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TITLE: Sympathetic reinnervation in cardiac transplant recipients: Prevalence, time course and association with long-term survival

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Standard abbreviations: CTRs = Cardiac Transplant Recipients; HRV= Heart Rate Variability; LF=Low Frequency

Key Words: cardiac transplant, cardiac reinnervation, prevalence, sympathetic reinnervation, survival, time course

1 | ABSTRACT

Background: Partial cardiac sympathetic reinnervation after cardiac transplant has been extensively investigated and evidenced. However, there have been no large-scale, long-term studies evaluating the prevalence, time-course, and association with long-term survival of sympathetic reinnervation of the heart.

Methods: Cardiac transplant recipients (n=232) were recruited from outpatient clinic at a single transplant centre in the United Kingdom. Participants were each tested once for the presence of sympathetic reinnervation of the sinus node using the low frequency component of power spectral analysis of heart rate variability, with a cut-off defined as 2 standard deviations above the mean for denervated participants (those tested <56 days post-transplant). Time-course was calculated based on the timing of testing post-transplant. Patients were then followed-up over a period of up to 27 years after transplant for survival analysis.

Results: The overall prevalence of cardiac sympathetic reinnervation in the 225 patients tested >56 days post-transplant was 64.9%. Sympathetic reinnervation primarily occurred in the first 18 months after transplant, with a plateau thereafter. The prevalence in participants tested >18 months post-transplant was 69.6%. In Kaplan-Meier survival analysis, sympathetic reinnervation was associated with significantly improved survival (Log-rank P=0.019), with a median survival time for reinnervated patients of 19.9 years compared to 14.4 years for the denervated group.

Conclusions: Sympathetic reinnervation of the sinus node occurs mostly within 18 months of transplant, is found in 70% of cardiac transplant recipients tested >18 months post-transplant, and is associated with significantly improved long-term survival.

2 | INTRODUCTION

The first human cardiac transplant was performed by Christian Barnard in 1967.[1] Since then the procedure has become recognised internationally as an effective treatment for end-stage heart disease. Over 5500 transplants are conducted annually worldwide.[2] The heart is extensively supplied by sympathetic and parasympathetic autonomic nerves which are severed during cardiac transplantation: causing a loss of efferent and afferent nerve function to and from the heart, most notably to the sinoatrial node.[3]

In the context of cardiac transplant, reinnervation refers to the return of nerve function and extra-cardiac control from the recipient by a process of spontaneous neurone regeneration and connection to the transplanted donor heart. It has been demonstrated in several studies that functional sympathetic reinnervation of the donor heart can occur in 40-70% of cardiac transplant recipients (CTRs), whereas there remains only limited evidence of functional parasympathetic reinnervation. [4-6]

Sympathetic reinnervation when it occurs in CTRs is thought to be anatomically heterogenous across regions of the heart, as evidenced by immunohistochemical techniques and catecholamine analogue tracing, as well as functionally incomplete; for this reason it has been described as partial.[4,7-9]

Methods of detecting sympathetic cardiac reinnervation include but are not limited to: nerve stimulated intra-cardiac norepinephrine release,[4,6,10] measurement of heart rate variability (HRV) with stimulus,[4,6] power spectral analysis of low frequency signals of HRV,[4,6,10-13] and mapping of novel sympathetic nerve pathways using radiolabelled catecholamine analogues.[4-6,8,10-20]

Power spectral analysis of HRV is a non-invasive technique that allows autonomic balance to be defined at any given time and for the intensity of the spectral components of HRV to be determined.[21,22] Low Frequency (LF) components have been shown to relate to efferent sympathetic activity on the sinus node.[4,22-24] Power spectral analysis has been shown to detect sympathetic reinnervation in CTRs and has been shown to correlate well to the "gold standard" invasive measure of sympathetic reinnervation of the sinus node, intracoronary tyramine injection.[4,13,25,26]

Many studies have demonstrated physiological differences between transplant recipients with and without functional sympathetic reinnervation, including: an improved exercise tolerance;[4,6,7,27-29] improved peak oxygen uptake;[4,7,27] improved ejection fraction and improved contractile response to exercise,[4,30,31] greater HRV and systolic blood pressure change in response to exercise.[6,7,27-33] These results indicate potential beneficial effects of reinnervation.

Existing cardiac reinnervation prevalence studies have relatively low numbers for reliable prevalence estimation and have used a wide array of different methods.[4] This has led to limited data on the long-term outcomes for CTRs with partial sympathetic reinnervation and there has yet to be a survival study with a sizeable cohort and a follow-up period representative of current CTR life expectancy.

2. 1 │ Objectives

This study sought to use large-scale non-invasive testing with HRV measurement to assess the time-course and prevalence of sympathetic reinnervation of the sinus node, and to compare survival rates between CTRs with and without evidence of reinnervation over long-term follow-up.

