ORIGINAL ARTICLE

Revisiting iron overload status and change thresholds as predictors of mortality in transfusion-dependent β-thalassemia: a 10-year cohort study

Short title: Iron overload and mortality in TDT

Khaled M. Musallam[1], Susanna Barella[2], Raffaella Origa[3], Giovanni Battista Ferrero[4], Roberto Lisi[5], Annamaria Pasanisi[6], Filomena Longo[7], Barbara Gianesin[8], Gian Luca Forni[8]; on behalf of the Webthal® project

[1]Center for Research on Rare Blood Disorders (CR-RBD), Burjeel Medical City, Abu Dhabi, United Arab Emirates

[2]S.C. Centro delle Microcitemie e Anemie Rare, ASL Cagliari, Cagliari, Italy

[3]Università di Cagliari, S.C. Centro delle Microcitemie e Anemie Rare, ASL Cagliari, Cagliari, Italy

[4]Hemoglobinopathies and Rare Anemia Reference Center, San Luigi Gonzaga University Hospital, Department of Biological and Clinical Sciences, University of Turin, Turin, Italy

[5]Thalassemia Unit, ARNAS Garibaldi, Catania, Italy

[6]Centro della Microcitemia A.Quarta, Hematology Unit, A. Perrino Hospital, Brindisi, Italy

[7]Day Hospital della Talassemia e delle Emoglobinopatie, Azienda Ospedaliero Universitaria S. Anna, Ferrara, Italy

[8]ForAnemia Foundation, Genoa, Italy

Correspondence

Gian Luca Forni, MD

ForAnemia Foundation

Via Garibaldi 7, 3C, 16124 Genoa, Italy

Tel: +393281503364; Email: gianlucaforni14@gmail.com

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ORCID ID:

Khaled M. Musallam: 0000-0003-3935-903X

Susanna Barella: 0000-0002-1952-7836

Raffaella Origa: 0000-0002-2346-9616

Giovanni Battista Ferrero: 0000-0002-3793-5788

Filomena Longo: 0000-0002-0434-0382

Gian Luca Forni: 0000-0001-9833-1016

ABSTRACT

Data on iron overload status and change thresholds that can predict mortality in patients with transfusion-dependent β-thalassemia (TDT) are limited. This was a retrospective cohort study of 912 TDT patients followed for up to 10 years at treatment centers in Italy (median age 32 years, 51.6% female). The crude mortality rate was 2.9%. Following best-predictive threshold identification through receiver operating characteristic curve analyses, data from multivariate Cox-regression models showed that patients with *Period Average Serum Ferritin (SF)* >2145 vs ≤2145 ng/mL were 7.1-fold (*P* <0.001) or with *Absolute Change SF* >1330 vs ≤1330 ng/mL increase were 21.5-fold (*P* <0.001) more likely to die from any cause. Patients with *Period Average Liver Iron Concentration (LIC)* >8 vs ≤8 mg/g were 20.2-fold (*P* <0.001) or with *Absolute Change LIC* >1.4 vs ≤1.4 mg/g increase were 27.6-fold (*P* <0.001) more likely to die from any cause. Patients with *Index (first) cardiac T2* (cT2*)* <27 vs ≥27 ms were 8.6-fold (*P* <0.001) more likely to die from any cause. Similarly, results at varying thresholds were identified for death from cardiovascular disease. These findings should support decisions on iron chelation therapy by establishing treatment targets, including safe iron levels and clinically meaningful changes over time.

Keywords: serum ferritin; liver; heart; MRI; iron overload; iron chelation.

INTRODUCTION

Improved survival in patients with transfusion-dependent β-thalassemia (TDT) over the years is largely attributed to the introduction of safe transfusion practices and advances in iron overload monitoring and management [1-5]. Beyond serial measurement of serum ferritin (SF) levels, it is now possible to estimate iron concentration in the liver and heart by non-invasive magnetic resonance imaging (MRI) techniques (R2 and T2*) which have been validated against biopsies, showed international reproducibility, and have now become standards of care [6-12]. This has allowed tailoring of iron chelation therapy to the patient's heart/liver iron overload profile, as informed by data on organ-specific efficacy of different types and doses of iron chelators [13].

The iron overload thresholds used today to aid clinical decision making are primarily based on the associated mortality risk. For instance, SF levels ≥1000 ng/mL and ≥2500 ng/mL have been associated with cardiovascular disease and death; and are commonly used to indicate the need for treatment initiation and intensification, respectively [3, 13-16]. Liver iron concentration (LIC) values of >7 and >15 mg/g [dry weight] and cardiac T2* (cT2*) values of <20 and <10 ms have also been associated with cardiovascular disease and death in TDT [16-23]. Despite wide use of such thresholds, evidence mostly stems from very few, small, single-center, outdated (using liver biopsy for LIC), or short-term follow-up studies that often used pre-assigned thresholds; and they have been rarely revisited [24]. More importantly, evidence on the relationship between 'changes' (rather than spot measurements) in

iron parameters and long-term outcomes is limited; and thus, clinically meaningful changes remain undefined.

Correlations between SF and LIC or cT2* have also shown mixed results in various cross-sectional and longitudinal studies; and have been shown to vary based on the type of iron chelator and dynamics of iron loading and unloading in various organs [25-27]. Knowledge of such correlations and the ability of SF to predict cardiac/hepatic iron concentrations remain essential, both in resource-limited settings where MRI availability can be limited as well as in settings when MRI is available but patient prioritization is required.

With this background, the current study aims to revisit and identify iron overload status and change thresholds that can predict long-term all-cause and cardiovascular disease-related mortality in a large cohort of patients with TDT followed for 10 years, and to further investigate correlations between various iron parameters.

METHODS

This was a retrospective cohort study of β-thalassemia patients attending treatment centers in Italy, using pooled data from DB-INTHEM. DB-INTHEM is a database that automatically collects data of Italian patients with β-thalassemia who are followed by centers that use Webthal®, a computerized medical record software currently owned by the Italian Society of Thalassemias and Hemoglobinopathies (SITE), which was developed in 2000 to aid in standardized clinical, laboratory, and imaging data

recording across participating centers. A Secure Socket Layer system and passwords are used to ensure data safety and confidentiality. At each center, an Ethics Committee approval is obtained and written informed consents for data collection and use are retrieved from patients.

For this study, we retrieved data for a 10-year period starting 01 January 2010 for all β-thalassemia patients attending participating centers and followed until 31 December 2019, death, transplant, or loss to follow-up. This period was chosen to represent a long-term observation, during a timeframe that reflects modern management of the disease with all advances in MRI monitoring and oral iron chelation therapy being routinely available, and before the onset of the Covid-19 pandemic which may have disrupted standard patient management. Among these, we selected patients who had an original diagnosis of β-thalassemia major and had received an average of at least ten red blood cell units per year during the 10-year observation period, in keeping with recent definitions of transfusion-dependence in clinical trials [28]. Two patients who had been receiving luspatercept therapy were excluded. A total of 912 patients were eventually included in the analyses.

For each patient, we retrieved data on age, sex, center of treatment, pretransfusion hemoglobin (observation period average), and status at last follow-up date (dead, alive, transplant, lost to follow-up). We also retrieved data on SF, LIC, and cT2*. For these, we considered the annual average of all available records for each consecutive year during the 10-year observation period. All LIC values were retrieved using hepatic MRI T2* with standard calibration techniques.

