Lipoprotein Risk Factors for Ventricular Tachyarrhythmias in a Cohort of ICD Patients

By

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ABSTRACT

Objective

The purpose of this investigation is to determine whether or not unhealthy levels of lipoprotein subfractions, such as LDL and HDL, serve as risk factors for ventricular fibrillation (VF) and ventricular tachycardia (VT). This study uses a cohort of 200 patients from an original dataset obtained from a randomized, double-blind, placebo-controlled trial performed at 6 US medical centers with enrollment from February 1999 until January 2003. This initial 1999-2003 trial aimed to determine whether omega-3 polyunsaturated fatty acids (PUFAs) may have beneficial antiarrhythmic effects in patients with a history of sustained VT or VF. It found that among patients with a recent episode of sustained ventricular arrhythmia and an ICD, fish oil supplementation does not reduce the risk of VT/VF and may be proarrhythmic in some patients.[1]

Context

Clinical studies have shown that the balance between LDL and HDL levels not only determines the level of atherosclerosis in the coronary vasculature, but may also serve to indicate the level of cardiac inflammation. These inflammatory processes may play a role in inducing potentially fatal arrhythmias that can lead to sudden cardiac death (SCD).

SCD is a cardiovascular affliction that carries the heaviest burden on the United States public health system, more than any other disease' [2,3,4,5]. Over 450,000 people in the U.S. die from sudden cardiac arrest (SCA) each year [1], more than death from stroke [2], lung cancer [3], breast cancer [3], and AIDS [4] combined. The most widely-used therapeutic and preventive modality against SCD is ICD. Recent clinical studies have shown that patients most at risk for the fatal arrhythmias that cause SCD receive the most potential benefit from ICDs if they have a

left ventricular ejection fraction less than 35%. However, it is now clear that current indications for ICD implantation are not adequate in that patients at the highest risk for ICDs are not being properly identified to benefit from the lifesaving-prevention that ICDs provide. As a result, a larger emphasis is placed on non-invasive risk stratification measures such as lipoprotein subfraction levels in order to refine criteria for ICD implantation for primary and secondary prevention of SCD.

Main Outcome Measures

There were two main outcome measures of interest: (1) time to first episode of ICD shock for VF and (2) time to first episode of ICD shock for VT, treated separately. ICD shock for VF and VT served as a proxy for the actual, sensed VF and VT events, which were chosen as the main outcome measures based on their high clinical significance for the induction of SCD and for their different outcomes from fish oil treatment in the original fish oil investigation.

Methods

The statistical analysis was an intent-to-treat-analysis. The baseline characteristics of patients randomized to receive fish oil vs. placebo were compared using the t-test. Significant differences in lipid subfraction values of total cholesterol, LDL, HDL, triglycerides, and total cholesterol:HDL ratio over time were determined using a mixed-model repeated measures analysis of variance approach. The initial value was used as a covariate to control for any differences at baseline, with the most appropriate covariance structure selected using the Akaike information criterion. Least square-adjusted means were estimated and compared for all analysis

of variance effects. All analyses will be performed with SAS software, versions 8 and 9, and STATA 10 software.

Primary time to event analyses were performed using the Kaplan-Meier method, and continuous, discrete lipid panel subfractions were subjected to univariate Cox proportional hazards regression and quartiled lipid panel subfractions were subjected to the log-rank test. A Cox proportional hazards model was used to assess the significance of the primary outcome controlling for other baseline characteristics. Variable selection was performed with these baseline characteristics using all possible regression models with the score statistic and stepwise addition of variables. Treatment group was then added to the best model to determine if it was a significant predictor after controlling for significant baseline characteristics. Interactions were tested for significance among the significant variables at the multivariate level. The resulting Cox proportional hazards model was tested for the proportionality assumption by using the Schoenfeld and scaled Schoenfeld residuals. The goodness of fit of the final model was be evaluated using Cox-Snell residuals. Post hoc power analysis showed that this investigation had only 28% power and 68% power to detect a 33% difference in VF rate and VT rate, respectively.

Results

After adjusting for fish oil treatment allocation group, each 1 mg/dl increase in LDL level was associated with a 2% increase in the hazard ratio of ventricular fibrillation ($p = 0.027$). An ejection fraction less than 40% (hazard ratio 1.5; 95% CI 1.029 – 2.241) and VT as the qualifying arrhythmia (hazard ratio 2.5; 95% CI: 1.555 – 4.163) were significant independent predictors of time to ICD therapy for VT. When treatment assignment was added to this model, the fish oil group had a hazard ratio of 1.5 (95% CI 1.019 – 2.209). This finding is not

unexpected, as the original fish oil RCT found similar results for the combined endpoint of VT/VF and was reported in the original publication. None of the lipoprotein nor nonlipoprotein subfractions were significant predictors of time to first ICD therapy for VT.

Conclusion

Among patients with a recent episode of sustained ventricular arrhythmia and an ICD, LDL level is a significant predictor for ventricular fibrillation before adjusting for treatment allocation. Neither LDL nor HDL were significant predictors for the risk of VT. Moreover, the significance of ejection fraction as a predictor for ventricular tachycardia point to different pathologic mechanisms between the two ventricular tachyarrhythmias most responsible for sudden cardiac death, information that may be useful when risk stratifying patients for ICD implantation for secondary prevention of sudden cardiac death.

INTRODUCTION

Sudden Cardiac Death

Sudden cardiac death (SCD) is a problem of epidemic proportions in the United States today. SCD is death related to an abrupt loss of heart function, also known as sudden cardiac arrest (SCA). The 2006 American College of Cardiology/American Heart Association/Heart Rhythm Society (ACC/AHA/HRS) drafting of established data standards for electrophysiology included the following definitions of SCA and SCD: "[Sudden] cardiac arrest is the sudden cessation of cardiac activity so that the victim becomes unresponsive, with no normal breathing and no signs of circulation. If corrective measures are not taken rapidly, this condition progresses to sudden death. Cardiac arrest should be used to signify an event as described above, that is reversed, usually by CPR and/or defibrillation or cardioversion, or cardiac pacing. Sudden cardiac death should not be used to describe events that are not fatal." Only a small percentage of patients indicated for implantable cardioverter defibrillator (ICD) therapy are successfully identified and referred for ICD implantation. The solution to this problem lies in better ICD therapy education, awareness, and accessibility, as well as successful screening and patient identification for ICD therapy. Over 450,000 people in the U.S. die from sudden cardiac arrest (SCA) each year [1], more than death from stroke [2], lung cancer [3], breast cancer [3], and AIDS [4] combined. Worldwide, there are 3 million cases of SCD per year with less than 1% survival rate.[5] In the U.S., the survival rate of SCD is 5%.

Most SCD involves a malignant arrhythmia. Underlying causes of these fatal arrhythmias are coronary artery disease (80%), cardiomyopathy (15%), valvular heart disease (5%), and congenital conditions such as Long QT syndrome (<5%).[6] The underlying arrhythmias of SCD

are ventricular tachycardia (VT), bradyarrhythmias, Torsades de Pointes, and primary ventricular fibrillation (VF) .[7] VF consists of very rapid, chaotic ventricular beats, thereby reducing cardiac output to zero, and can be fatal in four minutes. Bradyarrhythmias, such as high-degree AV block with asystole, may be involved, which is considered to be an underestimated pathophysiology. 88% of SCD is of arrhythmic causes, while 12% consist of other cardiac causes.[8]

Risk factors for SCD include smoking, inactivity, obesity, advancing age, hypertension, elevated serum cholesterol levels, glucose intolerance (diabetes), prior myocardial infarction (MI), angina pectoris, impaired left-ventricular (LV) dysfunction, low socioeconomic status, methadone use, and vigorous physical activity.[9, 10, 11, 12] Potential protective effects include increased left ventricular wall thickness.[13] Factors that initiate and maintain VT/VF include triggers such as changes in autonomic nervous system tone, metabolic demands, electrolyte disturbances, ion channel abnormalities and myocardial ischemia/infarction. Acute processes such as ischemia, toxins, and infections can cause myocardial scarring that result in electro-mechanical remodeling leading to VT/VF.

Current treatment alternatives include drug therapy (ie, EP-guided, non-invasive guided, or empiric anti-arrhythmic therapy), which have been shown in previous studies to have no difference or increased mortality rate, or device therapy (ie, ICD), which have consistently shown significant decreases, up to 30%, in all-cause mortality.

The Role of Implantable Cardioverter Defibrillators on Sudden Cardiac Death

Implantable cardioverter defibrillators (ICDs) are small battery-powered electrical impulse generators that are able to constantly monitor the rate and rhythm of the heart and can deliver therapies, by way of an electrical shock, when the electrical manifestations of the heart activity exceeds the present number. Many modern ICDs use a combination of various methods to determine if a fast rhythm is normal, VT, or VF. Through rate, rhythm, and morphology discrimination, ICDs are able to provide highly reliable real-time monitoring the patient's native rhythm. According to the Heart Rhythm Society, ICDs are 99% effective in stopping life-threatening arrhythmias and are the most successful therapy to treat VF, the major cause of SCD.

While it is the current mainstay of primary and secondary prevention of sudden cardiac death, ICD therapy has been recently observed to have disappointing results with regard to decreasing the enormous public health burden posed by SCD, the leading cause of mortality in the United States. Preliminary 2007 national data indicate that while the total number of cardiovascular deaths continues to decline, the proportion of cardiovascular mortality due to sudden cardiac death has climbed to 70%. The MADIT I, MUSTT, MADIT II, and SCD-HeFT trials showed a significant benefit of using ICDs for the primary prevention of SCD and have been described elsewhere.[14-22] As a result, MADIT criteria for ICD implantation include Q-wave MI > 3 weeks, LVEF < 35%, and asymptomatic, unsustained VT. MUSTT criteria for ICD implantation include post-MI patients with LVEF < 40%. The AVID, CIDS, and CASH trials showed a significant benefit of using ICDs for the secondary prevention of SCD.[23-25] Unfortunately, recent studies have shown that 70-80% of SCDs occur in people who do not meet standard indications for an ICD for primary prevention of SCD, and of those who do get ICDs,

about 70% won't experience the lifesaving shock in the first 4-5 years. The use of ICDs for secondary prevention of SCDs also has not had a major public health impact. It has been estimated that ICDs implanted for secondary prevention of SCD save approximately 500 lives per year, or 0.1% of all SCD. Such a poor outcome may be due to the poor survival rate from the first out-of-hospital cardiac arrest.

Myocardial Inflammation and Sudden Cardiac Death

Recently there has been a heightened emphasis on the evaluation of inflammatory pathophysiology and its role in sudden cardiac death. It was recently found that inflammation and malnutrition are significant risk factors for sudden cardiac death for those with end-stage renal disease (ESRD).[26] Compared with those who had low levels of high-sensitivity C-reactive protein or interleukin-6, those with elevated levels of either of the inflammatory markers were 1.65 and 1.84 times as likely, respectively, to die of sudden cardiac death ($p < 0.0001$ for both). It has been hypothesized that inflammation may trigger SCD through the development of premature atherosclerosis or through direct effects on the myocardium and the electrical conduction system.

The Role of Low-density Lipoproteins (LDL) and Cardiovascular Pathophysiology

LDL is a type of lipoprotein that transports cholesterol into the peripheral tissues and is removed by the liver. LDL is usually not measured, but calculated from the Friedewald equation, LDL cholesterol = total cholesterol – HDL cholesterol – triglycerides / 5. Of all the plasma lipoproteins, LDL has been the most investigated in terms of its role in atherosclerosis and myocardial infarction. LDL consists of a surface monolayer of phospholipids and free

cholesterol and a single molecule of apolipoprotein (apo) B, which encircles the lipoprotein. This surface monolayer surrounds a hydrophobic core of mainly cholesterol esters, but also some triglycerides. In itself, LDL is almost certainly not proinflammatory, but the particle can become modified in many ways. It is the modified LDL particle that is proinflammatory and proatherogenic because of its high cholesterol content which attracts macrophages to ingest it.[27]

LDL enters the artery wall by crossing the endothelial membrane. The LDL receptor plays a significant role in the pathogenesis of atherosclerosis. The cell-surface receptor recognizes the apoprotein B100, which is embedded in the phospholipid outer layer of LDL particles. They allow LDL to be bound and internalized in nucleated cells (mainly in the liver) and prevent LDL from simply diffusing around the membrane surface. Although the liver is quantitatively the most important organ for the removal of LDL from plasma, the relative rate of uptake is greatest in certain endocrine tissues that have a high capacity for the synthesis of steroid hormones for which cholesterol contained in LDL serves as an important precursor. Clinical and epidemiologic studies have shown a strong positive relation between elevated levels of LDL cholesterol and an increased risk of cardiovascular morbidity and mortality.

Once in the arterial wall, if LDL accumulates, it is subject to a variety of modifications. The best known of these is oxidation, both of the lipids and of the apo B. LDL is also subject to aggregation, and its phospholipids are subject to hydrolysis by phospholipases to form lysophosphatidylcholine. Several other chemical modifications have also been reported. The net effect of these changes is the production of a variety of modified LDL particles, and the evidence is now very strong that these modified LDL particles are proinflammatory.[28]

Modified LDL is involved in the process that leads to the development of atherosclerosis. It activates endothelial cells to express monocyte chemotactic protein 1 (MCP-1), which attracts monocytes from the vessel lumen and into the subendothelial space,[17] and promotes the differentiation of monocytes into macrophages,[29] a key step in the inflammatory process of atherosclerosis. The macrophages release a variety of chemicals, including cytokines. Of these cytokines, tumor necrosis factor alpha (TNF-alpha) and interleukin-1 (IL-1) activate endothelial cells to express adhesion molecules that bind monocytes,[30] making them available for recruitment into the subendothelial space by MCP-1. The activated macrophages also express a variety of scavenger receptors, several of which recognize the different forms of modified LDL. The macrophages take up the LDL cholesterol resulting in LDL degradation through these scavenger receptors, accumulate the cholesterol, and are converted into the lipid-rich foam cells that are the hallmark of atherosclerosis.[31] Factors that affect the plasma LDL concentrations include diet and genetic factors.

The Role of HDL and its Cardioprotective Effect

The major function of HDL is to transport cholesterol from peripheral tissues back to the liver. Thus, high levels of HDL, with normal delivery of HDL lipids to the liver (and kidney), may enhance removal of cholesterol from tissues, including the arterial wall, and protect against the development of atherosclerosis. This is the main reason why HDL-bound cholesterol is sometimes called “good cholesterol,” or HLD_C. A high level of HDL-C seems to protect against cardiovascular diseases and low-HDL levels (less than 40 mg/dL) increase the risk of heart disease. Variations in the concentration of HDL2 subfraction show the strongest inverse correlation with cardiovascular risk. Thus, increased concentrations of HDL^2 are viewed as

protective, whereas low concentrations may be detrimental. Factors that have been shown to increase the concentrations of HDL include sustained regular exercise, correction of hypertriglyceridemia, and certain pharmaceutical preparations. Decreases in the plasma concentrations of HDL may be seen in the association with weight gain, cigarette smoking, and hypertriglyceridemia.

A Link Between LDL-induced Cardiac Pathophysiology and Ventricular Tachyarrhythmias?

The incidence of sudden death is relatively high in the postinfarction period for months after an MI. Abnormal rapid stimulation of the ventricles can lead to fibrillation. This can occur during VT or in conditions, such as Wolff-Parkinson-White syndrome, when atrial fibrillation or flutter waves pass rapidly through a bypass tract to the ventricular musculature. Severe left ventricular dysfunction, a variety of cardiomyopathies, and acquired or idiopathic long QT syndrome also increase the risk of fibrillation.

Ventricular tachycardia (VT) is a general term that includes any rapid rhythm, faster than 100-120 beats per minute, arising in the ventricle. It most commonly occurs in patients with prior myocardial infarction or impaired left ventricular function. A prior history of myocardial infarction strongly suggests ventricular tachycardia. The most common mechanism is reentry. During VT, cardiac output is reduced due to the rapid heart rate and lack of a properly timed or coordinated atrial contraction. Ischemia and mitral insufficiency may also contribute to hemodynamic intolerance. Hemodynamic collapse is more likely when underlying left ventricular dysfunction is present or with very rapid rates. Diminished cardiac output may result

in diminished myocardial perfusion, worsening inotropic response, and degeneration to VF, resulting in SCD.