3 | MATERIALS AND METHOD

3.1 | Ethical approval

All patients provided written informed consent. Ethical approval was obtained from the Joint Ethical Committee of the University of Newcastle Upon Tyne and Newcastle Health Authority in November 1997. This study complied with the International Society for Heart and Lung Transplantation statement on transplant ethics.[34]

3. 2 | Subjects

Consecutive adult CTRs (age >18 years) attending the cardiac transplant outpatient department for routine clinic visits at Freeman Hospital, Newcastle upon Tyne, United Kingdom between December 1997 and May 1998 were considered for inclusion. Patients were excluded if they had received a combined heart and lung transplant, were not in sinus rhythm at the time of recruitment, or if they refused consent to take part in the study.

3. 3 | Assessment for sympathetic reinnervation

Sympathetic reinnervation was assessed using HRV testing, which was performed at the time of routine outpatient attendance from 1997 to 1998. Each participant only underwent HRV testing on one occasion. Measurement of HRV was performed following the protocol of Lord et al.[13] All therapy, including antihypertensive therapy, was maintained in subjects throughout the study. Antihypertensive agents in common usage included dihydropyridine calcium channel-blockers such as Nifedipine and Lacidipine and ACE-inhibitors such as Lisinopril, none of which would be expected to affect HRV measurement; however, beta-

blockers and rate-limiting calcium channel-blockers were not routinely used. The subjects were asked to lie supine for a 10-minute rest period to mitigate the effect of the arterial baroreflex, whilst a three-lead ECG monitor was attached. Two sets of magnetometer coils were used to monitor chest and abdominal wall movements. After the rest period, the subjects remained supine and their respiratory rate was regulated using verbal instruction at 10 breaths per minute. Data were collected for a 10-minute recording period.

3.4 | Analysis of heart rate variability

Power spectral analysis of recorded ECGs was performed for HRV using custom software developed at Freeman Hospital, as previously described in detail by Lord et al.[13]

In brief: using the software, R waves were identified. Ectopic beats were removed and replaced by interpolated beats at the midpoint of adjacent R waves. A power spectrum was produced which resampled the ECG trace at 4Hz using the method laid out by Berger et al.[35] The RR interval spectrum was then calculated from each of three overlapping 5-minute segments by multiplication using the Hanning function and Fourier transformation, then summed resulting in a final estimate. The software then calculated the area under the spectrum in three regions: total area, low frequency (LF) content (0.04 - 0.15Hz) and high frequency content (0.15 – 0.4Hz), as defined by the European Society of Cardiology and the North American Society of Pacing Electrophysiology.[24] The separation into high and low frequency reflects parasympathetic and sympathetic activity respectively, although as there is some debate as to the occurrence and functional significance of parasympathetic activation in the context of CTRs, [4] only sympathetic reinnervation was further analysed. As the distribution of HRV has been found to be skewed,[13] the natural logarithm of the LF component (LnLF) was taken. The LnLF value of the RR interval spectrum obtained during

the 10 minutes of metronomic breathing was used as a measure of sympathetic reinnervation of the sinus node.

3. 5 │ Definition of sympathetic reinnervation

To determine the prevalence of sympathetic reinnervation in the cardiac transplant population, individuals were categorised as being either reinnervated or having continued denervation by deriving a cut-off value using the results of HRV measurement. Recipients less than eight weeks (56 days) post-transplant can be assumed to be denervated and were therefore taken as a reference against which reinnervation was judged. The cut-off LnLF value for evidence of partial sympathetic reinnervation was accordingly set as two standard deviations above the mean for this denervated group.

In order to determine the time-course of sympathetic reinnervation, participants tested >56 days post-transplant were grouped into 1-year and subsequently 6-month windows according to the number of days post-transplant at the time of testing.

3.6 │ Survival analysis

Medical records of participants were reviewed between January – March 2023 to assess long-term survival. The follow up period was determined from the date of transplant to date of death or the end date of the study in March 2023. Date of death was determined for the majority of patients from the Freeman Hospital transplant recipient database and a minority (n=3) using the United Kingdom's government general registry office.

3.7 | Statistical analysis

Normally-distributed continuous variables are expressed as mean (SD) and were compared using Student's t test. Data that was not normally distributed was expressed as the median (interquartile range) and compared using the Mann-Whitney U test. Categorical variables are summarized as counts (percentages) and were compared with the chi-square or Fisher exact test as appropriate.

The relationship between sympathetic reinnervation using HRV and time after transplantation was investigated using linear regression analysis. Subjects were grouped by time post-transplant and pairs of mean values were compared using two-tailed Student's t-tests.

