ABSTRACT

THE RELEVANCE OF HEART RATE VARIABILITY CHANGES AFTER HEART TRANSPLANTATION

by
Sheeba Arnold

Heart transplantation has become an established treatment for end-stage heart disease. However, the shortage of donor organs is a major problem and long-term results are limited by allograft rejection. Heart rate variability (HRV) has emerged as a popular non-invasive research tool in cardiology. Analysis of HRV is regarded as a valid technique to assess the sympathovagal balance of the heart. The primary goal of this study was to investigate the relevance of heart rate variability changes after heart transplantation. It was found that spectral analysis of HRV is useful in detecting rejection episodes. Heart transplantation leaves the donor heart denervated. Spectral analysis of HRV was found appropriate to detect functional autonomous reinnervation. Extensive literature review was done to validate the findings.

The paper is divided into two parts. The first part of the paper deals mainly with the techniques and current status of heart transplantation. The second part, deals with the relevance of heart rate variability and reinnervation after heart transplantation. The results of the study suggest that heart rate variability analysis is a valuable tool in assessing the cardiovascular status after heart transplantation.

INVESTIGATION OF THE RELEVANCE OF HEART RATE VARIABILITY CHANGES AFTER HEART TRANSPLANTATION

by
Sheeba Arnold

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APPROVAL PAGE

INVESTIGATION OF THE RELEVANCE OF HEART RATE VARIABILITY CHANGES AFTER HEART TRANSPLANTATION

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To my beloved family

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GLOSSARY

AR	Acute transplant Rejection
CAD	Coronary Artery Disease
ECG	Electrocardiogram
HF	High Frequency
HRV	Heart Rate Variability
HTX	Heart Transplantation
ISHLT	International Society for Heart and Lung Transplantation
LF	Left Frequency
LV	Left Ventricle
LVAS	Left Ventricular Assist Device Support
NE	Norepinephrine
OPO	Organ Procurement Organization
RSA	Respiratory Sinus Arrhythmia
SD	Standard Deviation
SN	Sinus Node
UNOS	United Network for Organ Sharing
VAD	Ventricular Assist Device
VLF	Very Low Frequency
ULF	Ultra Low Frequency

CHAPTER 1

INTRODUCTION

1.1 Objective

The objective of this thesis is to try to unravel the possible role of heart rate variability in determining the incidence and functional significance of reinnervation after heart transplantation. Cardiac transplantation, first introduced in 1967[1], has emerged as the most effective treatment for end-stage heart disease. Total cardiac denervation occurs after transplantation. Although previously this state was thought to be permanent, recent studies have provided evidence for reinnervation. However, the extent and time course of reinnervation still remains a matter of debate. The primary aim of this study is to resolve this controversy by using heart rate variability as a reflection of the relative activities of sympathetic and parasympathetic systems.

This study focuses on the predictive power of heart rate variability to detect the incidence of reinnervation, which is a measure of the extent of recovery after heart transplantation. The history and current status of heart transplantation, and the insights from the study of heart rate variability are reviewed in this study. An extensive literature review was performed for finding the evidence for reinnervation and for assessing relevance of heart rate variability changes after heart transplantation.

1.2 Back Ground

The development of heart transplantation as a clinical tool for the treatment of heart failure necessitated the contributions of scientists and clinicians whose work has taken generations. The fields of vascular and thoracic surgery, immunology, pathology, medical ethics, engineering, and medicine have contributed to the development and success of this procedure. The contributions of people such as Alexis Carrel, Norman Shumway, and Christian Barnard paved the way for the modern era of transplantation in which the major obstacle to heart transplantation is no longer surgical mortality or rejection but donor supply. In the following paragraphs the history, survival statistics, cost and rejection issues of heart transplantation are summarized.

The following table gives the milestones in the development of clinical heart transplantation[1].

Table 1.1 Milestones in the Development of Clinical Heart Transplantation

1905	Vascular techniques	Carrel
1933	Recognition of graft rejection	Mann
1948	Cardiopulmonary bypass	Gibbon
1958	Myocardial preservation	Shumway
1960	Technique heart transplantation	Lower/Shumway
1967	First human heart transplantation	Barnard
1968	Definition of brain death	Harvard
1972	Modern immunosuppression	Borel

1.3 The History of Heart Transplantation

James D. Hardy attempted the first human heart transplantation at the University of Mississippi Medical Center in January 1964. A 68-year-old man with ischemic cardiovascular disease was the recipient patient[2]. As no human donor heart was available, the heart of a chimpanzee was transplanted. The donor heart was preserved by the retrograde gravity flow of cold, oxygenated blood through the coronary sinus. The operative technique was that described by Lower, Stofer, and Shumway. The transplanted heart functioned immediately, but the small donor heart could not handle the large venous return of the recipient, and the patient died 1 hour after transplantation of the heart.

The first human-to-human heart transplantation was performed in 1967 in Groote Shuur Hospital, South Africa. A 54-year-old man with intractable heart failure secondary to ischemic heart disease was the recipient. The donor heart was that of a woman, an accident victim, who died due to massive cerebral injuries. Using cardiopulmonary bypass, arterial perfusion, hypothermia of the donor heart, and the surgical technique of Lower and Shumway, the heart was implanted successfully in the waiting recipient. The patient did well until day 18, when Pseudomonas pneumonia developed. Dr. Christian Barnard was the surgeon who performed this first successful human-to-human heart transplantation.

Denton Cooley performed the first successful heart transplantation on an infant at the Texas Children's Hospital in Houston in 1984. The infant, an 8-month-old girl with sub endocardial fibrosis, received the heart of a brain-dead 2-year-old infant.

Adults up to 70 years old have been transplanted with good results. Patients with transplants are surviving longer (the longest survivor received a transplant in January 1970, and died of non-cardiac causes in 1994.

1.4 Organization of Heart Transplantation in the U.S.

The procurement and distribution of organs for transplantation in the U.S. are the contractual responsibility of the United Network for Organ Sharing (UNOS). UNOS was established in 1972 as a voluntary association intended to serve the needs of the renal transplant community. Because of its demonstrated commitment to organ procurement, UNOS was awarded a federal contract in 1986 to establish and operate the Organ Procurement and Transplantation Network. In 1987, UNOS was also awarded a separate contract to maintain the scientific registry for organ transplantation. UNOS maintains a national computerized list of patients waiting for kidney, heart, heart-lung, liver and pancreas transplants. An "Organ Center" number allows 24-hr access to the computer system by all transplant programs in the U.S. The Scientific Registry collects and maintains data pertaining to patients waiting for transplants, donors, and recipients of donated organs, donor-recipient matching and organ allocation and donor-recipient histocompatibility.

1.5 Current Status of Heart Transplantation

The annual U.S. transplantation volume currently is limited by donor availability and has been so limited since the mid-1980s. Conservative analysis of donor availability (by review of causes of death listed on death certificates) indicates that approximately 5,200

donor hearts (20.8 per million population) could be available annually, but this figure is as high as 8,200 (32.9 per million population) if the criteria are only slightly liberalized[6]. These estimates suggest that only 26% to 42% of available donor organs are utilized. Intensification of efforts toward public education, effective legislation and improved training of procurement coordinators are needed to reduce the number of potential donors that remain unavailable for transplantation.

1.6 Survival Statistics

Survival statistics obtained from the general registry of International Society for Heart and Lung Transplantation (ISHLT) as per April 1999 is shown in figures 1.1 and 1.2.

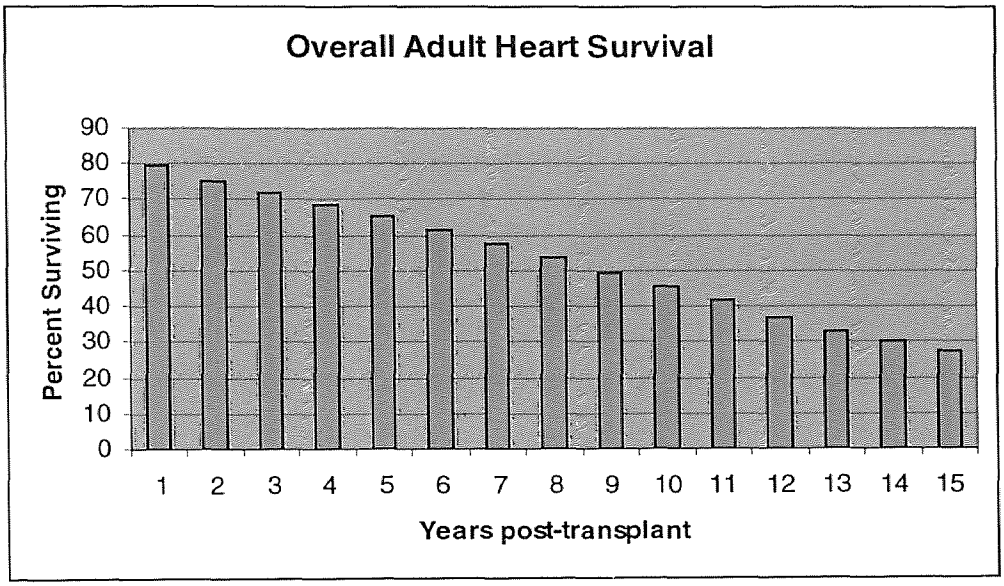

Figure 1.1 Overall adult heart survival (N=43,936)

The above statistics suggest that the number of people surviving decrease with the number of years after transplantation. The time taken for the initial heart transplant population to become half (half-life) was estimated as 8.8 years.

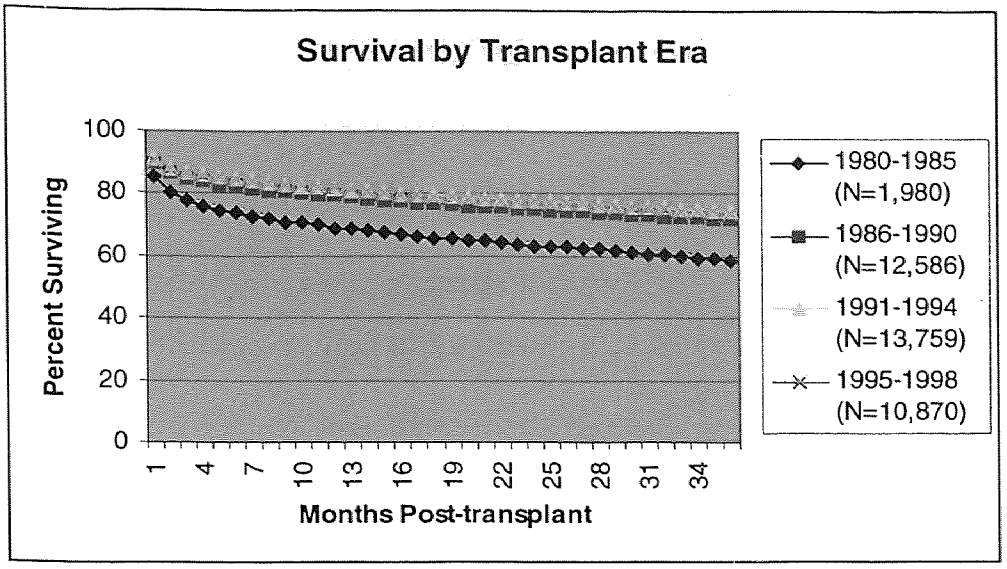

Figure 1.2 Survival by transplant era

The half-life for 1980-1985 was found to be 5.3 years and the half-life for 1986-1990 was 8.9 years. Even though the survival rate drops with time after transplantation, a comparison of the different transplant era suggests that the survival rate has improved from one transplant era to the other. This can be attributed to the advances in detection techniques for rejection and other malignancies after transplantation.

1.7 Cost Issues

Total transplant charges vary according to several factors. For example, patients aged <= 18 years incurred higher charges than did patients aged > 18 years. Retransplant procedures were more costly than primary grafts. Charges for patients who required an artificial device for cardiac support were much higher than those who did not. Patients on life support or in intensive care before transplantation incurred much higher post-operation charges than did patients who were hospitalized outside the intensive care unit

or at home[6]. The range of heart transplantation procedure charges by category and average length of hospital stay is shown in the following table.

Table 1.2 Range of Heart Transplantation Procedure Charges by Category and Average Length of Hospital Stay

Category of Charges	Minimum	25th percentile	50th percentile	75th percentile	Maximum
Hospital charges	$8,173	$44,139	$62,463	$101,849	$1,290,033
Surgeon fees	2000	9000	10,000	12,500	40,391
Other professional fees	232	4,236	6,529	11,606	75,216
Donor organ acquisition charges	390	9,859	12,578	16,124	60,000
Length of stay (days)	1.0	15.0	23.0	42.0	554.0

The data suggest that the average charge (50th percentile) for transplantation is about $91,570 (sum of the mean charges for hospital stay, donor organ acquisition, surgeon fees and other professional fees) and the average length of stay is about 23 days.

CHAPTER 2

METHODS AND PROCEDURES

2.1 Introduction

Since the introduction of cardiac transplantation in 1967, the growth in the number of transplant operations have been directly dependent on the identification and procurement of cardiac allograft donors. The medical criteria for donor selection, the surgical procedure, the recipient prioritization and donor allocation criteria, the means for increasing donor supply, the complications and quality of life after transplantation and some of the general statistics of heart transplantation will be discussed in this chapter.

2.2 Medical Criteria for Donor Selection

The initial step in donor procurement is the recognition and declaration of brain death of the donor. It is the attending physician, not the members of the heart transplant or procurement team, who diagnoses brain death. The clinical diagnosis of brain death requires 1) that there be a loss of function of the entire brain and 2) that the loss of brain function is irreversible.

The screening of potential cardiac donors is accomplished in three phases. Primary screening is done by organ procurement specialists who work for the nonprofit organ procurement agencies. Information pertinent to all organ donation is obtained initially, including body size, ABO blood type, hepatitis B and human immunodeficiency virus (HIV) sero-logic data, information on cause of death and clinical course and routine laboratory data. Secondary screening is done by cardiac surgeons or cardiologists.

relevant to cardiac donation includes determination of the circumstances leading to severe brain injury, extent of other (especially thoracic) injuries, the extent of treatment required to sustain an acceptable hemodynamic status, baseline electrocardiogram (ECG), chest X-ray film, arterial blood gas analysis and echocardiogram. The purpose of this secondary screen is to provide enough information to decide whether to implement the tertiary screen, which is inspection of the heart by a "harvesting" surgeon.

In heart transplantation, an effort is made to match the muscle mass of the donor heart to the expected cardiac output requirements of the recipient. A common practice is to accept hearts from donors with a donor/recipient weight ratio of 0.8 or higher[3]. Donor height is another variable that is considered because cardiac output often parallels body height as well as weight. Because of a profound shortage of cardiac organ donors and high pretransplantation mortality rates of patients listed for heart transplantation, it is clear that donor-screening strategy should be liberal. Conversely, because primary graft failure is almost always associated with death of the heart transplant recipient, the screening strategy must identify hearts that will reliably support the circulation in the immediate post transplantation period.

2.3 Surgical Procedure

There are two different surgical techniques for heart transplantation: standard surgical techniques, which leaves most of the recipient atria intact and the bicaval technique in which the whole recipient heart including the entire atrial junctions of both superior and inferior venaecavae are removed and substituted with equivalent components of the donor heart. Two different heart transplant methods employed are heterotopic and

orthotopic. In heterotopic heart transplantation, the donor heart is connected either in a parallel or non-parallel way to the recipient heart, which is left in place[4]. In orthotopic heart transplantation, the recipient heart is removed and the donor heart replaces the recipient heart (figure 2.1).

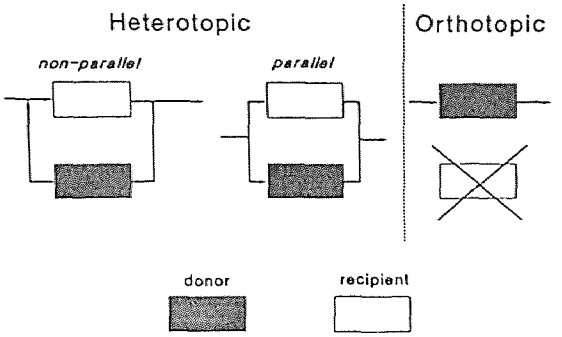

Figure 2.1 Schematic representations of experimental heart transplant models. (Shumway and Shumway, *Thoracic Transplantation*, 1995.)

The operative technique employed for orthotopic heart transplantation[4] is summarized in the following paragraphs.

The donor heart is removed from its sterile travel container and is placed in a basin of cold saline solution. The aorta and pulmonary artery are separated. The pulmonary veins are identified and connected first along their short axis and then along their long axis, to form a single large posterior atrial cuff. After further adjustments, which include the identification of mitral valve (the valve between the left atrium and left ventricle), inferior vena cava, and superior vena cava, the donor heart is submerged in cold saline solution until it is time for implantation.

For excising the recipient's native heart, the heart is exposed and cannulation is carried out. The great vessels are divided just above the semilunar valves. The right

atrium is incised along the atrioventricular groove. After the native heart has been excised, the pericardial space is irrigated with cold saline solution. The prepared donor heart is positioned to start the left atrial anastomosis, which is followed by right atrial anastomosis. Once the atrial anastomoses are completed, the great vessel anastomoses are done. First, the donor and recipient pulmonary arteries are sewn end-to-end and then the donor and recipient aortas are sewn end-to-end. The different steps in the operative procedure are given in the following figures.

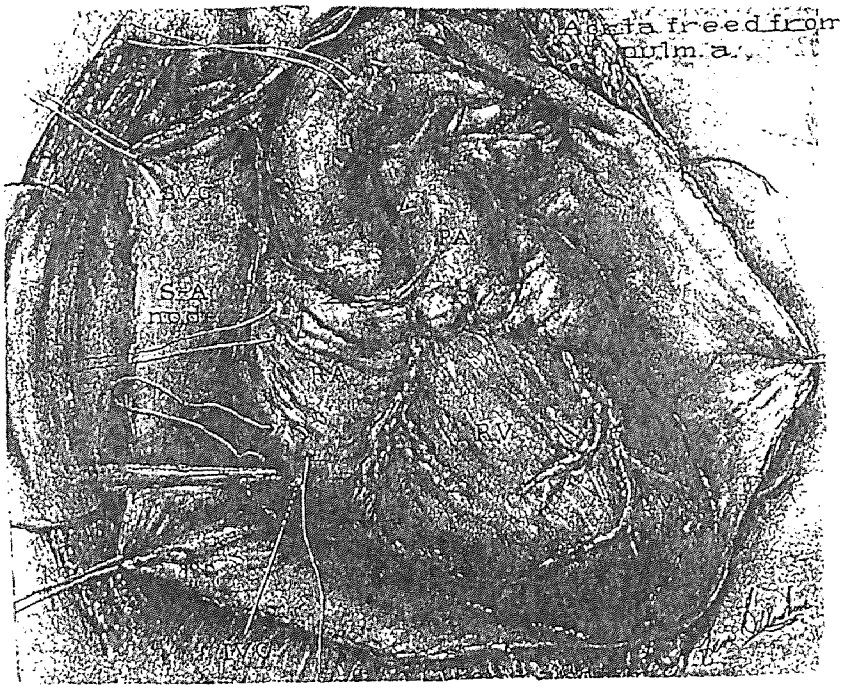

Figure 2.2 The recipient's native heart is exposed and cannulation is carried out. (Shumway and Shumway, *Thoracic Transplantation*, 1995.)