The etiology of VF remains incompletely understood. It often occurs in the setting of acute cardiac ischemia or infarction, and acute MI is diagnosed in up to half of SCA cases. One etiology is mechanical or electrical stimulation of the myocardium during the early phase of repolarization (R-on-T phenomenon). VF may be induced when this premature beat during a vulnerable period of the cardiac cycle occurs in the setting of a worsened “dispersion of refractoriness,” which is the unequal levels of resting membrane potentials as well as unequal depolarization potentials in myocardial and pacemaker cells during systole and diastole. It is hypothesized that cardiac inflammation plays a significant role in exacerbating “dispersion of refractoriness,” which can cause conditions to be ideal for VF.

METHODS

Overview

This investigation is a secondary analysis of lipoprotein subfractions and ventricular tachyarrhythmias using data from a randomized controlled trial of fish oil vs. placebo to determine whether omega-3 polyunsaturated fatty acids (PUFAs) have beneficial antiarrhythmic effects in patients with a history of sustained VT or VF. Data was collected from study participants at six U.S. medical centers with enrollment from February 1999 until January 2003. The risk of ventricular tachyarrhythmias, specifically VT and VF, was estimated with the hazard ratio calculated by the Cox Proportional Hazards regression. Lipoprotein panels were obtained from all study participants at baseline, month 3, year 1, and year 2, which were used in the statistical analysis.

Study Population

Data from 200 study participants from the fish oil randomized controlled trial were used to create the study population. One participant did not have ICD follow-up data, therefore, no arrhythmia data was available. The final study population included 199 individuals, as this subject could not be included in the outcome assessment

Fish Oil Supplementation and Risk of VT and VF in Patients with ICDs

As the dataset from this randomized controlled trial was used in lipoprotein analysis, the methods and results will be summarized. This investigation sought to determine whether omega-3 PUFAs have beneficial antiarrhythmic effects in patients with a history of sustained VT or VF. Patients at 6 U.S. medical centers, mainly Oregon Health & Science University, Portland VA

Medical Center, and Providence St. Vincent Medical Center, were eligible for entry if they were receiving an implantable cardioverter defibrillator for an electrocardiogram-documented episode of sustained VT or VF that was not the result of acute myocardial infarction or a reversible cause or who had a preexisting ICD and had received ICD therapy for an episode of ECG-documented VT/VF within the previous 3 months.

200 patients were randomly assigned to receive fish oil, 1.8 g/d, 72% omega-3 PUFAs, or placebo and were followed up for 2 years with monthly clinic visits at the enrolling center for the first 3 months and every 3 months thereafter. 100 patients were allocated to receive fish oil and 100 to receive olive oil. We calculated that 100 patients per group or 200 total would be required for 92% power to detect a 33% reduction in event rate with treatment using a 2-tailed level of 0.05. This is based on an estimated 75% incidence of these arrhythmias in placebo patients during 2 years of follow-up and a 15% dropout rate. Time to first ICD treatment for VT/VF was the main outcome measure. At all visits, the ICD memory was checked for occurrence of episodes of ICD therapy. Blood was drawn for RBC membrane lipid analysis at baseline and months 1, 2, 3, 6, 12 18, and 24.[31]

The investigation found that among patients with a recent episode of sustained ventricular arrhythmia and an ICD, fish oil supplementation does not reduce the risk of VT/VF. In addition, in those whose presenting arrhythmia was VT with an ICD, there was a proarrhythmic effect in developing VT/VF among those who took fish oil compared to placebo. Enrollment has terminated and the study follow-up has completed. The findings from the randomized control trial were published in the Journal of the American Medical Association in 2005,[31]

Data Collection and Variable Definition

Plasma lipid lipoprotein panels were collected at baseline, months 3, 12, and 24 and were used for analysis in this investigation.

Independent Variables

All independent variables would be treated as continuous, discrete variables and as quartiles. There were two primary independent variables for this investigation, the lipoproteins from the lipid panels:

1. LDL – calculated in cases where triglycerides were < 400 mg/dL. There were 4 study subjects who had triglycerides > 400 mg/dL, in which cases the LDL was more directly measured by ultracentrifugation.

    Quartile 1: 32 – 78 mg/dL

    Quartile 2: 79 – 93 mg/dL

    Quartile 3: 94 – 115 mg/dL

    Quartile 4: 116 – 171 mg/dL

2. HDL – measured

    Quartile 1: 13 – 26 mg/dL

    Quartile 2: 27 – 33 mg/dL

    Quartile 3: 34 – 40 mg/dL

    Quartile 4: 41 – 83 mg/dL

There were three secondary independent variables for this investigation, the nonlipoproteins from the lipid panels:

1. Total cholesterol – measured

Quartile 1: 85 – 139 mg/dL

Quartile 2: 140 – 160 mg/dL

Quartile 3: 161 – 184 mg/dL

Quartile 4: 185 – 264 mg/dL

2. Triglycerides – measured

Quartile 1: 45 – 102 mg/dL

Quartile 2: 103 – 134 mg/dL

Quartile 3: 135 – 212 mg/dL

Quartile 4: 213 – 849 mg/dL

3. Total cholesterol:HDL ratio – calculated

Quartile 1: 2.10 – 3.95

Quartile 2: 3.96 – 4.72

Quartile 3: 4.73 – 6.03

Quartile 4: 6.04 – 11.57

Study Outcome

There were two main study outcomes:

1. Time to first ICD therapy for VF

2. Time to first ICD therapy for VT

At all follow-up visits, the ICD memory was checked for occurrence of episodes of ICD therapy. Specifically, a printout of each episode of ICD therapy was reviewed by the local investigator and by a member of the electrogram committee, both of whom were blinded to the treatment assignment of the patient. Episodes of ICD therapy were classified as VF using

methods previously reported.[33, 34] When there was disagreement between the investigator and the committee member on the interpretation of the tracings, the tracings were reviewed by the entire committee and classified by consensus. For the outcome of VF, patients were considered censored when they experienced their first ICD therapy for VF after enrollment. For the outcome of VT, patients were considered censored when they experienced their first ICD therapy for VT after enrollment. As VT and VF were considered separate outcomes, reaching the endpoint for VT did not eliminate the subject from reaching the endpoint of VF, and vice versa. There were 16 first episodes of VF and 106 first episodes of VT. During follow-up, patients received ICD therapy for a total of 45 VF episodes and 901 VT episodes.

Potential Confounders

Demographic, clinical, and study-specific patient characteristics were assessed for confounding (Appendix A).

Statistical Analysis

Descriptive statistics and Cox Proportional Hazards models were used to explore the relationship between lipoprotein levels and ventricular tachyarrhythmias. Hazard ratios were used as the primary measure of association. The statistical analysis is an intention-to-treat analysis.

Statistical analyses were performed with Microsoft Office Excel 97 (Microsoft Corp., Bellevue, Washington), SAS software, version 8 and 9 for Windows (SAS Institute Inc, Cary, NC) and STATA 10 for Windows (StataCorp, College Station, TX).

Treatment of Missing Data

One study subject died before the 3 month visit. No lipid panels were available for analysis. This subject, did, however, have arrhythmic endpoint data and baseline demographic/clinical information available. As a result, these baseline demographic and clinical information of the study subject will be used in the baseline analysis but will not be included in the model building due to lack of baseline lipid panel data.

Descriptive Statistics

Means, standard deviations, and ranges were presented for continuous variables. Proportions were presented for categorical variables. Unpaired t-tests were used to assess for differences at baseline in the lipoprotein and nonlipoprotein subfractions among the study participants randomized into the fish oil and placebo treatment allocation groups. Differences in lipoprotein and nonlipoprotein levels over time were determined using separate mixed model analysis of variance models. The initial value was used as a covariate to control for any differences at baseline, with the most appropriate covariance structure selected using the Akaike information criterion.[35]

Univariate Analysis

Kaplan-Meier curves were constructed for all the categorical predictors (Appendix C-F), in order to provide insight into the shape of the survival function for each group and give an idea of whether or not the groups are proportional. Tests of equality across strata were used to explore whether or not to include the predictor in the final model. For the categorical variables, the log-rank test of equality across strata was used, which is a non-parametric test. For the

continuous variables, univariate Cox proportional hazard regression was used, which is a semi-parametric model.

For the lipoproteins, HDL and LDL, associations with ventricular tachyarrhythmias with a p-value of less than 0.05 were considered statistically significant. For the nonlipoproteins, total cholesterol, triglycerides, and total cholesterol:HDL, associations with ventricular tachyarrhythmias with a p-value of less than 0.05 were considered statistically significant.

Assessment of Confounding

Univariate models of the lipoprotein and nonlipoprotein subfractions and ventricular tachyarrhythmias were assessed for confounding by gender, treatment allocation, NYHA Class, ejection fraction, documented coronary artery disease, presenting arrhythmia, statin use, history of myocardial infarction, and diabetes at enrollment. All these factors are known to be associated with both lipoprotein and nonlipoprotein subfractions and with ventricular tachyarrhythmias.

A difference of at least 10% in the odds ratios of the lipid subfraction variable in bivariate and full models, with and without these adjustment covariates was considered evidence of confounding by the covariate being assessed.[32]

Construction of a Main-Effects Cox Proportional Hazards Model

A Cox proportional hazards model was used to assess the significance of the primary outcome controlling for other baseline characteristics. Variable selection was performed with these baseline characteristics using all possible regression models with the score statistic and

stepwise addition of variables.[36-38] Treatment group was forced into the equation to account for design effects in the original randomized controlled trial.

Variables that were significant in the univariate analysis with $p < 0.05$ for lipoprotein and nonlipoprotein variables and $p < 0.25$ for demographic and clinical variables were selected for the multivariate Cox proportional hazards model. Fish oil vs. placebo treatment allocation was forced into the final model based on its importance in the overall design of the randomized control trial.

Exploration of Non-Linear or Interaction terms to Include in Final Model

Proper scaling of the continuous variable (age) was assessed visually with Lowess Smoothing curve, scatter plots, and histograms, as well as in tertiles, quartiles, and dichotomous splitting. The most statistically significant categorization scheme of age in the main effects model was used.

Possible interactions between each lipoprotein and nonlipoprotein variable and gender, treatment allocation, ejection fraction, statin use, history of myocardial infarction, and diabetes at enrollment. Interaction terms that were significant when added individually to the main effects Cox proportional hazards model were added simultaneously to the main effects model. Interaction terms significant at Wald statistic $p < 0.05$ were retained in the final model.

Assessment of the Proportionality Assumption

Proportionality of the Cox proportional hazards model was assessed using time-dependent covariates in the model, which are interactions of the predictors and time. If a time-dependent covariate is significant at $p < 0.05$, this indicates a violation of the proportionality assumption for that specific predictor.

Proportionality was also assessed by using Schoenfeld and scaled Schoenfeld residuals for each predictor. If the tests in the table are not significant (p-values over 0.05), then the proportionality assumption cannot be rejected and it is assumed that there is no violation of the proportional assumption. A horizontal line in the graphs further indicates that the predictors do not violate the proportionality assumption.

Evaluation of Collinearity between Variables

In the event of multicollinear trends between variables, correlation matrices will be assessed and regression analyses will be performed to evaluate betas (standardized regression coefficients). Variance Initiation Factors (VIFs) and tolerance of the regression analyses will be assessed for evidence of collinearity between variables. VIFs greater than 10 are generally seen as indicative of severe collinearity and tolerance of 1.0 is generally seen as indicative of absence of multicollinearity.

Assessment of the Final Model

The final model's goodness-of-fit was assessed using Cox-Snell residuals. If the model fits the data well, then the true cumulative hazard function conditional on the covariate vector has an exponential distribution with a hazard rate of one. The Nelson-Aalen cumulative hazard

function is graphed with the Cox-Snell residual variable in order to compare the hazard function to the diagonal line. If the hazard function follows a 45 degree line, then it is confirmed that it approximately has an exponential distribution with a hazard rate of one and the model fits the data well.

RESULTS

Study Population Characteristics

200 subjects were randomly allocated to receive fish oil or placebo treatment for 2 years. The demographic characteristics (Table 1) and clinical characteristics (Table 2) were similar for both treatment groups, which can be attributed to the success of the randomization procedure. 54 study participants in the fish oil treatment group were taking statin medication at baseline compared to 41 study participants in the placebo group (Table 3).

Those randomized into the fish oil treatment group had a total cholesterol level 8 mg/dL above those from the placebo group, LDL level 2.3 mg/dL above those from the placebo group, and an HDL level 0.4 mg/dL below those from the placebo group (Table 4). In addition the average LDL level among those who took statins was 89.5 mg/dL compared to 98.1 mg/dL among those who were not taking statins upon enrollment.

At baseline, there was no statistically significant difference in the total cholesterol, LDL, HDL, triglyceride levels, and total cholesterol:HDL ratio between fish oil and placebo groups. In addition, there was no statistically significant difference in the total cholesterol, LDL, HDL, triglyceride, and total cholesterol:HDL ratio over time in the study population. These findings allow the use of baseline lipoprotein and nonlipoprotein subfractions in the univariate analysis and Cox proportional hazard model building.

Nelson-Aalen cumulative hazard estimates and Kaplan-Meier survival curves shows the visual trend that 40% of first episodes of ICD therapy for VF occurred within the first 100 days

after randomization in the study (Figure 1, Figure 2) and 50% of first episodes of ICD therapy for VT occurred within the first 100 days after randomization in the study (Figure 3, Figure 4).

Univariate Analysis

Independent Predictors for Ventricular Fibrillation

We analyzed LDL as a continue and discreate variable. Treated as a continuous variable at the univariate level, LDL was significantly associated with ventricular fibrillation ($p = 0.027$; hazard ratio, 1.02; 95% CI, 1.002 – 1.039). There were no ventricular fibrillation events in study subjects with LDL less than 64 mg/dL. In addition, HDL was not a significant predictor of arrhythmic events, treated continuously and categorically as quartiles, although as expected, its hazard ratio of 0.973 is indicative of an antiarrhythmic effect (95% CI, 0.928 – 1.020). Those in the highest LDL quartile were observed to have more VF events overall and earlier in the course of the longitudinal study ($p = 0.417$) compared to the other three quartiles (Appendix C). No such trend was observed for HDL, as no one particular quartile appeared to have an increased risk for developing VF. The nonlipoprotein subfractions of total cholesterol, triglycerides, and total cholesterol:HDL ratio were also not not significant as independent predictors of VF at the univariate level (Table 5, Table 6).

Among the demographic and clinical variables, gemfibrozil use, coronary artery disease, and entry arrhythmia were significant independent covariates for VF at the univariate level with p-values < 0.25. (Table 9).

Independent Predictors for Ventricular Tachycardia

None of the lipoprotein and nonlipoprotein subfractions – treated continuously and categorically as quartiles – were significant univariate predictors for ventricular tachycardia (Table 7, Table 8). Those in the lowest two LDL quartiles tended to have more VT events and also had them earlier ($p = 0.077$) compared to the highest two LDL quartiles (Appendix E). The second lowest HDL quartile had more VT events ($p = 0.501$), but they did not occur earlier compared to the other three HDL quartiles. Those in the lowest total cholesterol quartiles had more VT events ($p = 0.343$) but not earlier ones (Appendix F).

Among the demographic and clinical variables, fish oil status, NYHA class, ejection fraction, gender, history of myocardial infarction, diabetes mellitus, coronary artery disease, and entry arrhythmia were significant univariate predictors for ventricular tachycardia at $p < 0.25$ (Table 9).

Assessment of Confounding and Associations among Covariates

Absence of Confounding

Neither treatment allocation, NYHA class, ejection fraction < 40%, age, gender, use of statin medication, history of MI, hypertension at enrollment, diabetes at enrollment, documented CAD, nor presenting arrhythmia confounded the relationship between lipid subfractions and ventricular tachyarrhythmias.