Survival analysis was conducted using the Kaplan Meier estimator and compared using the Log-Rank test. All tests were 2-sided, and a P value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS (version 29, IBM Corp., Armonk, NY).

4 | RESULTS

4. 1 | Subjects

At the time of the recruitment period, the total population of CTRs was approximately 300 and predominately male (~85%) with an average age of age of ~50 years. 268 CTRs were approached to take part of whom 4 (1.5%) were excluded as they were not in sinus rhythm and 32 (11.9%) refused consent, resulting in recruitment of 232 subjects to the study.

Subjects ranged from 8 days to 12.9 years post-transplant at the time of HRV testing. Seven CTRs were tested within 56 days of transplantation and therefore formed the denervated reference group from which the LnLF cut-off value for reinnervation was derived. The remaining 225 participants were included in the final data set.

4.2 | Heart rate variability

Linear regression of LnLF against time post-transplant showed a significant relationship (r^2=0.0521, P<0.001).

4.3 | Cut-off value for sympathetic reinnervation

Calculation of the LnLF cut-off value for reinnervation (set as 2 standard deviations above the mean for the 7 patients tested <56 days post-transplant) is shown in Table 1. The cut-off value was calculated as 0.347.

4.4 | Time-course of sympathetic reinnervation

To assess time-course, after excluding those tested <56 days, the 225 subjects included in the analysis were grouped by the number of years post-transplant at the time of testing (Figure 1). There was a significant increase in the mean LnLF of subjects tested at >1 but <2 years post-transplant compared to that of subjects tested <1 year post-transplant (1.21 (SD 1.60) vs. -0.40 (SD 1.51), P=0.0006) and also a significant increase in the proportion of subjects meeting the reinnervation cut-off (69.2% vs. 25.0%, P=0.002). However, there were no other significant differences between later groups.

To more precisely clarify the time-course of sympathetic reinnervation in its early phase, subjects were divided into six-month groups over the first 4 years. Figure 2 shows the mean LnLF and proportion of subjects meeting the reinnervation cut-off value. A non-significant difference was seen in the mean LnLF values between subjects tested 6-12 months post-transplant (n=7) compared to subjects tested 12-18 months post-transplant (n=10) (-0.38 (SD 0.90) vs 1.14 (SD 1.89), P=0.032), with a trend towards an increased proportion of reinnervated subjects (28.6% vs. 70.0%, P=0.153). This non-significant difference was likely due to low numbers (n=7) in the 6 – 12-month group. Further comparison was therefore made between those tested 56 days – 12 months post-transplant (n=24) and subjects tested 12 – 18 months post-transplant, finding a significant difference in both mean LnLF (P=0.010) and proportion reinnervated (P=0.047). There were no significant differences in mean LnLF or proportion reinnervated between other six-month groups, indicating that sympathetic reinnervation primarily occurs during the first 18 months after transplantation.

4.5 | Prevalence of sympathetic reinnervation

Of the total cohort of subjects tested >56 days post-transplant (n=225), 146 (64.9%) had a LnLF value above the cut-off threshold. However, as analysis of the time-course of sympathetic reinnervation showed that this primarily occurs in the first 18 months after transplantation, meaning that some patients tested earlier than 18 months might have gone on to develop reinnervation, prevalence was reassessed in patients tested >18 months post-transplant. Of these 191 subjects, 133 (69.6%) showed evidence of sympathetic reinnervation.

4.6 | Demographics of cohort for survival analysis

Of the 191 CTRs tested for sympathetic reinnervation after the 18-month cut-off, the majority were male. The reinnervation group had a greater proportion of female subjects (P =0.045) and were on average younger (P=0.028). The two groups were comparable with respect to eGFR and diabetes prevalence at the time of HRV testing (Table 2).

4.7 | Survival analysis

Five CTRs known to be deceased were censored at the date of their last clinical contact because it was not possible to confirm their exact date of death. Of these, four were reinnervated and one denervated at the time of testing.

The overall follow-up period for the cohort of 191 CTRs was 27 years and the overall mean time from transplant until an endpoint of death or study completion was 18.5 years (SD 8.4). Data on survival was available for all subjects. The proportion of subjects who had survived at the end of the study was not different between groups (reinnervated: 22/133 [16.5%] vs.

denervated: 7/58 [12.1%], *P*=0.428). However, due to the length of the follow-up period, which was substantially greater than the average life expectancy of CTRs,[2] this finding was not unexpected.

A more precise Kaplan-Meier survival analysis (Figure 3) showed significantly improved survival in patients with cardiac sympathetic reinnervation (Log-rank *P* =0.019), with median survival times of 19.9 years for the reinnervation group and 14.4 years for the denervation group. When diabetic patients were excluded from survival analysis the results were unchanged, with significantly improved survival in those with sympathetic reinnervation (Log-rank *P*=0.009*)* and a longer median survival time of 20.3 years for the reinnervation group versus 14.6 years for the denervation group, similar results to the total cohort.