At least one annual average was available for SF in 892 patients, for LIC in 412 patients, and for cT2* in 454 patients. For these, the median number of years with an annual average available was 10 (min: 1, max: 10) for SF, 4 (min: 1, max: 10) for LIC, and 4 (min: 1, max: 10) for cT2*. The median number of records available per individual year ranged between 6-8 (min: 1, max: 28) over the 10 years for SF, and was 1 (min: 1, max: 4) for LIC, and 1 (min: 1, max: 4) for cT2*. Other than being in the period following patient death/loss to follow-up, absence of records would be attributed to missing documentation (rare), no assessment due to management recommendations (e.g., monitoring frequency >1 year), limited access (patients living in remote areas), a physician decision, or a patient choice.

For the purposes of this analysis, we assigned the following for each patient for SF, LIC, and cT2*: $Y_{1\text{-}10}$ value (annual average of corresponding year), *Index* value (first annual average during the 10-year observation period; to represent a spot measurement of iron status at the beginning of observation and its association with mortality within 10 years), *Period Average* (average of all annual averages during the 10-year observation period; to represent average iron status patient was maintained on during 10 years), *Absolute Change* (the difference between last and first values; to represent the magnitude of change in iron status during 10 years), *Relative Change* (the difference between last and first values divided by first value and multiplied by 100; to represent the magnitude of change in iron status during 10 years as a function of initial iron status). With such interpretations, it is assumed that the last observation is carried forward and first observation carried backward to represent the full 10 years.

All patients were receiving iron chelation therapy. For each patient, we retrieved data on the annual chelation type received for each consecutive year during the 10-year observation period, which was grouped as: deferoxamine (DFO), deferiprone (DFP), deferasirox (DFX), DFO-DFP, DFO-DFX, DFP-DFX, DFO-DFP-DFX; based on all iron chelators used during the individual year. Combinations reflected simultaneous or alternating chelation. We used a majority rule to assign a chelation type for the entire 10-year observation period (type used in most years, or a combination in case of equal number of years).

For each patient, we also retrieved data on active morbidities at study start. These included the following morbidities which were diagnosed per local standards: heart disease, liver disease, and diabetes mellitus. These morbidities were selected owing to their direct association with iron overload and impact on mortality [29].

Statistical analysis

Data for SF, LIC, and cT2* did not follow a normal distribution (Shapiro Wilk test P <0.001), hence nonparametric tests were used. Descriptive statistics are represented as median (interquartile range [IQR], min, max) or percentages. Bivariate correlations were done using the Mann Whitney U t-test and Spearman's correlation coefficient (rs). Receiver operating characteristic (ROC) curves were used to identify the best thresholds to predict all-cause and cardiovascular disease-related mortality within 10 years, using the highest Youden Index (sensitivity + specificity – 1), and areas under the curve (AUC) were estimated. Kaplan-Meier survival curves were constructed to estimate cumulative survival, and the Log-rank test was used for comparisons of survival curves. Cox regression analyses were used to estimate

hazard ratios (HR) and 95% confidence intervals (CI) of all-cause and cardiovascular disease-related mortality. Multivariate forward stepwise models were used to adjust for confounding effects. For all analyses on mortality, missing SF, LIC, and cT2* values for dead patients were imputed with dead patients' median to avoid losing events in outcomes analyses. Sensitivity analyses were also carried out for *Index LIC* and *Period Average LIC* by replacing missing values for the entire cohort with ones imputed from the linear regression formula as predicted from SF values. All *P*-values were two-sided with the level of significance set at <0.05.

RESULTS

A total of 912 TDT patients were included in this analysis, with 471 (51.6%) being female. The median age at study start was 32 years (IQR: 24.1-36, min: 0.1, max: 61), including 133 (14.6%) children (<18 years) and 779 (85.4%) adults. The median pretransfusion hemoglobin level (observation period average) was 9.7 g/dL (IQR: 9.5-10, min: 7.5, max 11.8), reflecting optimal transfusion per international guidelines (9.5-10.5 g/dL) [13]. Among evaluated morbidities 149 (16.3%) patients had single and 18 (2%) patients had multiple active morbidities at study start including heart disease n = 112, liver disease n = 10, diabetes mellitus n = 64. The distribution of iron chelation used over the observation period was: DFO (n = 84, 9.2%), DFP (n = 151, 16.6%), DFX (n = 428, 46.9%), DFO-DFP (n = 176, 19.3%), DFO-DFX (n = 25, 2.7%), DFP-DFX (n = 23, 2.5%), DFO-DFP-DFX (n = 25, 2.7%). Evaluated iron parameters are summarized in **Table 1**, which generally showed a low iron burden in our patient population and a slight decline or stable values over the 10 years.

Correlations between iron parameters

Cross-sectional correlations between iron parameters for each individual year are summarized in **Supplementary Table S1**, with Spearman's rs ranging from 0.587 to 0.772 for SF vs LIC, -0.091 to -0.495 for SF vs cT2*, and -0.109 to -0.432 for LIC vs cT2* (*P* <0.05 for all except Y_1 *SF* vs *cT2** and Y_1 *LIC* vs *cT2**). When looking at longitudinal changes over time (**Supplementary Table S2**), numerically higher rs values were also observed for the correlation between *Absolute Change SF* vs *LIC* (0.508, *P* <0.001) than for *Absolute Change SF* vs *cT2** (-0.124, *P* = 0.025) or *Absolute Change LIC* vs *cT2** (-0.186, *P* = 0.001). Similar trends were also noted for *Relative Change*. When longitudinal correlations were stratified per iron chelation type in patients who remained on the same monotherapy for most of the observation period, correlations between SF vs LIC were only significant for DFO and DFX and rs values were numerically higher than for DFP (**Supplementary Table S1**).

Iron overload status and change thresholds for predicting mortality

All patients were followed for the full 10 years, except for 20 patients who were lost to follow up/transferred outside the Webthal® network, 7 patients who underwent bone marrow transplantation, and 26 (2.9%, 95%CI: 1.9-4.2) patients who died during the observation period at a median age of 40.5 years (IQR: 36.8-44, min: 28, max: 49). These included 17 (1.9%, 95%CI: 1.1-3) patients who died of cardiovascular disease, and 9 who died of other causes (cirrhosis/hepatocellular carcinoma n =3, sepsis n = 3, renal failure n = 1, accident n = 2). The death rates in patients without any SF (n = 20), LIC (n = 500), and cT2* (n = 458) records were 0%, 3% (95%CI: 1.7-4.9), and 3.3% (95%CI: 1.8-5.3), respectively; comparable to the overall population.

Table 2 summarizes differences in median SF, LIC, and cT2* according to death status. ROC curve analyses for the prediction of all-cause and cardiovascular disease-related mortality within 10 years by evaluated iron parameters (*Index* and *Period Average* values to represent status, and *Absolute Change* and *Relative Change* values to represent change over time) are summarized in **Table 3**. Upon considering AUCs and *P*-values, we considered *Period Average SF*, *Absolute Change SF*, *Period Average LIC*, *Absolute Change LIC*, and *Index cT2** as best predictors for both all-cause and cardiovascular disease-related mortality. Thresholds with the highest Youden Index (sensitivity + sensitivity – 1) for all-cause mortality were: *Period Average SF* (>2142 ng/mL, sensitivity: 65.4%, specificity: 74.1%; **Figure 1A**), *Absolute Change SF* (>1329 ng/mL increase, sensitivity: 61.5%, specificity: 92.4%; **Figure 1B**), *Period Average LIC* (>8.2 mg/g, sensitivity: 80.8%, specificity: 79%; **Figure 1C**), *Absolute Change LIC* (>1.4 mg/g increase, sensitivity: 88.5%, specificity: 75%; **Figure 1D**), and *Index cT2** (<27.2 ms, sensitivity: 72.7%, specificity: 80.8%; **Figure 1E**). Thresholds with the highest Youden Index (sensitivity + sensitivity – 1) for cardiovascular disease-related mortality were: *Period Average SF* (>3261 ng/mL, sensitivity: 70.6%, specificity: 87.7%; **Figure 2A**), *Absolute Change SF* (>764 increase ng/mL, sensitivity: 64.7%, specificity: 87.1%; **Figure 2B**), *Period Average LIC* (>8.2 mg/g, sensitivity: 88.2%, specificity: 77.7%; **Figure 2C**), *Absolute Change LIC* (>1.4 mg/g increase, sensitivity: 88.2%, specificity: 73.1%; **Figure 2D**), and *Index cT2** (<27.2 ms, sensitivity: 71.3%, specificity: 82.4%; **Figure 2E**). The sensitivity and specificity of conventional thresholds are also indicated in **Figure 1** and **Figure 2**.