Final Cox proportional hazard model for Ventricular Fibrillation

The final Cox proportional hazard model for VF included two covariates: LDL treated as a continuous variable, and fish oil treatment allocation (Table 10). The interaction of LDL vs. treatment allocation was assessed and was not significant. As such, no interaction terms were included in the final Cox proportional hazards model. Although fish oil treatment allocation was not significant at the univariate level, it was forced into the model based on the importance of treatment allocation in the design of the randomized control trial. A summary on the analysis of the proportionality assumption and goodness-of-fit of the final VF model is discussed in Appendices G-H.

Final Cox proportional hazard model for Ventricular Tachycardia

The final Cox proportional hazard model for VT included three covariates, ejection fraction less than 40%, presenting arrhythmia, and fish oil treatment allocation (Table 11). Neither LDL nor HDL were significant at the univariate level to warrant inclusion into the final model. Even after adjusting for LDL and HDL, neither of these lipoprotein subfractions was significant in the multivariate model. The interactions of ejection fraction vs. presenting arrhythmia, ejection fraction vs. fish oil treatment allocation, and presenting arrhythmia vs. fish oil treatment allocation were assessed, none of which were significant. As such, no interaction terms were included in the final Cox proportional hazards model. A summary on the analysis of the proportionality assumption and goodness-of-fit of the final VT model is discussed in Appendices I-J.

Interpretation of the Final Models

After adjusting for fish oil treatment allocation group, each 1 mg/dl increase in LDL level was associated with a 2% increase in the hazard ratio of ventricular fibrillation ($p = 0.027$). HDL was not a significant predictor of VF.

Neither LDL nor HDL was a significant predictor for VT. An ejection fraction less than 40% (hazard ratio 1.5; 95% CI, 1.03 – 2.24) and VT as the qualifying arrhythmia (hazard ratio 2.5; 95% CI, 1.55 – 4.16) were significant independent predictors of time to ICD therapy for VT. This indicates that study subjects in our cohort with an ejection fraction < 40% had a 50% increased risk of VT compared to those with an ejection fraction < 40%. When treatment assignment was added to this model, the fish oil group had a hazard ratio of 1.5 (95% CI, 1.02 – 2.21). This finding is not unexpected, as the original fish oil RCT found similar results for the combined endpoint of VT/VF and has been reported.[20]

Limitations of the study

This study was underpowered in its ability to detect differences in the risk of ventricular fibrillation and ventricular fibrillation, independently. The design of the original randomized control trial was to analyze VT and VF as a combined event. Post hoc power analysis showed that this study had only 28% power for ventricular fibrillation and 65% power for ventricular tachycardia to detect an effect size difference if the true hazard ratio is 1.33. The low power was due to low numbers of first episodes of ventricular fibrillation (16) and ventricular tachycardia (106). The findings of this investigation need to be confirmed in a larger, higher powered study.

Data was available regarding statin use at the time of enrollment was available but the dose and frequency of statin administration was not reliable. Although statin use was statistically insignificant in the multivariate Cox proportional hazards model, statins have been found to have antiarrhythmic properties.[65,71] It is unknown the magnitude of effect that it has on ventricular tachyarrhythmia based on type of statin, dosage, and frequency of administration.

The analysis did not control for all known risk factors of ventricular tachyarrhythmias, such as family history and excessive alcohol use. Inclusion of these potential confounders may have demonstrated a different relationship between lipoprotein risk factors and ventricular tachyarrhythmias.

DISCUSSION

Sudden cardiac death currently accounts for 5.6% of annual mortality based on death certificate data from Multnomah County, Oregon.[39] With the alarming human toll of SCD, much effort has been made in the recent years to refine and enhance the risk stratification approach for implantation of ICDs for primary and secondary prevention of SCD. Recent data showing that 70% of those who die from SCD with available echocardiograms do not meet current indications for ICD implantation.[59] Furthermore, a history of heart failure was present in only 12% of 492 consecutive patients with out-of-hospital SCD in Maastricht, Netherlands.[60] These findings highlight the need for refining current ICD implantation guidelines and better risk stratification for SCD. Currently, noninvasive techniques for risk stratification include QRS duration[40] and signal averaged ECG [41,42] for slowed conduction, QT interval and T-wave alternans for repolarization abnormalities[40-44], heart rate variability (HRV) [45-47], heart rate turbulence (HRT) [48], HRR baroreceptor for ANS tone changes, LVEF[49-50] and MRI to assess myocardial damage, and Holter monitoring for ventricular ectopy. The circumstances surrounding cardiac death from arrhythmic activity require the definition of certain electrophysiologic parameters. Alterations in autonomic nervous system activity involve heart rate variability and baroreflex sensitivity (BRS) [51-52]. Myocardial vulnerability can be delineated through ejection fraction, the presence or absence of atrial fibrillation, QRS duration, QT interval[53-54] and T wave variability.[55-58]

Currently, there are no data to support the use of any single technique for prediction of SCA risk. The optimal strategy should identify the vast majority of those who will experience sudden cardiac death and a minimal number who will not. Most current risk stratification techniques predict overall mortality rather than arrhythmic death. The most useful strategy will

likely involve a combination of clinical, demographic, and noninvasive techniques. Whether these should be applied simultaneously or sequentially is also unclear. Only further prospective trials such as CARISMA may better elucidate a strategy that truly identifies “at risk” populations who are vulnerable to sudden cardiac death.

As a result of this paradigm shift, there is heightened emphasis on improving noninvasive risk stratification for ICD placement. The most optimal SCD risk stratification approach should follow a stepwise process, starting from evaluating patient demographic factors (age, EF, NYHA) to clinic setting (cardiac arrest, CHF, post MI, CMP, prior CAD) and ending with noninvasive testing (echocardiogram, EKG, Holter, ETT, or MTWA). There are emerging techniques that may also enhance the process of SCD risk stratification. Such emerging techniques include cardiac MRI, electrophysiology studies, J point elevation, serum biomarkers, CRP, and multivariable techniques deemed plausible from the CARISMA study.

In the midst of heightened efforts to identify significant predictors of SCD, it may be useful to investigate the association between LDL, an inducer of arterial inflammation and atherosclerosis, and the ventricular tachyarrhythmias responsible for SCD. This investigation primarily focuses on the role of LDL in its ability to risk stratify patients for secondary prevention of SCD. All 200 patients in this cohort study were enrolled either upon ICD implantation for an ECG-documented episode of sustained VT or VF or had a preexisting ICD and received ICD-therapy for an ECG-documented episode of sustained VT or VF.

This investigation sheds new light on the significance of LDL levels and its potentially predictive role in the induction of the ventricular tachyarrhythmias in a cohort of individuals who have already experienced and survived a prior VT or VF event. The finding that ejection fraction less than 40% serves as an independent predictor of time to ICD therapy for VT may be reflective of potentially different mechanisms between VT and VF. This observation is also reflected in the quartile trends of the Kaplan-Meier survival curves among the lipid subfractions (Appendices C-F). It was expected that there were more VF events in those with LDL levels in the highest quartile. However, it was unexpected that there were more VT events in those with LDL levels in the lowest half and in those with total cholesterol in the lowest quartile. It would be difficult to discern the cause behind these differing trends with regard to LDL without knowing the corresponding HDL level, as the level of atherosclerosis and potentially ischemia is influenced by the delicate balance between LDL and HDL levels. However, these trends support the finding that LDL is a significant independent predictor for VF and not for VT, before adjusting for treatment allocation. If these findings can be confirmed in an adequately powered investigation, they would be supportive of observations that lipid-lowering through statin pharmacotherapy lead to a statistically significant benefit on the reduction of risk of ventricular arrhythmia among patients with coronary artery disease and an ICD.[61]

Public Health Implications

If these findings can be confirmed in an adequately-powered study, there are significant public health implications from the finding that high LDL levels are a risk factor for VF and EF < 40% is a risk factor for VT. Among those who have survived a prior episode of VT or VF, they may be the focus of heightened emphasis for lipid-lowering to prevent recurrent ventricular

tachyarrhythmias that may lead to sudden cardiac death. In addition, the significance of LVEF < 40% as an independent predictor for VT would strengthen the evidence that those who survive initial episodes of VT or VF are indicated for echocardiographic evaluation for cardiac structure/function abnormalities that are associated with ventricular tachyarrhythmias.

There have been several observational studies with sound analytical approaches and adequate controlling of confounders that have shown the significant benefit of statin pharmacotherapy for lipid-lowering in reducing the risk of ventricular arrhythmias. An analysis of 281 consecutive ICD patients from the Cleveland Clinic showed that statins conferred a significant protective effect among patients with CAD and an ICD from ventricular tachyarrhythmias compared to those not taking statins.[62] Statins have also been shown to decrease mortality in patients with ICDs implanted for ventricular tachyarrhythmias and LV systolic dysfunction.[63] Both of these study populations are different from the fish oil cohort in that the fish oil study population have only 73% of patients with CAD and 52% of patients with LVEF < 40% indicative for LV systolic dysfunction. An observational study in 78 patients with CAD and life-threatening ventricular tachyarrhythmias treated with ICD showed that the use of lipid-lowering therapy is associated with a reduction in recurrences in ventricular arrhythmias in patients with CAD and ICD implants.[64] While our findings do not support the use of statins for the reduction of the risk of recurrent ventricular tachyarrhythmias, they do show a significant association between LDL levels and ventricular tachyarrhythmias in a cohort of ICD patients who have experienced a prior episode of VT or VF. The role of statins and its association with ventricular tachyarrhythmias for secondary prevention of SCD could be a future analytical direction. Current guidelines regarding target LDL levels are < 100 mg/dL in high risk cardiac

patients and < 70 mg/dL for those at very high risk (ie, those who have experienced myocardial infarction). LDL is presumed to cause MI and CAD patients through its role in the development of atherosclerosis via the deposition of cholesterol-rich foam cells in coronary artery walls, and induction of cytokines such as TNF-alpha, interleukin 1, and interleukin 6. There are no known etiology regarding LDL causing ventricular fibrillation, but it is hypothesized that the combination of cardiac inflammation through cytokine release and resulting increase in the dispersion of refractoriness would aid the precipitation of the microreentry circuits responsible for VT in the right conditions (ie, ischemia, increased vagal tone, increased sympathetic tone).

A number of factors contribute to high LDL-cholesterol, including a high-saturated fat, high-cholesterol diet, family history/genetics, lack of physical activity, age, gender, and other concurrent health conditions such as hypothyroidism, hypertension and diabetes. If these findings can be confirmed, there may be a greater emphasis in diet control of LDL levels in addition to pharmacologic lowering of LDL levels in those who have survived an episode of VT or VF.

Future Directions

Confirmation in an Adequately-Powered Investigation

The most significant limitation of this investigation was that it was underpowered in its ability to detect a 33% change in the hazard ratio for VT and VF as the arrhythmic outcome. Further confirmation of this study's findings is needed and this can be pursued through the enrollment of more ICD patients in order to increase the number of study subjects who experience ventricular fibrillation during the follow up period.

New Questions Arise

There are several new interesting questions arising from the conclusion of this study that would warrant future investigation. If high LDL is associated with induction of VF, can statin treatment prevent future ICD shocks due to VF? If low EF is associated with induction of VT, can improving EF with cardiac medications or cardiac resynchronization therapy prevent ICD shock due to VT? The multivariate significance of LDL with VF sheds new light on the utility of using high-sensitivity C-reactive protein (a marker for cardiac inflammation) to monitor patients at highest risk for VF and basic natriuretic peptide (a marker of ventricular stretch) to monitor patients at highest risk for VT.

Generalizability

The findings from this study come from a cohort of individuals that are not representative of the general population. In particular, a higher proportion of these ICD cohort patients have nonischemic cardiomyopathy, lower total cholesterol levels, and lower LDL levels compared to the general population. The most recent NHANES findings showed that the average total cholesterol in the general population from 1999-2002 was 203 mg/dL and the average LDL was 123 mg/dL, values that are higher than those observed for this cohort. These findings may be attributed to the demographic characteristic that the majority of the patients enrolled in this study were being cared for in tertiary care institutions and may be receiving frequent medical care because of their ICDs. In addition, the average HDL level and triglyceride level in the general population from NHANES from 1999-2002 was 43 mg /dL and 123 mg/dL, respectively. The lower HDL level observed in this cohort may be attributed to the higher levels of triglycerides, which, in turn, may be attributed to relative nutritional deficiencies leading to increased

mobilization of adipose triglycerides releasing free fatty acids bound to albumin, causing a FFA influx to the liver. It would be worthwhile to not only increase the power of the study by increasing the number of study subjects, but to also select from a population more representative of the clinical cardiovascular profiles of the general populace. This could be achieved by enrolling patients from primary and secondary care facilities in addition to tertiary care medical centers.

A Different Research Design

The most significant limitation of this investigation is the underpowered nature based on the relatively few events of time to first ICD therapy for VF (16). Further investigation on the significance of LDL as a predictor for ventricular tachyarrhythmias is needed, particularly using a larger sample size of study subjects who experienced ventricular fibrillation. It is possible to combine the outcomes of VF and VT into one, as in the original fish oil investigation to increase power. However, even in the original study, post hoc power analysis showed that, although the study was designed to have a 92% chance of detecting a 33% reduction in event rate, the total event rate in the placebo group and the difference between placebo and fish oil were less than predicted and that the study only had 70% power to detect a 33% reduction in event rate.

In addition, there are other research designs that can answer the question of lipoprotein risk factors for arrhythmic events in a cohort of ICD patients. An example is a nested case-control study of whether higher levels of LDL and total cholesterol were risk factors for breast cancer. Our investigation utilized a survival analysis to evaluate the main outcome of time to ICD therapy. A nested case-control study would identify cases at the end of the 2-year follow-up

as those who had experienced a ventricular tachyarrhythmia during the follow-up period. Then controls could be selected from those who did not experience a ventricular tachyarrhythmia. The analysis would center on the measured lipoprotein predictors on the baseline samples from the identified cases and controls.

This nested case-control design preserves all the advantages of cohort studies that result from collecting predictor variables before the outcomes have happened, and it avoids the potential biases of conventional case-control studies that draw cases and controls from different populations and cannot make measurements on cases and controls who have died. The chief disadvantage of this design is that many research questions and circumstances are not amenable to the strategy of storing materials for later analysis on a sample of study subjects, although this would not be an issue with the lipoprotein investigation as there are no such perishable samples. Also, when data are available for the entire cohort at no additional cost, nothing is gained by studying only a sample of controls, as the entire cohort should be used.

Another alternative study design is to analyze the time to each VF or VT event in a time to repeated events analysis. This would be especially helpful in elucidating the risk of ventricular tachyarrhythmias by taking into account that such arrhythmic events can occur in “storms” and can clarify the risk of developing these arrhythmias in a short period of time.

SUMMARY AND CONCLUSIONS

This study was undertaken to better understand the effect of plasma lipoproteins and lipids on the risk of developing ventricular tachyarrhythmias responsible for sudden cardiac

death. Among patients with a recent episode of sustained ventricular arrhythmia and an ICD, LDL level was a significant predictor for ventricular fibrillation before adjusting for fish oil or placebo treatment allocation. However, none of the lipoproteins were significant predictors for ventricular tachycardia. Moreover, the significance of ejection fraction as a predictor for ventricular tachycardia after adjusting for entry arrhythmia may point to different pathologic mechanisms between the two ventricular tachyarrhythmias most responsible for sudden cardiac death. This information may be useful when risk stratifying patients for ICD implantation.