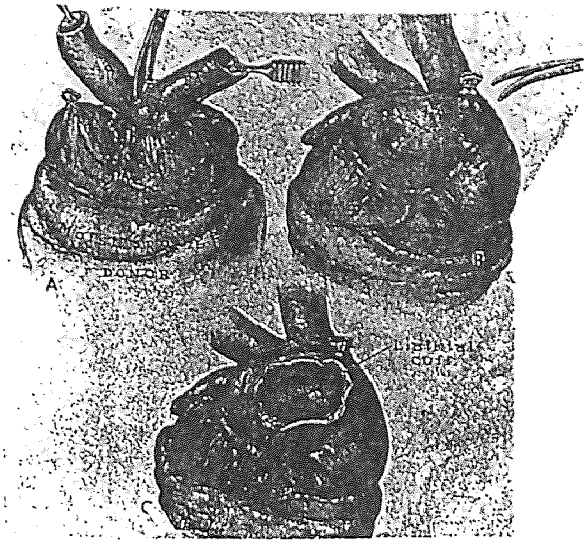

Figure 2.3 The donor heart is prepared. The great vessels are separated (A). The left atrial cuff is created by connecting the pulmonary veins (B) and (C). (Shumway and Shumway, *Thoracic Transplantation*, 1995.)

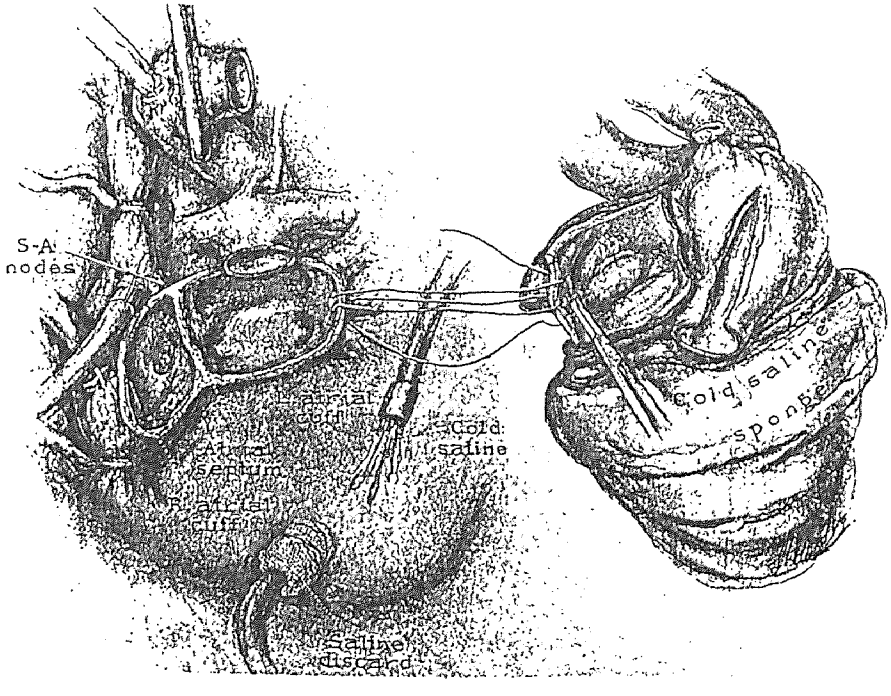

Figure 2.4 The native heart has been excised. (Shumway and Shumway, *Thoracic Transplantation*, 1995.)

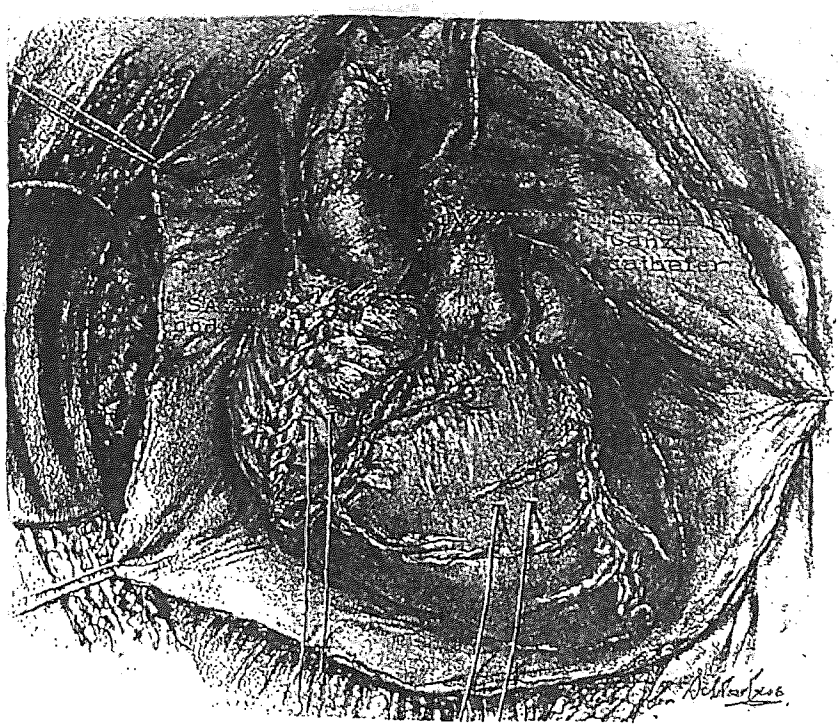

Figure 2.5 The recipient heart as it appears with all anastomoses completed. (Shumway and Shumway, *Thoracic Transplantation*, 1995.)

2.4 Means of Increasing Donor Supply

Little information is available regarding donor-specific parameters that predict success or failure after heart transplantation. Furthermore, with the increasing number of patients awaiting heart transplantation, there is tremendous pressure to expand the donor pool by stretching the margins of donor acceptability. To gain insight into donor-related and donor-recipient interrelated predictors or death after transplantation, 1719 consecutive primary transplantations performed at 27 institutions between Jan. 1, 1990, and June 30, 1992, were analyzed by Young et al[3]. Mean follow-up of survivors was 13.9 months, and actuarial survival was 85% at 1 year. There were 248 deaths during the period of follow up. The three most common causes of death were infection (22%), acute rejection (18%),

and early graft failure (18%). By multivariable analysis, risk factors for death included younger recipient age, older recipient age, ventilator support at time of transplantation, higher pulmonary vascular resistance, older donor age, smaller donor body surface area (female donor heart placed into larger male patient), greater donor inotropic (inotropes are administered to increase the force of contraction of the heart) support, donor diabetes mellitus, longer ischemic time, diffuse donor heart wall motion abnormalities by echocardiography, and, for pediatric donors, death from causes other than closed head trauma. The overall 30-day mortality rate was 7% but increased to 11% when donor age exceeded 50 years and was 12% when inotropic support exceeded 20μg/kg/min dopamine plus dobutamine and 22% with diffuse echocardiographic wall motion abnormalities. The 30-day mortality rate for older recipients were greater as compared to younger recipients. The interaction of donor risk factors was such that the heart of a smaller female donor given high-dose inotropes placed into a larger male recipient produced a predicted 30-day mortality rate of 26% and the heart of a 25 year old male donor given high dose inotropes with diffuse echocardiographic wall motion abnormalities transplanted into a 50 year old male recipient led to a predicted 30 day mortality rate of 17%. This analysis supports cautious extension of criteria for donor acceptance but with an anticipated greater risk in the presence of diffuse echocardiographic wall motion abnormalities and long anticipated ischemic time, particularly in older donors given inotropic support.

Table 2.1 Activity on Cadaveric Heart Waiting List in the Entire United States

	1/1/1998 – 12/31/1998	1/1/1999 - 3/31/1999
Number Waiting At Start of Period	3,883	4,167
Number Added to the Waiting List	3,931	857
Number Transplanted	2,320	494
Number Died on Waiting List	773	185
Number Removed For Other Reasons	554	139
Number on Waiting List at end of period	4,167	4,206

(Source: ISHLT general registry[5])

2.5 Current Medical Indications for Heart Transplantation

The success of heart transplantation has resulted in a critical limitation of donor supply, expanding waiting lists and increasing numbers of new transplant centers. The current indications for transplantation have been described as end-stage heart disease including heart failure, ischemic heart disease and arrhythmias[6]. Clinicians usually examine clinical factors such as escalating medical requirements or frequent hospitalizations, and list patients for heart transplantation when medical therapy seems doomed.

Ischemic heart disease and idiopathic dilated cardiomyopathy are the primary underlying diseases leading to congestive heart failure severe enough to serve as indications for heart transplantation in adults. Cardiomyopathies constitute a group of diseases in which the dominant feature is the involvement of heart muscle itself. Ischemic cardiomyopathy is a condition in which coronary artery disease results in severe myocardial dysfunction. Patients with advanced valvular heart disease or congenital heart disease are also suitable candidates at times. Heart transplantation has been considered in

some centers to be a lower risk form of surgical intervention than high-risk vascularization procedures or valve replacement and, hence, is recommended to some patients rather than more conventional surgical interventions.

2.5.1 Recipient Priority for Heart Transplantation

The current means of recipient categorization in the U.S. includes an estimation of the recipient's severity of illness and determination of ABO blood type, body size and length of time on the waiting list[3]. Because of the expanding number of potential recipients, the primary determinants of organ allocation have come to be severity of illness and time on the waiting list[7]. The physically small adult recipient or recipients in AB or B blood groups will usually be on a smaller waiting list, but donor availability is also limited for these patients. Current prioritization systems include a local allocation system and a national listing, both of which are supervised by UNOS[8] (United Network for Organ Sharing).

2.5.2 Current Recipient Status Criteria of UNOS

Status I

Patients who require cardiac and/or pulmonary assistance with one or more of the following devices:

1. Total artificial heart
2. Left and/or right ventricular assist systems
3. Intra-aortic balloon pump
4. Ventilator

Or, patients meeting both of the following criteria:

1. Patient in an intensive care unit and
2. Patient requires inotropic agents to maintain adequate cardiac output

Or, patients less than six months old.

Status II

All other waiting patients who do not meet the Status I criteria.

2.6 Current Allocation Criteria of UNOS

Hearts are allocated locally in the following sequence. For every thoracic donor, the choice will be made locally whether to use the heart for a heart (without lung) transplant for a Status 1 patient, or for a heart-lung combination transplant. If the heart is to be used for a heart (without lung) transplant, the heart will be allocated first to local Status 1 patients according to length of time waiting. If the organ is not allocated to a Status 1 heart patient, then the heart will be allocated to a local patient awaiting a heart-lung combination transplant who has a blood type that is identical to the donor, according to length of time waiting. If the heart is not allocated to a local patient awaiting a heart-lung combination transplant who have a blood type that is identical to the donor, then the heart will be allocated to the local patients awaiting heart-lung combination transplantation who have a blood type that is compatible with that of the donor, according to length of time waiting. If the heart is not allocated to a Status 1 heart patient or a heart-lung patient, then the heart is allocated to a local Status 2 (ABO identical) patient according to length of time waiting.

Local conflicts regarding allocation of hearts, lungs, heat-lung combinations, locally unresolved inequities or conflicts that arise from prevailing OPO (Organ Procurement Organization) boundaries or policies may be submitted to any interested local member for review and adjudication to the UNOS Thoracic Organ Transplantation Committee, Organ Procurement and Distribution Committee and Board of Directors[8].The distance of the recipient hospital from the donor hospital will also be used to prioritize the recipient list for hearts, lungs and heart-lung combination not used by the local OPO.

2.7 Quality of Life After Heart Transplantation

Heart transplantation was previously considered an appropriate option only in patients unlikely to survive 6 months, but patients today sometimes are considered candidates for heart transplantation if they have 50% survival likelihood at 24 months. As more patients are placed on waiting lists for heart transplantation and waiting times lengthen, the acuity of illness of these patients may be lessening. Assessing quality of life variables after heart transplantation, therefore, is important particularly when comparing the physiological outcome after transplantation[9]. Some of the post transplant events that are indicative of the quality of life after transplantation are given in the following tables. These data where obtained from a follow up of patients in the US (from April 1994-December 1998) by ISHLT.

Table 2.2 Functional Status

Heart	1 Year Follow-up		2 Year Follow-up		3 Year Follow-up		4 Year Follow-up	
	No.	%	No.	%	No.	%	No.	%
No Activity Limitations	5515	89.7	3779	92.2	2120	93.6	706	93.9
Performs with Assistance	525	8.5	278	6.8	131	5.8	40	5.3
Total Assistance	92	1.5	41	1.0	15	0.7	6	0.8

Table 2.3 Employment Status

Heart	1 Year Follow-up		2 Year Follow-up		3 Year Follow-up		4 Year Follow-up	
	No.	%	No.	%	No.	%	No.	%
Working Full Time	1494	27.6	1104	31	631	32	217	32.9
Working Part Time	447	8.2	308	8.6	157	8	47	7.1
Not Working	2589	47.8	1465	41.1	763	38.8	234	35.6
Retired	892	16.5	684	19.2	418	21.2	161	24.4

Table 2.4 Re-hospitalization Post Transplantation

Heart:	1 Year Follow-up		2 Year Follow-up		3 Year Follow-up		4 Year Follow-up	
	No.	%	No.	%	No.	%	No.	%
No Hospitalization	3684	56.8	3180	75.9	1900	81.7	641	82.8
Hosp., Not Rejection/Not Infection	621	9.6	313	7.5	146	6.3	54	7.0
Hosp, Rejection	701	10.8	156	3.7	68	2.9	20	2.6
Hosp, Infection	1026	15.8	472	11.3	193	8.3	55	7.1
Hosp, Rejection + Infection	454	7.0	69	1.6	19	0.8	4	0.5

Table 2.5 Malignancy - Post Heart Transplantation

1 Year Follow-up:	No: 96.1%	
	Yes: 3.9% ---------->	Lymph - 78 (31.8%)
		Not Reported - 17 (6.9%)
		Other - 66 (26.9%)
		Skin - 84 (34.3%)
4 Year Follow-up:	No: 91.5%	
	Yes: 8.5% ---------->	Lymph - 8 (12.1%)
		Not Reported - 2 (3.0%)
		Other - 20 (30.3%)
		Skin - 36 (54.5%)

The statistics indicate that the most of the heart transplant recipients could lead a normal life after heart transplantation. However, the quality of life judgments are frequently subjective and if a patient's premorbid quality of life is poor, it is unlikely to be significantly changed by a heart transplant procedure. As compared to the total number of patients undergoing heart transplantations, the number of reported cases of post transplantation malignancy is relatively less. However, the statistics suggest that malignancy increases with time after transplantation. This can be attributed to the immunosuppressive agents administered to the transplant patients to suppress rejection. Thus, transplant patients with malignancy can be at a higher risk of graft failure as compared to transplant patients with no sign of malignancy.

2.8 Complications After Heart Transplantation

Many of the early postoperative complications like primary graft failure can be avoided by proper donor selection. Another complication, right ventricular failure secondary to high pulmonary vascular resistance, can usually be avoided by careful screening of the potential recipient before transplantation[11].

One of the leading causes of death in the first year after heart transplantation is cardiac allograft rejection[12]. Some of the factors that are associated with an increased risk of rejection are human leukocyte antigen (HLA) mismatch, female gender, younger donor hearts, non-O blood types, etc. The main reason for the rejection of female hearts may be the weight and height mismatch as compared to that of the recipient. The same reason can be attributed to the rejection of small (younger) donor hearts[13].

Since the introduction of the endomyocardial biopsy by Caves et al. in 1973, it has been considered the reference standard for the detection of cardiac rejection and assessment of the adequacy of antirejection therapy. Endomyocardial biopsy is an invasive and expensive procedure, in which the clinician obtains a tissue sample from the right or left ventricle using a bioptome via a transvenous or transarterial approach. Five or six tissue samples (average size 1-4 mm) are often required to be certain of a given histological finding since pronounced topographic variations may be found in the myocardium[14]. There has been an intensive search for an accurate and reproducible noninvasive method to detect acute rejection. Electrocardiography, echocardiography, nuclear magnetic resonance imaging, nuclear scintigraphy[15] etc. are some of the noninvasive methods to detect cardiac rejection. Heart rate variability (HRV) analysis,

which will be discussed later in this paper, is another noninvasive tool to detect cardiac rejection.

Table 2.6 Cause of Death Vs Timing of Death After Heart Transplantation

Cause of Death	1 Year	2 Years	3 Years	4 Years	5 Years
Cardiac allograft vasculopathy	4.6	19.3	20	24.7	25
Malignancy, Other	1.9	8.8	12	13.3	18.6
Lymphoma	0.9	4.1	4.5	3.4	2.9
Cytomegalovirus	1.5	1.5	0.6	0.5	--
Acute Rejection	13.3	12.7	8.8	5.7	2.2
Infection, Other	20.3	13.3	12.6	10.9	7.9

(Source: ISHLT general registry[5])

The above data suggests that acute transplant rejection (which occurs as a result of the body's resistance to foreign tissue), and infection is the main cause of death in early years after transplantation. Immunosuppressive drugs are administered to suppress the immune system to prevent rejection, but they also prevent the body from reacting to infection so that even the mildest infection may be fatal. New immunosuppressive drugs are being developed, and rejection is becoming less of a problem, but because of their toxicity malignancy develops and it is one of the main causes of death in later years of transplantation.

2.9 General Statistics

Following are some of the general statistics of interest. These data were collected from the sixteenth annual data report published by ISHLT[5] in April 1999.

Table 2.7 Number of Transplants and Centers

Organ	Number of Transplants	Number of Centers
Heart	48,541	304
Heart-Lung	2,510	124
Single Lung	5,347	153
Bilateral/Double Lung	3,751	140

The figures in the above gives the total accumulated number of transplants and transplant centers as of April 1999. The graph given below shows the number of thoracic procedures by year. Even though the plot shows an increase in the number of procedures as compared to last decade, a recent drop has been found in the number. This can be attributed to economic factors and scarcity of donor organs.

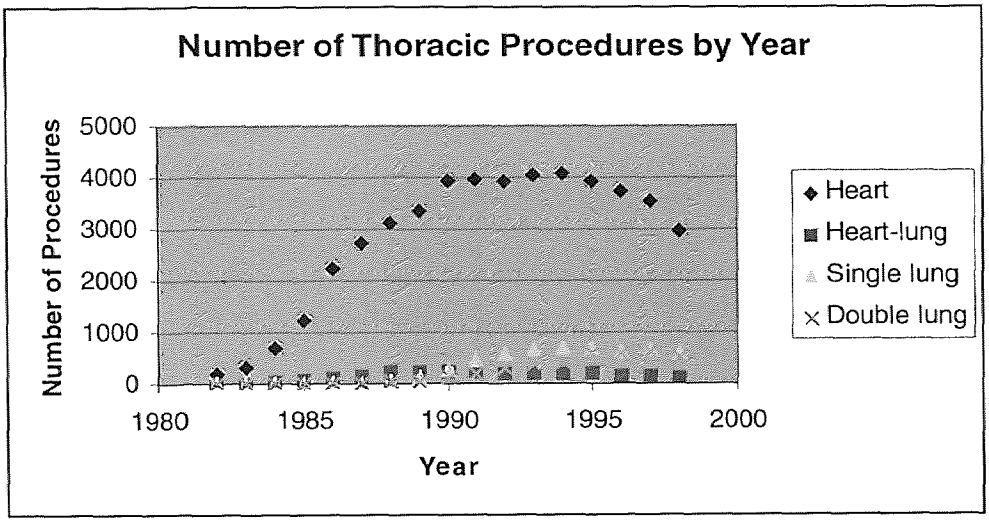

Figure 2.6 Number of thoracic procedures vs. number of procedures

Table 2.8 Adult Heart Transplant Indications

Disease Category	Number	Percent
Cardiomyopathy	18,602	45.6
Coronary Artery Disease	18,599	45.6
Valvular Disease	1,398	3.4
Retransplant	891	2.2
Congenital Disease	646	1.6
Miscellaneous	646	1.6

The above data indicates that cardiomyopathy and coronary artery disease are the leading causes for heart transplantation. A plot of the percent of cases for heart transplant indications of cardiomyopathy and coronary artery disease is given below. Even though there has been a decrease in the incidence of cardiomyopathy with time, percent cases for coronary artery disease is found to increase. This may be due to the change in life style of people as compared to the past decade.