Sensitivity analyses for *Period Average LIC* imputed using a linear regression formula for correlation with SF [LIC = SF*0.0026 + 1.9376; R = 0.716, *P* <0.001) yielded similar results (n = 892): AUC for all-cause mortality 0.711, *P* <0.001; AUC for cardiovascular-disease related mortality: 0.803, *P* <0.001.

Survival according to iron overload status and change thresholds

Identified iron overload status and change thresholds were carried forward for survival analyses. Kaplan-Meier survival curve data and comparisons are summarized in **Table 4**. For all-cause mortality, survival was significantly shorter in patients with *Period Average SF* >2145 vs ≤2145 ng/mL (**Figure 3A**), *Absolute Change SF* >1330 vs ≤1330 ng/mL increase (**Figure 3B**), *Period Average LIC* >8 vs ≤8 mg/g (**Figure 3C**), *Absolute Change LIC* >1.4 vs ≤1.4 mg/g increase (**Figure 3D**), and *Index cT2** <27 vs ≥27 ms (**Figure 3E**), *P* <0.001 for all comparisons. For cardiovascular disease-related mortality, survival was also significantly shorter in patients with *Period Average SF* >3265 vs ≤3265 ng/mL (**Figure 4A**), *Absolute Change SF* >765 vs ≤765 ng/mL increase (**Figure 4B**), *Period Average LIC* >8 vs ≤8 mg/g (**Figure 4C**), *Absolute Change LIC* >1.4 vs ≤1.4 mg/g increase (**Figure 4D**), and *Index cT2** <27 vs ≥27 ms (**Figure 4E**), *P* <0.001 for all comparisons.

We constructed multivariate Cox regression models with the outcome of death as the dependent variable, and iron overload status and change thresholds as independent variables. Unadjusted HR and 95%CI for all-cause and cardiovascular disease-related mortality are summarized in **Table 5**. We further adjusted the associations for potential confounders that may affect iron parameters as well as the outcome of death, whether directly or as a reflection of overall patient management including

age, sex, center of treatment, pretransfusion hemoglobin level (observation period average), iron chelation type, and active morbidity at study start. Iron overload status and change thresholds remained independently associated with all-cause and cardiovascular disease-related mortality (**Table 5**).

Patients with *Period Average SF* >2145 vs ≤2145 ng/mL were 7.1-fold (*P* <0.001) or with *Absolute Change SF* >1330 vs ≤1330 ng/mL increase were 21.5-fold (*P* <0.001) more likely to die from any cause. Patients with *Period Average LIC* >8 vs ≤8 mg/g were 20.2-fold (*P* <0.001) or with *Absolute Change LIC* >1.4 vs ≤1.4 mg/g increase were 27.6-fold (*P* <0.001) more likely to die from any cause. Patients with *Index cT2** <27 vs ≥27 ms were 8.6-fold (*P* <0.001) more likely to die from any cause. Similarly, patients with *Period Average SF* >3265 vs ≤3265 ng/mL were 37.2-fold (*P* <0.001) or with *Absolute Change SF* >765 vs ≤765 ng/mL increase were 19.6-fold (*P* <0.001) more likely to die from cardiovascular disease. Patients with *Period Average LIC* >8 vs ≤8 mg/g were 34.1-fold (*P* <0.001) or with *Absolute Change LIC* >1.4 vs ≤1.4 mg/g increase were 33.9-fold (*P* <0.001) more likely to die from cardiovascular disease. Patients with *Index cT2** <27 vs ≥27 ms were 8.6-fold (*P* = 0.001) more likely to die from cardiovascular disease (**Table 5**).

DISCUSSION

In this study, we have established iron overload status and change thresholds that are associated with increased mortality in patients with TDT, though analysis or a large cohort of patients followed for 10 years. Patients who remained on average at SF levels >2145 and >3265 ng/mL had increased risk of all-cause and

cardiovascular disease-related mortality, respectively. At the moment, a SF level >1000 ng/mL is generally used to indicate the need for iron chelation therapy, while levels >2500 ng/mL are commonly used to indicate severe iron overload requiring treatment intensification [13]. These values primarily stem from few older studies. In one analysis from Italy on 720 patients (receiving deferoxamine therapy) born after 1970 and followed until the end of 1999, with SF values available from 1991 onwards, both pre-assigned cut-offs of 1000 ng/mL and 2500 ng/mL (average prior to event) were prognostic for heart failure (HR: 3.2 and 4.8, respectively) and mortality (HR: 2.6 and 4.4 respectively) [15]. In an updated analysis of the same cohort, the SF value of 1140 ng/mL (observation period average) was the best predictor of death on ROC analysis on a subset of 417 patients [3]. In an older study of 97 patients from the US born between 1954 and 1975 and followed for a median of 12 years after the initiation of iron chelation with deferoxamine, a pre-assigned SF level >2500 ng/mL (measured as proportion of values above this cut-off) was associated with poorer cardiovascular disease-free survival (HR: 19.1) [14]. In a smaller study of 32 patients from the UK who received a liver biopsy between 1984 and 1986 and followed for a median of 13.6 years afterwards, there was a statistically significant difference in cardiovascular disease-free survival in patients with pre-assigned SF (period average) >2500 ng/mL (12.5%) and 1500-2500 ng/mL (78.6%) compared with <1500 ng/mL (90%) [16]. A small, more recent study from Greece on 75 patients with TDT aimed to revisit established iron overload thresholds and identified a SF level >1700 ng/mL (patients stratified around population mean) as prognostic for mortality (HR: 3.8) [24].

Our study comes in to confirm findings of an increased risk of death with high SF levels in TDT and provides predictive SF thresholds that were not pre-assigned. It is evident that SF levels >2000-3000 ng/mL are a ‘red flag’ and indicate uncontrolled iron overload requiring immediate treatment optimization. Target levels, however, may need to be lower (<1000-1500 ng/mL) to keep patients in a safe zone, in keeping with previous literature indicating some degree of risk above such lower levels, and in view of their association with other morbidities like endocrinopathies [30]. Our study has also determined clinically meaningful change thresholds for SF (765-1330 ng/mL), which to our best knowledge, were not available in the literature.