Table 1: Baseline Demographic Profile of the Study Population

Demographic Variable	Placebo (100)	Fish Oil (100)
Age, mean (SD), in years	62 (13)	63 (13)
Male	86	86
White	97	94
VT at entry	69	64
Enrolled at ICD implantation	56	58

Table 2: Baseline Cardiac Profile of the Study Population

Cardiac Variable	Placebo (100)	Fish Oil (100)
Coronary artery disease	71	75
Myocardial infarction	56	55
Nonischemic cardiomyopathy	31	35
LVEF, %, quantitative mean (SD) (n = 169)	34 (15)	36(16)
LVEF, %, qualitative mean (n = 195) < 40	56	57
Hypertension	55	46
Diabetes	23	24
NYHA functional class 1	28	25
NYHA functional class 2	14	13
NYHA functional class 3	50	48
NYHA functional class 4	8	14

Table 3: Baseline Medication Profile of the Study Population

Medications	Placebo (100)	Fish Oil (100)
Beta-blocker	73	74
ACE inhibitor	66	66
Calcium channel blocker	13	9
Statin	41	54
Digoxin	33	29
Diuretic	54	52

Table 4: Baseline Lipid Subfraction Profile of the Study Population

Lipid Subfraction	Fish oil (100)	Placebo (99)*
Total cholesterol mean (SD)	158.4 (34)	166.3 (37)
LDL mean (SD)	92.8 (27.1)	97.5 (29.7)
HDL mean (SD)	35.1 (15.6)	34.7 (10.8)
VLDL mean (SD)	30.4 (14.6)	33.9 (23.6)
Triglycerides mean (SD)	161.4 (93.3)	182.9 (140.7)

* one study subject did not have a baseline lipid panel available for analysis

Table 5: Univariate Results (Cox proportional hazards model) for Continuous Lipid Panel Subfractions using VF as the Outcome

Lipoprotein Subfractions	Hazard ratio	Standard error	P-value	95% CI
LDL	1.02	0.009	0.027	1.002 - 1.038
HDL	0.97	0.023	0.264	0.928 - 1.020
Nonlipoprotein Subfractions	**Hazard ratio**	**Standard error**	**P-value**	**95% CI**
Total cholesterol	1.01	0.006	0.288	0.993 - 1.020
Triglycerides	0.99	0.002	0.488	0.992 - 1.003
Total cholesterol:HDL	1.18	0.124	0.101	0.966 - 1.457

Table 6: Univariate Results (Log-rank test) for Quartiled Lipid Panel Subfractions using VF as the Outcome

Lipoprotein Subfractions	Chi Square	P-value	95% CI
LDL	2.84	0.416	0.993 – 1.004
HDL	1.34	0.719	0.989 – 1.019
Nonlipoprotein Subfractions	**Chi Square**	**P-value**	**95% CI**
Total cholesterol	0.8	0.849	0.986 – 1.029
Triglycerides	1.28	0.733	0.987 – 1.018
Total cholesterol:HDL	1.18	0.590	0.994 – 1.006

Table 7: Univariate Results (Cox proportional hazards model) for Continuous Lipid Panel Subfractions using VT as the Outcome

Lipoprotein Subfractions	Hazard Ratio	Standard Error	P-value	95% CI
LDL	0.99	0.003	0.365	0.990 - 1.004
HDL	1.01	0.007	0.888	0.987 - 1.014
Nonlipoprotein Subfractions	**Hazard Ratio**	**Standard Error**	**P-value**	**95% CI**
Total cholesterol	0.998	0.003	0.603	0.992 - 1.004
Triglycerides	1.000	0.001	0.864	0.998 - 1.001
Total cholesterol:HDL	0.933	0.051	0.203	0.839 - 1.037

Table 8: Univariate Results (Log-rank test) for Quartiled Lipid Panel Subfractions using VT as the Outcome

Lipoprotein Subfractions	Chi Square	P-value	95% CI
LDL	6.86	0.076	0.999 – 1.002
HDL	2.36	0.501	0.987 – 1.021
Nonlipoprotein Subfractions	**Chi Square**	**P-value**	**95% CI**
Total cholesterol	3.33	0.343	0.991 – 1.009
Triglycerides	1.85	0.603	0.981 – 1.029
Total cholesterol:HDL	1.17	0.519	0.984 – 1.023

Table 9: Univariate P-values for Demographic and Clinical Variables

Baseline Variables	Continuous Lipids		Quartiled Lipids	
	VT	VF	VT	VF
Fish oil status	**0.111**	0.975	**0.111**	0.975
NYHA Class	**0.006**	0.538	**0.006**	0.538
Smoking	0.794	0.322	0.794	0.322
Ejection fraction	**0.005**	0.365	**0.005**	0.365
Age	0.333	0.279	0.333	0.279
Gender	**0.081**	0.937	**0.081**	0.937
Statin use	0.791	0.332	0.791	0.332
Bile acids use	0.533	0.752	0.533	0.752
Niacin use	0.473	0.591	0.473	0.591
Gemfibrozil use	0.877	**0.209**	0.877	**0.209**
Myocardial infarction	**0.119**	0.513	**0.119**	0.513
Hypertension	0.274	0.676	0.274	0.676
Diabetes Mellitus	**0.195**	0.926	**0.195**	0.926
Coronary artery disease	**0.196**	**0.111**	**0.196**	**0.111**
Entry arrhythmia	**0.184**	**0.001**	**0.184**	**0.001**

Table 10: Final Cox proportional hazard model for VF, with and without adjusting for treatment allocation

Variable	Hazard Ratio	Standard Error	P-value	95% Confidence Interval
Cox proportional hazard model's overall p-value: 0.0268				
LDL	1.02	0.009	0.027	1.002 - 1.038
Cox proportional hazard model's overall p-value: 0.0841				
Treatment allocation	1.12	0.563	0.824	0.416 - 3.003
LDL	1.02	0.009	0.027	1.002 - 1.039

Table 11: Final Cox proportional hazard model for VT, with and without adjusting for treatment allocation and lipoproteins

Variable	Hazard Ratio	Standard Error	P-value	95% Confidence Interval
Cox proportional hazard model's overall p-value: 0.0001				
Ejection fraction < 40%	1.51	0.301	0.036	1.027 - 2.241
Entry arrhythmia	2.54	0.639	0.0001	1.555 - 4.163
Cox proportional hazard model's overall p-value: 0.0001				
Treatment allocation	1.51	0.296	0.04	1.019 - 2.209
LDL*	0.99	0.003	0.844	0.993 - 1.006
HDL*	1.01	0.007	0.842	0.987 - 1.016
Ejection fraction < 40%	1.47	0.293	0.052	0.995 - 2.176
Entry arrhythmia	2.71	0.687	0.0001	1.652 - 4.461

* - not included in the final model

Figure 1: Nelson-Aalen Cumulative Hazard Estimate for Ventricular Fibrillation

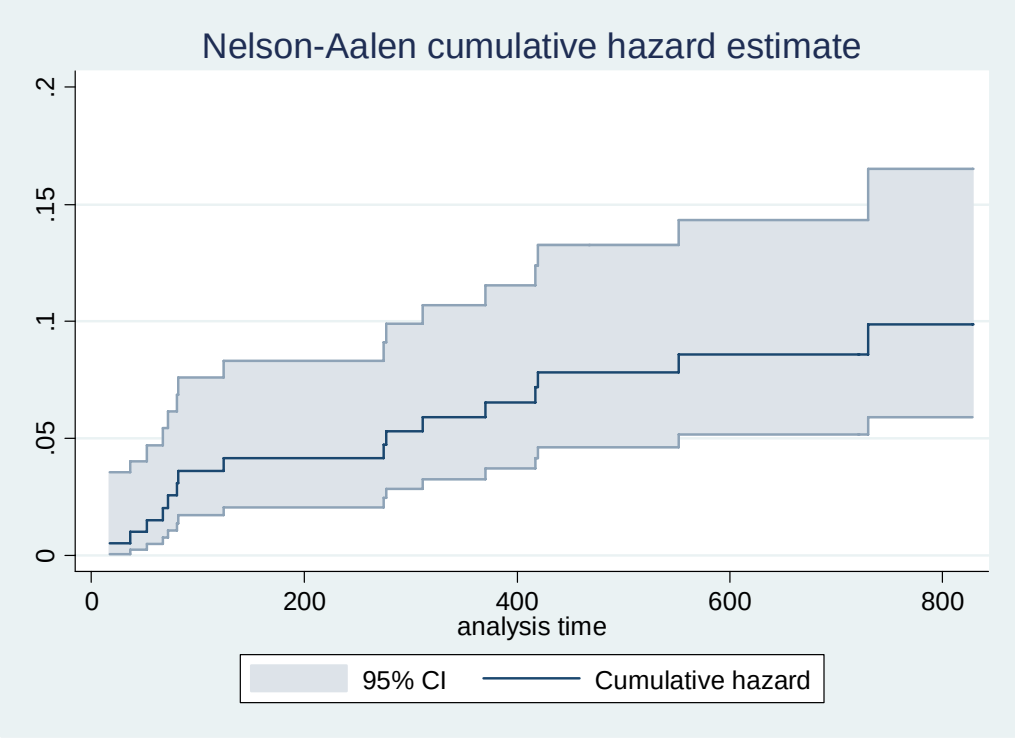


Figure 2. Kaplan-Meier Survival Curve for Ventricular Fibrillation

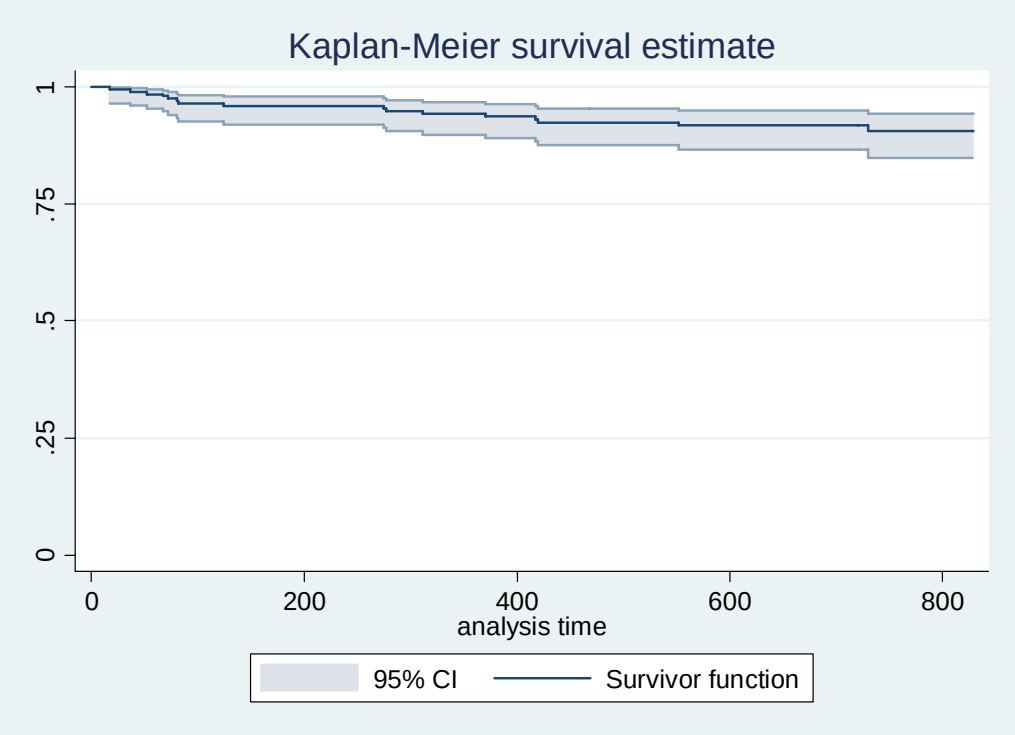


Figure 3. Nelson-Aalen Cumulative Hazard Estimate for Ventricular Tachycardia

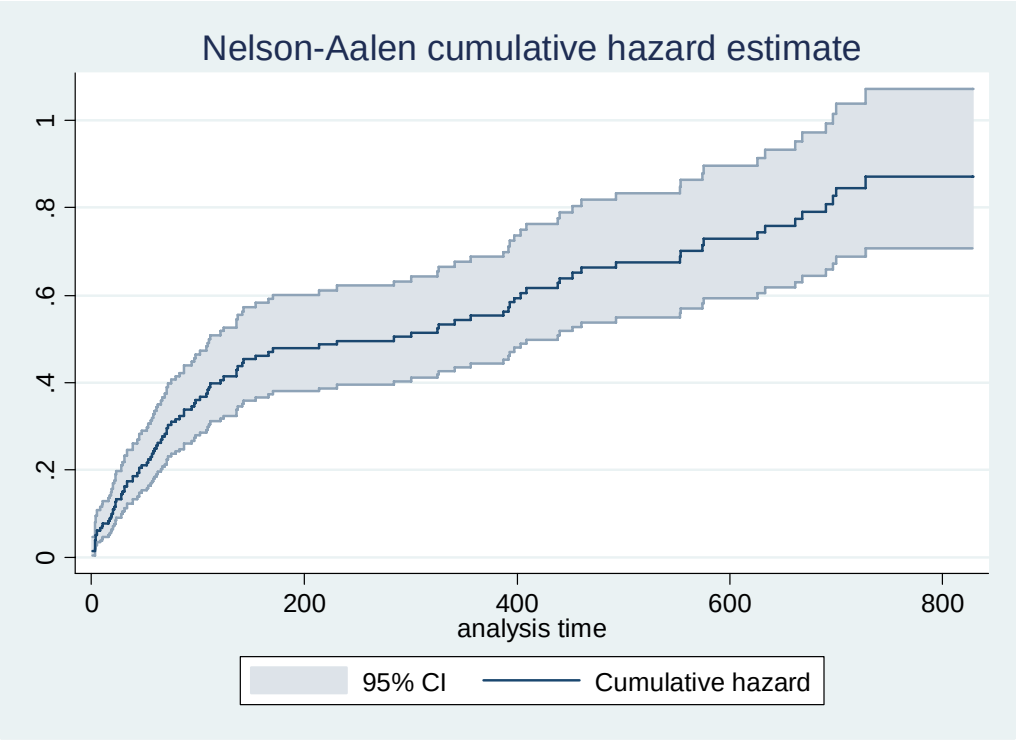
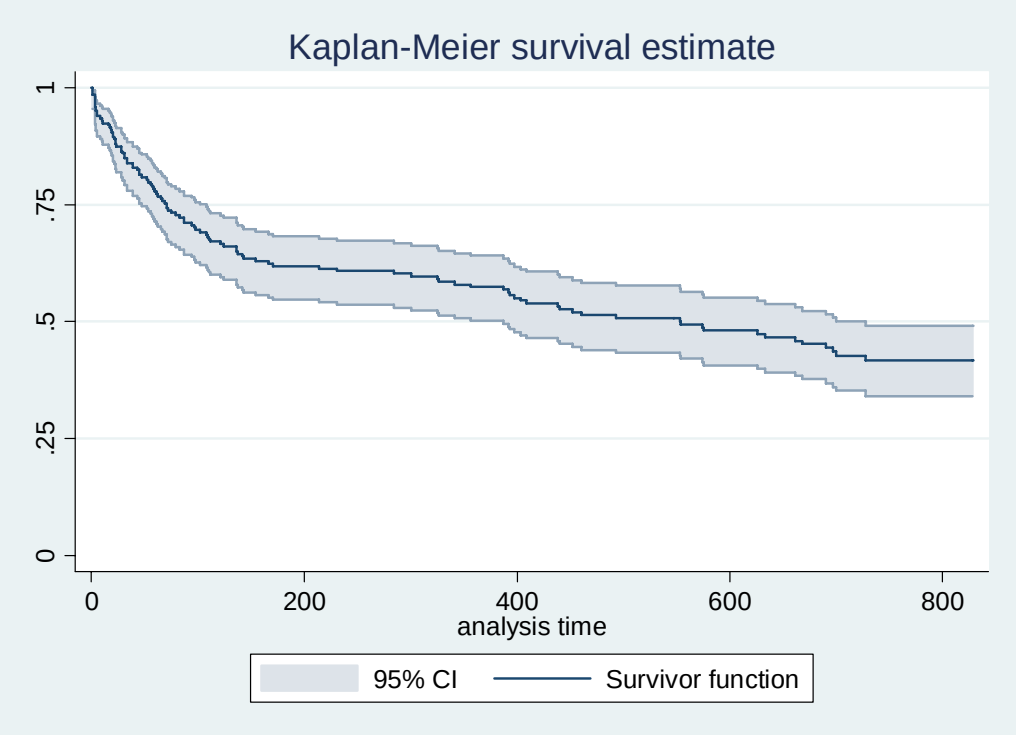