5 | DISCUSSION

This study is the largest and longest evaluation of the time-course, prevalence and association with long-term survival of cardiac sympathetic reinnervation of the sinus node to date. The main study findings are:

- Sympathetic reinnervation primarily occurs in the first 18 months after cardiac transplantation
- The prevalence of sympathetic reinnervation in the overall population tested >56 days post-transplant was 64.9%, rising to 69.6% in those tested after the 18-month period during which reinnervation primarily occurs
- Sympathetic reinnervation is associated with significantly improved survival compared to CTRs without evidence of sympathetic reinnervation when tested >18 months post-transplant (P=0.019)

5.1 | Definition of sympathetic reinnervation

In this study, the cut-off LnLF value used to define evidence of sympathetic reinnervation of the sinus node was derived from denervated (early post-transplant) individuals. This was done in preference to using values from normal subjects because post-transplant sympathetic reinnervation is a progressive and partial process rather than an “all or nothing” phenomenon. Therefore, comparing CTR subjects with controls from a non-transplant population with full cardiac innervation would have underestimated the prevalence of partial reinnervation.

5.2 | Time-course of sympathetic reinnervation

Multiple studies in the literature show that sympathetic reinnervation increases over time both in population prevalence and in individual cardiac nerve supply. [4-6,8,11,15,16,30] However, there is currently no clear consensus on the period in which the majority of cardiac sympathetic reinnervation occurs. The nature of this study, with a large sample size and testing performed at a random time-point post-transplant, allowed analysis of the time-course of sympathetic reinnervation by comparing sequential tranches of patients grouped by time of testing post-transplant. The results suggest that reinnervation primarily occurs during the first 18 months post-transplant. This finding is informative for future studies as it indicates an ideal period for identifying sympathetic reinnervation to limit the incorrect grouping of CTRs. This value is relevant to power spectral analysis and does not take into account the variable sensitivity of different methods at detecting sympathetic reinnervation.

5.3 | Prevalence of sympathetic reinnervation

The prevalence of sympathetic reinnervation in the overall CTR population in this study (65%), rising to almost 70% in CTRs tested >18 months post-transplant, is similar to some but higher than other prior studies where estimated reinnervation prevalence was between 40-70%.[4] However, previous prevalence studies, which utilised a range of methods for assessing for sympathetic reinnervation, many of which were invasive, had relatively small study populations.[4-7,23,36-38] In this study, a simple, non-invasive measurement tool was used, allowing the largest population to date to be assessed for sympathetic reinnervation.

5.4 | Association between sympathetic reinnervation and survival

Only one study to date has addressed long-term outcomes of sympathetic reinnervation, following up 77 CTRs who had been assessed for sympathetic reinnervation using catecholamine uptake on PET-CT imaging for a period of 7.3 years (SD 4.2) after transplantation.[17] Neither 'hard' endpoints (cardiac death or re-transplantation: n=6), or 'soft' endpoints (revascularisation or allograft failure: n=10) were associated with reinnervation status. However, this study was under-powered for survival analysis (n=77) considering the event rate and follow-up period, and the inclusion criteria were selective for various risk factors that may have influenced both reinnervation and survival, including absence of rejection, coronary artery disease and diabetes. Finally, reinnervation was measured by volumetric sampling of PET-CT catecholamine uptake, which does not assess functional sinus node reinnervation. The present study is therefore the first to evaluate long-term outcomes associated with sympathetic reinnervation of the sinus node, with sufficient power to address this.

It is difficult to attribute the median survival benefit to sympathetic reinnervation alone with so many potential unknown variables. There is, however, a plausible physiological basis for an improved median survival outcome in the reinnervation cohort. Multiple studies have shown that reinnervated CTRs have significantly better clinical response to exercise training in a variety of parameters and improved overall exercise capacity than recipients with continued denervation.[4,6,7,11,12,27,29,33] Reinnervated hearts have been shown to have significantly increased ejection fraction during exercise, especially in the antero-septal territory.[27,30,31] Other haemodynamic benefits have been identified such as a faster heart rate response to exercise, improved peak oxygen uptake and cardiac contractility.[27,33] It has been proposed that denervated hearts develop greater dependence on circulating catecholamines,[39]

however the balance of these hormones with autonomic supply to the heart is likely to be considerably more complex.[4]

Exercise tolerance has been linked to improved survival which is thought to be attributed to both the reduction in visceral fat and the anti-inflammatory effect of exercise itself;[40] these factors have both been shown to reduce the incidence of coronary artery disease or cardiac allograft vasculopathy,[29,41] the cause of approximately 10% of deaths in transplant recipients after the first year.[42] One previous study also found a lower incidence of cardiac allograft vasculopathy in reinnervated transplant recipients.[19]

5.5 │ Clinical implications

Sympathetic reinnervation after cardiac transplant has been documented since the early 1970s and there is now ample evidence of its presence.[4,6] Although many haemodynamic effects of reinnervation have been demonstrated, there has until now been no clear evidence of benefit on long-term outcomes associated with this phenomenon.