With respect to LIC, evidence on association with long-term outcomes and mortality is even more limited. The original values of 7 mg/g and 15 mg/g where in fact ‘inherited’ from studies on hereditary hemochromatosis owing to their association with hepatic fibrosis [7, 18, 31]. In the aforementioned study from the UK following 32 patients after liver biopsy, these thresholds were revisited and there was a statistically significant difference in cardiovascular disease-free survival in patients with LIC (single measurement) >15 mg/g (50%) and 7-15 mg/g (71.4%) compared with <7 mg/g (93.3%) [16]. In another study of 211 post-transplant (1983-1989) thalassemia patients followed for a median of 64 months following liver biopsy, a LIC of 16 mg/g was identified as a threshold for hepatic fibrosis progression [19]. Our study is the first to establish an association between LIC, measured by MRI, and mortality in TDT patients and revealed the threshold of 8 mg/g as most predictive of death within 10 years. Changes in LIC by 1.4 mg/g were also associated with mortality risk. Clinically meaningful changes for LIC have not been previously established in TDT, although in one trial, a change of 3 mg/g was used to establish

iron chelation response based on expert opinion [32]. In patients with non-transfusion-dependent thalassemia, changes of 1 mg/g LIC have been associated with the risk of multiple morbidities [33].

Data on the association of cT2* values with long-term outcomes are more robust, with levels <20 ms associated with increased risk of arrythmia and heart failure, and levels <10 ms associated with increased risk of heart failure and death in several studies [16-23], although studies were mainly looking at cross-sectional associations or event occurrence within 1 year of assessment. Our study identified a higher threshold of <27 ms for the prediction of death within 10 years, which may reflect that lower iron overload levels in the heart may lead to cardiovascular disease-related death in the long-term (vs more acutely). Similar findings were also noted in the aforementioned study from Greece which identified a threshold of cT2* ≤34 ms as a better prognostic factor for cardiac mortality or hospitalization than 20 ms [24]. Accumulation of toxic iron species within myocytes is not necessary to induce cardiac dysfunction, and only initial exposure to non-transferrin-bound iron may be enough to cause damage to cardiac tissue. Thus, even without evidence of cardiac iron overload by MRI, patients may still be at risk of cardiovascular disease [34-36]. It should also be noted that non-iron related cardiovascular disease is also common, especially in older adults with thalassemia [37].

Our study confirmed a good correlation between SF and LIC, yet a weaker correlation between SF (or LIC) and cT2* on both cross-sectional (spot measurements) and longitudinal (change over time) analysis, which is in general agreement with earlier studies using both liver biopsy and MRI for the assessment of

LIC [20, 25-27, 38-50]. This may be attributed to several factors. Both SF and LIC are considered indirect measures of systemic and total body iron [51], while cT2* primarily reflects organ-specific (heart) iron levels. More importantly, it has been previously established that longitudinal iron changes in the heart significantly lagged behind those in the liver and may partially explain the weak association between these parameters. Although a correlation between SF and LIC is noted, it should be acknowledged that it is not perfect. Cross-sectionally, correlation was shown to be poor at SF levels >4000 ng/mL, which we could not assess in this study owing to the generally low iron burden [25, 50, 52]. Longitudinally, a discordance between response to iron chelation therapy as measured by changes in SF vs LIC is noted in up to 30% of patients [25, 49, 50]. We have also identified variation in the correlation between changes in SF and LIC by iron chelator type, where such correlation was weaker for deferiprone than for deferoxamine and deferasirox. This may be attributed to deferiprone's slower effects on hepatic iron stores compared with deferoxamine or deferasirox [13, 26], although other studies with smaller patient numbers did not observe such variation [43]. Collectively, these findings highlight the added value of MRI monitoring of iron overload status over reliance on serial serum ferritin levels alone. It is understandable that this may not be conducted at the regularity recommended by international guidelines [13, 45], and patient prioritization through practical algorithms may be needed in resource-limited countries where access and affordability remain an issue [53]. It is often the combination of information from two or more iron indices that allows the understanding of the patient's overall iron overload 'profile' and subsequent treatment optimization needs. Other measures of iron overload such as non-transferrin-bound iron and labile plasma iron have also been used in clinical trials of iron chelation therapy but their

wide routine use in clinical practice has been hampered by the limited number of laboratories that can perform such studies [13].

Our study has two key limitations. First, data may not be generalizable to the global TDT population and may be more relatable to patients managed in Western countries with adequate resources, where mortality is more attributable to disease progression or lack of response to therapy rather than challenges in access to optimal care. The overall iron overload burden and mortality rate in our cohort was low, and thus the observed associations between iron parameters and outcomes may only be relevant in this context. Second, a large proportion of patients did not have MRI measurements for iron overload. Although the remaining samples used for various analyses were still considerably large compared to previous studies, a potential bias could not be dismissed. We cannot fully ascertain if absence of MRI assessment was fully random. In a previous study from Webthal, we have identified low iron intake (transfusion burden), low serum ferritin level, and young age as the main risk factors for not having LIC or cT2* assessment by MRI [45], implying that patients who had less severe iron burden were less likely to get an MRI. On the other hand, it may also be assumed that such patients will go on to develop higher iron burden in the liver/heart as a consequence of lack of MRI assessment and subsequent tailoring of iron chelation therapy. Irrespectively, patients who did not have MRI assessment in this study had a comparable rate of mortality to the overall population, rendering any over- or underestimation of mortality risk for evaluated iron overload status and change thresholds minimal.

Our findings should add to the evidence base supporting decisions on iron chelation therapy by establishing treatment targets, including safe iron levels and clinically meaningful changes over time. These thresholds may also be used, individually or in combination, to develop prognostic scoring models along with other risk factors that moderate mortality risk.

STATEMENTS AND DECLARATIONS

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Author contribution

Study conception and design: KMM, GLF. Data collection: SB, RO, GBF, RL, AP, FL, BG, GLF. Statistical analysis: KMM, BG. Review and interpretation of results: KMM, SB, RO, GBF, RL, AP, FL, BG, GLF. Manuscript drafting: KMM, GLF. Manuscript review for important intellectual content: SB, RO, GBF, RL, AP, FL, BG. All authors approved the manuscript before submission.

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Competing interests

KMM reports consultancy fees from Novartis, Celgene Corp (Bristol Myers Squibb), Agios Pharmaceuticals, CRISPR Therapeutics, Vifor Pharma, and Pharmacosmos; and research funding from Agios Pharmaceuticals and Pharmacosmos. SB reports being on the advisory board of Vertex Pharmaceuticals and Bristol Myers Squibb and receiving speaker honoraria from Bristol Myers Squibb and Chiesi. RO reports being on the advisory board of Chiesi and Bristol Myers Squibb and consultancy fees from Vertex Pharmaceuticals and Bristol Myers Squibb. GBF reports consultancy fees from Bristol Myers Squibb, Agios Pharmaceuticals, FORMA Therapeutics, Vertex Pharmaceuticals. RL reports receiving speaker honoraria from Bristol Myers Squibb. AP reports receiving speaker honoraria from Bristol Myers Squibb. FL reports being on the advisory board of Vertex Pharmaceuticals and Bristol Myers Squibb. GLF reports receiving speaker honoraria and being on the advisory board Vertex Pharmaceuticals, Bristol Myers Squibb, Hemanext and Garuda Pharmaceuticals. The remaining authors have no relevant financial or non-financial interests to disclose.

Data Sharing and data availability

Data can be made available upon reasonable request to the corresponding author.

Ethics approval

An Ethics Committee approval was obtained at each participating center.

Consent to participate

Written informed consents for data collection and use were retrieved from all patients.

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Table 1. Evaluated iron parameters.