Figure 4. Kaplan-Meier Survival Curve for Ventricular Tachycardia



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APPENDICES

Appendix A. Variable Definitions of Potential Confounders

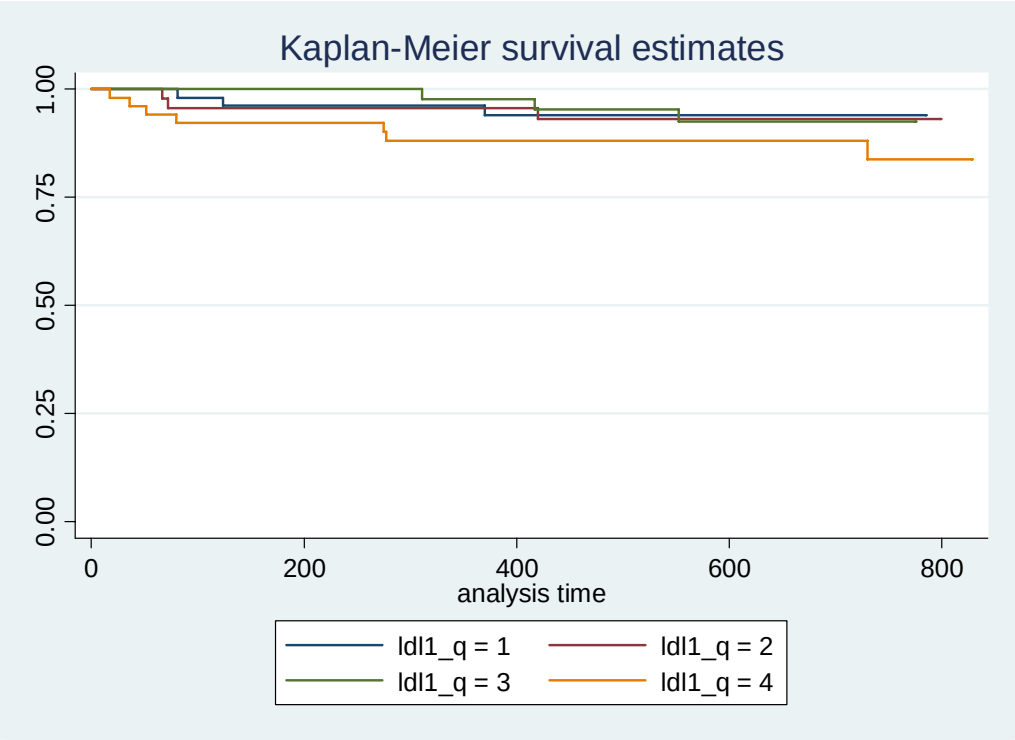
Potential Confounding Variable	Values	Analysis
Treatment allocation	Fish oil Placebo	Categorical
New York Heart Association Class	No heart failure Class I Class II Class III Class IV	Categorical
Smoking	Yes No	Categorical
Ejection fraction	≥ 40% < 40%	Categorical
Age	Age in years	Continuous
Gender	Male Female	Categorical
Use of statin medications	Yes No	Categorical
History of myocardial infarction	Yes No	Categorical
Hypertension at enrollment	Yes No	Categorical
Diabetes at enrollment	Yes No	Categorical
Documented coronary artery disease	Yes	Categorical

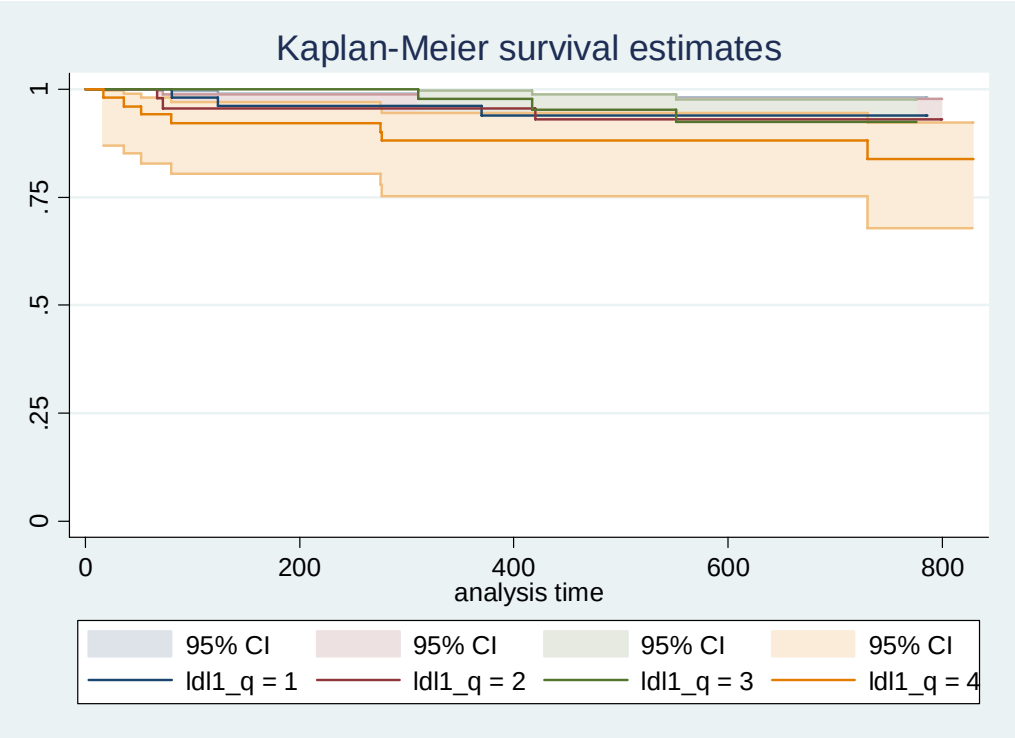
	No	
Presenting arrhythmia	VF VT	Categorical

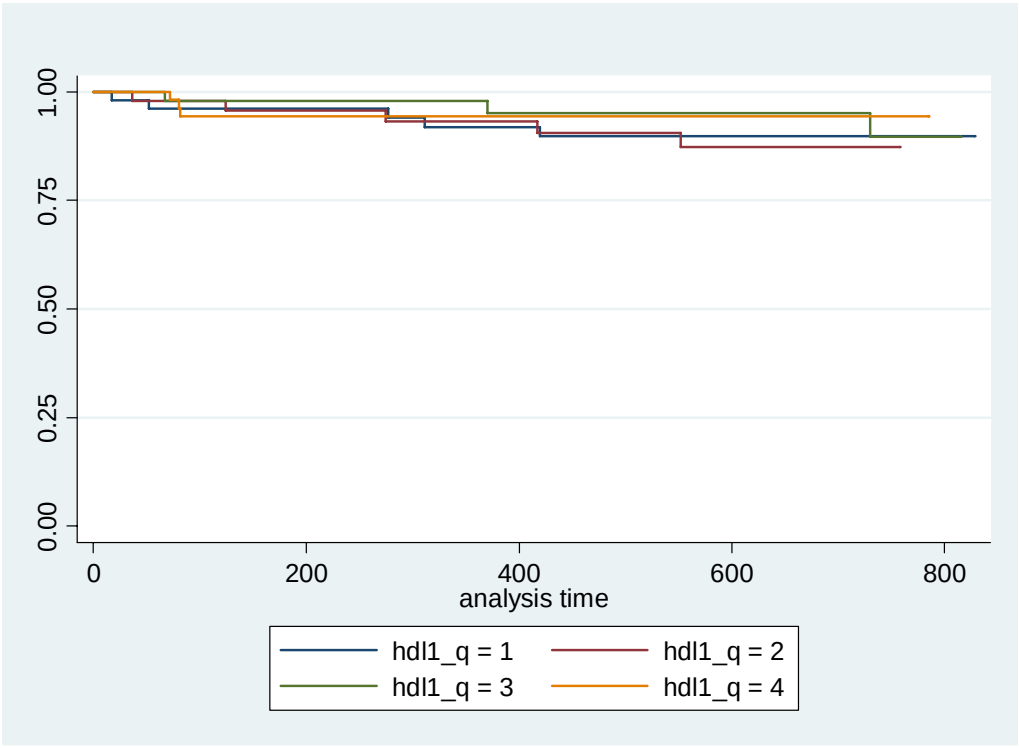
Appendix B – Akaike Information Criterion for Selection of Best Model for Repeated Measures ANOVA

	1st Order Autoregressive	Compound symmetry	Unstructured	Huynh-Feldt
Total cholesterol	6173.3 (AIC)	6187.4 (AIC)	**6153.4 (AIC)**	6167.5 (AIC)
LDL	**5838.4 (AIC)**	5845.7 (AIC)	5846.7 (AIC)	5846.9 (AIC)
HDL	**4852.7 (AIC)**	4859.7 (AIC)	4857.7 (AIC)	4865.3 (AIC)
Triglycerides	7691.2 (AIC)	7695.7 (AIC)	**7633.3 (AIC)**	7663.1 (AIC)
VLDL	5433.6 (AIC)	5448.7 (AIC)	**5341.9 (AIC)**	5366.1 (AIC)

Appendix C – Kaplan Meier curves for lipoprotein subfractions for VF






1.00
0.75
0.50
0.25
0.00
0
200
400
600
800
analysis time
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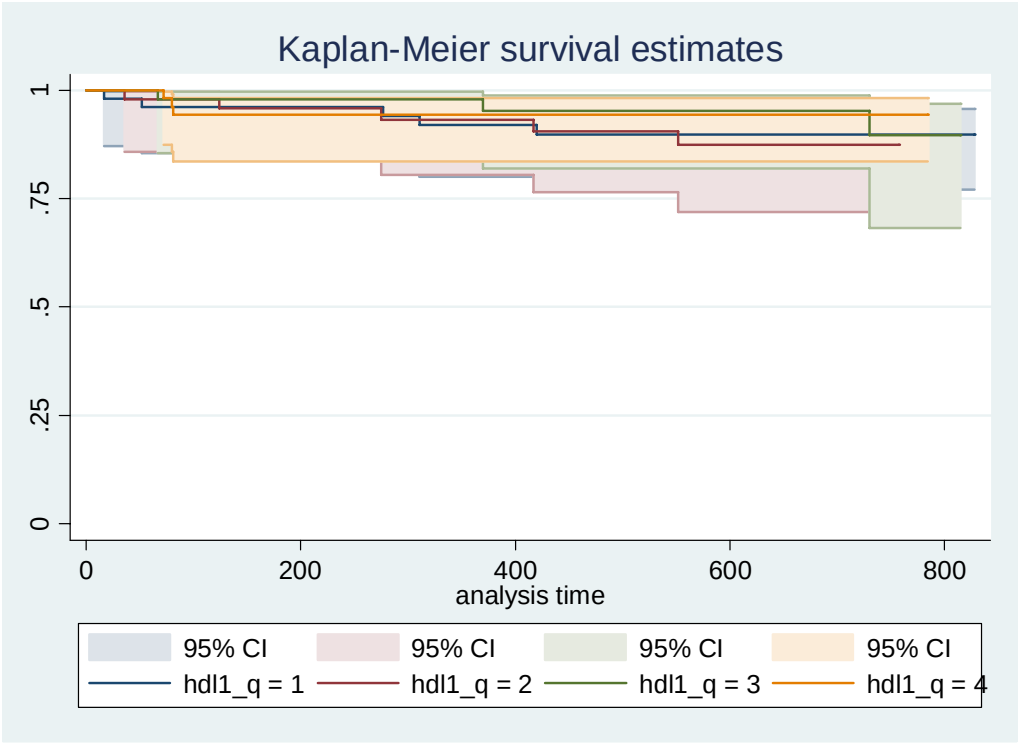
hdl1_q = 2
hdl1_q = 3
hdl1_q = 4


Kaplan-Meier survival estimates
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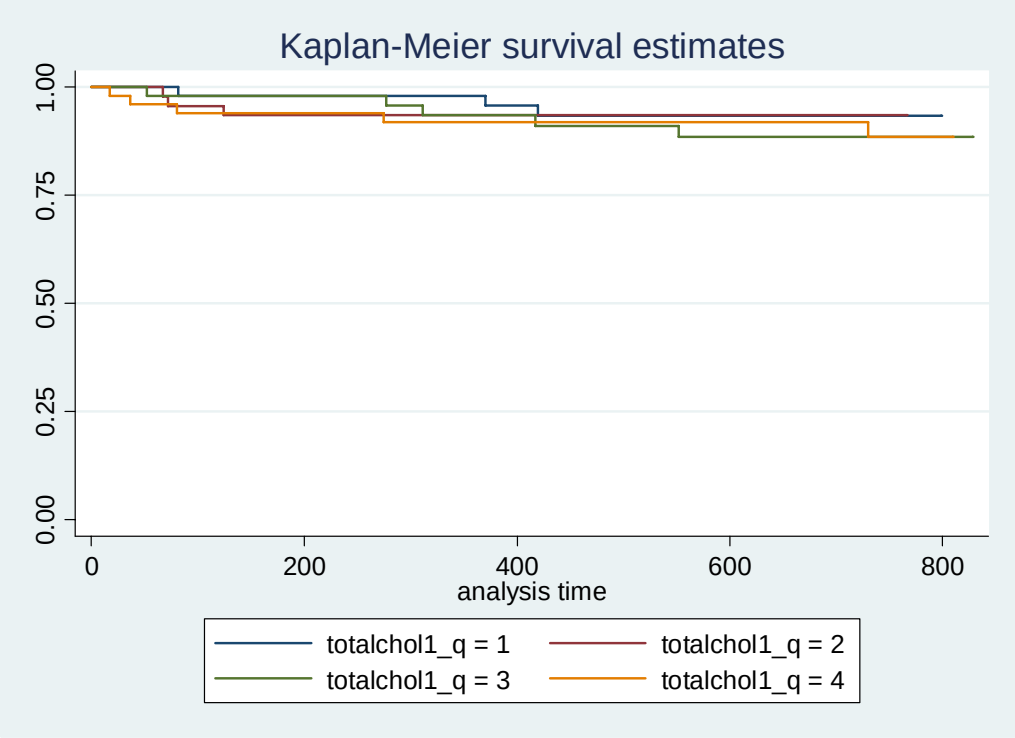
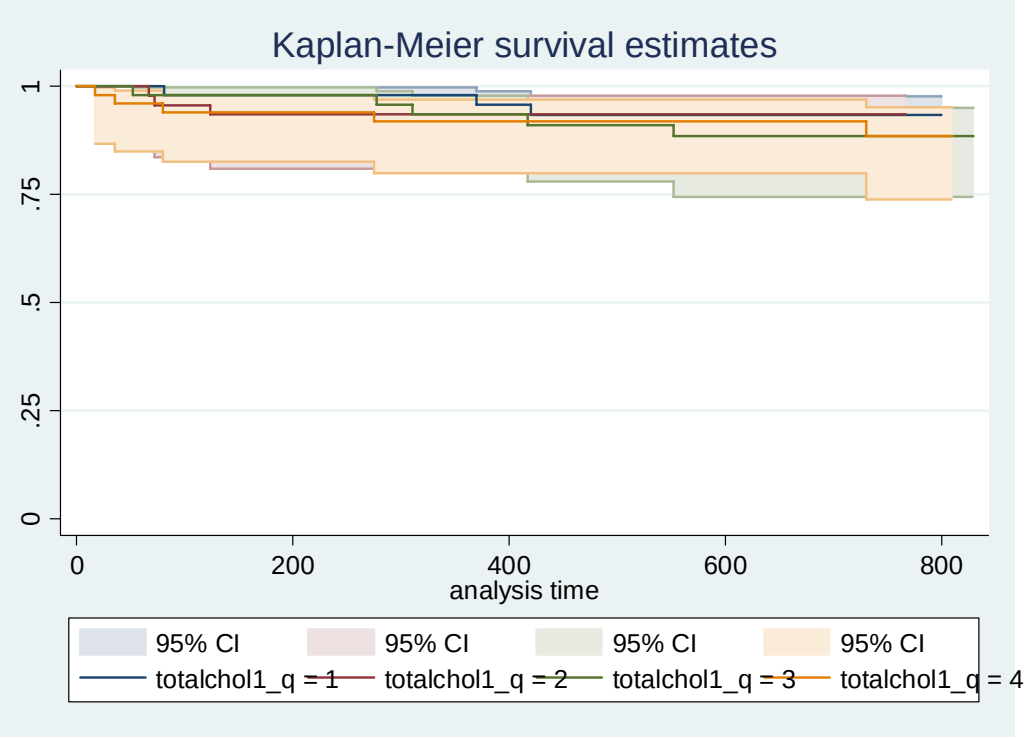
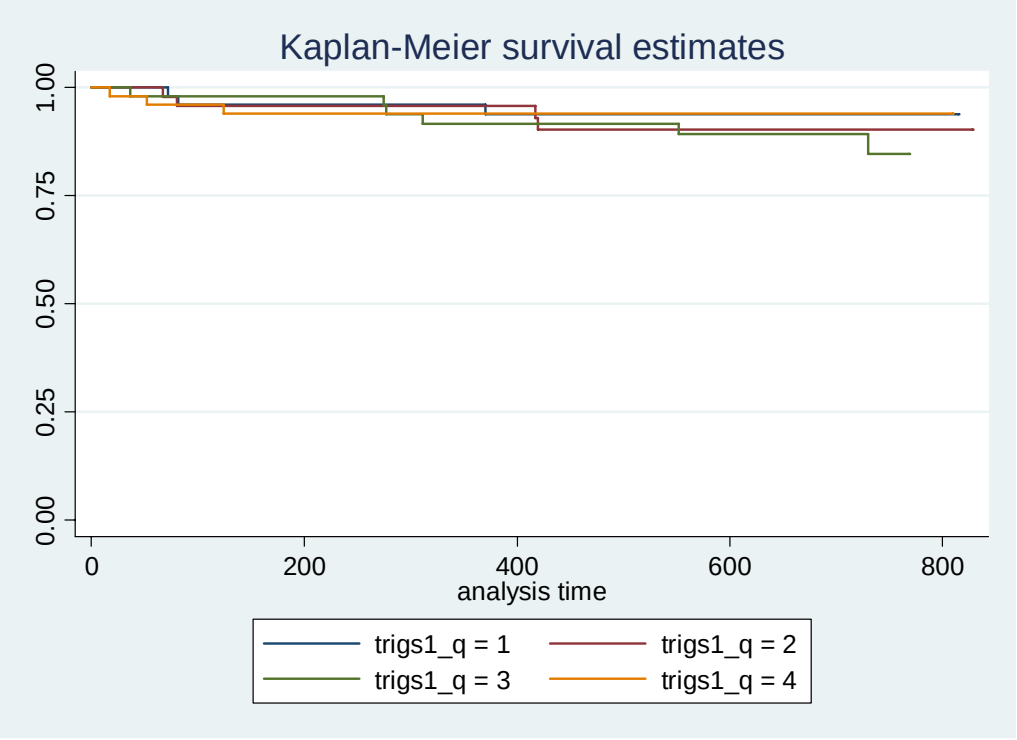
.75
.5
.25
0
0
200
400
600
800
analysis time
95% CI
95% CI
95% CI
95% CI
hdl1_q = 1
hdl1_q = 2
hdl1_q = 3
hdl1_q = 4

