabiel thuisfront, met de nodige afleiding. Jullie veel dank voor alle steun en begrip.

Curriculum Vitae

De auteur van dit proefschrift werd geboren op 6 augustus 1957 te Driebergen-Rijsenburg. Hij behaalde in 1975 het diploma Atheneum-B aan het Revius Lyceum te Doorn. In datzelfde jaar begon hij met de studie Geneeskunde aan de Universiteit Utrecht. Tijdens de studie werkte hij als student-assistent bij de vakgroep Medische Anatomie en Embryologie. Na het behalen van het artsexamen op 25 juni 1982, volgde de militaire dienstplicht. Deze bracht hij door in het Militair Hospitaal A. Mathijssen te Utrecht, als arts-assistent Inwendige geneeskunde (dr. M van Zoeren) en als arts-assistent cardiologie (dr. B.K. Bootsma). Van 1984 tot 1986 volgde hij de vooropleiding Inwendige geneeskunde in het Sophia ziekenhuis te Zwolle (dr. T. Tjabbes). In 1986 werkte hij als arts-assistent cardiologie in hetzelfde ziekenhuis (dr. R. Enthoven). Daarna volgde hij van 1986 tot 1989 de opleiding Cardiologie in het Academisch Ziekenhuis Utrecht (Prof. dr. E.O. Robles de Medina). Sinds oktober 1989 is hij werkzaam als staflid cardiologie in het Academisch Ziekenhuis Utrecht, met als aandachtsgebied hartfalen en harttransplantatie.