Parameter	n	Median	IQR	min	max
SF					
Y_1 (ng/mL)	760	1466.1	782.2-2431.9	53.1	19,032
Y_2 (ng/mL)	755	1470	701-2651.4	64.6	22,711.3
Y_3 (ng/mL)	774	1417.3	732-2682.2	38.6	19,003
Y_4 (ng/mL)	764	1281.4	638.6-2476.2	66.7	17,773.5
Y_5 (ng/mL)	768	1233.4	598.7-2323	49.6	17,673.9
Y_6 (ng/mL)	689	1040.5	504.9-2046	74.4	13,390
Y_7 (ng/mL)	708	1050.5	537.7-2130.6	92.8	20,358
Y_8 (ng/mL)	713	997.9	515.5-1956.7	89	14,951
Y_9 (ng/mL)	722	996.3	506.5-1921	68	14,645
Y_{10} (ng/mL)	775	841.5	434.7-1800.5	68.3	16,026.5
Index (ng/mL)	892	1380.5	723.4-2392.8	53.1	19,032
Period Average (ng/mL)	892	1253.8	674.4-2212.3	99	15,101.3
Absolute Change (ng/mL)	820	(-)276	(-)938.5-(+)212.2	(-)11,129.9	(+)9707.4
Relative Change (%)	820	(-)26	(-)57-(+)22	(-)97.1	(+)2881.4
LIC					
Y_1 (mg/g)	130	3.7	1.9-8.3	0.8	26.9

Y_2 (mg/g)	140	4.3	1.9-8.6	0.9	31.2
Y_3 (mg/g)	154	4.4	1.9-9.3	0.8	32
Y_4 (mg/g)	169	4.2	1.5-9.2	0.6	29.7
Y_5 (mg/g)	182	3	1.4-6.3	0.6	32.4
Y_6 (mg/g)	144	2.8	1.6-7.3	0.5	35.5
Y_7 (mg/g)	147	3.1	1.9-8.2	1	33.2
Y_8 (mg/g)	191	3.8	2.2-9.1	0.5	37.6
Y_9 (mg/g)	138	3.5	2-6.6	0.6	46.4
Y_{10} (mg/g)	145	2.9	1.8-7	1	44
Index (mg/g)	412	3.7	1.8-8.4	0.7	31.2
Period Average (mg/g)	412	3.9	2.1-8.1	0.8	34.2
Absolute Change (mg/g)	306	0	(-)2.3-(+)1.5	(-)18.9	(+)27.8
Relative Change (%)	306	(-)0.3	(-)41.9-(+)66.3	(-)92	(+)1983.2
cT2*					
Y_1 (ms)	140	34.5	20.3-41.8	6.6	61.1
Y_2 (ms)	157	35.9	25.5-43	8	69.7
Y_3 (ms)	198	34	23-43	4.4	88.6
Y_4 (ms)	197	32.4	24.7-40.9	1.8	70
Y_5 (ms)	188	34.7	26.3-43.7	6.3	70

Y_6 (ms)	143	36.1	28-42.7	6.3	62.2
Y_7 (ms)	158	37.5	24.8-43.3	6.6	78.6
Y_8 (ms)	195	36	24.7-44	5.1	67.1
Y_9 (ms)	147	38	30.5-43.6	6	62.6
Y_{10} (ms)	148	37.9	30.3-42	5.5	67.5
Index (ms)	454	35	24-41.4	5	88.6
Period Average (ms)	454	36	27.3-41.5	5	57.9
Absolute Change (ms)	326	(+)1.8	(-)4.5-(+)8.9	(-)49.4	(+)39.2
Relative Change (%)	326	(+)5.5	(-)14.9-(+)29	(-)76.2	(+)426.3

IQR, interquartile range; SF, serum ferritin; LIC, liver iron concentration; cT2*, cardiac T2*.

Table 2. Iron parameters according to death status.

Parameter	All-cause mortality					Cardiovascular disease-related mortality				
	Yes		No		*P*-value	Yes		No		*P*-value
	n	Median	n	Median		n	Median	n	Median	
SF										
Index (ng/mL)	26	2705.1	866	1368.6	0.050	17	2935.7	875	1367.5	**0.011**
Period Average (ng/mL)	26	2899.7	866	1242.9	**0.001**	17	4118.5	875	1241.3	**<0.001**
Absolute Change (ng/mL)	26	(+)1554.3	798	(-)309.5	**<0.001**	17	(+)1771.9	805	(-)295	**<0.001**
Relative Change (%)	26	(+)36.2	798	(-)27.8	**<0.001**	17	(+)38.8	805	(-)26.7	**<0.001**
LIC										
Index (mg/g)	26	6.4	401	3.6	**0.027**	17	6.4	410	3.7	**0.021**
Period Average (mg/g)	26	8.3	401	3.8	**<0.001**	17	8.3	410	3.9	**<0.001**
Absolute Change (mg/g)	26	(+)1.4	300	(-)0.02	**<0.001**	17	(+)1.4	309	(+)0.1	**0.002**
Relative Change (%)	26	(+)9.2	300	(-)0.3	0.140	17	(+)9.2	309	(+)3.6	0.274
cT2*										
Index (ms)	26	27	443	35.1	**0.003**	17	27	452	34.9	**0.022**
Period Average (ms)	26	33.1	443	36	0.160	17	33.1	452	35.9	0.180
Absolute Change (ms)	26	(+)2	319	(+)1.8	0.614	17	(+)2	328	(+)1.9	0.784
Relative Change (%)	26	(+)7.4	319	(+)5.1	0.731	17	(+)7.4	328	(+)7	0.644

SF, serum ferritin; LIC, liver iron concentration; cT2*, cardiac T2*.

Table 3. Receiver operating characteristic curve analyses for the prediction of mortality within 10 years.

Parameter	n	All-cause mortality			Cardiovascular disease-related mortality		
		AUC	95%CI	*P*-value	AUC	95%CI	*P*-value
SF							
Index (ng/mL)	892	0.601	0.460-0.743	0.078	0.670	0.501-0.838	**0.017**
Period Average (ng/mL)	892	0.681	0.547-0.815	**0.002**	0.787	0.643-0.931	**<0.001**
Absolute Change (ng/mL)	824	0.767	0.651-0.882	**<0.001**	0.768	0.626-0.909	**<0.001**
Relative Change (%)	824	0.759	0.681-0.836	**<0.001**	0.787	0.705-0.870	**<0.001**
LIC							
Index (mg/g)	427	0.642	0.548-0.735	**0.016**	0.677	0.584-0.770	**0.014**
Period Average (mg/g)	427	0.749	0.669-0.828	**<0.001**	0.796	0.742-0.851	**<0.001**
Absolute Change (mg/g)	326	0.735	0.672-0.798	**<0.001**	0.729	0.657-0.801	**0.002**
Relative Change (%)	326	0.587	0.518-0.656	0.140	0.579	0.506-0.652	0.274
cT2*							
Index (ms)	469	0.687	0.595-0.780	**0.001**	0.675	0.572-0.779	**0.015**
Period Average (ms)	469	0.611	0.515-0.707	0.059	0.625	0.521-0.729	0.082
Absolute Change (ms)	345	0.470	0.395-0.546	0.615	0.520	0.444-0.595	0.784
Relative Change (%)	345	0.480	0.399-0.560	0.731	0.533	0.450-0.617	0.645

AUC, area under the curve; CI, confidence interval; SF, serum ferritin; LIC, liver iron concentration; cT2*, cardiac T2*.

Table 4. Survival according to identified iron overload status and change thresholds.