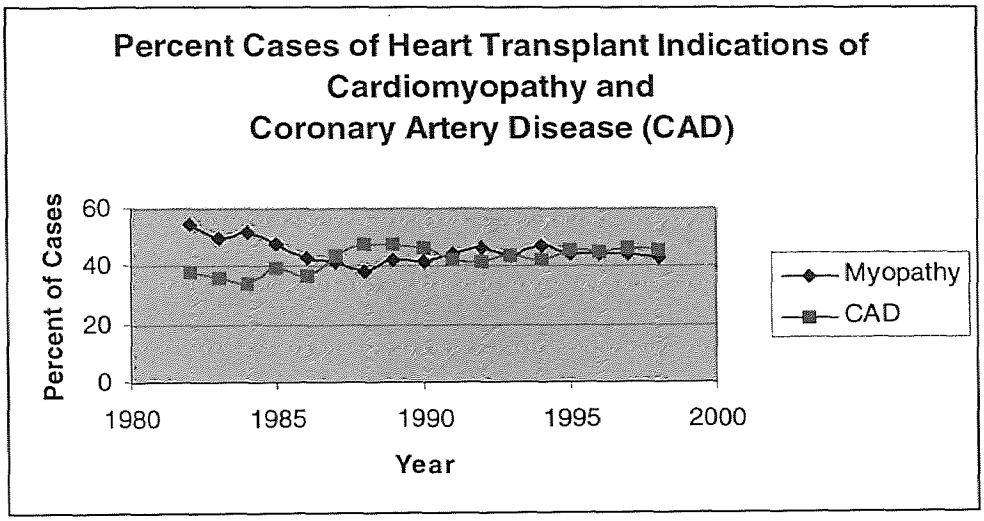

Figure 2.7 Percent cases of heart transplant indication vs. percent of cases

Table 2.9 Age Distribution of Heart Transplantation

Age	Percent of Transplants
<1	2.44
1-5	1.82
6-10	1.17
11-17	3.17
18-34	9.77
35-49	27.46
50-64	47.31
>65	3.5

The above data suggests that the people in the age group of 35-65 constitute about 75% of the total population of people undergoing heart transplantation. The plot given below compares the percent of thoracic procedures in the east and west hemispheres. The percent of double-lung transplants have been compared with that of heart-lung transplants and has been found to increase with time. The west hemisphere holds a greater share of transplantation as compared to the east hemisphere.

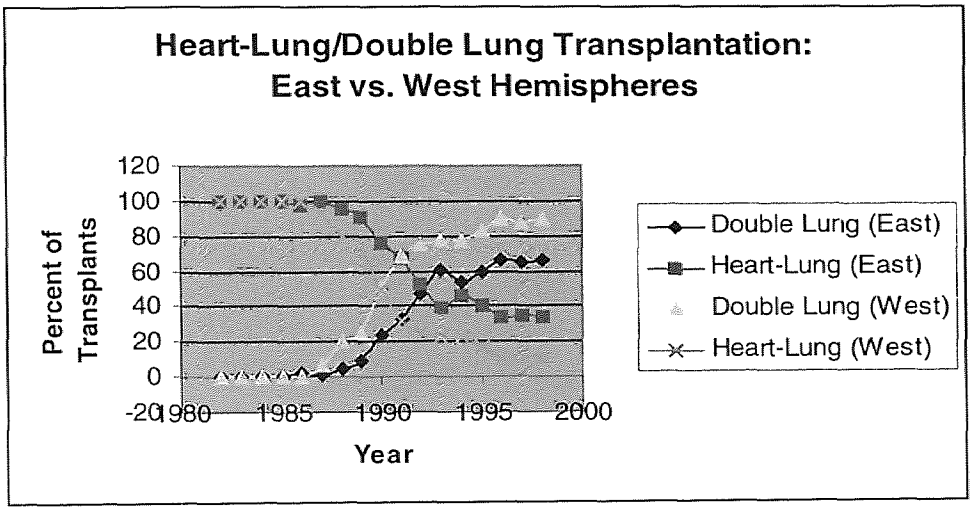

Figure 2.8 Heart-lung/Double-lung transplantation vs. percent of transplants

CHAPTER 3

FUTURE OF HEART TRANSPLANTATION

3.1 Introduction

Over the past decade heart transplantation has evolved from a rarely performed experimental procedure to an accepted therapy for end-stage heart disease. Despite the escalation in the number of procedures and progressive improvement in postoperative outcome, two formidable obstacles, the issues of rejection and shortage of organ donors, continue to challenge the success of heart transplantation[13].

Contemporary immunosuppressive strategies[11] have reduced the incidence of acute rejection but have severe limitations. These include lack of specificity, inability to reduce the risk of cardiac allograft vasculopathy (the major factor limiting long-term recipient survival), significant toxicity and an increased risk of opportunistic infections and malignancy. The understanding of the immune response at increasingly fundamental levels and the ability to combine data from in vitro assays of immune function with information from in vivo models of organ transplantation and autoimmune disease have enabled rapid advances in the discovery and development of new immunosuppressive modalities.

Shortage of donor organs is another major problem facing heart transplantation. Because the pool of potential candidates continues to expand while donor organs remain critically scarce, the discrepancy between the number of patients who would benefit from heart transplantation and the number of patients who actually receive a transplant

continues to increase at an alarming rate. It is estimated that as many as 30% of patients awaiting heart transplantation die before a suitable donor organ becomes available. The severe shortage of donor organ emphasizes the crucial need for identifying alternatives to allotransplantation. Potentials solutions include 1) xenotransplantation, 2) the application of transformed skeletal muscle, and 3) mechanical assist and replacement devices[17].

3.2 Permanent Ventricular Assist Device Support Versus Cardiac Transplantation

Among heart transplant candidates, UNOS status II, O blood type, and weight >180 lb, older age and preformed antibodies are negative factors for receipt of donor hearts. Of patients transplanted, women and nonwhites have poorer outcomes[16]. Success with wearable left ventricular assist device support (LVAS) suggests some of these patients might be better served with an LVAS than with cardiac transplantation.

The Novacor N-100 wearable LVAS with biological valved conduits (Baxter Healthcare/Novacor, Oakland, CA) and the Heartmate LVAS (Thermocardiosystems Inc, Woburn, MA) are currently in use as electrically powered devices that fulfill all the criteria for completely implantable systems except for their requirement for an externalized drive system[16]. The N-100 LVAS system has the capacity to become a completely implantable system, the proposed model of which is shown in figure 3.1. It employs an integrated pump and drive unit, a compliance chamber that is to be housed in the pleural space, an internalized battery system that can provide a backup energy source, and a method of transmitting energy across the skin by inductive coupling from a primary to a secondary coil.

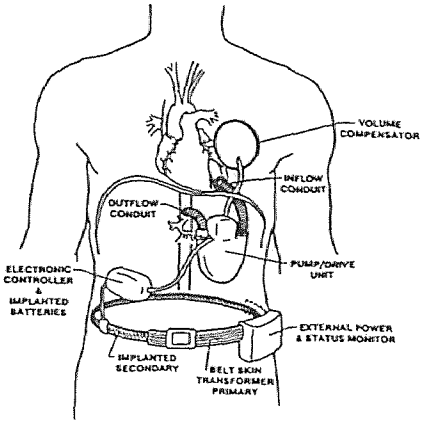

Figure 3.1 The proposed totally implantable Novacor LVAS. (*Thoracic Transplantation*; Shumway and Shumway, 1995)

The strategy of transplantation in which patients may or may not receive a donor heart is inadequate to serve the large number of potential candidates (figure 3.2). In 1995, for instance, the mean waiting time for cardiac transplantation was over 200 days and more than 40% of the patients waited more than 1 year for transplantation. Because only about 4,000 new patients are listed for cardiac transplantation each year, about 28,000-30,000 patients are apparently not considered viable candidates to be placed on the list. Of those not placed on the list, it is possible that as many as half of them (13,000) would be candidates for a permanent ventricular assist device (VAD). An important deterrent to listing may be advanced age, because the estimate of 30,000 is made with an upper age limit of 75 years, and the estimate of 60,000 is made with an upper age limit of 85 years. It is also possible that current VAD technology using externalized drivelines might offer a reasonable alternative to cardiac transplantation.

Because LVAS devices could be made readily available without the need for a waiting list, they might compete well with the strategy of heart transplantation. The most obvious advantage of VAD systems over transplantation is their immediate availability.

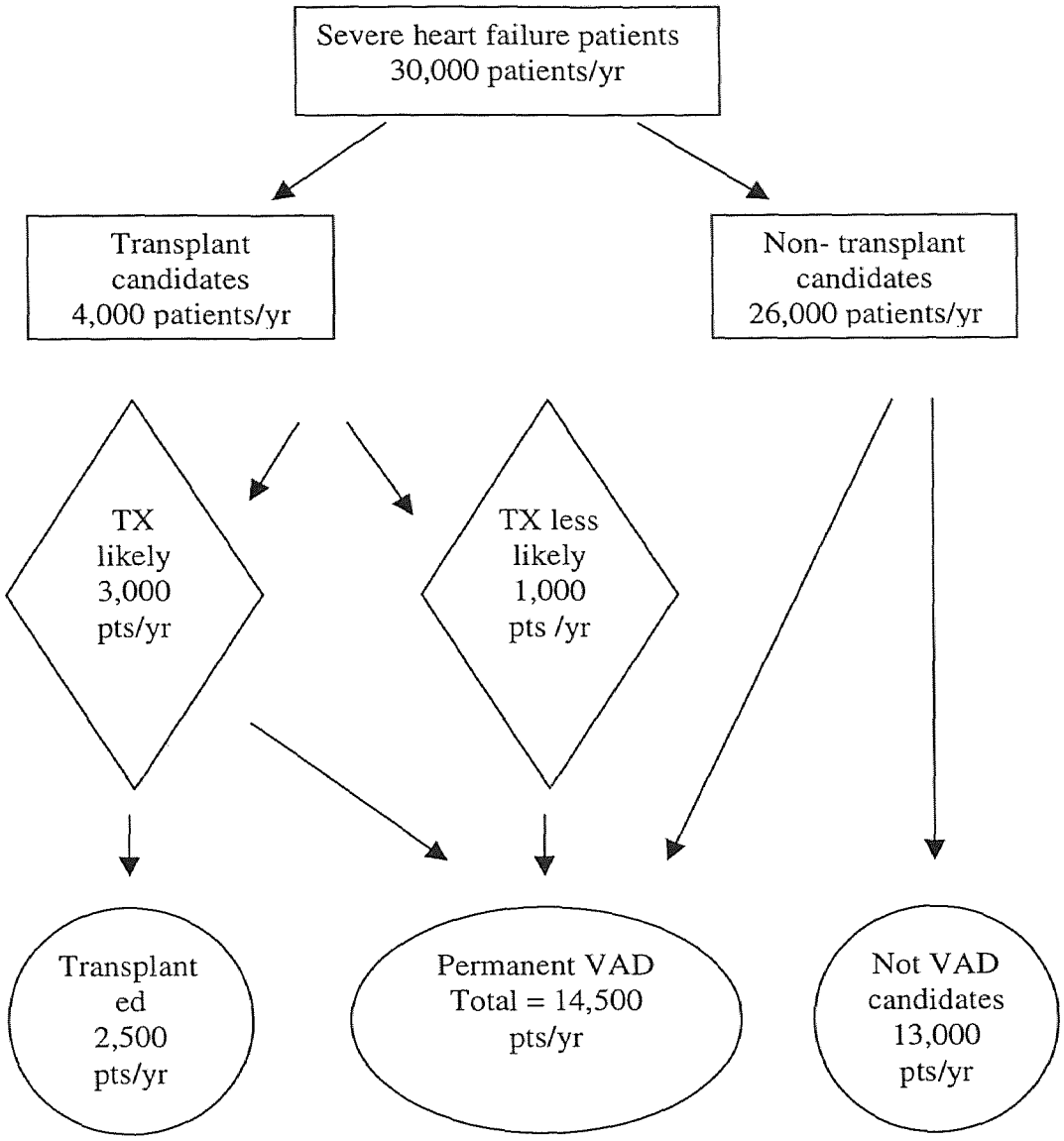

Figure 3.2. Representation of severe heart failure patients that could benefit from a permanent VAD on an annual basis. (Pennington et al., "Permanent Ventricular Assist Device Support Versus Cardiac Transplantation," *Ann Thorac Surg*, 1999.)

A positive psychological feature is the fact that LVAS insertion does not necessitate removal of the natural heart, which might be able to temporarily support the circulation, or recovers sufficiently to allow for device removal. The disadvantages include issues of thromboembolism, requirements for platelet deaggregating drugs and issues of device failure.

Thus LVAS may offer the opportunity to improve the quality of life in patients who face a high mortality during their last six months of life. Although transplantation will be important to maintain a small core of congestive heart failure patients, it is apparent from many studies that unless some major changes occur in immunosuppressive therapy or organ donor supply, LVAS devices hold the best promise for the future in making any real impact on this huge public health problem.

3.3 Cardiac Retransplantation

Since the first report of cardiac retransplantation in 1977, sequential orthotopic transplantation has become a frequent treatment for heart failure[17].

To evaluate the risk of cardiac retransplantation and to better establish selection criteria, Michler et al reviewed the records of all patients who underwent retransplantation at the Colombia-Presbyterian Medical Center. Of 431 patients who underwent transplantation between February 1977 and March 1991, 408 underwent the procedure in the era of cyclosporine-based immunosuppression. Thirteen of these 408 patients underwent retransplantation (including one patient who received a third graft). Indications for the 14 retransplantations included transplant coronary artery disease (n=8), rejection (n=5), and intraoperative graft failure (n=1). Immunosuppression and follow-up protocol used in this cohort were similar to those in the primary transplantation

population. No significant differences were found (figure 3.2) in either actuarial survival between primary transplant recipients and patients who underwent retransplantation or in linearized rates of rejection and actuarial freedom from rejection between the two groups. The results of this study indicated that the prognosis of patients undergoing cardiac retransplantation is identified more than 30 days after initial transplantation.

Whether to use donor organs for retransplantation remains controversial. The alternative of using ventricular assist devices should be considered whenever possible. The clinician ultimately must balance a sense of therapeutic obligation to the transplant recipient in question with the current scarcity of donor supply.

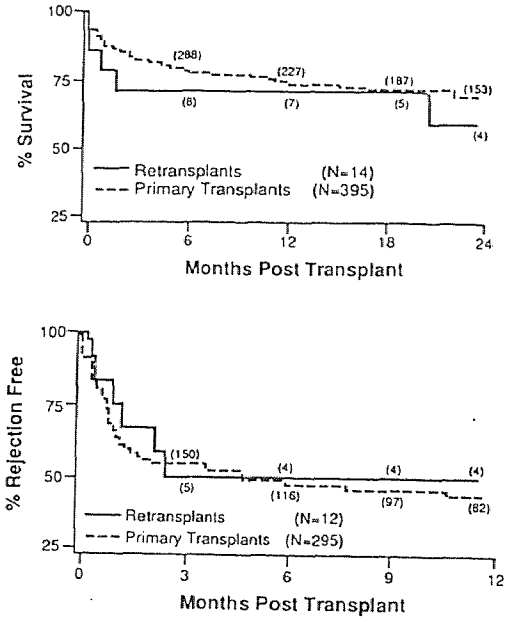

Figure 3.3. Percentage survival (top) for 395 and 14 patients who underwent primary transplantation and retransplantation, respectively. Differences in percentage of survival between these two groups were not statistically significant. Actuarial freedom from rejection (bottom) for 295 and 12 patients who underwent primary transplantation and retransplantation, respectively. Differences in actuarial freedom from rejection between these two groups were not statistically significant. (Michler et al., "Clinical experience with cardiac retransplantation", *JAMA*, 1998.)

The one-year survival statistics of adult heart retransplanatation from the ISHLT registry is given below.

Table 3.1 Adult Heart Retransplantation One-Year Survival

Inter-transplant Interval (Months)	1-Year Patient Survival (%)		
	High	Low	Close ((High + Low)/2)
1	54.4	38.3	46.3
2	53.7	43.5	48.6
3-6	49.3	19.1	34.2
7-12	74.5	44.7	59.6
13-24	70.4	46.3	58.4
25-36	85.2	61.2	73.2
37-48	91.3	67.2	79.2
49-60	87.3	60.8	74
61-84	88.4	61.6	75
85+	85	69.7	77.3

(Source: ISHLT general registry[5])

The statistics show that there is a much better chance of survival if the inter-transplant interval is greater than 6 months.

CHAPTER 4

HEART RATE VARIABILITY AS AN ASSESSMENT OF CARDIOVASCULAR STATUS

4.1 Background

A complex signal that is commonly recorded in cardiovascular medicine is, the electrocardiogram (ECG). Since the original description by William Einthoven in 1903, the signal has traditionally been regarded as containing much significant information about the heart. This information has, in the past, been derived from the interpretation of short-term portions of the signal, i.e., one or two beats, or at most a short rhythm strip. However, the ECG yields a complex signal that has additional information derivable from longer time period analyses. The information in these longer segments of data forms the basis of heart rate variability study.

In the following sections of this chapter, the neural control mechanisms of the heart, the measurement techniques of heart rate variability, and its role in assessing cardiovascular status, especially after heart transplantation, will be described.

4.2 Neural Control Mechanisms of the Heart

4.2.1Autonomic Nervous System

The autonomic nervous system (ANS) is that portion of the nervous system that controls the visceral functions of the body. This system helps control arterial pressure, gastrointestinal motility and secretion, urinary bladder emptying, sweating, body

temperature, and many other activities, some of which are controlled almost entirely and some only partially.

The ANS is activated mainly by centers located in the spinal cord, brain stem and hypothalamus. Portions of the cerebral cortex and especially of the limbic system can transmit impulses to the lower centers, and in this way influence autonomic control[18]. The autonomic signals are transmitted to the body through two major subdivisions

1. Sympathetic nervous system
2. Parasympathetic nervous system

Sympathetic Nervous System

Figure 4.1 shows the general organization of the sympathetic nervous system.

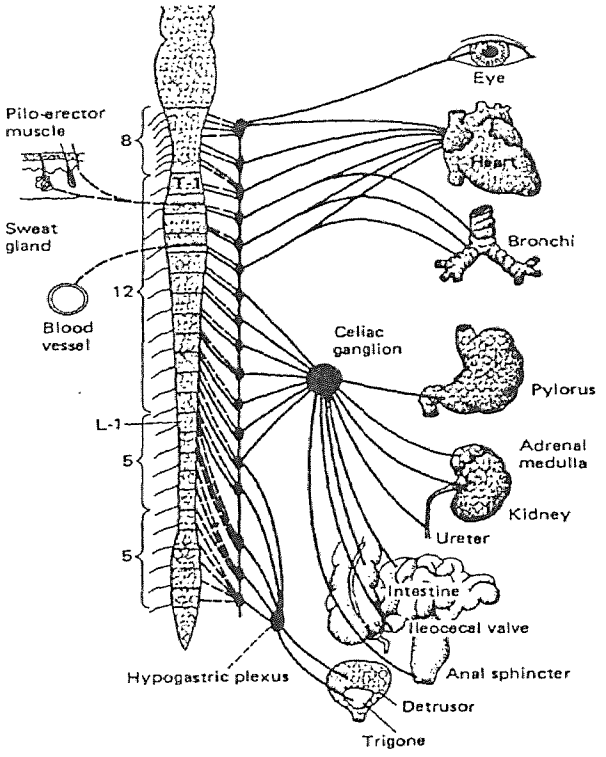

Figure 4.1 The Sympathetic Nervous System. Dashed lines represent postganglionic fibers. (*Basic Neuroscience: Anatomy and Physiology*; Guyton, 1987.)