Appendix D – Kaplan Meier curves for nonlipoprotein subfractions for VF



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0.75
0.50
0.25
0.00
0
200
400
600
800
analysis time
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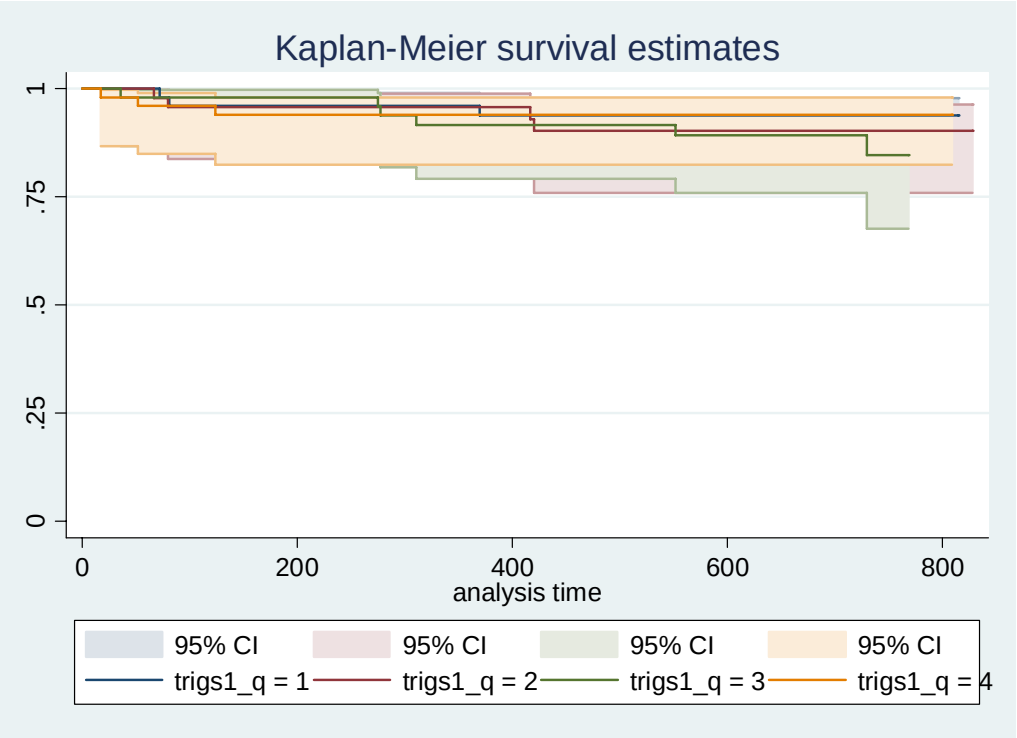
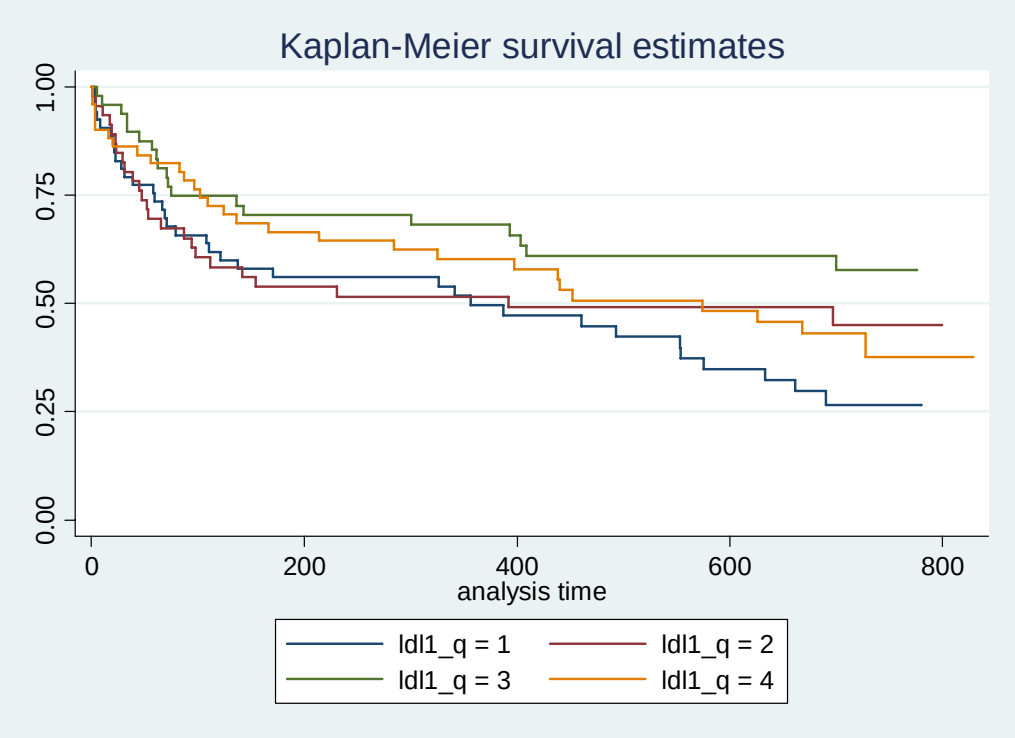
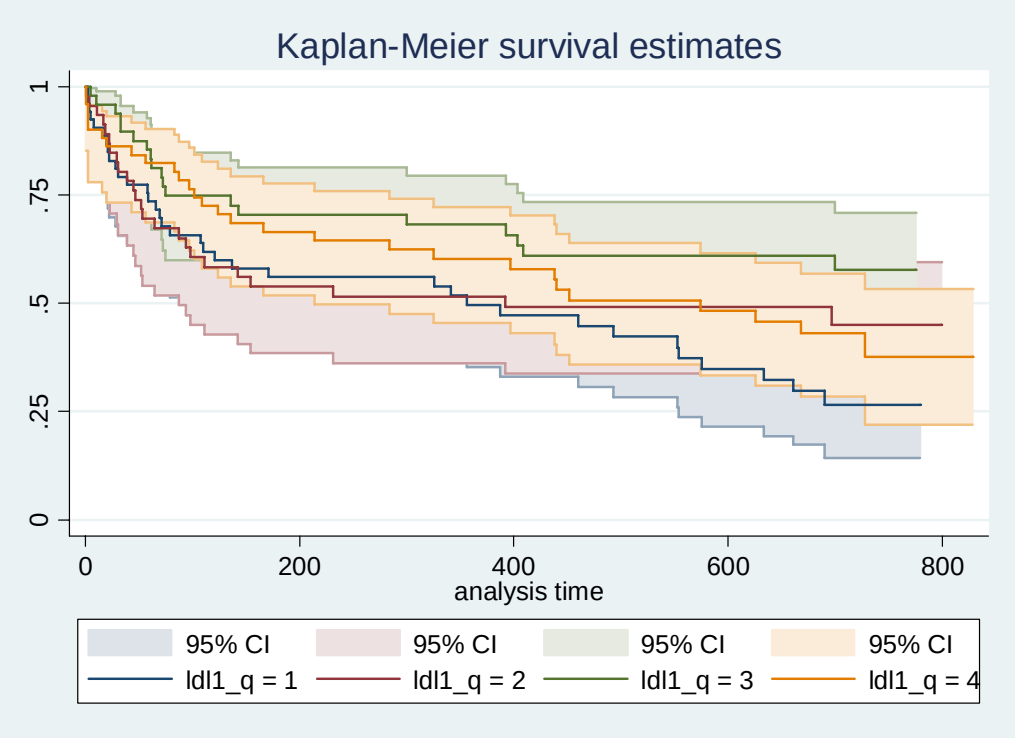
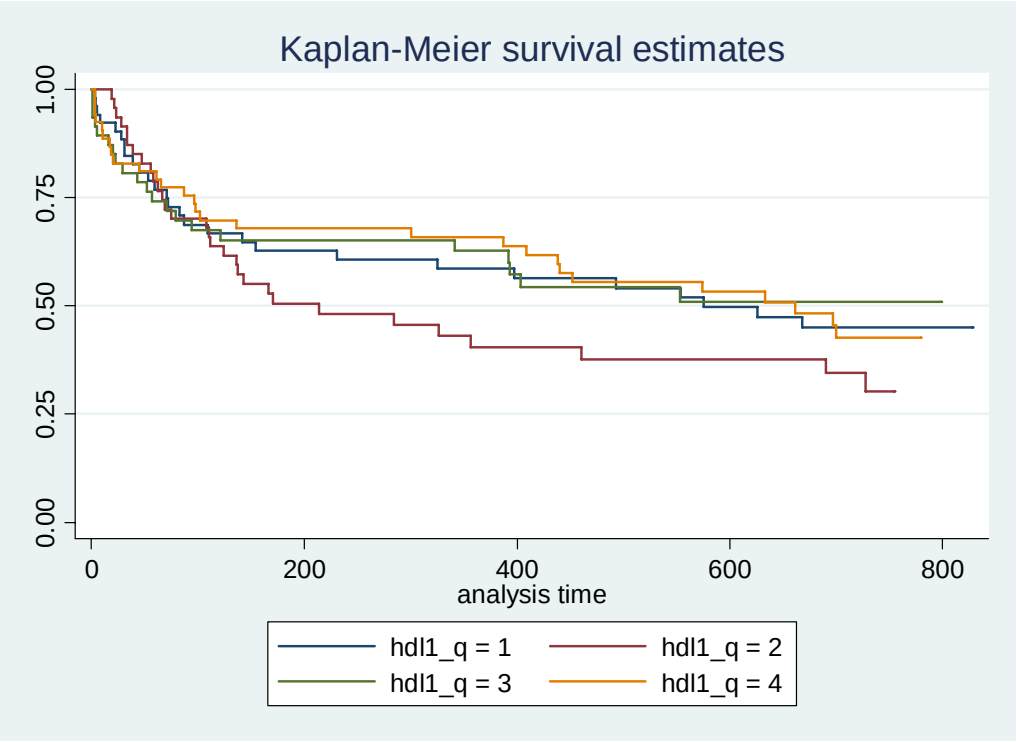
trigs1_q = 2
trigs1_q = 3
trigs1_q = 4

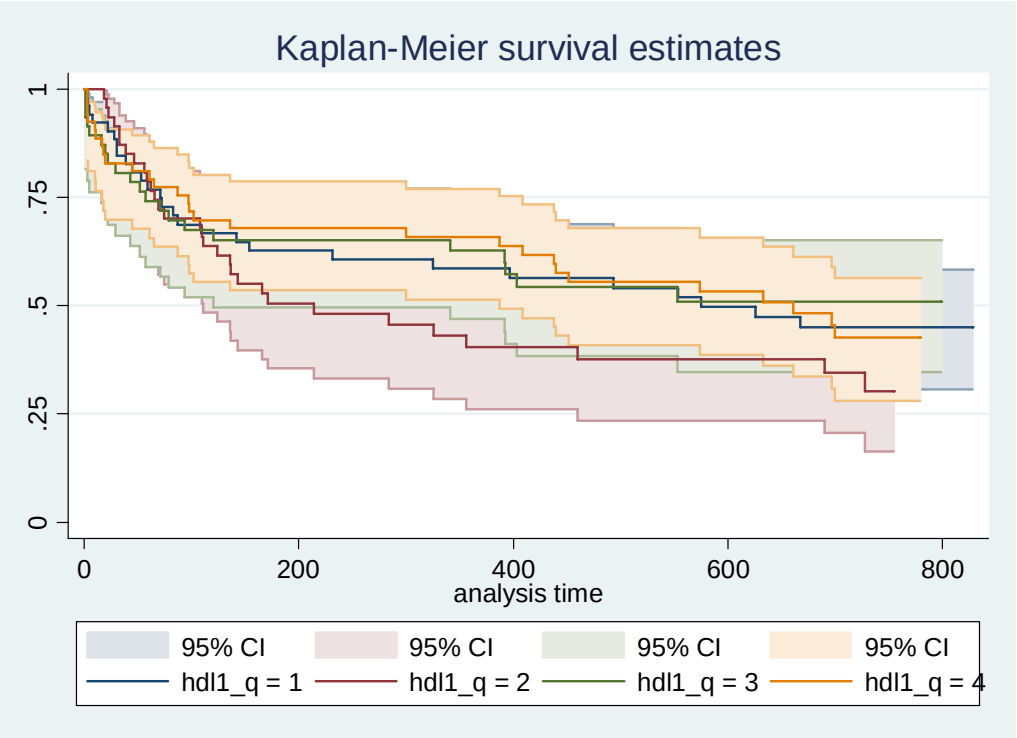

Kaplan-Meier survival estimates
1
.75
.5
.25
0
0
200
400
600
800
analysis time
95% CI
95% CI
95% CI
95% CI
trigs1_q = 1
trigs1_q = 2
trigs1_q = 3
trigs1_q = 4

Appendix E – Kaplan Meier curves for lipoprotein subfractions for VT






Kaplan-Meier survival estimates
1.00
0.75
0.50
0.25
0.00
0
200
400
600
800
analysis time
hdl1_q = 1
hdl1_q = 2
hdl1_q = 3
hdl1_q = 4