Parameter	All-cause mortality				Cardiovascular disease-related mortality			
	Deaths n (%)	**5-year survival**	**10-year survival**	**Log-rank Chi-square (*P*-value)**	**Deaths n (%)**	**5-year survival**	**10-year survival**	**Log-rank Chi-square (*P*-value)**
SF								
Period Average (ng/mL)								
≤2145 (n = 659)	10 (1.5)	99%	98%	17.497 (**<0.001**)				
>2145 (n = 233)	16 (6.9)	97%	93%					
≤3265 (n = 776)					5 (0.6)	100%	99%	52.812 (**<0.001**)
>3265 (n = 116)					12 (10.3)	95%	89%	
Absolute Change (ng/mL)								
≤1330 increase (n = 747)	10 (1.3)	99%	99%	93.439 (**<0.001**)				
>1330 increase (n = 77)	16 (20.8)	92%	79%					
≤765 increase (n = 709)					6 (0.8)	99%	99%	40.438 (**<0.001**)
>765 increase (n = 115)					11 (9.6)	97%	90%	
LIC								
Period Average (mg/g)								
≤8 (n = 313)	5 (1.6)	99%	98%	44.363 (**<0.001**)	2 (0.6)	100%	99%	37.763 (**<0.001**)
>8 (n = 114)	21 (18.4)	88%	81%		15 (13.2)	92%	86%	

Absolute Change (mg/g)								
≤1.4 increase (n = 228)	3 (1.3)	99%	99%	48.904 (**<0.001**)	2 (0.9)	100%	99%	32.361 (**<0.001**)
>1.4 increase (n = 98)	23 (23.5)	87%	76%		15 (15.3)	91%	83%	
cT2*								
Index (ms)								
≥27 (n = 320)	5 (1.6)	99%	98%	30.932 (**<0.001**)	3 (0.9)	100%	99%	21.688 (**<0.001**)
<27 (n = 149)	21 (14.1)	92%	86%		14 (9.4)	95%	90%	

SF, serum ferritin; LIC, liver iron concentration; cT2*, cardiac T2*.

Table 5. Multivariate Cox regression analyses for the outcomes of all-cause mortality and cardiovascular disease-related mortality.

Parameter	All-cause mortality				Cardiovascular disease-related mortality			
	Unadjusted HR	**95% CI (*P*-value)**	**Adjusted HR[a]**	**95% CI (*P*-value)**	**Unadjusted HR**	**95% CI (*P*-value)**	**Adjusted HR[a]**	**95% CI (*P*-value)**
SF								
Period Average (ng/mL)								
≤2145 (n = 659)	1.00 (referent)	2.100-10.196 (**<0.001**)	1.00 (referent)	2.857-17.524 (**<0.001**)				
>2145 (n = 233)	4.627		7.076					
≤3265 (n = 776)					1.00 (referent)	5.956-48.001 (**<0.001**)	1.00 (referent)	11.406-121.029 (**<0.001**)
>3265 (n = 116)					16.909		37.154	
Absolute Change (ng/mL)								
≤1330 increase (n = 747)	1.00 (referent)	7.781-37.813 (**<0.001**)	1.00 (referent)	9.266-49.835 (**<0.001**)				
>1330 increase (n = 77)	17.153		21.488					
≤765 increase (n = 709)					1.00 (referent)	4.563-33.375 (**<0.001**)	1.00 (referent)	6.520-58.660 (**<0.001**)
>765 increase (n = 115)					12.341		19.557	
LIC								
Period Average (mg/g)								
≤8 (n = 313)	1.00 (referent)	4.877-34.334 (**<0.001**)	1.00 (referent)	7.509-54.478 (**<0.001**)	1.00 (referent)	5.352-102.428 (**<0.001**)	1.00 (referent)	7.492-155.596 (**<0.001**)
>8 (n = 114)	12.940		20.226		23.413		34.144	
Absolute Change (mg/g)								

≤1.4 increase (n = 228)	1.00 (referent)	6.135-68.123 (**<0.001**)	1.00 (referent)	8.185-92.794 (**<0.001**)	1.00 (referent)	4.649-88.999 (**<0.001**)	1.00 (referent)	7.541-152.296 (**<0.001**)
>1.4 increase (n = 98)	20.443		27.560		20.341		33.889	
cT2*								
Index (ms)								
≥27 (n = 320)	1.00 (referent)	3.604-25.354 (**<0.001**)	1.00 (referent)	3.140-23.673 (**<0.001**)	1.00 (referent)	3.071-37.198 (**<0.001**)	1.00 (referent)	2.398-30.870 (**0.001**)
<27 (n = 149)	9.559		8.621		10.688		8.603	

SF, serum ferritin; LIC, liver iron concentration; cT2*, cardiac T2*; HR, hazard ratio; CI, confidence interval.
[a]Adjusted for age, sex, center, pretransfusion hemoglobin (observation period average), baseline morbidity, iron chelation type.

FIGURE LEGENDS

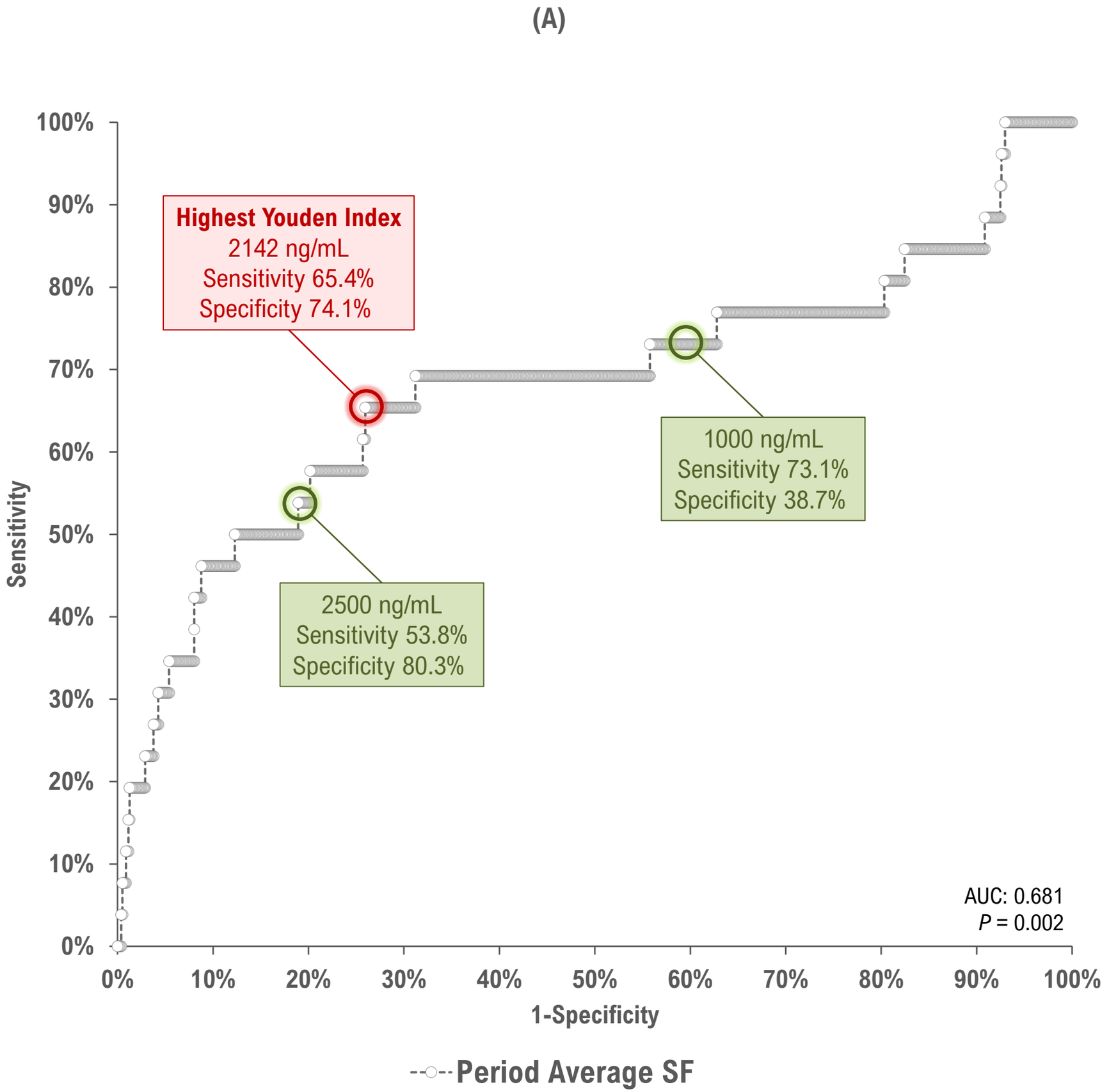
Figure 1. Receiver operating characteristic (ROC) curves for the prediction of all-cause mortality within 10 years by: (A) *Period Average SF*, **(B)** *Absolute Change SF*, **(C)** *Period Average LIC*, **(D)** *Absolute Change LIC*, and **(E)** *Index cT2**. Red circles indicate thresholds with the highest Youden Index (sensitivity + specificity – 1). Green circles indicate commonly used conventional thresholds. AUC, area under the curve; SF, serum ferritin; LIC, liver iron concentration; cT2*, cardiac T2*.