The sympathetic nerves originate in the spinal cord between the spinal segments T1 and L2 and pass from here into the sympathetic chain, then to the tissues and organs that are stimulated by the sympathetic nerves. Each sympathetic pathway from the cord to the stimulated tissue is composed of two neurons, a preganglionic neuron and a postganglionic neuron. The cell body of the preganglionic neuron lies in the spinal cord and its fiber passes through an anterior root of the cord into a spinal nerve. Immediately after the spinal nerve leaves the spinal column the preganglionic sympathetic fibers leave the nerve and pass through the white ramus into one of the ganglia of the sympathetic chain. The postganglionic neuron originates either in one of the sympathetic chain ganglia or in one of the outlying ganglia. From here the postganglionic fibers travel to their destinations in the various organs. The sympathetic pathways originating in the different segments of the spinal cord are not necessarily distributed to the same part of the body as the spinal nerve fibers from the same segment. The sympathetic fibers from T1 generally pass up the sympathetic chain into the head; from T2 into the neck; T3, T4, T5 and T6 into the thorax; T7, T8, T9, T10 and T11 into the abdomen; T12, L1 and L2 into the legs[18].

Parasympathetic Nervous System

Parasympathetic fibers leave the central nervous system through several 10 out of the 12 cranial nerves and occasionally the first and fourth sacral nerves. About 75% of all parasympathetic nerve fibers are in the vagus nerves (vagus nerve is the tenth cranial nerve), passing to the entire thoracic and abdominal regions of the body. This is the reason parasympathetic activity is also referred to as vagal activity. The vagus nerve

supplies parasympathetic nerves to the heart, the lungs, the esophagus, the stomach, the small intestine, the liver, the gall bladder, the pancreas and the upper portion of the uterus. Parasympathetic fibers in the third cranial nerve flow to the pupillary sphincters and ciliary muscles of the eye. Fibers from the seventh cranial nerve pass to the lacrimal, nasal, and submandibular glands, and fibers from the ninth cranial nerve pass to the parotid gland. The sacral parasympathetic fibers congregate and leave the sacral plexus on each side of the cord and distribute their peripheral fibers to the descending colon, rectum, bladder, and lower portions of the uterus. The sacral group of fibers also supplies the external genitalia to control various sexual functions. The parasympathetic nervous system is shown in figure 4.2.

The sympathetic and parasympathetic nerve endings secrete one of the two synaptic transmitter substances, acetylcholine and norepinephrine. Those fibers that secrete acetylcholine are called cholinergic and those that secrete norepinephrine are called adrenergic. In general, the terminal nerve endings of the parasympathetic system secrete acetylcholine and most of the sympathetic nerve endings secrete norepinephrine. These hormones, in turn, act on the different organs to cause the respective parasympathetic and sympathetic effects[18].

Sympathetic stimulation causes excitatory effects in some organs but inhibitory effects in the others. Likewise, parasympathetic stimulation causes excitation in some organs but inhibition in others. Also, when sympathetic stimulation excites a particular organ, parasympathetic stimulation sometimes inhibits it, illustrating that the two systems usually act reciprocally to each other.

The sympathetic and parasympathetic systems are continually active, and the basal rates of activity are known respectively as sympathetic tone or parasympathetic tone. The value of the "basal" tone is that it allows a single nervous system to increase or decrease the activity of the stimulated organ. The heart is the principal organ or driver of the human body, which is responsible for supplying blood to the different regions of the body, thus maintaining the blood pressure. The following sections attempt to explain the relation between the heart and the autonomic nervous system and the latter's role in influencing the performance of the former.

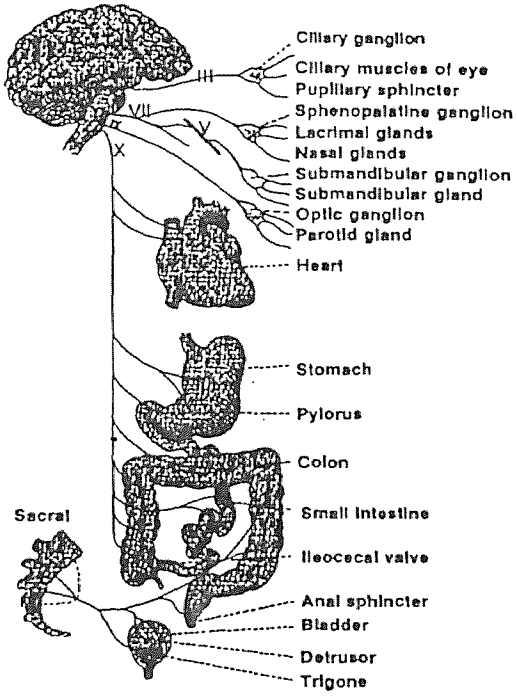

Figure 4.2 The Parasympathetic Nervous System (*Basic Neuroscience: Anatomy and Physiology*; Guyton 1987.)

A model suggested by Fleisher LA for cardiovascular regulation is shown in figure 4.3. This model is a schematic representation of opposing feedback mechanisms that, in addition to central integration, sub serves neural control of the cardiovascular system. Baroreceptors and vagal afferents exert a negative feedback mechanism, whereas sympathetic afferents exert a positive feedback mechanism.

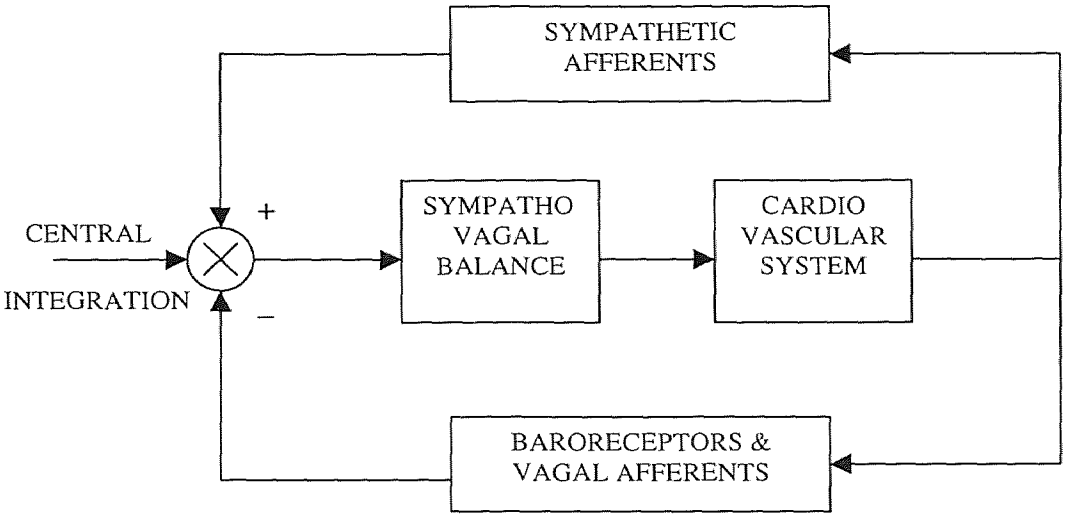

Figure 4.3. Neural control mechanism of the cardiovascular system (Fleisher L.A., "Heart rate variability as an assessment of cardiovascular status," *Journal of Cardiothoracic and Vascular Anesthesia*, 1996.)

4.2.2 Conductive System of the Heart

Figure 4.4 shows the specialized excitatory cells and conductive system of the heart that controls the cardiac contractions[19].

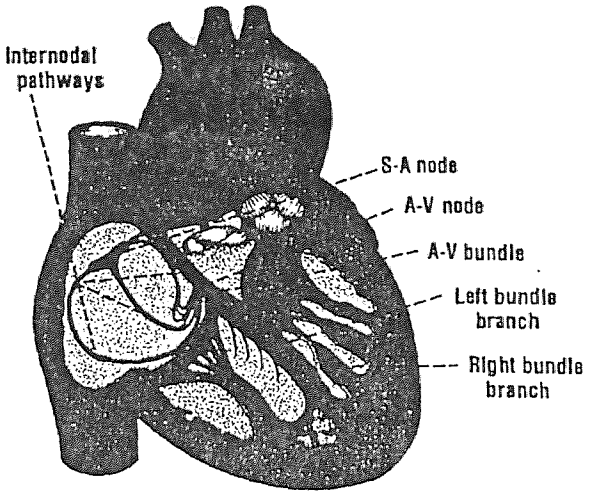

Figure 4.4 The SA node and the Purkinje system of the heart, showing also the AV node, the atrial internodal pathways and the ventricular bundle branches. (*Basic Neuroscience: Anatomy and Physiology*; Guyton 1987.)

The following are the main components that have a role to play in the excitation and conduction mechanism occurring in the heart:

1. *The S-A node*, which is a small, ellipsoid strip of specialized cells, located in the anterosuperior wall of the right atrium. The S-A node is the portion of the heart's specialized conducting system that displays self-excitation property at the highest frequency. The normal rhythmic impulse that paces the heart is generated here.
2. *The internodal pathways* that conduct the impulse from the S-A node to the A-V node

3. *The A-V node*, located in the septal wall of the right atrium that delays the transmission of the cardiac impulse from the atria into the ventricles before ventricular contraction begins, thereby facilitating a coordinated and smooth pumping action.
4. *The A-V bundle*, which conducts the impulse from the atria into the ventricles, and
5. *The left and right bundles of Purkinje fibers*, which conduct the cardiac impulse to all parts of the ventricles.

4.2.3 Autonomic Nervous System and the Heart

The heart is supplied with both sympathetic and parasympathetic nerves as shown in figure 4.5.

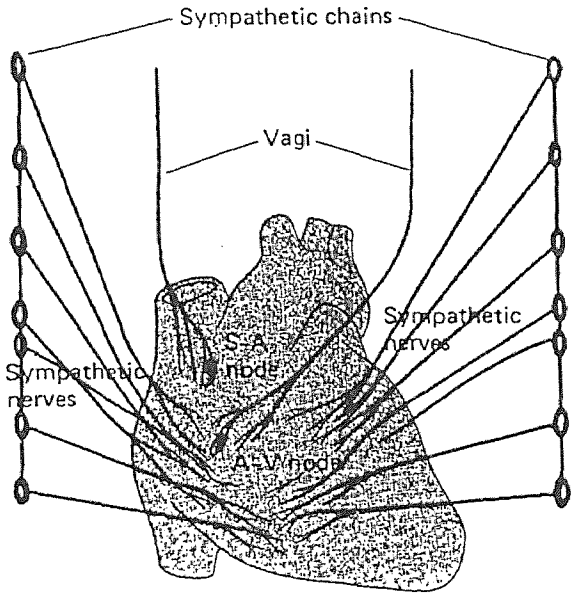

Figure 4.5 The Cardiac Nerves. (*Basic Neuroscience: Anatomy and Physiology; Guyton* 1987.)

The parasympathetic nerves are distributed mainly to the S-A and A-V nodes, to a lesser extend to the muscle of the two atria, and even less to the ventricular muscle. The sympathetic nerves, on the other hand, are distributed to all parts of the heart, with a strong representation to the ventricular muscle as well as to all other areas.

Autonomic Nervous System and Pumping Efficiency of the Heart

The sympathetic and parasympathetic nerves have a strong influence on the pumping efficiency of the heart. The amount of blood pumped by the heart each minute, i.e. the cardiac output (~5 liters per minute) can often be increased more than 200% by sympathetic stimulation.

Effect of Sympathetic Excitation

Strong sympathetic stimulation can increase the heart rate in the humans to as high as 200 to 250 beats/min in young people. Sympathetic stimulation can also increase the force with which the heart muscle contracts, thereby increasing the volume of blood pumped as well as increasing the ejection pressure. During vigorous exercise can even increase sevenfold.

Effect of Vagal Excitation

Strong vagal stimulation of the heart can even stop the heartbeat for a few seconds. Strong parasympathetic stimulation, in addition, can decrease the strength of heart contraction by as much as 20 to 30%. The relatively modest decrease in cardiac

contractility is primarily because the vagal fibers are distributed mainly to the atria and not much to the ventricles, where the power contraction of the heart occurs[19].

The effect of the sympathetic or parasympathetic stimulation on the cardiac output can be gauged from the cardiac function curve shown in figure 4.5.

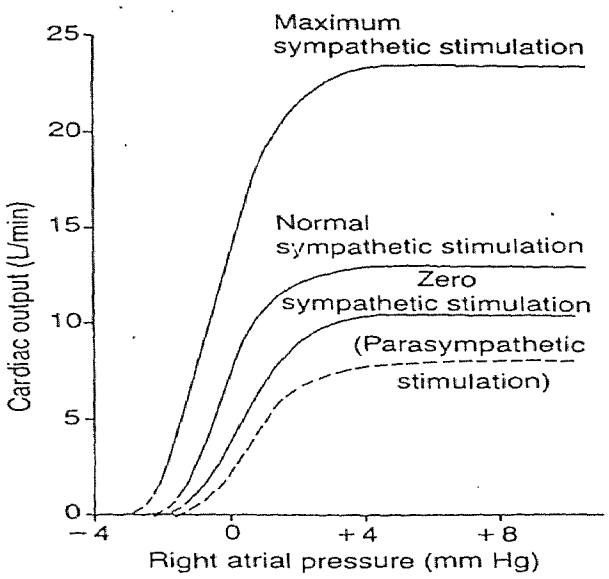

Figure 4.6 Effect on the cardiac output curve of different degrees of sympathetic and parasympathetic stimulation. (*Textbook of Medical Physiology*; Guyton; Eighth edition)

The curves demonstrate that at any given right atrial pressure, the cardiac output increases with increasing sympathetic stimulation and decreases with increasing parasympathetic stimulation. But it is also to be noted that nerve stimulation affects both the contraction strength of the heart as well as the heart rate, both of which play a role in determining the cardiac output. The following section examines the influence of ANS on heart rhythmicity and conduction.

Effect of ANS on the Heart Rhythmicity and Conduction

The parasympathetic nerves are distributed mainly to the SA node and AV node. Its presence in the muscles of the atria, as mentioned before, is less and to the ventricular muscles less yet. The sympathetic nerves on the other hand are distributed to all parts of the heart with a strong presence in the ventricular muscles.

Stimulation of the parasympathetic fibers to the heart results in the hormone acetylcholine being released at the vagal endings. This hormone has two major effects on the heart. First, it decreases the rhythm rate of the sinus node, and second it decreases the excitability of the A-V junctional fibers between the atrial musculature and the A-V node, thereby slowing the transmission of the cardiac impulse into the ventricles. This results in slowing down the heart rate.

Stimulation of the sympathetic nerves releases the hormone norepinephrine. This hormone, firstly, increases the rate of sinus nodal discharge. Secondly, it increases the rate of conduction as well as the level of excitability in all portions of the heart. Thirdly, as described earlier, it greatly increases the force of contraction of all the cardiac musculature, both atrial and ventricular. In short, sympathetic stimulation increases both the heart rate and the strength of contraction.

Systemic blood vessels are one of the other organs affected by the ANS. Most blood vessels, especially those of the abdominal viscera and the skin of the limbs, are constricted by sympathetic stimulation. Parasympathetic stimulation generally has no effect on blood vessels but does dilate vessels in certain restricted areas. Since the sympathetic stimulation can increase both, the propulsion by the heart and resistance to the flow of blood through the blood vessels, the arterial pressure can increase greatly in

response to sympathetic stimulation. On the other hand, parasympathetic stimulation decreases the pumping by the heart, which in turn lowers the arterial pressure by a moderate amount.

Due to the role played by the ANS in regulating all major organs in the body, diagnostic tests to assess the integrity of the ANS and its modulating effects on the heart have been developed. The basic objectives of such tests is to subject the ANS to a known stressor that activates a reflex and measure the response of the end organ, namely the heart. The heart response to such stresses as a change of position from supine to standing, deep breathing, valsalva (blowing into a sphygmomanometer to maintain a pressure of about 40 mm of Hg in it, for 10-15 seconds), emotional stress, and lower body negative pressure has emerged as an index of autonomic control.

4.3 Heart Rate Variability

Resting heart rate (HR) varies widely in different individuals. During various physiological stresses, particularly exercise, it can even increase by threefold. The average value of heart rate in normal individuals in the resting state is around 72 beats/min. This value varies in the range of 60-90 beats/min in a day. During exercise this value could reach as high as 220 beats/min. Age is a limitation to exercise capacity. By heart rate variability is meant the continuous changes occurring in the beat-to-beat interval. Heart rate variability (HRV) is mainly affected by respiration and by blood pressure. Respiration affects the heart rate through the parasympathetic nervous system only[20].

The clinical relevance of HRV was first appreciated in 1965 when Hon and Lee noted that fetal distress was preceded by alterations in interbeat intervals. During the

1970's Ewing et al devised a number of simple bedside tests of short-term RR differences to detect autonomic neuropathy in diabetic patients. Wolf et al first showed the association of higher risk of post infarction mortality with reduced HRV in 1977. In 1981, Akselrod et al, introduced power spectral analysis of heart rate fluctuations to quantitatively evaluate beat-to-beat cardiovascular control[21].

The clinical importance of HRV became appreciated in the late 1980s, when it was confirmed that HRV was a strong and independent predictor of mortality after an acute myocardial infarction. With the availability of digital, high frequency, 24-hour, multichannel ECG recorders, HRV has the potential to provide additional valuable insight into physiological and pathological conditions and to enhance risk stratification[22,23]. The following chapters will investigate the role of HRV analysis in predicting the extent of reinnervation after cardiac transplantation.

Heart rate is dependent, among other things, on the physical fitness, mental stage and age. In addition, even in the absence of external perturbations, the normal heartbeat is not characterized by clockwork regularity. Periodic changes in HR occur at a high frequency due to the influence of respiration throughout the day and night. In addition very slow circadian changes occur that are mediated by neural and hormonal influences and various uncertain influences. Both the basic heart rate and its modulation are primarily determined by alterations in autonomic tone. As was discussed before, increased parasympathetic or vagal tone slows the HR, and increased sympathetic tone increases the HR. It has been shown that normal aging results in a reduction in autonomic control of the heart.

Changes in HR (heart rate variability) may be measured by a number of techniques, and since changes in HR are autonomically mediated, these measurements, reflect autonomic tone. The techniques used for analysis involve both the frequency domain and time domain[24].

4.3.1 Time Domain Methods

In these methods, either the heart rate at any point in time or the intervals between successful normal complexes are determined. In a continuous ECG record, each QRS complex is detected, and the normal-to-normal intervals (NN) intervals (that is, all intervals between adjacent QRS complexes resulting from sinus node depolarization) or the instantaneous heart rate is determined. Simple time domain variables that can be calculated include the mean NN interval, the mean heart rate, the difference between the longest and the shortest NN interval, the difference between night and day heart rate, and so forth[24].

Table 4.1 Selected Time Domain Measures of HRV

Variable	Units	Description
SDNN	ms	Standard deviation of all NN intervals
SDANN	ms	Standard deviation of the averages of NN intervals in all 5-minute segments of the entire recording
RMSSD	ms	The square root of the mean of the sum of squares of differences between adjacent NN intervals
SDNN index	ms	Mean of the standard deviations of all NN intervals for all 5-minute segments of the entire recording
SDSD	ms	Standard deviation of differences between adjacent NN intervals
NN50 count		Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording
PNN50	%	NN50 count divided by the total number of all NN intervals
HRV triangular index		Total number of all NN intervals divided by the height of the histogram of all NN intervals measures on a discrete scale with bins of 7.8125 ms (1/128 seconds)

4.3.2 Frequency Domain Methods

Influences of HRV occur at different periodic oscillations. Frequency domain analysis is a mathematical tool to determine each of these oscillations on the overall heart rate pattern. The underlying principle is that any time series is made up of the combination of much simpler oscillations with differing amplitudes and phases. Fourier transformation algorithms and autoregressive (AR) techniques are used to separate these components. The primary difference between the two methods is that the Fourier transformation contains all of the information of the original time series, whereas the autoregressive technique uses modeling to depict a specific time series and is therefore less dependent on underlying periodicity[24]. The modeling results in smooth curves as an output, which allows better distinguishing of the different peaks.