The findings of this study therefore justify further research into the clinical determinants of sympathetic reinnervation, which is more prevalent than much of the existing literature suggests, with a view to finding modifiable factors that may improve the chance of reinnervation for CTRs.

The technique of power spectral analysis used to test for sympathetic reinnervation of the sinus node in this study is easily reproducible, inexpensive and non-invasive, and therefore more appropriate for larger population studies. More research is required comparing power spectral analysis to more invasive or expensive techniques and across more varied populations to confirm the validity of this study. Serial HRV testing over the first two years

from transplant may better determine a transition point in the timeline of functional sympathetic reinnervation. This study may justify future trials into therapies that induce physiological reinnervation,[43-45] or, with the advent of new technologies, even implanting artificial reinnervation of the heart.[46,47]

5.6 │ Limitations

Although well-established , it should be noted that there remains some controversy in the use power spectral analysis of HRV as a measure of sympathetic activity,[48] and its reproducibility.[49] Ideally measurements would have been repeated at a later date to ensure HRV results were accurate and reproducible. It is well recognised that the LF component corresponds with sympathetic stimulation of the sino-atrial node, however controversy arises regarding the co-contaminant effect of the arterial baroreflex, which in this study was mitigated by measurement in supine position.[48] It is acknowledged that parasympathetic innervation may also have happened in this cohort of patients and could directly or indirectly contribute to some of the variability in low-frequency power observed. Our original data suggest this effect is likely to be small.[13] In the absence of a gold standard measure of parasympathetic reinnervation to the sinus node we have been careful not to draw any conclusions about parasympathetic innervation.

Other markers of cardiac autonomic function have been evidenced such as LF/HF ratio and QT variability show promising results that may mitigate artifact and vagal influence on LF power measurement however further research is needed on the cardiac transplant population.[50,51]

Antihypertensive agents were continued on the basis that the drugs predominantly used at the time (dihydropyridine calcium channel-blockers and ACE-inhibitors) would not be expected

to affect HRV measurement. However, comprehensive recording of concurrent medication was not undertaken at the time of HRV testing and therefore, while beta-blockers and rate-limiting calcium channel-blockers (which could influence HRV measurement) are known to have not been used routinely, it is possible that a very small minority of patients were taking these agents.

It would have been preferable to have incorporated more variables known to affect survival to allow for further group comparison and adjustment for confounding factors but these data were not collected prospectively and could not be retrieved retrospectively. Certain conditions impair autonomic function to varying degrees such as,[52] Parkinson Disease, certain autoimmune conditions and most commonly Diabetes Mellitus. Despite this, prevalence of Diabetes at HRV testing showed no difference between groups and removal of these patients from survival analysis showed a persistent significant survival difference between groups. Other conditions were not recorded but would be sufficiently rare in this relatively young (median 51.4 years) and predominately male (84.5%) cohort to have a negligible impact on study outcomes.

Only 7 subjects fell within the arbitrary 56-day post-transplant period of presumed complete denervation whose LnLF measurements were used to create the cut-off value. Ideally, this number would have been higher for a more reliable mean and SD considering there is literature indicating HRV heterogeneity.[49]

Furthermore, the prevalence study was conducted from 1997-1998 and cardiac transplant technique, CTR demographic and post-transplant care have all changed over time, which may have an influence on the prevalence and degree of reinnervation. One previous study found that clinical determinants such as recipient age, rejection frequency and surgery time all influenced reinnervation,[17] whereas a recent study found that pre-transplant diagnosis of non-

ischaemic cardiomyopathy corresponded to higher indices of reinnervation,[39] which could potentially have contributed to the survival difference seen between the two groups in this study.

Survival analysis makes the assumption that reinnervation is a fixed baseline variable at 18 months after transplant which if flawed could lead to inaccuracy in grouping and therefore error in the Kaplan-Meier estimate. CTRs were tested at various time intervals from transplant and as those tested later may be more likely to have reinnervated there is potential for survivor bias leading over-estimate of the effect of reinnervation on survival.

6 | CONCLUSIONS

Sympathetic reinnervation of the sinus node occurs primarily in the first 18 months after transplant and is detectable using the non-invasive and easily reproducible technique of power spectral analysis in 70% of CTRs tested >18 months post-transplant. The presence of sympathetic reinnervation detected using LF heart rate variability is associated with significantly improved survival in CTRs and further research into this phenomenon for the benefit of a growing cardiac transplant population is therefore merited.