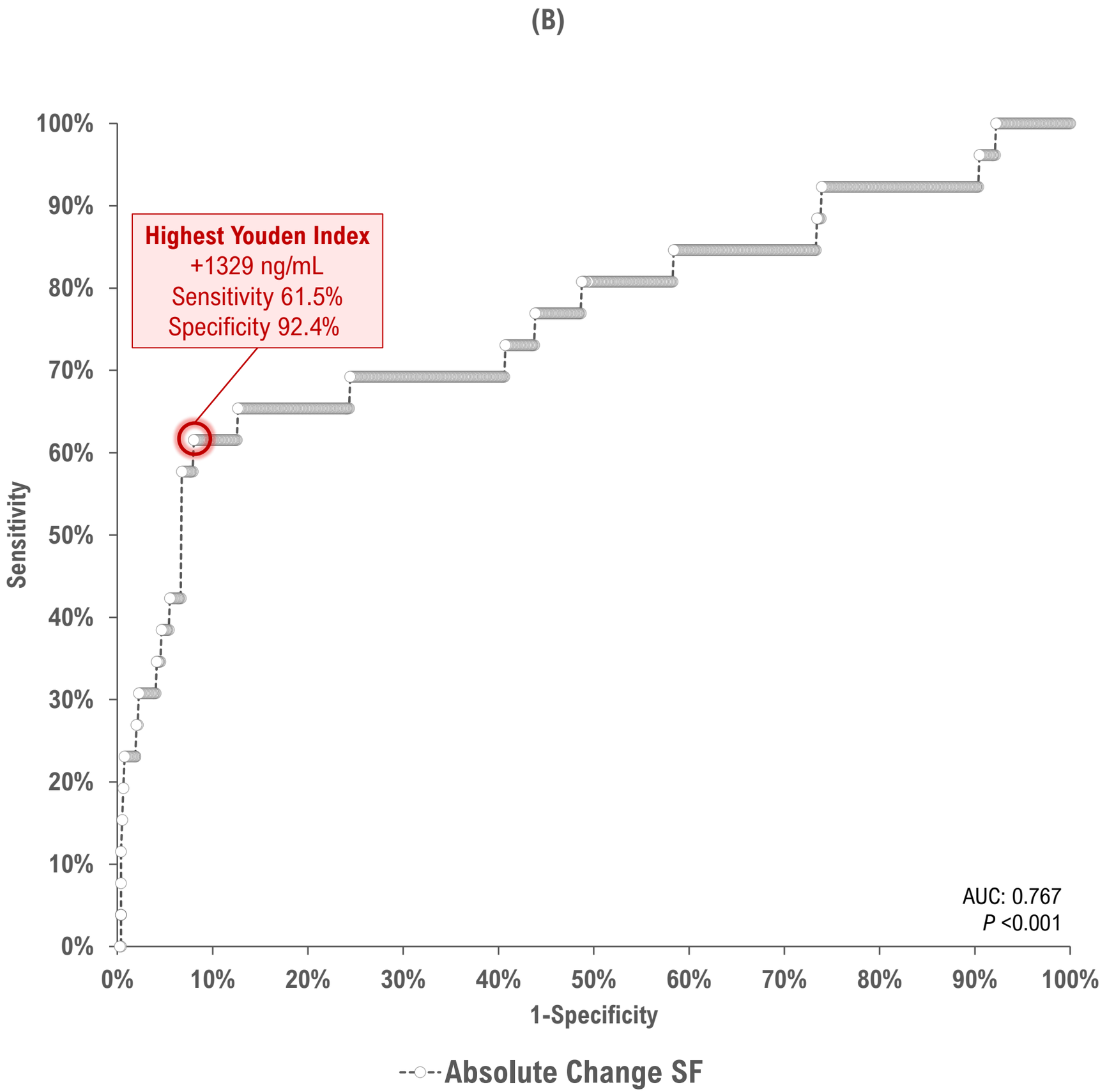
Figure 2. Receiver operating characteristic (ROC) curves for the prediction of cardiovascular disease-related mortality within 10 years by: (A) *Period Average SF*, **(B)** *Absolute Change SF*, **(C)** *Period Average LIC*, **(D)** *Absolute Change LIC*, and **(E)** *Index cT2**. Red circles indicate thresholds with the highest Youden Index (sensitivity + specificity – 1). Green circles indicate commonly used conventional thresholds. AUC, area under the curve; SF, serum ferritin; LIC, liver iron concentration; cT2*, cardiac T2*.