In order to calculate the Fast Fourier Transformation (FFT), RR intervals must be converted to a series of evenly spaced data points, which may be expressed as either RR intervals or as heart rate. This process is called interpolation. The influence of any ectopic beats must also be removed. Even rare ectopic beats can lead to significant changes in the power spectra, so periods surrounding ectopy are usually replaced with normal interval data. Data must also be stationary over the analysis period; therefore short periods from 2 to 5 minutes are usually analyzed.

If the model order is not too low, AR technique has better resolution than the FFT technique. Figure 4.7 shows the statistical stability (i.e., less noise) in the curve determined by AR estimation. If the model order is too low, there is too much noise and the resolution is not very good. Conversely, if the model order is too high, there is too much smoothing and the signals from the data are not easily detectable[20].

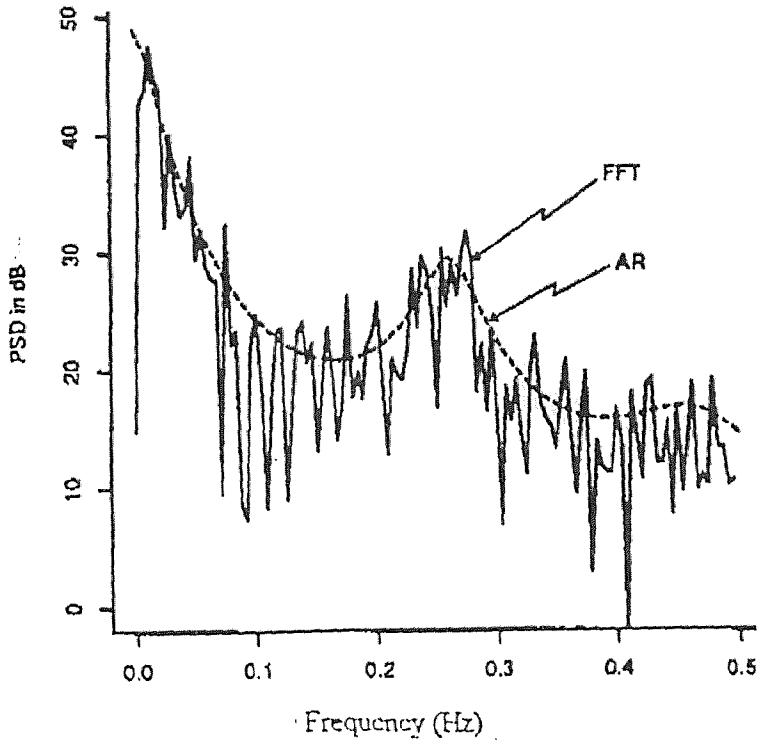

Figure 4.7 Power spectral density curves of the AR technique and the FFT technique using 478 segments of heart period. (Cowan M.J., "Measurement of heart rate variability," *Western Journal of Nursing Research*, 1995.)

The following are the steps involved in interpolation: first the point process signal is derived from the ECG by detecting the R waves. The point process signal consists of an impulse at every time instant that an R wave occurs. The samples between each impulse are then filled in with a constant value corresponding to the duration of the interval preceding that R wave. This is referred to as backward step interpolation. The samples may also be filled in with a constant equal to the inverse of the duration of the previous inter-beat-interval (IBI). Figure 4.8 shows the steps in deriving the backward step interpolated IBI signal.

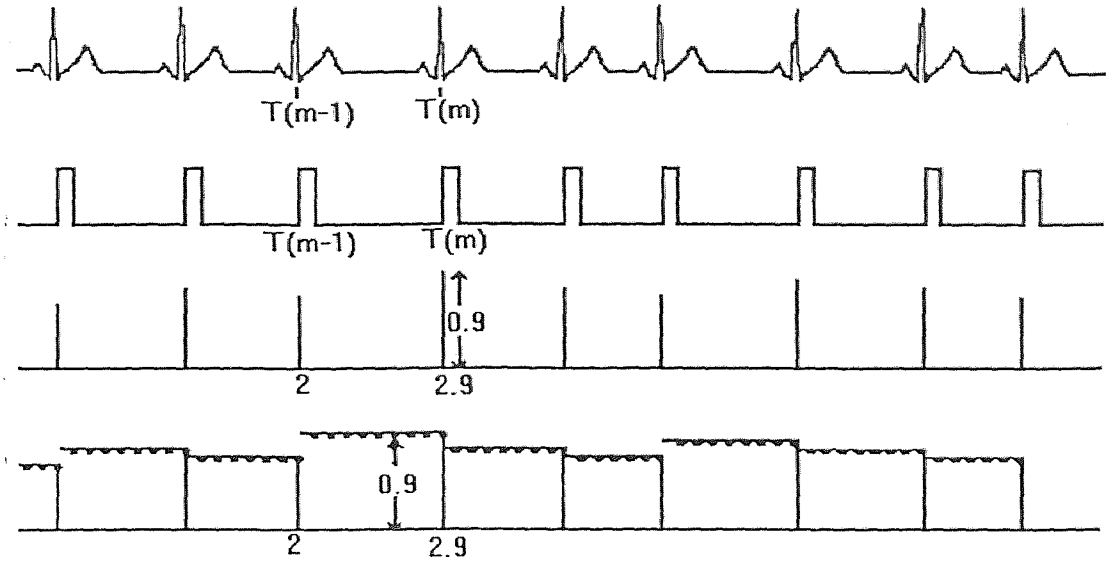

Figure 4.8 Backward step interpolation of the IBI (Shin S., et al., "Assessment of autonomic regulation by the method of complex demodulation," *IEEE Transactions in Biomedical Engineering*, 1983.)

Power spectral analysis of the heart rate has emerged as a powerful technique for the study of the ANS as it provides a window through which neurocardiac function can be assessed noninvasively. The power spectral analysis process transforms a signal from the time domain to the frequency domain. The power spectrum of either HR or BP variability yields three major bands. A low frequency peak that appears within the spectral band ranging from 0.04 Hz to 0.15 Hz is associated with baroreceptor-mediated blood pressure control. A high frequency peak in the range 0.15 to 0.4 Hz is strongly correlated with respiratory sinus arrhythmia. The major activity in this band is due to respiration and a predominant peak usually occurs at the respiration frequency. A very low frequency peak (VLF) between 0.0033 and 0.04 Hz has been linked with vasomotor control and/or temperature control. A fourth band called ultra low frequency (ULF) is also found between 1.15×10^{-5} and 0.0033. Measurement of ULF is based on the 24-hour

recording and reflects circadian rhythms and contains most of the variance in the 24-hour spectrum.[28]

The high frequency band (HF) has been linked with parasympathetic activity; the greater the area under the HF peak, the more active is the parasympathetic system. The low frequency band (LF) is related to both sympathetic and parasympathetic activities.

Selected Frequency Domain Measures of HRV in both short-term and long-term recordings are given in the following tables[24]. The long-term recordings include a ULF component. This is due to the circadian rhythm over the 24-hour period. In order to avoid the problem of "stationarity", spectral analysis performed on the entire 24-hour period as well as spectral results obtained from shorter segments are averaged over the entire 24-hour period. This provides averages of the modulations attributable to the LF and HF components. Such averages obscure the detailed information of the autonomic modulation of RR intervals that is available in shorter recordings. Thus the components of HRV provide measurement of the degree of autonomic modulations rather than the level of autonomic tone[24].

Table 4.2 Analysis of Short-term Recordings (5 min)

Variable	Units	Description	Frequency Range
5-min total power	ms^2	The variance of normal to normal (NN) intervals over the temporal segment	≈<=0.4 Hz
VLF	ms^2	Power in VLF range	<=0.04 Hz
LF	ms^2	Power in LF range	0.04-0.15 Hz
LF norm	nu	HF power in normalized units (nu) HF/(total power-VLF)*100	
HF	ms^2	Power in HF range	0.15-0.4 Hz
HF norm	nu	HF power in normalized units (nu) HF/(total power-VLF)*100	
LF/HF		Ratio LF [ms^2]/HF [ms^2]	

Table 4.3 Analysis of Entire 24 Hours

Variable	Units	Description	Frequency Range
Total power	ms^2	The variance of normal to normal (NN) intervals over the temporal segment	≈<=0.4 Hz
ULF	ms^2	Power in ULF range	<=0.003 Hz
VLF	ms^2	Power in VLF range	0.003-0.04 Hz
LF	ms^2	Power in LF range	0.04-0.15 Hz
HF	ms^2	Power in HF range	0.15-0.4 Hz
α		Slope of the linear interpolation of the spectrum in a log-log scale	≈<=0.04 Hz

The time domain measures correlate strongly with many of the frequency domain measures. HF correlates with RMSSD and pNN50. LF and VLF correlate with SDNN index. ULF correlates with SDNN and SDANN. (Refer to table 4.1).

The following is a typical power spectrum of the heart rate variability[25].

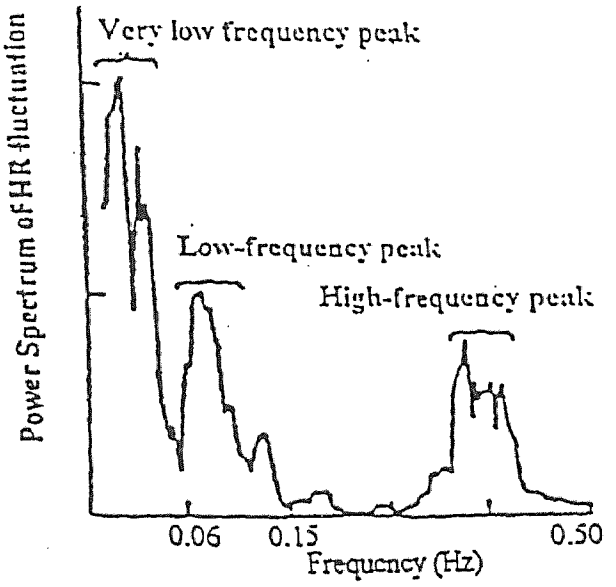

Figure 4.9 A typical power spectrum of the heart rate variability (Kamath M. and Fallen E., "Power spectral analysis of heart rate variability," *Crit. Rev. in Biomed. Eng.*, 1993.)

4.3.3 Standard Measurement of HRV

Standardization is needed particularly in studies investigating the physiological and clinical potential of HRV. To standardize physiological and clinical studies, both short-term and long-term recordings are used. Frequency domain methods should be preferred to time domain methods when short-term recordings are investigated. The time domain methods are ideal for the analysis of long-term recordings.

Equipment designed to analyze HRV in long-term recordings should implement time domain methods, including all four standard measurements (SDNN, SDANN, RMSSD, and HRV triangular index). In addition to other options, the frequency analysis should be performed in 5-minute segments (using the same analysis as with the precision of 5-minute ECGs). When spectral analysis of the total nominal 24-hour record is performed to compute the whole range of HF, LF, VLF, and ULF components, the analysis should be performed with a similar precision of periodogram sampling as suggested for the short-term analysis (with sampling frequency > twice the maximum frequency of the signal).

To ensure the quality of different equipment involved in HRV analysis and to find an appropriate balance between the precision essential to research and clinical studies and the cost of the equipment required, independent testing of all equipment is needed[24]. Because the potential errors of the HRV assessment include inaccuracies in the identification of QRS complexes, the testing should include all the recording, replay, and analysis phases.

The strategy of obtaining the data for the HRV analysis should use the design outlined in figure 4.10.

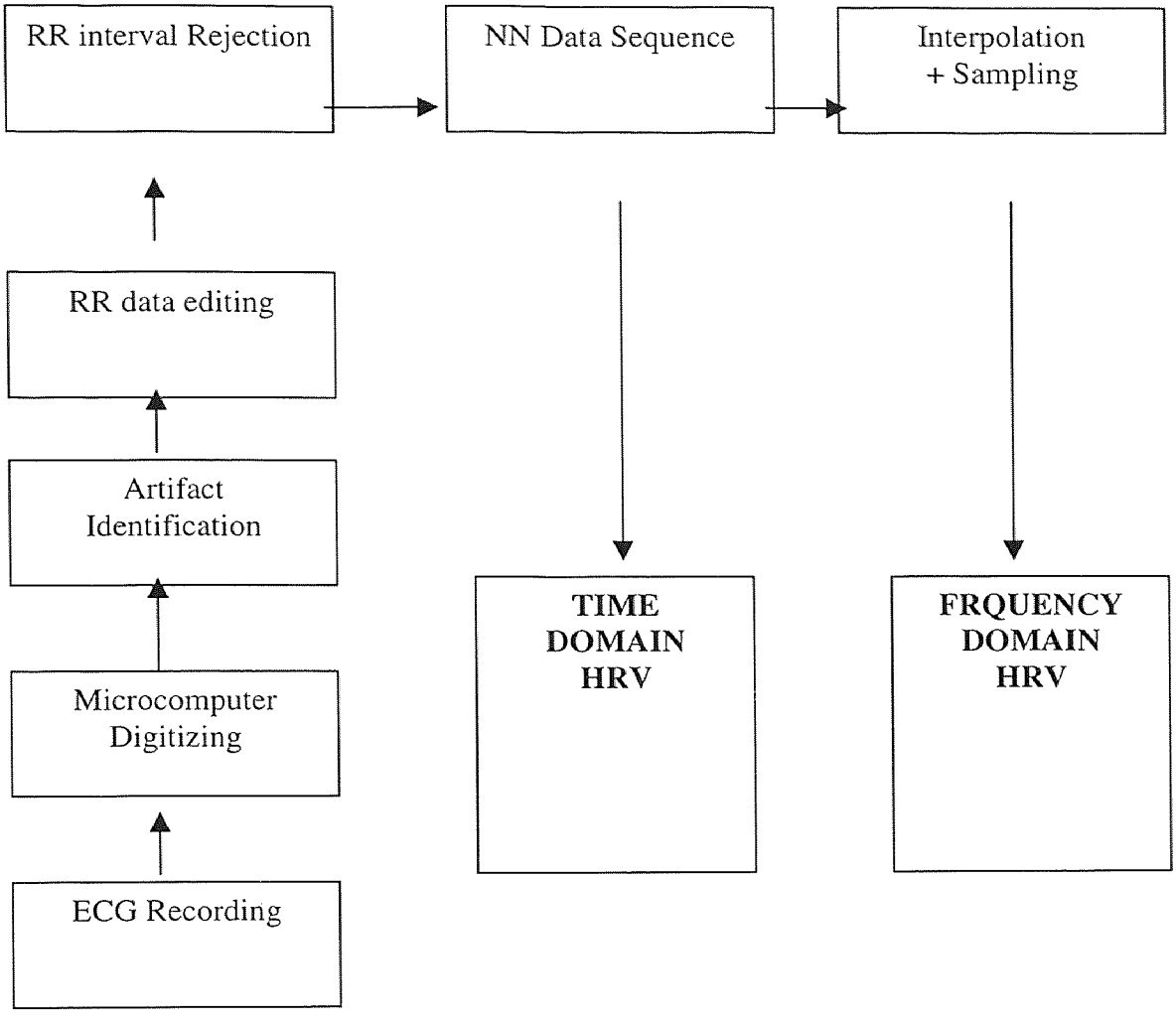

Figure 4.10 Flow chart summarizing individual steps used when recording and processing the ECG signal in order to obtain data for HRV analysis. (Camm A.J., et al. "Standards of heart rate variability," *Circulation*, 1996.)

4.4 Clinical Relevance of Heart Rate Variability

In recent years, a growing number of reports have shown that the analysis of HRV is a valid non-invasive tool capable of providing adequate information on autonomic modulation of the sinus node in normal subjects and in patients with a variety of cardiac and non-cardiac diseases[26]. The basic assumption behind this methodology is that sympathetic and vagal neural activities directed to the heart are largely responsible for the beat-to-beat fluctuations that also characterize heart period during resting controlled conditions[27]. Assessment of HRV may therefore furnish an indirect measure of sympathovagal interaction, which is one of the major determinants of the functional properties of the heart. To validate this hypothesis, an extensive literature search was performed, the results of which are discussed in the following paragraphs.

Power spectral analysis of heart rate has been used to examine the period before the onset of myocardial ischemia. Bigger et al[28] studied 14 patients enrolled in the Mulicenter Study of Silent Myocardial Ischemia. They found that both the LF and HF power decreased to half their control values during segments with ischemic episodes[28]. The ratio of LF/HF power did not change significantly during segments of ischemia. The authors suggest that the pattern of change suggested a predominant decrease in parasympathetic activity during ischemic episodes.

Vardas et al studied 15 patients with three-vessel coronary artery disease. They compared spectral power at five intervals before the start of ischemia and at the end of ischemia. They observed a significant decrease in HF power and an increase in the LF/HF power between the initial intervals versus the start of ischemia.

Beattie and Buckley studied episodes of nonhemodynamically related myocardial ischemia after noncardiac surgery. They observed a decrease in HF power before the onset of ischemia, suggesting a predominance of sympathetic tone over parasympathetic tone.

Both time and frequency domain measures of HRV are reduced in patients with congestive heart failure compared with normal subjects. The degree to which HRV is reduced depends on the severity of the disease. Saul et al studied patients with severe congestive heart failure. They observed only VLF components, usually centered around 0.015 Hz. They suggest that the preservation of the VLF component is due to 60- to 80-second oscillations of heart rate associated with a similar pattern in respiratory activity. This is an indicator of lack of autonomic control, followed by a diminished vagal activity during congestive heart failure.

Decreased HRV is associated with increase in mortality[29]. Kleiger et al. (1987) reported that the relative risk of death was 5.3 times higher in 850 patients followed 31 months after myocardial infarction with a decreased HRV in time domain (standard deviation of normal beat intervals (SDNN) <50 milliseconds) than that of the group with an HRV>100 milliseconds (figure 4.11). This definitive finding has favored wide use of this methodology in patients with diseases like myocardial infarction, congestive heart failure and hypertension. From a general point of view, HRV can be used in clinical practice to estimate 1) the integrity of cardiac autonomic innervation, 2) the physiological status of cardiac autonomic activity and 3) the vulnerability to various cardiac arrhythmias resulting from autonomic imbalance.

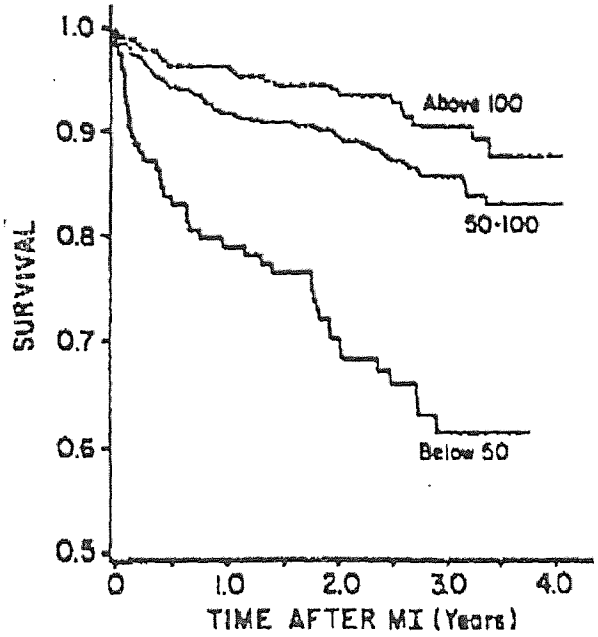

Figure 4.11 Cumulative survival follow-up as a function of heart rate variability (Fleisher L.A., "Heart Rate Variability as an Assessment of Cardiovascular Status," *Journal of Cardio thoracic and Vascular Anesthesia*, 1996.)

The denervated hearts of patients after cardiac transplantation provide a unique model for understanding cardiac autonomic innervation. As a result of the lack of innervation, there is a greatly reduced total spectral power and there are no distinct spectral peaks of HRV in the hearts of these patients. After an interval of more than a year, predominantly low-frequency spectral components might appear that suggest possible cardiac reinnervation. The most important observation for clinical practice seems to be the potential of HRV to detect rejection episodes in the transplanted heart. Reinnervation and HRV after heart transplantation are discussed in detail in the next two chapters.