7 | AUTHORS' CONTRIBUTIONS

The study design was developed by JM, SL, AM, RC and GP. Data extraction and analysis for the prevalence study was conducted by MD. Data extraction and analysis for survival analysis was conducted by OW with supervision from MD and SL. The manuscript was drafted by OW and MD with editing by SL. All authors have read and approved the contents of the manuscript.

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9 | CONFLICTS OF INTEREST

The authors of this study have no relevant conflicts of interest to disclose; financial, competitive or otherwise.

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11 | FIGURES AND TABLES

Table 1: Process for calculating cut-off value of 2SD above the mean

Figure 1 Mean HRV (LnLF ± 1 standard deviation) relative to the cut-off value grouped at yearly intervals from transplant date

Table 2 Demographics of reinnervation and continued denervation groups

Figure 2: Mean LnLF (top panel) and percentage of subjects with reinnervation (bottom panel) for subjects tested within 6.5 years of transplant and grouped by time-of-testing post-transplant in 6-month intervals

Figure 3 Kaplan-Meier survival graph for comparison of reinnervated and denervated cardiac transplant recipients

Table 1. Process for calculating cut-off value of 2SD above the mean.

	Number	**Mean**	**SD**	**Cut-off value (LnLF)**
Subjects tested <56 days post-transplant	7	-1.805	1.076	0.347*

*Value defining sympathetic reinnervation set at 2 SD above the mean LnLF of denervated CTRs

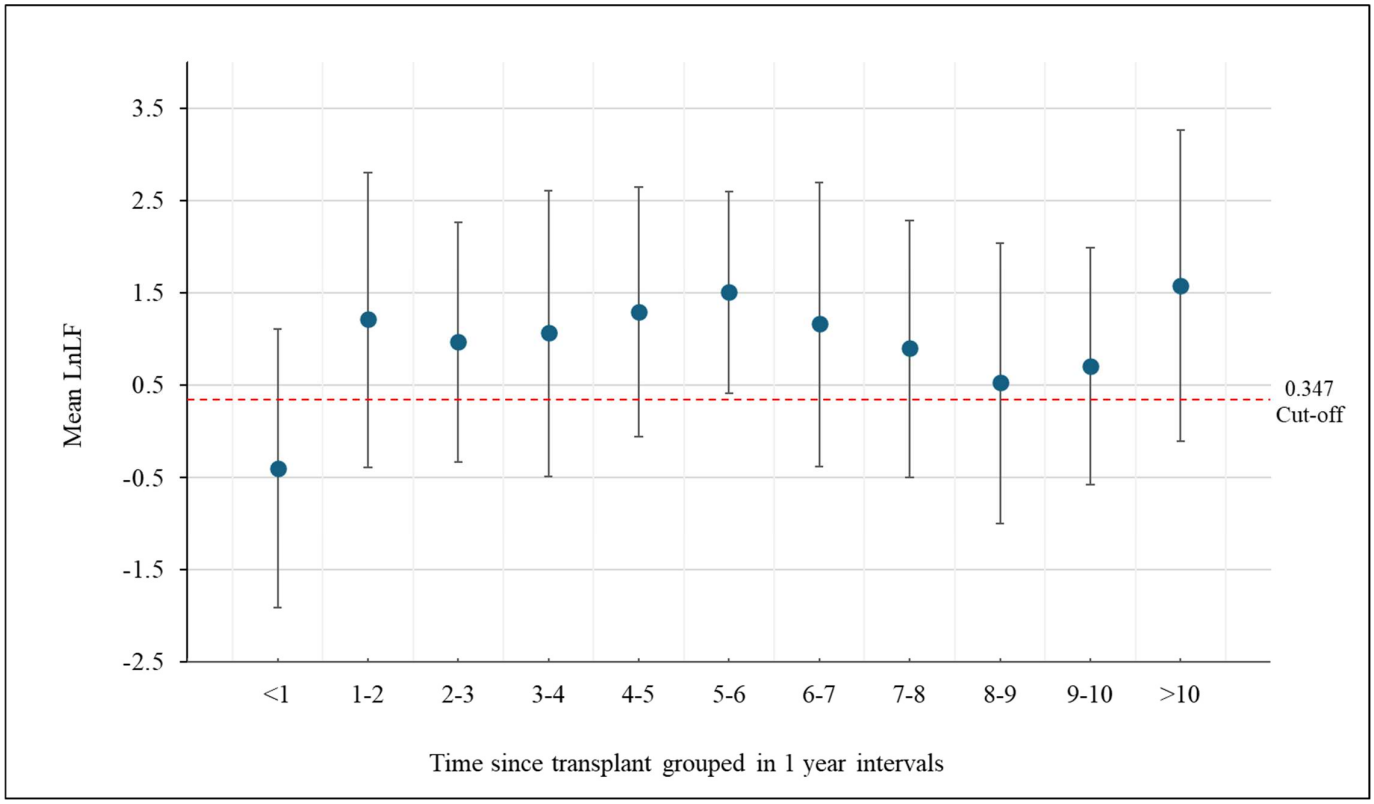


Figure 1 Mean HRV (LnLF± 1 standard deviation) relative to the cut-off value grouped at yearly intervals from transplant date