Kaplan-Meier survival estimates
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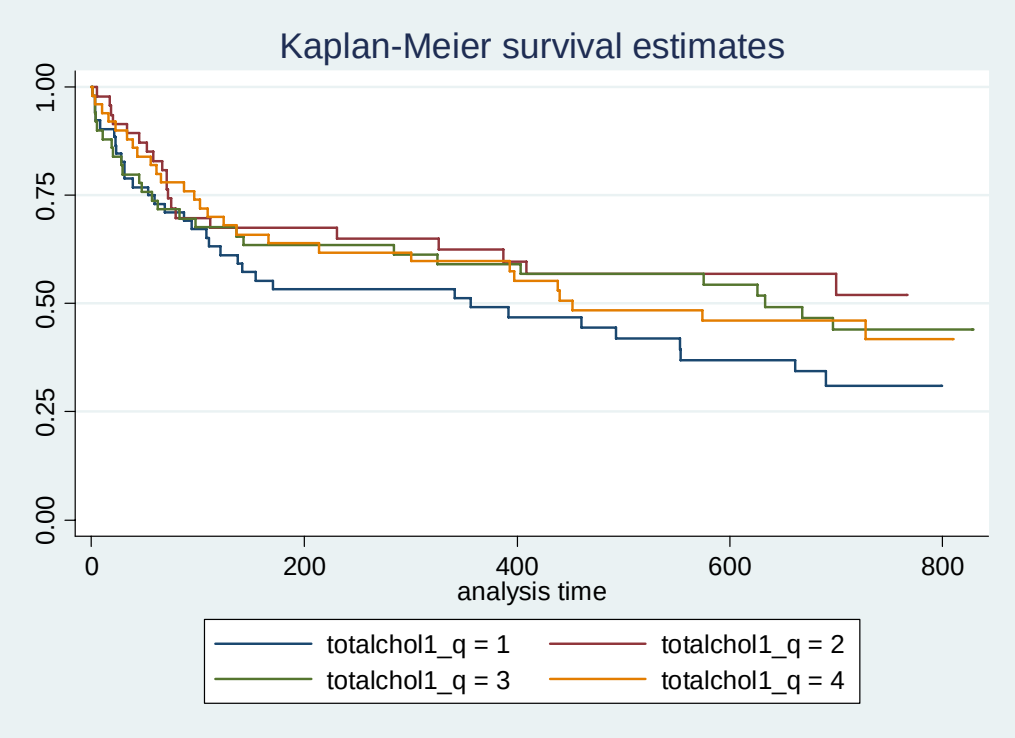
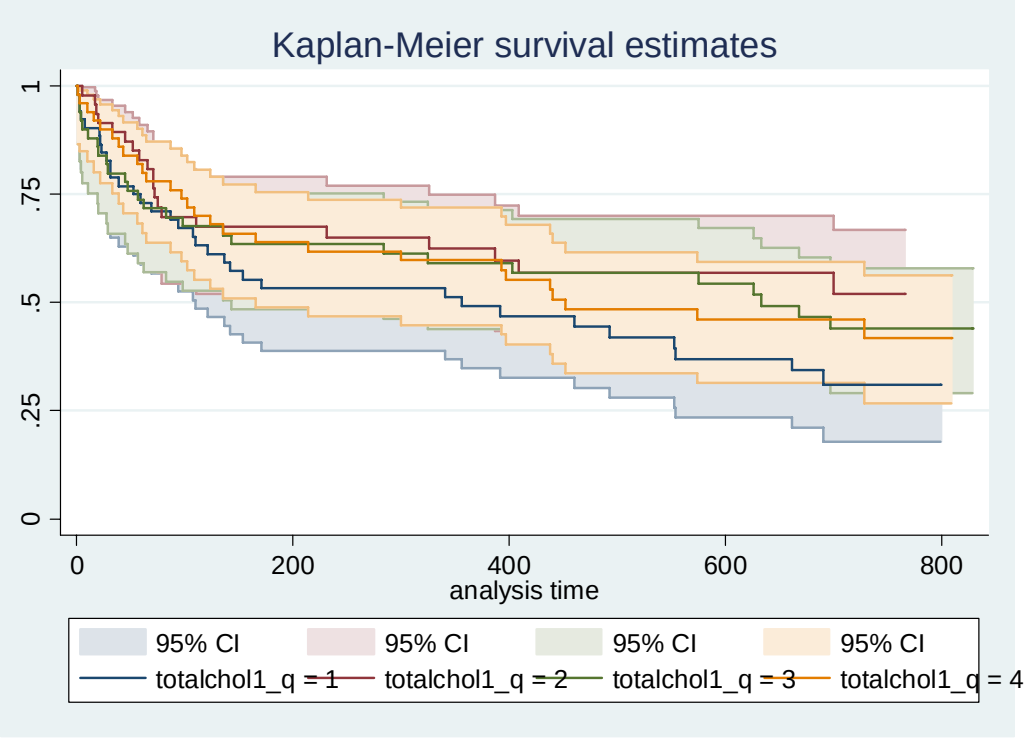
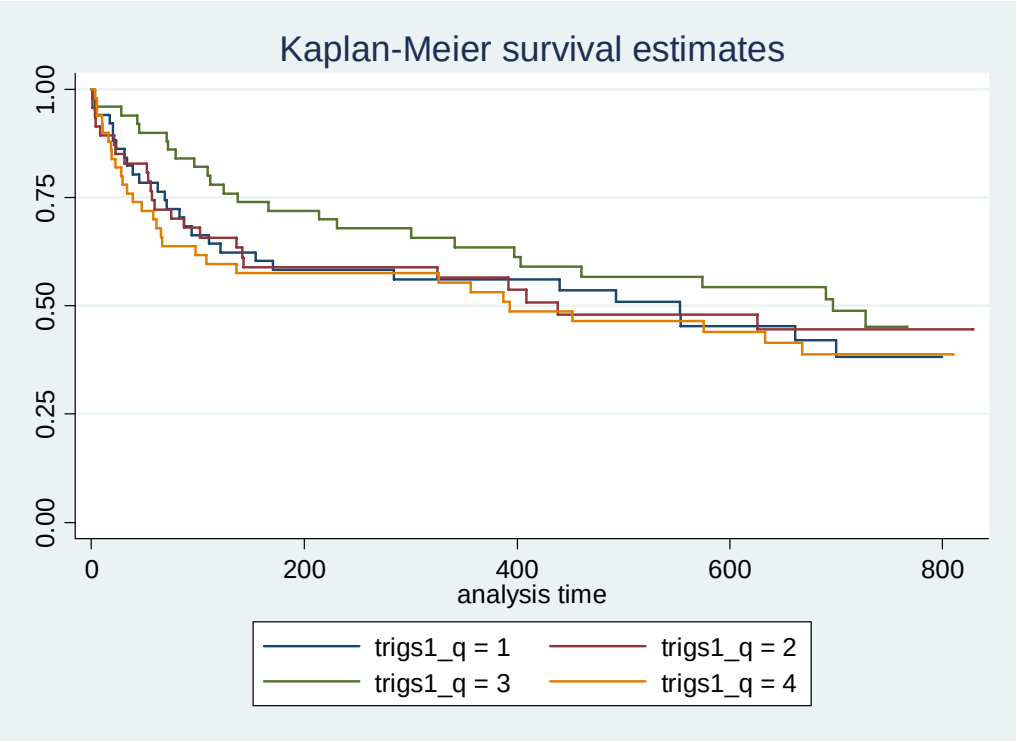
.75
.5
.25
0
0
200
400
600
800
analysis time
95% CI
95% CI
95% CI
95% CI
hdl1_q = 1
hdl1_q = 2
hdl1_q = 3
hdl1_q = 4

Appendix F – Kaplan Meier curves for nonlipoprotein subfractions for VT






Kaplan-Meier survival estimates
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0.75
0.50
0.25
0.00
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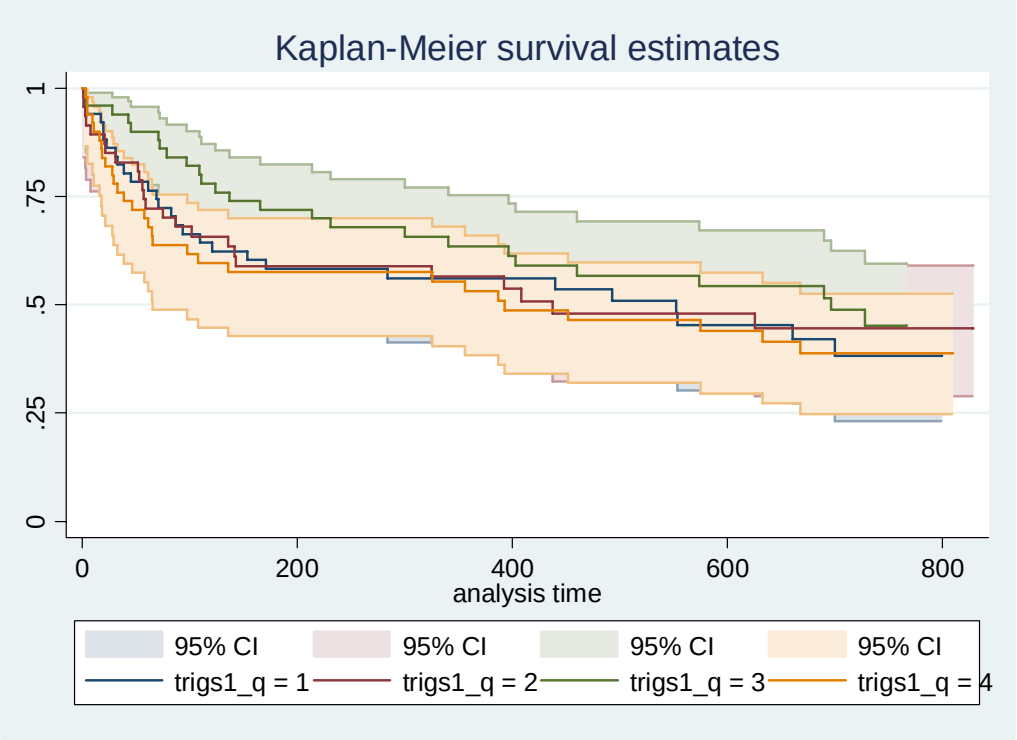
200
400
600
800
analysis time
trigs1_q = 1
trigs1_q = 2
trigs1_q = 3
trigs1_q = 4


Kaplan-Meier survival estimates
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.75
.5
.25
0
0
200
400
600
800
analysis time
95% CI
95% CI
95% CI
95% CI
trigs1_q = 1
trigs1_q = 2
trigs1_q = 3
trigs1_q = 4

Appendix G – VF Model Diagnostics – Assessment of the Proportionality Assumption

```
. stcox ldl1 status, nohr tvc (ldl1 status) texp(ln(_t))

         failure _d:  vfevent
   analysis time _t:  vfshockday

Iteration 0:   log likelihood = -81.885657
Iteration 1:   log likelihood = -79.045318
Iteration 2:   log likelihood = -79.039444
Iteration 3:   log likelihood = -79.039444
Refining estimates:
Iteration 0:   log likelihood = -79.039444

Cox regression -- no ties

No. of subjects =          199                     Number of obs   =       199
No. of failures =           16
Time at risk    =       116144
                                                    LR chi2(4)      =      5.69
Log likelihood  =   -79.039444                      Prob > chi2     =    0.2233
```

_t	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
rh						
ldl1	.0526784	.0417715	1.26	0.207	-.0291922	.1345491
status	-.5601818	2.419417	-0.23	0.817	-5.302152	4.181788
t						
ldl1	-.0065523	.0082756	-0.79	0.429	-.0227722	.0096676
status	.1326052	.4714441	0.28	0.778	-.7914082	1.056619

```
Note: second equation contains variables that continuously vary with respect
      to time; variables are interacted with current values of ln(_t).
```

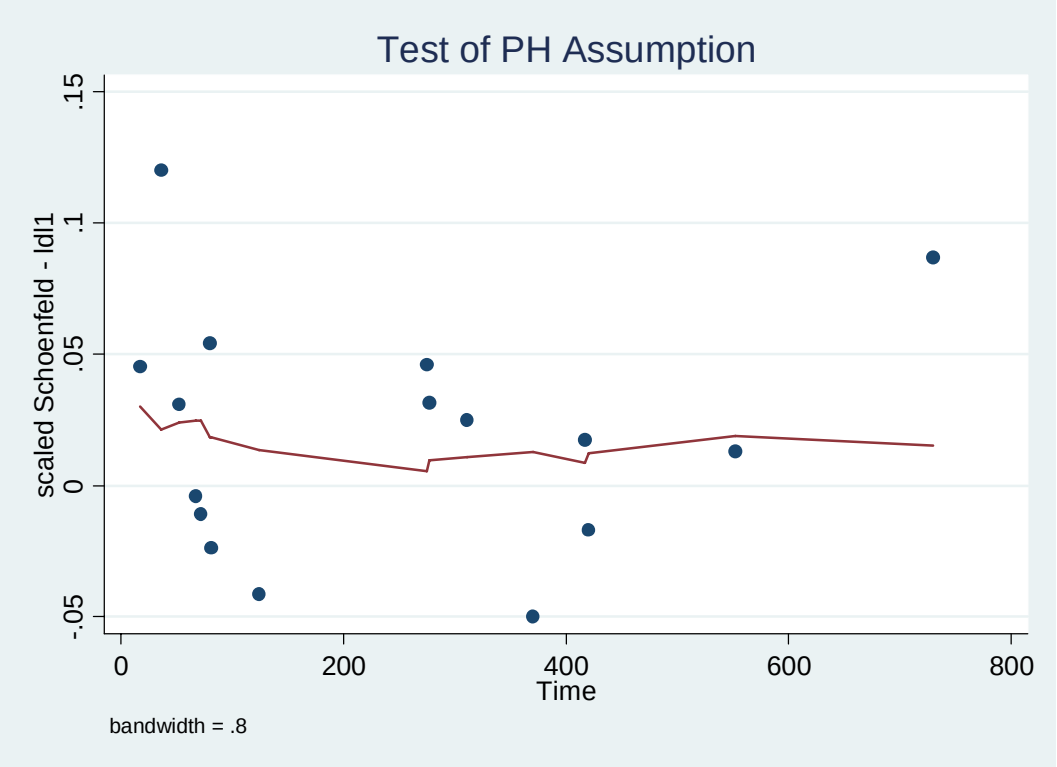
Proportionality was assessed by including time-dependent covariates in the model by using the tvc and the texp options in the coxreg command. In this analysis, we chose to use interactions with log(time) because this is the most common function of time used in time-dependent covariates. Time-dependent covariates were not significant, so there is no violation of the proportionality assumption neither for LDL nor for fish oil treatment allocation.

We also tested for the proportionality assumption by using the Schoenfeld and scaled Schoenfeld residuals. The tests were not significant, thus we could not reject proportionality and we assume that we do not have a violation of the proportional assumption. A horizontal line in the graphs below is further indication that there is no violation of the proportionality assumption. The **stphplot** command in STATA uses log-log plots to test proportionality and if the lines in these plots are parallel then we have further indication that the predictors do not violate the proportionality assumption.