CHAPTER 5

HEART RATE VARIABILITY AFTER HEART TRANSPLANTATION

5.1 Introduction

Analysis of heart rate variability is regarded as a valid technique to assess non-invasively the sympathovagal balance of the heart. Frequency domain analysis of heart rate fluctuations identifies the relative influence of the two neural limbs (sympathetic and parasympathetic) that regulate heart rhythm. Mortara et al conducted a study to test the hypothesis that power spectrum analysis of heart rate variability in congestive heart failure may identify patients with a more pronounced sympathovagal imbalance and who, as a consequence, could have a poorer prognosis[30]. Power spectral estimates of recipient sinus rhythm fluctuations were used to assess the sympathetic and parasympathetic modulation of the heart after hemodynamic function had been improved by heart transplantation. The methods, results, and conclusions of the study are discussed in the following paragraphs.

Spectral indices of heart rate variability were assessed in 30 patients in severe congestive heart failure (CHF) and in 13 patients after orthotopic heart transplantation[30]. A group of 15 age-matched subjects served as controls. Heart rate variability was assessed by standard ECG in patients in heart failure and by esophageal ECG in patients after heart transplantation. The subjects were in a supine position while recordings were taken. Compared with controls, the mean RR interval and total power of HRV were reduced in heart failure. The 30 CHF patients showed two different patterns of heart rate variability: in 14 no power was detected in the low frequency band (LF) and total power

was mainly concentrated in the high frequency band (HF), whereas in the remaining 16 patients power in the LF band was increased and power in HF band was reduced compared with the controls. Patients with undetectable LF had a lower mean RR interval and total power, higher concentration of plasma noradrenaline, and worse clinical status and prognosis (4 deaths Vs no deaths at 6 month follow up) than patients with a dominant LF band. In the post-transplant group, HRV of the RR interval sequences of the donor heart and the PP interval sequences of the recipient atrium were analyzed. Both the mean PP interval of the remnant atrium and total power resembled results in the patients with heart failure; in 7 of the 13 post-transplant patients no power was detectable in the LF band: when both HF and LF power were present the results resembled those in the 16 patients in heart failure. The rest of the transplant patients had a well-defined low frequency peak though it was of very low amplitude.

These data suggest that in more advanced stages of congestive heart failure, power spectral analysis of heart rate variability allows identification of a subgroup of patients with higher sympathetic activation and poorer clinical status who are at major risk of adverse events. In the short term after cardiac transplantation, the spectral profile of the rhythm variability of the remnant atrium was not improved, suggesting that parasympathetic withdrawal and sympathetic hyperactivity persist, despite the restoration of ventricular function. The lack of heart rate variability short term after transplantation is an indicator of autonomic imbalance, which usually improves with time after transplantation.

5.2 Heart Rate Reactivity Throughout the First Year after Heart Transplantation

Heart rate reactivity (the capacity of the heart to react to mental stress) is substantially blunted early after heart transplantation, suggesting that the loss of neural modulation, limits the cardiovascular response to mental stress. Heart rate reactivity could be used as a measure of reinnervation because better heart rate reactivity is an indication of better neural control of the heart. Shapiro et al tested whether reactivity to mental stress recovers during the first year after heart transplantation[31]. Hemodynamic and respiratory responses to mental arithmetic challenge were studied in 20 heart transplant recipients 3, 6 and 12 months after surgery. A normal comparison group was studied at equivalent intervals. Heart rate reactivity to mental arithmetic was significantly reduced in the cardiac transplant group compared to the normal subjects. This effect persisted up to 1 year after transplantation. Heart period variability in the heart transplant recipients was minimal in all three test sessions. The findings suggest that no functional reinnervation or other compensatory adaptation occurs up to 1 year after heart transplantation.

5.3 Long-term Follow up of HRV in Patients After Heart Transplantation

To evaluate HRV within 5 years of follow-up Folino et al studied 20 patients who underwent orthotopic heart transplantation[68]. Six measurements were taken: one in the first 3 weeks after transplantation, and the others once annually, for 5 years. Twenty healthy subjects constituted the control group. HRV increased in the first 3 years of follow-up. In the following years this trend slackened and values did not reach a statistically significant difference, suggesting that variations in HRV improved significantly during the first 3 years after transplantation as compared to later years. The mean standard deviation of HRV was invariably greater in the control group. These

findings show that sinus rhythm variability in the denervated heart progressively increased over 5 years of follow-up. The factors associated with this increase were increase in beta-adrenergic receptor density or affinity as well as sympathetic reinnervation.

5.4 Reinnervation of the Transplanted Heart

After heart transplantation the heart is denervated, so in principle, no heart rate variability should be present. However, accurate recordings with direct computer acquisition of ECG with increased time resolution (1-2 msec) demonstrate that the RR interval is not fixed, but fluctuates rhythmically, synchronous and coherent with the respiratory signal. The origin of this fluctuation might be a marker of reinnervation or else indicate another mechanism of non-autonomic modulation. Bernardi et al studied the significance of the spontaneous fluctuations in HRV in heart transplant recipients[34]. A brief review of respiration-related and non respiration-related changes in HRV and the responses to various physiological and pharmacological maneuvers are discussed in the following sections.

5.4.1Respiration-related Changes in HRV

Spontaneous Respiratory Sinus Arrhythmia

The heart rate of the transplanted heart is not fixed. Even the first recordings after heart transplantation show heart period fluctuations, though the extent amounts only about 1-4% of that observed in normal subjects. For at least the first year the only fluctuation present is clearly related to respiration, and can be considered as a special type of

respiratory sinus arrhythmia (RSA). However, the phase between the changes in the RR interval and the phase of respiration differs from that observed in normal subjects. It was found to be about 1.5 sec in normal subjects, but in transplanted patients it was significantly shorter, in the range of 0.5 sec.

Changes in Frequency and Depth of Breathing

In normal subjects the RSA has a maximum variability around a respiration rate of 0.1 Hz and decreases with increasing breathing frequency, but transplanted subjects increase their RSA variability with increasing respiratory frequency. Also, while controls show only a minor dependence of RSA on tidal volume, RSA power is much greater with increasing tidal volume in transplanted subjects. Taken together, these results suggest that it is the rate of change in ventilation that that determines the RSA in the denervated heart.

Changes in Heart Rate Induced by Valsalva maneuver

The valsalva maneuver acutely alters the intrathoracic pressure in a "square wave" fashion. This maneuver is divided into four phases. Phase I, a transient increase in systemic blood pressure with the onset of straining, reflects increased intrathoracic pressure. Phase II, a gradual decrease in pulse pressure, and stroke volume due to a decrease in venous return, is often referred to as the active phase of the valsalva maneuver. During phase III (initial release of straining), the blood pressure transiently decreases further as a result of abrupt decrease in intrathoracic pressure. This phase is rapidly followed by phase IV, characterized by an overshoot of the systemic pressure over baseline values. In normal subjects, a progressive tachycardia (increase in HR) and

a sudden bradycardia (decrease in HR) are observed during the strain and release phases of the valsalva maneuver, respectively. Minor or no changes are caused by this maneuver in patients early after heart transplantation.

Changes in Respiratory Components During Physical Exercise

In normal subjects, physical exercise is thought to be responsible for a sudden withdrawal of vagal activity and a progressive increase in sympathetic activity. As a consequence, one expects and sees a rapid decrease in RSA at the beginning of exercise; on the other hand, the increase in ventilation determines also an increase in respiratory-synchronous changes in venous return, which should increase the changes in stretch imposed on the right atrium, and increase RSA. In transplanted subjects, physical exercise actually increases RSA. In transplanted subjects, physical exercise actually increases RSA; the increase is directly correlated with that in ventilation, and inversely correlated with the increase in heart rate: as a consequence, the maximum increase in RSA occurs at the beginning of exercise. With progression of exercise, the slow increase in heart rate seems to counteract part of the effect of the increase in ventilation, and the RSA declines toward baseline levels.

Changes in Respiratory Components During Tilting

Tilting causes an absolute or relative decrease in vagal activity and it also causes a reduction in central venous volume; but the respiratory variations in venous return are unchanged or increased in the upright position. During head up tilt RSA is markedly reduced in normal subjects and this effect is clearly due to autonomic changes.

Conversely, in heart transplant subjects, RSA does not decrease, or in some subjects increases, showing that the behavior of the RSA in these subjects follows that of venous return rather than autonomic activity.

Changes in Respiratory Components During Neck Suction

Neck suction is a noninvasive technique, which selectively (i.e., without other effects other than autonomic) stimulates the carotid arterial baroreceptors. A sudden suction to the neck region normally causes a sudden bradycardia, due to increased vagal outflow to the heart. This response was absent in heart-transplanted subjects. Sinusoidal neck suction at a frequency similar to that of respiration was used to compare the effects of pure autonomic stimulus (i.e., neck suction) with that of a mixed autonomic and mechanical stimulus (i.e., respiration). In normal subjects both stimuli resulted in oscillations in RR interval that could be detected by power spectral analysis. In heart transplanted subjects, only respiration could change RR interval.

Changes in Respiratory Components After Parasympathetic Blockade

Although intravenous administration of atropine (at doses able to effectively block the vagus) greatly reduce overall HRV, there remains a residual RSA of the same order of magnitude as that observed in heart transplant subjects, in whom atropine has no effect. Neck suction at near respiratory frequency is unable to generate high frequency oscillations after atropine, while the respiratory fluctuation remains unchanged in both normal and transplanted subjects.

5.4.1Non Respiration-related Changes in HRV

Spontaneous Fluctuations

A non-respiratory fluctuation in RR interval, particularly in the range of 0.1 Hz, can only be due to autonomic activity, and in the case of heart transplantation this will imply reinnervation. Such non-respiratory fluctuations were found in about half of the subjects in this study who underwent heart transplantation at least 14 months before. The most likely explanation for this is partial autonomic reinnervation, but although non-respiration related LF is considered an acceptable marker of sympathetic activity, this observation is alone not sufficient to decide whether this is due to sympathetic or vagal activity or both.

Effect of Tilt

In most long-term heart transplant subjects the LF components, evident in the supine position, remained unchanged in absolute terms after tilting. This behavior is similar to that of normal subjects; during tilting the LF remain unchanged in absolute terms, while the respiration related fluctuations (HF) decrease, so that in absolute terms the LF increase and the HF decrease.

Effect of Physical Exercise

As physical exercise increases sympathetic activity, an increase in LF could be seen in normal subjects during moderate exercise. Furthermore, due to marked decrease in overall RR interval variability, this increase is evident only in relative terms. LF fluctuations not due to occasional slow breathings could be found only in a limited

number of heart-transplanted subjects and only in subjects after at least two years from transplantation.

Changes in LF Components During Neck Suction

While sinusoidal neck suction at near respiratory frequency was ineffective, stimulation at 0.1 Hz significantly increased the LF in those transplanted subjects who exhibited spontaneous LF at rest. In addition in a limited number of subjects who had no LF at rest, 0.1 Hz fluctuations could be induced by neck suction.

Changes Induced by Pharmacological Interventions

Amyl nitrite causes a sudden direct vasodilation, which elicits an immediate reflex tachycardia in normal subjects, thought to be of sympathetic origin. In this study only transplants with LF could increase their heart rate in response to the drug, further suggesting a sympathetic origin of their LF.

The results of this study[34] are summarized in the tables 5.1 and 5.2. Analyses of the manipulations, which affect the RR interval variability in heart transplant subjects, indicate that the respiratory components largely reflect non-autonomic changes. Amongst the various methods examined, sinusoidal neck suction appears optimal for noninvasive demonstration of the presence and the progress of reinnervation, as it can give information on both the sympathetic and vagal branches (by using different frequencies of neck suction).

Table 5.1 Respiratory Fluctuations (HF) of RR interval in Normal Vs Heart Transplanted Subjects

Stimulus	Normal subject	Transplanted subject
Spontaneous breathing	Present	Present but very small
Voluntary breathing -Frequency ↑ -Tidal volume ↑	 Decreases above 0.1 Hz Small increase	 Increases Big increase
Valsalva maneuver	Tachy/bradycardia	~No effect
Tilt	Marked reduction	No effect or increases
Physical exercise -Beginning -Peak -Early recovery	 Decreases Decreases further Increases	 Increases Remains high but drops Decreases
Neck suction -Impulsive -Sinusoidal, HF	 Bradycardia HF oscillation	 No effect No effect
Atropine	Marked reduction	No effect

Table 5.2 Non-respiratory Fluctuations (LF) of RR Interval in Normal Vs. Heart Transplanted Subjects

Stimulus	Normal subject	Transplanted subject
Spontaneous breathing	Present	Present but small, after > 1 year, in ~ 50%
Tilt	No change in power	No change in power
Physical exercise -Beginning	 Increases	 Might increase
Neck suction Sinusoidal – LF	 Increases LF oscillation	 Increases LF oscillation
Atropine -Alone -With LF neck suction	 Marked drop but present Does not block the increase in LF	 No change Does not block the increase in LF
Beta-Blockade -Alone -With LF neck suction	 Reduces LF (long term) Unknown	 Reduces LF Reduces the increase in LF
Amyl nitrite	Tachycardia	Tachycardia

CHAPTER 6

EVIDENCE FOR REINNERVATION OF THE HUMAN HEART

6.1 Introduction

The presence of cardiac reinnervation after cardiac transplantation in humans has been widely debated based on the application of differing methods for the assessment of neuronal function. Some of these techniques have been rather indirect; consequently, the time course and extent of cardiac reinnervation remains uncertain. In some postmortem studies of human cardiac allografts, nerves and ganglion cells have been demonstrated[58]. Histological studies performed in canine transplant models and dogs have demonstrated considerable reinnervation within the first year after surgery[59]. Wharton et al examined myocardial tissue obtained at surgery from patients undergoing retransplantation. In their study using immunohistochemical techniques, viable intrinsic nerves were demonstrated, but they were unable to demonstrate the presence of extrinsic nerves in the majority of tissues examined[72]. Studies that provide evidence for reinnervation will be discussed in the following paragraphs.

Tio et al conducted a study to find the evidence for differential sympathetic and parasympathetic reinnervation after orthotopic heart transplantation in humans. Heart rate variability analysis was used to investigate signs of reinnervation. Both sympathetic and parasympathetic activity was demonstrated by spectrum analysis of HRV. All HRV parameters were found to be lower in heart transplantation. Another important finding of this study was that heart rate variations were less when the donor was older, and higher in

case of a longer time after transplantation. A study by Koskinen et al also found that heart rate variability increases with post-transplantation time[67].

To evaluate the presence of reinnervation, Bernardi et al investigated the spontaneous variability in R-R interval, supine and after passive tilting in 23 heart transplant recipients and in 25 normotensive control subjects by autoregressive spectral analysis of low- and high- frequency spontaneous fluctuations in R-R interval and respiration[45]. Detectable LF oscillations, unrelated to respiration, were present in 13/23 heart transplant recipients, particularly in those who were transplanted at least 20 months earlier (11/14). The natural logarithm of the power of LF fluctuations was markedly lower than in control subjects. The LF but not the HF correlated with time since transplantation. The conclusion of this study was that signs of functional (reflex) reinnervation could be found in most heart transplant recipients.

To find the electrophysiological evidence of reinnervation of the orthotopic transplanted human heart Wesche et al studied the beat-by-beat heart rate (HR) changes during exercise in two young and fit heart-transplanted humans at different time intervals following transplantation[44]. Upon the start of the exercise, a slow gradual increase in HR was seen during the early experiments after the transplantation, whereas an immediate rapid increase in HR was observed during the later experiments, which suggests reinnervation. From standard ECGs obtained 32 months after transplantation, two P waves at somewhat different rates could be identified in both subjects, probably arising from donor and recipient sinoatrial nodes, respectively. The two P wave rate changes during and following exercise were very similar. The conclusion of this study was that

these changes in the HR pattern and ECG must be due to reinnervation of the donor hearts, most likely by parasympathetic cardiac fibers.

6.2 Sympathetic Reinnervation

In patients studied within 5 months following orthotopic cardiac transplantation, intravenous tyramine administration, which ordinarily causes release of norepinephrine from sympathetic nerve endings, produced a significant increase in systolic blood pressure but no change in HR and no intracardiac release of norepinephrine (Wilson et al, 1991). In transplant patients studied at 12 or more months following surgery, however, tyramine did stimulate a small increase in HR and a significant intracardiac release of NE and a blood pressure response. These findings provide evidence of sympathetic reinnervation in patients late after cardiac transplantation. When the same group of investigators compared heart transplant patients less than and more than 1 year following surgery, they found similar changes over time in the HR response to sinoatrial artery tyramine infusion and in norepinephrine release into the left main coronary artery. Thus, reinnervation is defined as a measure of cardiac norepinephrine release after intracoronary tyramine injection. Burke et al demonstrated evidence for functional sympathetic reinnervation of left ventricle and coronary arteries by tyramine injection. The implication of this study was that stimulation by tyramine of regenerating sympathetic neurons in transplanted hearts can affect both left ventricular contractility and vasomotor tone in a manner similar to that seen in healthy hearts.

Schwaiger et al (1991) reported evidence suggesting regional sympathetic reinnervation among transplant recipients studied more than 2 years after surgery but not among patients studied less than 1 year following surgery. Additional evidence for late

(more than 2 years after transplantation) sympathetic reinnervation of the heart derives from tyramine stimulation studies in two patients with angina pectoris 3 years after transplantation (Stark, McGinn, & Wilson, 1991) and studies of cardiac norepinephrine spillover and HR in a rest and exercise paradigm[57] (Kaye et al., 1993).

Cardiac norepinephrine spillover = $[(NE_{CS}-NE_A)+(NE_A*NE_{EX})]*CSPF$

Where NE_A and NE_{CS} are arterial and coronary sinus plasma concentrations of norepinephrine, NE_{EX} is the fractional extraction of tracer norepinephrine across the heart, and CSPF is the coronary sinus blood flow.

Doering et al[73] evaluated cardiac reinnervation early and late after heart transplantation. Handgrip and deep breathing tests, passive 80 degrees head-up tilt, and heart rate (HR) responsiveness of 33 transplant recipients (n = 16 at < 5 months and n = 17 at > 1 year after transplant) were compared with those of 16 age- and sex-matched control participants. The results of this study suggest that sympathetic reinnervation occurs late (>1 year) after transplantation. The results of this study also suggest that the time elapsed since transplant should be considered when caring for transplant recipients.

Other studies evaluating heart period variability following heart transplantation generally indicate that all measures of variability are dramatically and persistently reduced by cardiac transplantation (Bernardi et al., 1989, Sands et al., 1989, Shapiro et al., 1996). By studying the immediate cardiovascular responses upon active transition from the supine to standing posture in the early and late months after heart transplantation, Rudas et al demonstrated that the development of heart rate responsiveness is compatible with sympathetic reinnervation[54, 55]. Reinnervation has also

been confirmed using PET studies[46, 47] (Demarco et al., 1995, Estorch et al., 1999) and SPECT[48, 49] (Parry et al 1997, Toba et al., 1998, Bengel et al., 1999).

6.2.1 Functional Vs. Structural Reinnervation

The functional evidence for the recurrence of system dynamics late after transplantation addresses the issue of its structural representation. In the intact heart control of heart rate is governed by the extrinsic nervous system, i.e., branches of the central autonomic nervous system, and the intrinsic cardiac nervous system, located in the fat pads near the base of the heart and containing efferent post-ganglionic sympathetic and parasympathetic neurons, local circuit inter neurons, and afferent neurons. The intrinsic system, even after surgical extrinsic decentralization, has been demonstrated to retain some capacity to modulate the heart by administration of substances like isoprotenol, tyramine or nicotine, which stimulate cardiac norepinephrine release (Murphy et al., 1994). Other lines of pharmacological and physiological evidence suggest that this intrinsic system has functions very similar to those that occur with the neural superstructure intact (Armour et al., 1994; Huang et al., 1993; Murphy et al., 1994). Thus, functional occurrence of heart rate control are due to reinnervation of the transplanted allograft by the extrinsic nervous system, but there is also a possibility that the post-transplant recovery of heart rate variability may be due to new organizations and reorganizations of the viable intrinsic cardiac neurons.