Table 2. Demographics of reinnervation and continued denervation groups.

Empty Cell	**Reinnervation (n=133)**	**Continued denervation (n=58)**	**Difference (95% CI)**	**P Value**
Male (%)	109 (82%)	54 (93%)	11 (2 – 20)	0.045
Female (%)	24 (18%)	4 (7%)		
Median age at transplant (IQR)	49 (40 - 55)	52 (47 - 56)	4 (0 – 8)	0.028
Mean±SD eGFR at HRV testing	45±1.3	43±1.7	-2 (-6 – 2)	0.374
Diabetes at HRV testing (%)	19 (14%)	6 (10%)	-4 (-13 – 6)	0.451

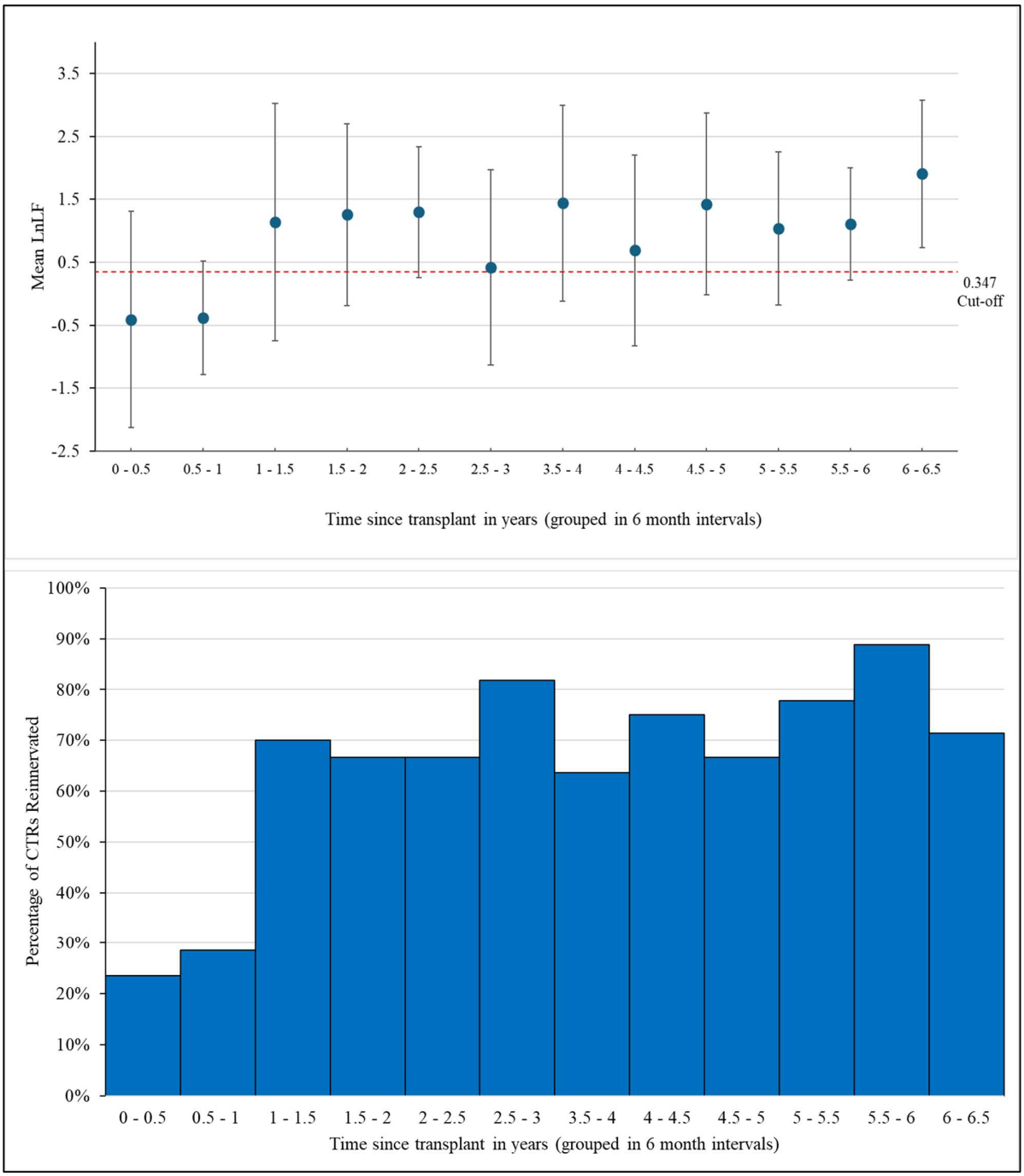
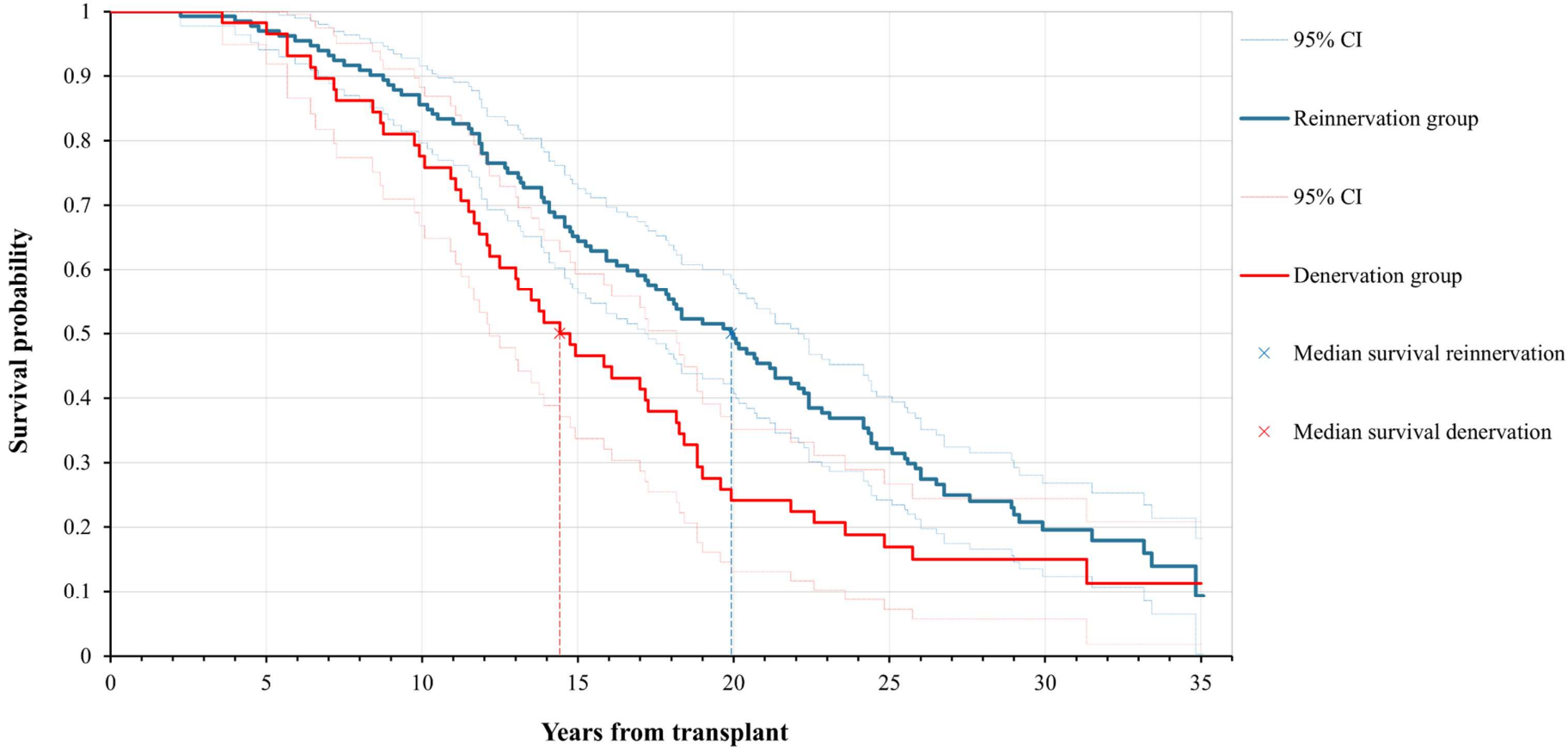


Figure 2: Mean LnLF (top panel) and percentage of subjects with reinnervation (bottom panel) for subjects tested within 6.5 years of transplant and grouped by time-of-testing post-transplant in 6-month intervals



	Number at risk							
Reinnervation group	133	128	113	86	65	41	16	1
Denervation group	58	57	45	27	14	9	4	1
Years from transplant	0	5	10	15	20	25	30	35