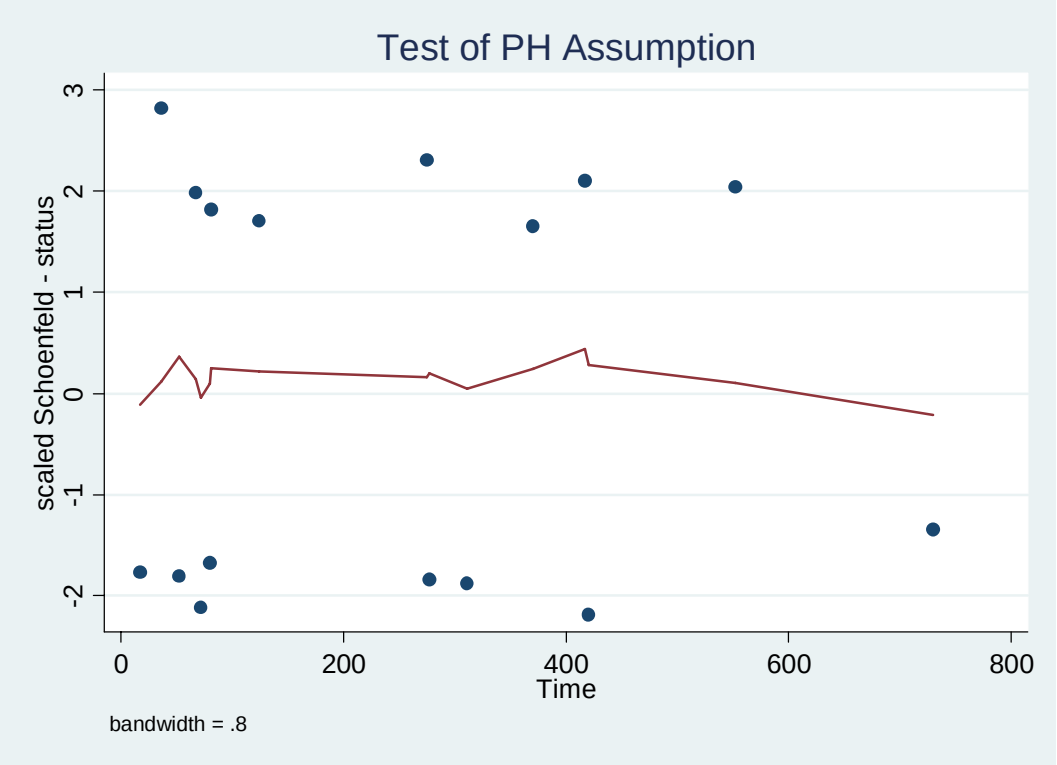
```
. quietly stcox ldl1 status, schoenfeld(sch*) scaledsch(sca*)

. stphtest, detail

      Test of proportional-hazards assumption

      Time:  Time
```

	rho	chi2	df	Prob>chi2
ldl1	0.04093	0.04	1	0.8459
status	0.00026	0.00	1	0.9992
global test		0.04	2	0.9810

For LDL

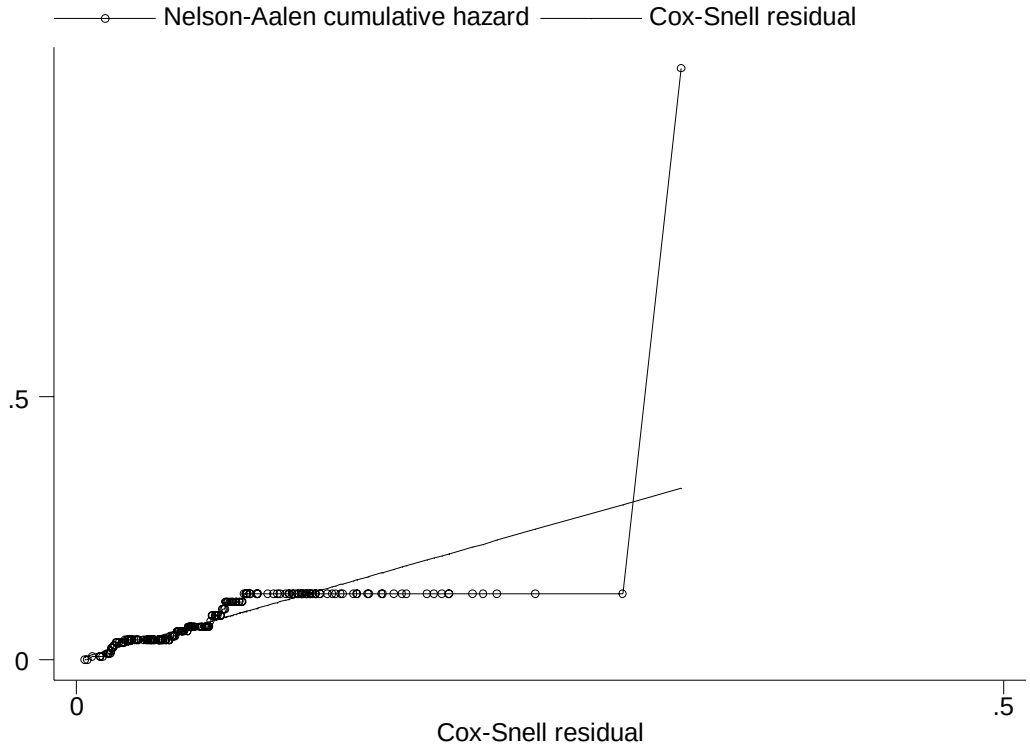


For treatment allocation



Appendix H – VF Model Diagnostics – Goodness of Fit of the Final Model

We evaluated the fit of the model by using the Cox-Snell residuals. If the model fits the data well then the true cumulative hazard function conditional on the covariate vector has an exponential distribution with a hazard rate of one. We graphed the Nelson-Aalen cumulative hazard function and the cs variable so that we could compare the hazard function to the diagonal line. If the hazard function follows the 45 degree line then we know that it approximately has an exponential distribution with a hazard rate of one and that the model fits the data well.



Appendix I – VT Model Diagnostics – Assessment of the Proportionality Assumption

Proportionality was assessed by including time-dependent covariates in the model by using the tvc and the texp options in the coxreg command. In this analysis, we chose to use interactions with log(time) because this is the most common function of time used in time-dependent covariates. Time-dependent covariates were not significant, so there was no violation of the proportionality assumption neither ejection fraction < 40%, entry arrhythmia, nor for fish oil treatment allocation.

```
. stcox ef40 entryarr status, nohr tvc(ef40 entryarr status) texp(ln(_t))

        failure _d:  vtevent
  analysis time _t:  vtshockday

Iteration 0:   log likelihood = -516.52562
Iteration 1:   log likelihood = -502.34519
Iteration 2:   log likelihood =  -502.1326
Iteration 3:   log likelihood = -502.13216
Iteration 4:   log likelihood = -502.13216
Refining estimates:
Iteration 0:   log likelihood = -502.13216

Cox regression -- Breslow method for ties

No. of subjects =          199                     Number of obs   =       199
No. of failures =          106
Time at risk    =        74247
                                                   LR chi2(6)      =     28.79
Log likelihood  =   -502.13216                     Prob > chi2     =    0.0001
```

_t	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
rh						
ef40	.184289	.5559804	0.33	0.740	-.9054125	1.273991
entryarr	.9376458	.7231481	1.30	0.195	-.4796984	2.35499
status	.6654023	.5594591	1.19	0.234	-.4311175	1.761922
t						
ef40	.047076	.1217914	0.39	0.699	-.1916307	.2857827
entryarr	.0123745	.1558255	0.08	0.937	-.2930378	.3177869
status	-.0602168	.1222903	-0.49	0.622	-.2999014	.1794679

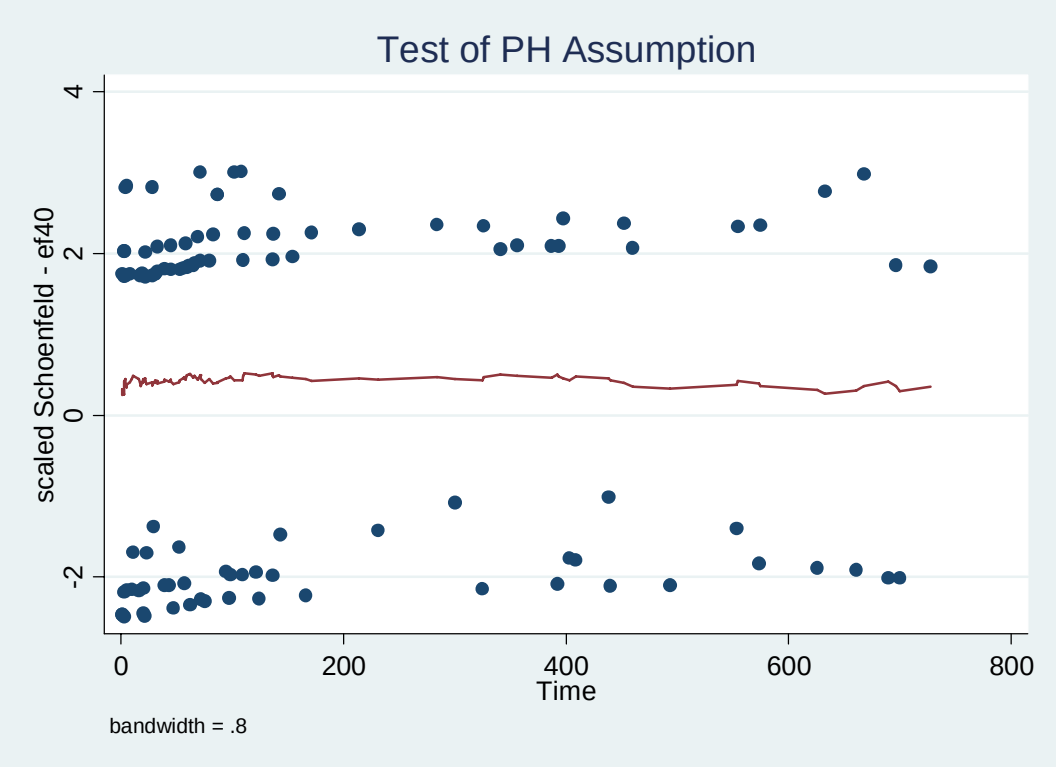
```
Note: second equation contains variables that continuously vary with respect
      to time; variables are interacted with current values of ln(_t).
```

We also tested for the proportionality assumption by using the Schoenfeld and scaled Schoenfeld residuals. The tests were not significant, thus we can not reject proportionality and we assume that we do not have a violation of the proportional assumption. A horizontal line in the graphs below was further indication that there is no violation of the proportionality assumption. The **stphplot** command uses log-log plots to test proportionality and if the lines in these plots are parallel then we have further indication that the predictors do not violate the proportionality assumption.

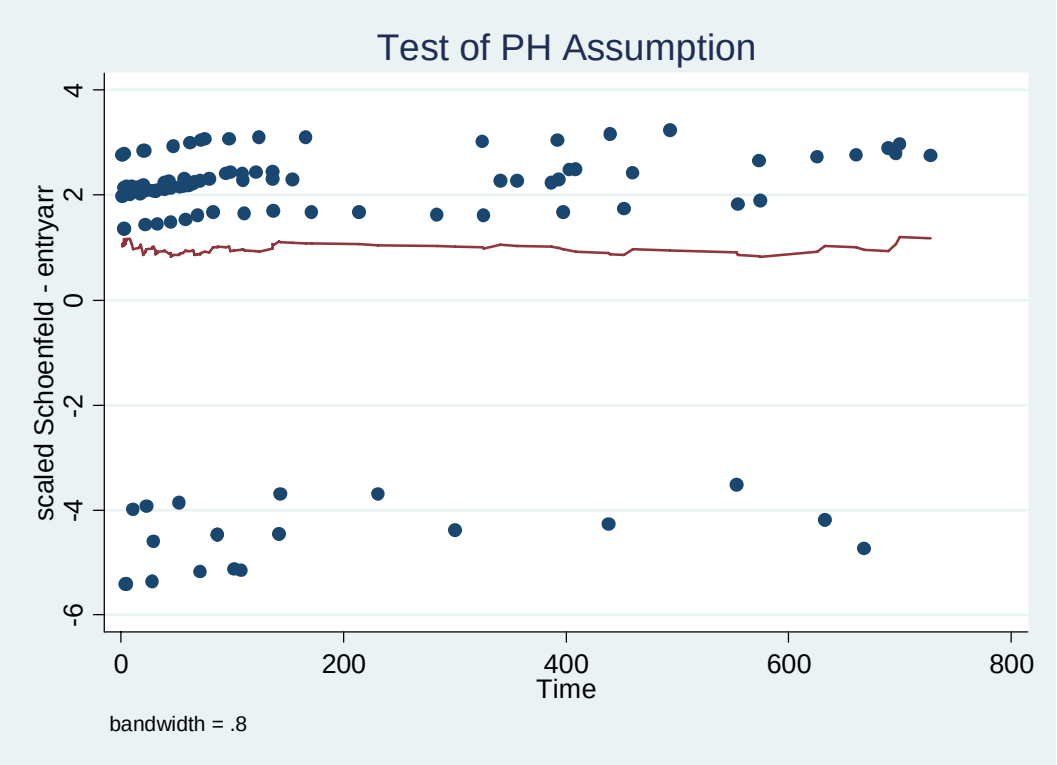
```
. quietly stcox ef40 entryarr status, schoenfeld(sch*) scaledsch(sca*)

. stphtest, detail

      Test of proportional-hazards assumption

      Time:  Time
```

	rho	chi2	df	Prob>chi2
ef40	-0.02608	0.07	1	0.7856
entryarr	0.04276	0.21	1	0.6490
status	-0.07220	0.56	1	0.4525
global test		0.93	3	0.8174

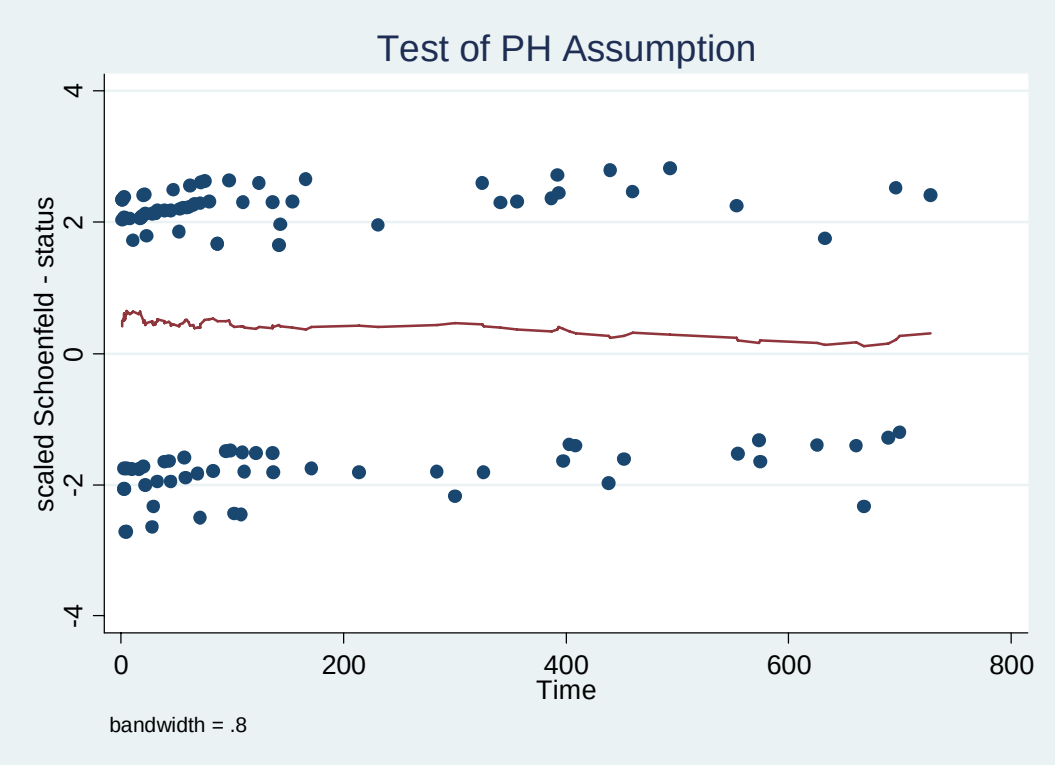
For ejection fraction < 40%



For entry arrhythmia



For treatment allocation



Appendix J – VT Model Diagnostics – Goodness of Fit of the Final Model

We evaluated the fit of the model by using the Cox-Snell residuals. If the model fits the data well then the true cumulative hazard function conditional on the covariate vector has an exponential distribution with a hazard rate of one. We graphed the Nelson-Aalen cumulative hazard function and the cs variable so that we can compare the hazard function to the diagonal line. If the hazard function follows the 45 degree line then we know that it approximately has an exponential distribution with a hazard rate of one and that the model fits the data well.