Fagard et al conducted a study to find signs of functional reinnervation. Short-term heart rate and blood pressure variability were assessed in 62 patients, studied within 1 month, at 1, at 2 or at 3-5 years after transplantation and in 13 healthy control

subjects[63]. Means and total variances were calculated and the powers of the LF and of HF components were lower in the transplanted patients than in the controls. The total variance and the LF and HF components were lower in the transplanted patients than in the controls. The total variance and the LF and HF powers differed significantly among the groups of transplanted patients and intergroup comparison showed significantly higher values in patients 3-5 years after transplantation than in those studied within 1 month. The results of this study suggest that partial functional reinnervation of the sinus node occurs after heart transplantation.

6.2.2 Regional Differences Sympathetic Reinnnervation After HTX

In the majority of humans ≥ 1 year after cardiac transplantation, cardiac norepinephrine (NE) stores reappear, suggesting late sympathetic reinnervation. To determine whether there are regional differences in reinnervation, Wilson et al studied markers of sympathetic reinnervation of the sinus node (SN) and left ventricle (LV) in five early transplant recipients (<= 4 months after cardiac transplantation), 45 late transplant recipients (≥ 1 year after cardiac transplantation), and 7 normally innervated control patients[49]. SN reinnervation was defined as an increase in heart rate by more than five beats per minute after injection of tyramine into the artery supplying the SN. LV reinnervation was defined as a measurable LV NE release after left main coronary injection of 8 μg/kg tyramine. In 13 patients with previously known LV reinnervation, regional LV reinnervation was assessed by NE release after sub selective injection of tyramine (4 μg/kg) into the proximal left anterior descending and circumflex arteries. Five of five patients <= 4 months after cardiac transplantation had no change in heart rate

and no LV NE release, confirming early, total denervation. In contrast, ≥ 1 year after cardiac transplantation, tyramine caused a heart rate increase (8 to 49 beats per minute) in 32 of 45 patients and LV NE release in 33 of 45. Although LV NE release was correlated with the change in heart rate in late cardiac transplantation recipients, 8 of 45 had only heart rate response, 9 had only LV NE release, and 4 had neither. In late cardiac transplantation recipients with LV reinnervation, tyramine caused NE release from both the anterior descending and circumflex perfusion fields in 10 of 14, but one of 14 patients released NE only after circumflex tyramine and three of 14 only after anterior descending tyramine stimulation. Tyramine caused a marked heart rate increase and LV NE release in all control patients.

Thus this study demonstrated that sympathetic reinnneravtion after cardiac transplantation is regionally heterogeneous. Reinnervation of the sinus node did not imply necessarily that the left ventricle is reinnervated and the reverse, although there was a weak relation between left ventricular and sinus node reinnervation. Similarly, reinnervation within the left ventricle occurred with approximately equal overall frequency in the perfusion fields of the circumflex and left anterior descending arteries, but in individual patients, norepinephrine release was found in the posterior or anterior walls separately or together. The time course of sinus node reinnervation was similar to that of left ventricular reinnervation, becoming detectable about 1 year after transplantation and increasing in magnitude thereafter.

6.3 Sympathetic Reinnervation and Heart Rate Variability

Halpert et al evaluated HRV after cardiac transplantation in an attempt to test if reinnervation occurred in the post-transplant period[65]. HRV was measured using 24-hour Holter recordings performed on 37 ambulant patients 1 to 122 months after cardiac transplantation. All patients were free of histological rejection and were taking no medication likely to influence HRV. Time and frequency domain were analyzed. HRV was found to increase with time after the transplant. Compared with patients in the early post-transplant period, patients >36 months after transplant had lower 24-hour heart rates, an increased average of all 5-minute SD's of NN intervals and higher low- and high-frequency power. Ten of the 27 patients >3 years after transplantation had evidence of functional cardiac reinnervation. The conclusion of this study was that patients late after transplantation have HRV evidence for an increase in sympathetic control of heart.

Heart rate variability (HRV) is reduced immediately after heart transplantation, but is found to increase with time suggesting that reinnervation occurs. Lord et al conducted a study to examine the relationship between sympathetic and parasympathetic reinnervation of the heart, and the various components of HRV using the cardiac transplant recipient as a model. Twenty-four cardiac transplant recipients at the time of routine surveillance coronary angiography two or more years after cardiac transplantation, and 10 controls with normal coronary arteries were used for the study.

Sympathetic effector function at the sinus node was assessed by measuring the fall in RR interval for two minutes after injection of tyramine to the artery supplying the sinus node. HRV was measured from three-minute RR interval sequences at rest, during metronomic respiration, and before and after injection of tyramine. Example patterns of

response to tyramine are shown in figure 6.1 for a control subject, a transplant recipient with no evidence of reinnervation, and a transplant recipient with evidence of reinnervation. Figure 6.1 also shows examples of spectra derived from the resting RR interval sequences in the same subjects. Although there is a dramatic response to tyramine in the transplant recipient with evidence of reinnervation, this is reflected only in an attenuated LF peak, and the continuing absence of an HF peak. Both LF and HF components of the spectrum were significantly lower in transplant recipients compared with controls.

The results of this study showed that the logarithm of the low frequency component of HRV during metronomic respiration was linearly related to the logarithm of the change in cycle length after injection of tyramine. Absolute units more accurately reflected sympathetic effector function than did normalized units or the ratio of low frequency to high frequency. Atropine did not affect HRV in transplant recipients. The conclusion of this study was that the low frequency component of HRV is directly related to sympathetic reinnervation of the sinus node.

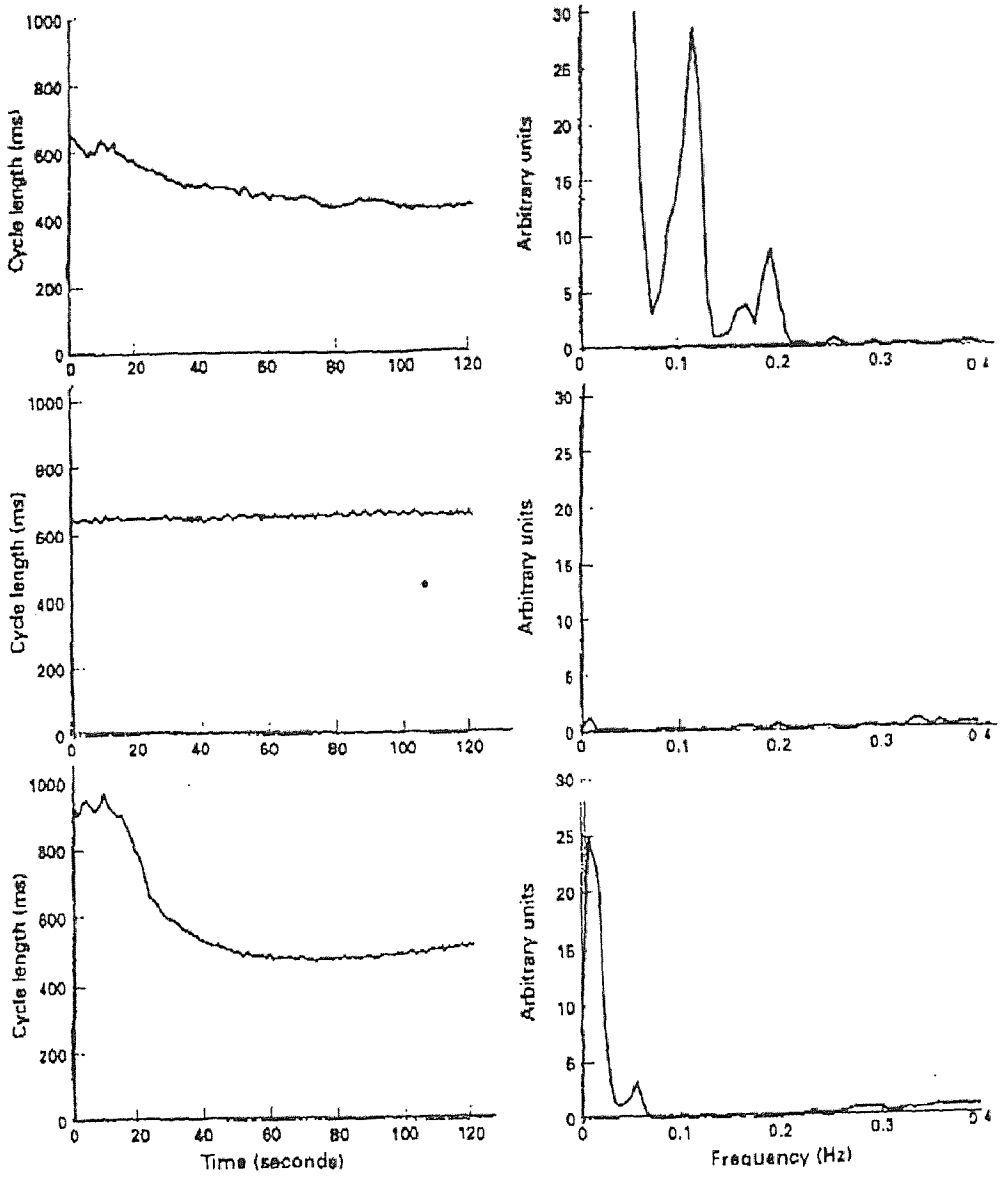

Figure 6.1 Comparison of results from a control subject (upper panel), a transplant recipient in whom there was no evidence of reinnervation (middle panel), and a transplant recipient in whom there was evidence of sympathetic reinnervation (lower panel). The cardiac cycle length response to intracoronary injection of tyramine is shown on the left, and the spectrum from a resting RR interval sequence on the right. (Lord S. W., et al., "Sympathetic reinnervation and heart rate variability after cardiac transplantation," *Heart*, 1997.)

6.4 Exercise Capacity and Reinnervation After Heart Transplantation

Although successful cardiac transplantation improves the patient's quality of life, the cardiovascular responses to exercise remain abnormal for long periods, and the exercise capacity in terms of maximum power output or maximum oxygen uptake is reduced by about 25-50% both in adults and in children. The presence or absence of cardiac reinnervation may therefore be pertinent to the improvement in exercise capacity late after cardiac transplantation. The results from a study by Meyer et al have provided functional evidence for the reemergence of the "dimensionality" of cardiac control, but the time course for recovery to normal appears to be on the order of about 10 years.

Many studies have shown that different components of the heart interact better with time after transplantation. Thus, cardiac mechanics is related to time after surgery. Lord et al conducted a study to investigate the relation between sympathetic efferent reinnervation and exercise response after cardiac transplantation[51]. 25 long-term cardiac transplant recipients and 11 normal controls were used for the study. Intracoronary tyramine was given to the transplant recipients and the percent heart rate change was measured. Exercise tests were performed in patients and controls according to the chronotropic assessment exercise protocol[74], and the percent heart rate reserve (HRR) measured at peak exercise and 6 minutes afterwards to estimate the recovery rate. The following formulas were used for the purpose.

HRR = (HR at peak exercise – resting HR)/(Age predicted maximum HR – resting HR)

6 min recovery = (HR at peak exercise – HR 6 min later)/(Age predicted maximum HR – resting HR)

Mean chronotropic response to exercise workload, and response to tyramine were compared using statistical methods (*t* test). Relation between response to tyramine and exercise variables were quantified using linear regression analysis. The results of the study demonstrated that chronotropic competence and response to intracoronary tyramine vary widely among transplant recipients at more than two years after transplantation. While both techniques measure the changing physiological responses after transplantation, the fact that there is a significant correlation between them suggests that they are both related to sympathetic efferent sinus node reinnervation. In those patients in whom sinus node reinnervation was demonstrated using tyramine, exercise time and total workload were increased as compared to heart transplant patients who had lesser signs of reinnervation. HRR and 6 min recovery were also high in patients with suggested reinnervation by increase in HR after tyramine administration. In normal controls the values for exercise time, HRR, 6 min recovery, and total workload were higher than that for transplant recipients. Thus, this study demonstrates that sympathetic reinnervation has clinical consequences.

To assess the development of HR responses to exercise, Rudas et al studied HR changes in response to orthostasis and treadmill exercise in 52 orthotopic cardiac transplant recipients[53]. HR response to standing in normal subjects was compared with HR in transplant recipients the results of which are shown in figure 6.2 (RR interval (ms) Vs. cardiac cycle). In early heart transplant patients (group 1) there was no significant change in heart rate for up to 100 cycles after standing. Patients 1-2 yr after transplantation (group 2) showed an increase in HR by the sixteenth to twentieth cardiac cycle after standing. Late transplant patients (group 3) were found to have significant

shortening of the RR interval by the fifth cardiac cycle after standing. HR responses on standing, during early exercise, and after exercise are shown in figure 6.3. Marked differences in HR recovery of the three groups where seen early after exercise. HR deceleration 1 minute after exercise was also related to the time after transplantation. The results of this study indicate development of functional reinnervation after orthotopic heart transplantation. The phenomenon of early acceleration of the HR after orthostasis (standing up from a supine position) and rapid deceleration after exercise in transplant recipients implies a local cardiac mechanism rather than response to circulating catecholamines.

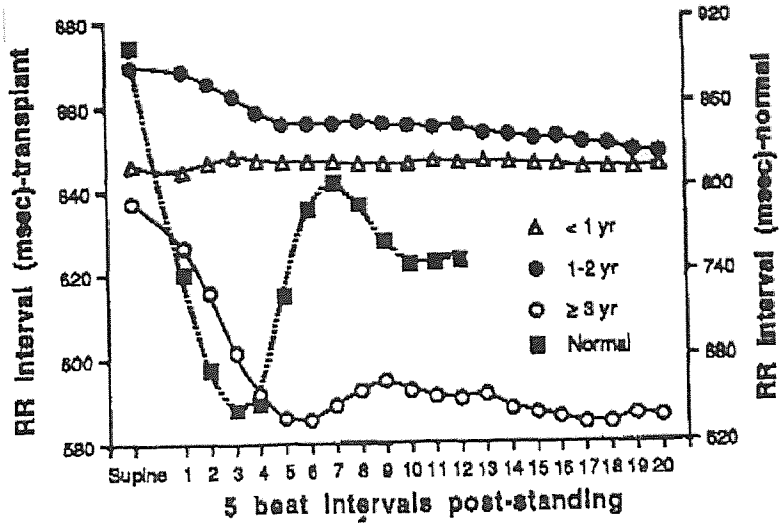

Figure 6.2 Changes in RR interval after standing in normal subjects and heart transplant recipients at varying times after heart transplantation. Different scale in RR interval is used for normal (right) and transplant (left) subjects. (Rudas L., et al., "Evolution of heart rate responsiveness after orthotopic cardiac transplantation," *The American Journal of Cardiology*, 1991.)

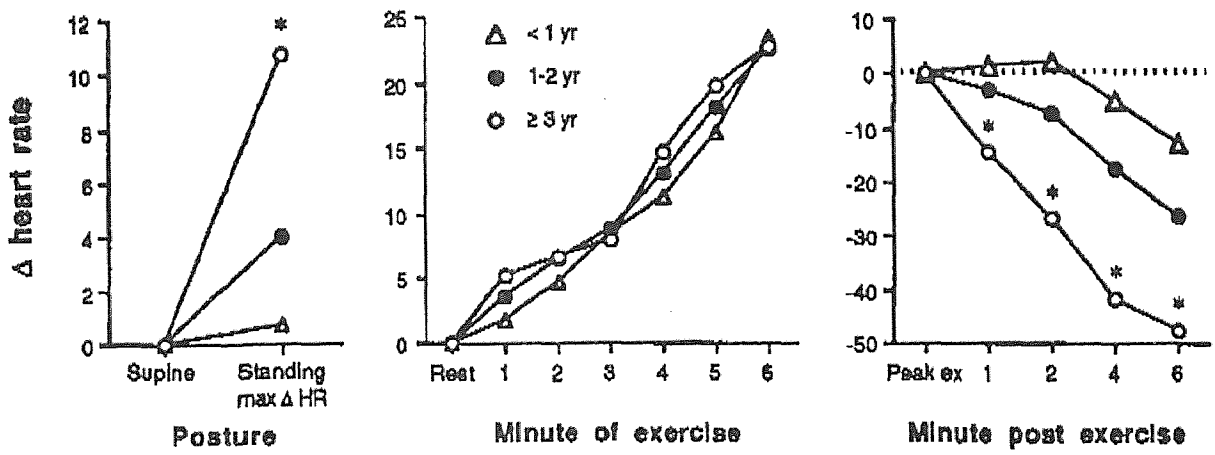

Figure 6.3 HR responses on standing (left), during early exercise (middle), and after exercise (right) in heart transplant recipients at varying times after heart transplantation. (Rudas L., et al., "Evolution of heart rate responsiveness after orthotopic cardiac transplantation," *The American Journal of Cardiology*, 1991.)

6.5 Detecting Acute Graft Rejection in Patients After Orthotopic Heart Transplantation: Analysis of HRV in the Frequency Domain

Acute transplant rejection (AR) is a major complication after heart transplantation. To treat patients with rejection, early detection is necessary before fatal heart dysfunction. Because of its invasive aspect, endomyocardial biopsy is limited with respect to its frequency and complication rate. Frey et al investigated the potential of HRV as a non-invasive tool for continuous follow-up of patients who have undergone heart transplantation to detect AR at an early stage. In an attempt to test this hypothesis, Sands et al. have demonstrated an increased HRV during AR. However, no respiratory sinus arrhythmia could be observed. In contrast to that, Zbilut et al. have shown respiration induced periodicity in HRV and report a decrease in HRV during AR when approaching the respiration frequency. The study of Frey et al., which will be described in the following paragraphs, was designed to establish which HRV-frequency components reliably detect AR and to gain an insight into possible underlying mechanisms of HRV changes during AR.

After endomyocardial biopsy in 117 heart transplant recipients (age 49±13 years, time after transplantation 6 to 48 months), electrocardiography results were recorded with the patient in a supine position at a controlled respiration rate (0.1 and 0.25 Hz) over 10 minutes. To minimize the effects of medication, only patients who underwent HTX greater than 6 months were enrolled. Patients with an artificial pacemaker or severe arrhythmia were excluded. Following these criteria, a total of 156 electrocardiographic recordings for both controlled respiration rates were retained. The spectra of RR intervals were calculated, and different spectral components were evaluated: LF, HF, LF+HF, LF+HF-RP (RP=respiratory peak), and the total power.

On the basis of the 156 electrocardiograms analyzed and endomyocardial biopsy reports, three groups of patients were defined: Group I-patients without rejection (AR0, n=73), group II-patients with mild rejection (AR1a, n=51), and group III-patients with severe rejection (AR1b to AR4, n=32). To investigate the performance of HRV in discriminating between groups of patients with respect to AR, statistical measures such as sensitivity and specificity were used. For each frequency domain parameter, sensitivity and specificity curves as function of varying threshold value were calculated. All the frequency domain parameters except HF were significantly higher in patients with severe AR than in patients without AR. The comparison between groups I and II and groups II and III showed a significant difference only in LF.

To compare the changes of HRV during AR with mechanically induced changes during a controlled respiration frequency, the electrocardiograms monitored at the low respiration rate (0.1 Hz) were analyzed. During this slow respiration rate, LF was significantly higher in patients with severe AR than without AR. For heart transplant recipient groups I and II, LF was compared at respiratory rates of 0.1 and 0.25 Hz. As expected a significant decrease of LF was observed in patients with no AR when shifting the respiratory rate from 0.1 to 0.25 Hz. However, in heart transplant recipients with severe AR the decrease of LF when increasing the respiratory rate was not statistically significant.

Thus the results of this study suggest that power spectral analysis of HRV is a promising tool for the quantification of acute graft rejection in patients after heart transplantation. With this simple and noninvasive technique, it was shown that the power spectrum density of HRV increases during AR and thus mild-to-severe acute graft

rejection could be detected (figure 6.4). Compared to heart transplant recipients with no evidence of AR, a significant increase in HRV was observed in those showing severe AR. On the basis of this study, the increase of HRV power is due to an increase in signal noise rather than in the amplitude of periodic components. The evolution of different parameters in the frequency domain of HRV with respect to AR was analyzed. The calculation of sensitivity-specificity relation showed that the most reliable parameter in the frequency domain for AR detection is LF+HF-RP.

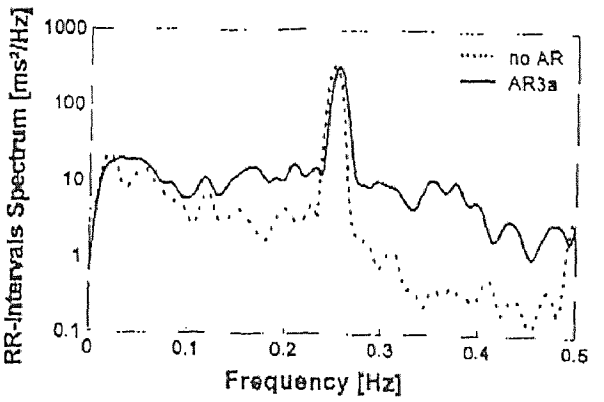

Figure 6.4 HRV power spectral density for same heart transplant recipient during no AR episode (dashed line) and during AR episode (solid line). (Frey A.W., et al., "Detecting acute graft rejection in patients after orthotopic heart transplantation," *Journal of Heart and Lung Transplant*, 1998.)

The study conducted by Uberfuhr et al to estimate the predictive power of HRV in detecting AR yielded similar results[63]. The conclusion of this study was that during rejection the variability increases significantly both in the time domain and in the frequency domain. This was attributed to an irregular increase of the amplitude of the beat-to-beat variability of QRS distances.

6.6 Parasympathetic Reinnervation

The presence of parasympathetic reinnervation is still controversial and no unequivocal evidence has been reported with conventional transplantation. Many authors have not observed any sign of parasympathetic reinnervation. Arrowood et al conducted a study to determine if reinnervation of chemosensitive endings subserved by cardiac vagal afferents (endings connected to the central nervous system by fibers running alongside vagal fibers) occurs after human orthotopic heart transplantation[66]. Two cardiac groups were studied: an "early" group (n=18, <24 months after transplant) and a "late" group (n=18, >43 months after transplant); these groups were compared with a control group with intact innervation (n=18). The reflex response of the recipient sinus node (RSN) in the remnant right atrium, which remains innervated after transplantation, was observed during selective right coronary artery and left coronary artery injection of a radiographic contrast, to stimulate ventricular chemosensory nerve endings. A decrease in the rate of RSN was expected if reinnervation of chemosensory endings had occurred and the afferent limb of the cardiac depressor reflex was intact. With injection, the RSN rate of both transplant groups did not decrease but increased compared with the expected decrease in control patients. The results obtained from this study suggest that reinnervation of ventricular chemosensory endings subserved by vagal afferents in cardiac transplant patients does not occur up to 74 months after transplantation.

Raczak et al conducted a study to test the presence of parasympathetic reinnervation by assessing the baroreflex sensitivity in 30 patients 1-24 months after transplantation[69]. The results of this study suggest that vagal efferent reinnervation of the donor heart does not occur up to 24 months in patients who underwent heart

transplantation by the Lower and Shumway method. The study also suggested that analysis of baroreceptor reflexes is a more specific method in the examination of cardiac parasympathetic reinnervation.

Fitzpatrick et al (1993) found evidence for vagal reinnervation in transplant recipients. Among 10 patients studied at a mean post surgical interval of 22±12 months, 7 had a vasovagal response to tilt testing and 3 had slowing of the graft heart (which can be due to parasympathetic activity). Some studies assessing heart period variability have demonstrated a trend toward increasing HF power over time (Fallen et al., 1988, Kaye et al., 1993) and correlation with exercise studies has led to the suggestion that parasympathetic reinnervation begins to have a functional effect only two or more years following heart transplantation (Kaye et al., 1993).

To find the signs of vagal reinnervation, Uberfuhr et al investigated the spectral analysis of HRV in 13 patients after heart transplantation[63]. 18 healthy volunteers were selected as control group. For each patient two recordings were performed which took part 14±5 months and 42±8 months respectively after heart transplantation. ECG was recorded simultaneously for 5 min in supine position during controlled respiratory rate of 12 or 15 cycles/min. No graft rejection has been detected in the endomyocardial biopsy performed right after the recordings. Power spectral densities were calculated for the beat-to-beat time series of RR intervals.

The figure 6.5 illustrates the power spectral densities from a patient at its first (16 months) and second (30 months) recording and visualizes the increase of power spectral densities in the low and high frequency band. The differences of the values of the power spectral densities between transplanted patients and control subjects are striking, in

particular, in the low frequency band. A significant increase of LF and HF power during a mean period of 28 months could be found.

The conclusion of this study was that an increase of HF band suggests parasympathetic reinnervation. This study also confirms that the LF band increases 4 years after transplantation, suggesting a possible sympathetic reinnervation of the heart.

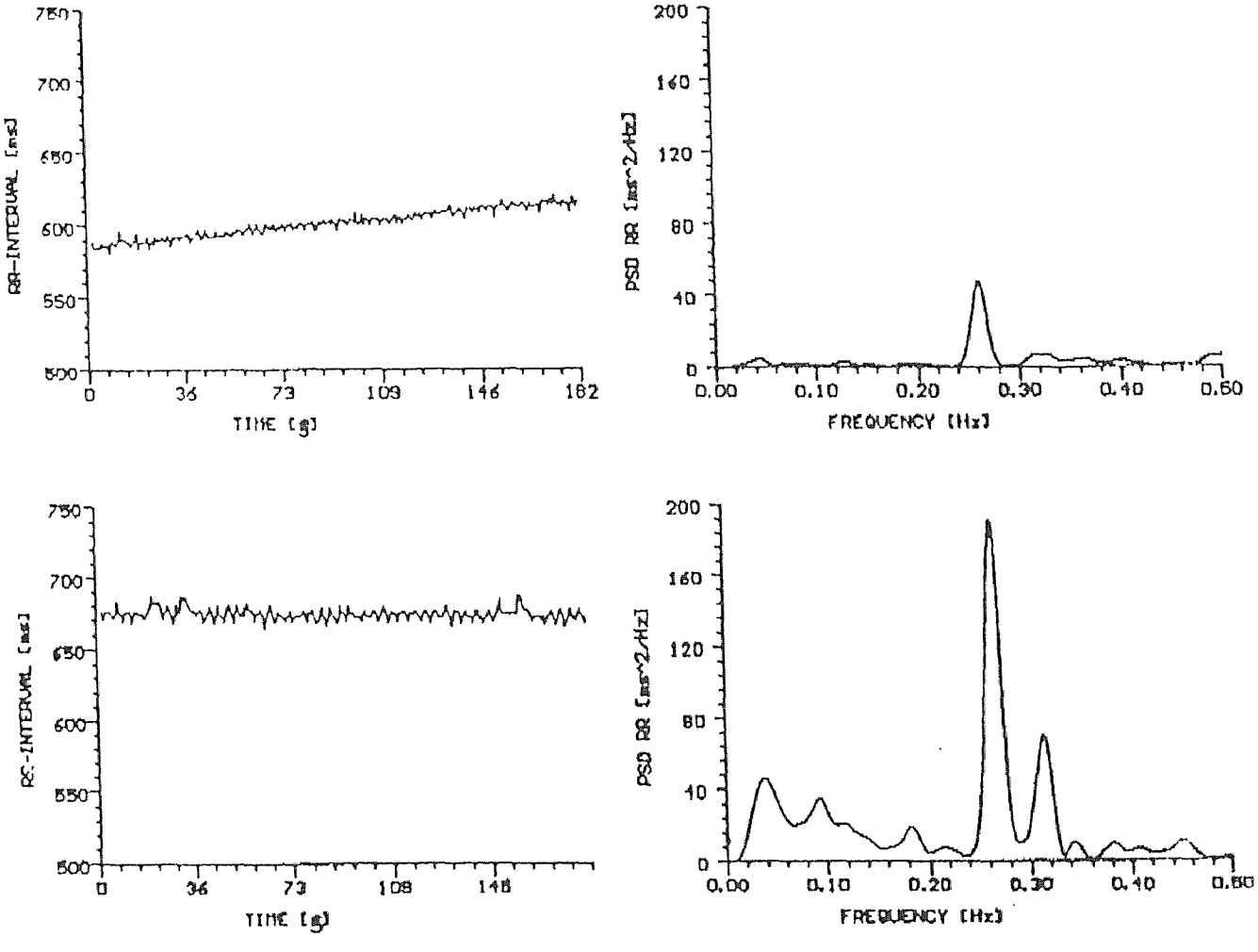

Figure 6.5 Top: tachogram and power spectrum of a patient 16 months after HTX; Bottom: recordings of the same patient 30 months after HTX. (Uberfuhr et al, "Signs of vagal reinnervation 4 years after transplantation in spectra of heart rate variability," *European Journal of Cardiothoracic Surgery*, 1997.)

6.7 Influence of Type of Surgery on the Occurrence of Parasympathetic Reinnervation After Cardiac Transplantation

Standard surgical techniques leave most of the recipient atria intact and most of the parasympathetic axons also remain intact and thus might not be stimulated to regenerate. Conversely, the bicaval technique (by which the whole recipient heart including the entire atrial junctions of both superior and inferior venaecavae are removed (orthotopic transplantation) and substituted with equivalent components of the donor heart) cuts 100% of both sympathetic and parasympathetic fibers and can therefore stimulate both branches to regenerate[70].

To investigate the influence of type of surgery on the occurrence of parasympathetic reinnervation after cardiac transplantation Bernardi et al studied 89 orthotopic heart transplant recipients, 10 with bicaval and 79 with standard surgery[70]. Changes in the R-R interval power spectrum induced by sinusoidal modulation of arterial baroreceptors by neck suction at different frequencies were used to detect both sympathetic and parasympathetic reinnervation. In 24 subjects (17 standard and 7 bicaval), the protocol was repeated 6 and 11 months after transplantation. Neck suction at 0.2 Hz produced a component at 0.2 Hz in the RR-interval spectrum not due to respiration (fixed at 0.25 Hz), which suggested parasympathetic reinnervation, in 4 of 10 bicaval but in only 2 of 79 standard transplant subjects (whose recipient atria underwent >50% resection to remove scars of previous interventions). In only 1 (bicaval) transplant subject was parasympathetic reinnervation present 6 months after transplantation (confirmed 3 months later); in 4 subjects, it was absent at 6 months but appeared after 11 months after transplantation. Atropine abolished the HF response to fast (0.02 Hz) and

reduced that to slow stimulation, confirming the presence of parasympathetic reinnervation (4 subjects).

The present study confirms that a rudimentary sympathetic reinnervation is relatively frequent, occurring in ≈ 50% of the heart transplant recipients. Thus, whereas, after standard surgery, only sympathetic reinnervation is common, after bicaval surgery the frequencies of parasympathetic and sympathetic reinnervation are similar (4 of 10 versus 6 of 10, respectively, 9 months after transplantation). All this indicates that the surgical technique plays a major role in the probability of subsequent development of parasympathetic reinnervation.

The distributions of neural endings in the two surgical techniques are shown in figure 6.6.

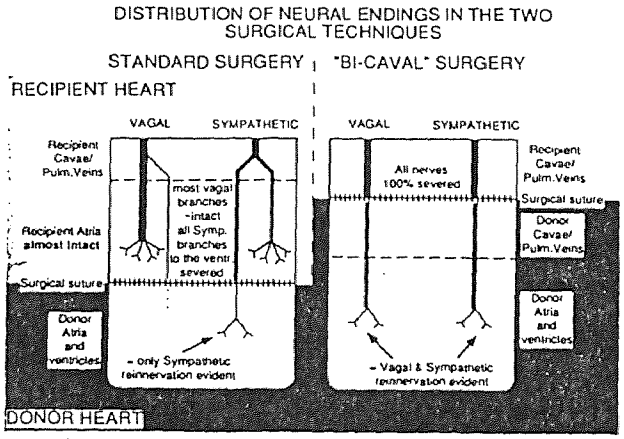

Figure 6.6 Schematic of different distribution of neural endings and possibility of reinnervation in bicaval vs. traditional surgery (Bernardi et al., "Influence of type of surgery on the occurrence of parasympathetic reinnervation after cardiac transplantation," *Circulation*, 1998.)

6.8 Relative Frequency of Functional Sympathetic and Parasympathetic Reinnervation After Heart Transplantation

Tio et al performed a study to investigate the sympathetic and parasympathetic reinnervations and the results of the study showed that parasympathetic reinnervation could occur with or without the signs of sympathetic reinnervation; sympathetic reinnervation without parasympathetic reinnervation was not found.

Brunner-La Rocca et al. conducted a study to determine the relative frequency of sympathetic and parasympathetic reinnervation[71]. The study subjects comprised 65 patients 3 to 110 months after undergoing orthotopic heart transplantation and 16 healthy volunteers. At the time of the study, patients were free from moderate or severe graft rejections, had no features of heart failure, and were in a stable sinus rhythm. Control subjects were free from heart medication and had no history, symptoms, or signs of cardiovascular disease. The heart rate response to various maneuvers (standing up, handgrip exercise, phase 2 of Valsalva maneuver for sympathetic function, carotid sinus massage, phase 4 of Valsalva maneuver, and atropine for parasympathetic function) was examined in the study subjects and in the control subjects. Reinnervation was defined as either one normal (>50% of control group) and at least one partial (>33% of control group) heart rate response or partial responses in all three tests of the respective part of the autonomic nervous system.

Changes of heart rate to the different maneuvers were overall markedly diminished in patients after heart transplantation as compared with healthy volunteers. However, a considerable number of patients had a partial or a normal heart rate response. Thirty-five (54%) patients had sympathetic reinnervation, but only 16 (25%) had parasympathetic reinnneravtion; earliest reinnervation was found 11 months after

transplantation, and all but one patient with parasympathetic reinnneravtion also had sympathetic reinnnervation. The frequency and extent of parasympathetic reinnneravtion, late after transplantation (>5 years) were markedly lower as compared with sympathetic reinnneravtion (74% Vs 30%). As the type of surgical technique (bicaval or standard) used for heart transplantation in this study was not clearly indicated in the literature, this difference in reinnervation can be attributed to the type of surgery[70].

CHAPTER 7

CONCLUSIONS AND FUTURE STUDIES

In recent years, analysis of the HRV signal in time and frequency domain has revealed important physiologic and prognostic information. Extensive literature review of articles that describe reinnervation and HRV after heart transplantation was performed, the conclusions of which are summarized in the following paragraphs.

From the survival statistics obtained from ISHLT and UNOS databases it is evident that heart transplantation has emerged as a successful treatment of end stage heart disease. The major continuing limitation to the volume of heart transplantation has been that of donor availability. Because left ventricular assist devices (LVAD) could be made readily available without the need for a waiting list, they might compete well with the strategy of heart transplantation. Although further study is required to overcome the limitations of LVADs, the recent advances made in this field are promising.

Heart rate variability was found to be substantially blunted early after heart transplantation. Long-term follow up studies after heart transplantation, which were reviewed in the course of this study, have shown that HRV improves with time after transplantation. This can be attributed to the reinnervation after heart transplantation. Thus, HRV is an efficient tool in measuring the extent of reinnervation.

Some of the studies that were reviewed in the course of this project are given in table 7.1. HRV was the tool used in most of the studies.

Table 7.1 A Review of Methods and Conclusions of Various Studies on Patients After Heart Transplantation

Authors	No: of patients	Technique Used	Conclusion
Rudas et al, 1991	52	HR responses to treadmill exercise	Functional reinnervation
Kaye et al, 1992	15	HR responses to exercise + Neurochemical studies	Restoration of cardiac sympathetic nerve function
Mortara et al, 1994	30	HRV	HRV can predict extend of recovery
Bernardi et al, 1994	26	HRV	Reinnervation
Burke et al, 1994	56	Tyramine injection	Reinnervation
Fagard et al, 1995	62	HRV	Reinnervation
Shapiro et al, 1996	20	Heart rate reactivity + Heart period variability	No functional reinnervation occurs upto 1 year after heart transplantation
Lord et al, 1996	25	HRV+ Tyramine injection	Sympathetic reinnervation
Koskinen et al, 1996	38	HRV	Reinnervation increases with post transplantation time
Halpert et al, 1996	38	HRV	Reinnervation
Lord et al, 1997	24	HRV + Tyramine injection	Reinnervation
Uberfuhr et al, 1997	13	HRV	Parasympathetic reinnervation
Tio et al, 1997	16	HRV	Reinnervation
Estorch et al, 1998	31	SPECT	Reinnervation
Bengal et al, 1999	30	PET	Reinnervation is heterogeneous

The main conclusion of this research was that sympathetic reinnervation of the transplanted human heart can occur > 1 year after transplantation. Reinnervation was found to increase with time after heart transplantation and was seen more frequently 2 years post-transplantation. Also, complete reinnervation of the transplanted heart was not found to occur even up to 12 years post-transplantation. Reinnervation was found to be regionally heterogeneous. The findings of this study also suggested that reinnervation is

less likely to occur in patients with a pretransplantation diagnosis of idiopathic cardiomyopathy than in those with other etiologies of congestive heart failure. Studies have shown that vast majority of graft rejection episodes, which is one of the major complications after heart transplantation, occur before cardiac reinnervation. So HRV analysis can be applied in assessing the prognosis after heart transplantation.

Exercise response of the transplanted heart was found to improve with reinnervation. It was found that sympathetic reinnervation enables an increased peak oxygen uptake, which means that reinnervation precedes functional recovery. Hence, reinnervation can be used a promising measure of the cardiovascular status.

The type of surgical technique employed in the heart transplant procedure was found to influence parasympathetic reinnervation. Most of the studies suggested that the extent of parasympathetic reinnnervation, late after transplantation (>5 years) were markedly lower as compared with sympathetic reinnnervation (74% Vs 30%). Also, a higher donor age was found to reduce signs of reinnervation.

Despite the fact that HRV has been applied in a broad spectrum of cardiovascular diseases, general agreement has been reached so far only in two clinical conditions: 1) Impaired HRV can be used alone or in combination with other factors to predict risk of arrhythmic events after acute myocardial infarction, and 2) decrease in HRV is a successful clinical marker of evolving diabetic neuropathy. Recently, its role in the evaluation and management of heart failure has also been recognized. Although several earlier studies have reported on the clinical and prognostic value of heart rate variability analysis in the cardiovascular assessment of patients after heart transplantation, this technique has not been incorporated into clinical practice. The determination of exact

sensitivity, specificity, and predictive value of HRV still requires further investigation before standards can be set for applying this technique in finding the reinnervation after heart transplantation. The measurement of HRV appears to be a well-established research tool and is useful in the assessment of the function of central and peripheral modulators involved in the control of the heart's beat-to-beat variability. Its clinical applicability still remains limited because of the complex methodology and lack of standardization.

Large population studies with follow-up are needed to establish normal HRV standards for various age and sex subsets. If standardization is followed, HRV has considerable potential to assess reinnervation after HTX. Also, HRV analysis could be used as a tool to evaluate the chances of survival in patients HTX. Follow-up studies on a large group of patients are necessary to confirm the conclusions of this research.

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