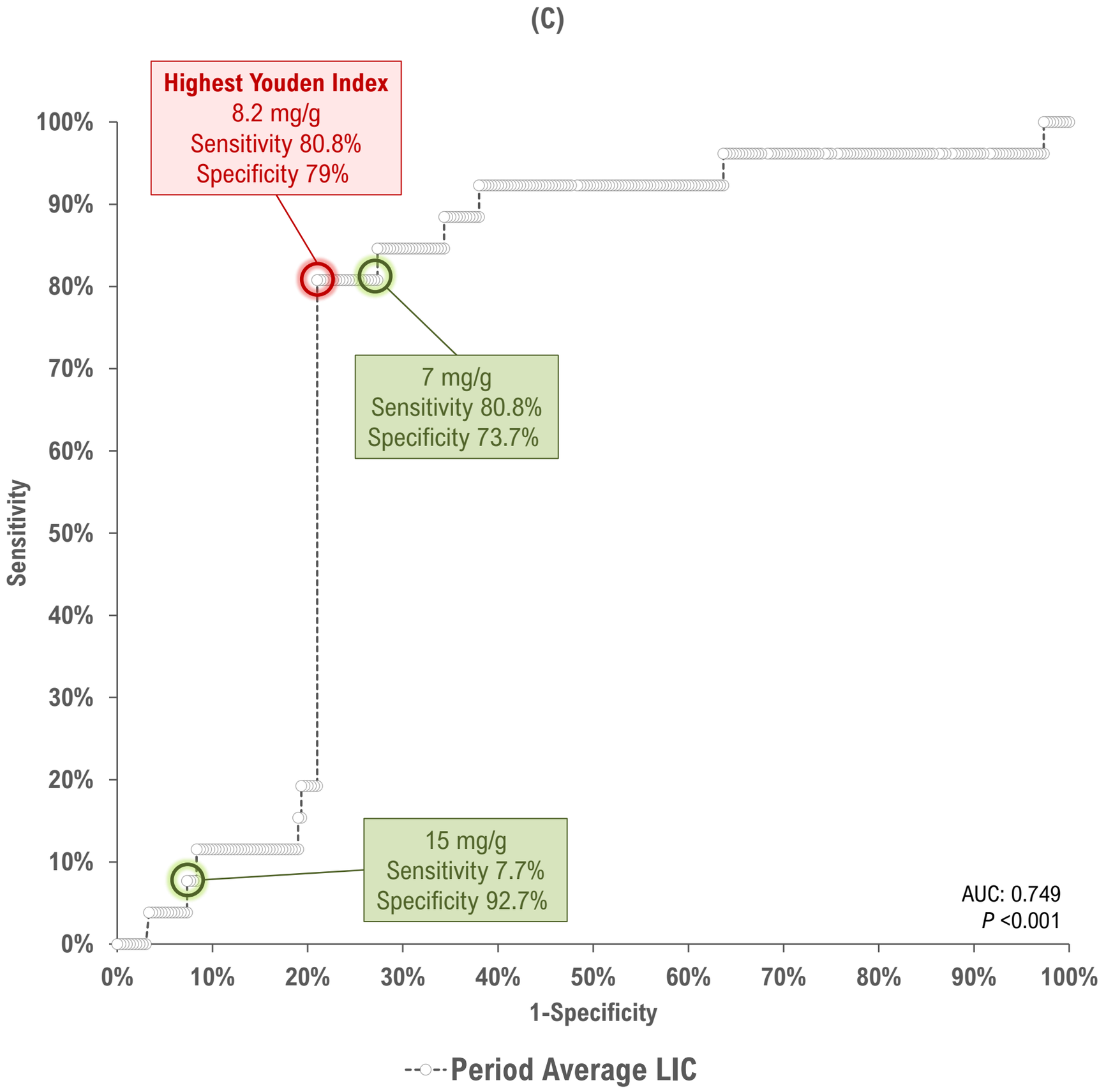
Figure 3. Kaplan-Meier survival curves for all-cause mortality by: (A) *Period Average SF*, **(B)** *Absolute Change SF*, **(C)** *Period Average LIC*, **(D)** *Absolute Change LIC*, and **(E)** *Index cT2**. SF, serum ferritin; LIC, liver iron concentration; cT2*, cardiac T2*.

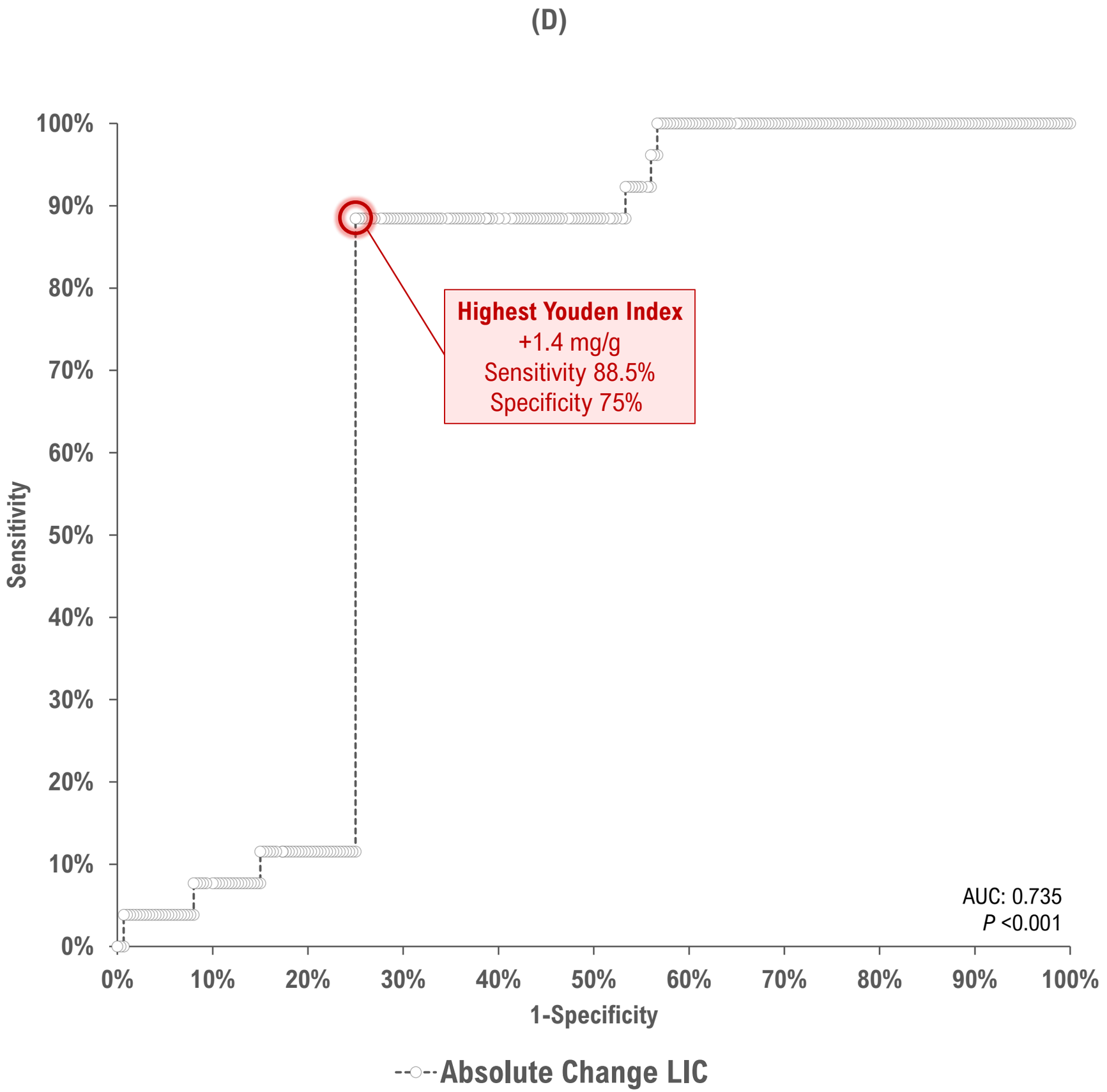
Figure 4. Kaplan-Meier survival curves for cardiovascular disease-related mortality by: (A) *Period Average SF*, **(B)** *Absolute Change SF*, **(C)** *Period Average LIC*, **(D)** *Absolute Change LIC*, and **(E)** *Index cT2**. SF, serum ferritin; LIC, liver iron concentration; cT2*, cardiac T2*, CVD, cardiovascular disease.

Figure 1


(A)
Highest Youden Index
2142 ng/mL
Sensitivity 65.4%
Specificity 74.1%
1000 ng/mL
Sensitivity 73.1%
Specificity 38.7%
2500 ng/mL
Sensitivity 53.8%
Specificity 80.3%
AUC: 0.681
P = 0.002
Sensitivity
1-Specificity
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Period Average SF


(B)
Highest Youden Index
+1329 ng/mL
Sensitivity 61.5%
Specificity 92.4%
AUC: 0.767
P <0.001
Sensitivity
1-Specificity
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Absolute Change SF


(C)
Highest Youden Index
8.2 mg/g
Sensitivity 80.8%
Specificity 79%
7 mg/g
Sensitivity 80.8%
Specificity 73.7%
15 mg/g
Sensitivity 7.7%
Specificity 92.7%
AUC: 0.749
P <0.001
Sensitivity
1-Specificity
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Period Average LIC


(D)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Sensitivity
Highest Youden Index
+1.4 mg/g
Sensitivity 88.5%
Specificity 75%
AUC: 0.735
P <0.001
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
1-Specificity
Absolute Change LIC

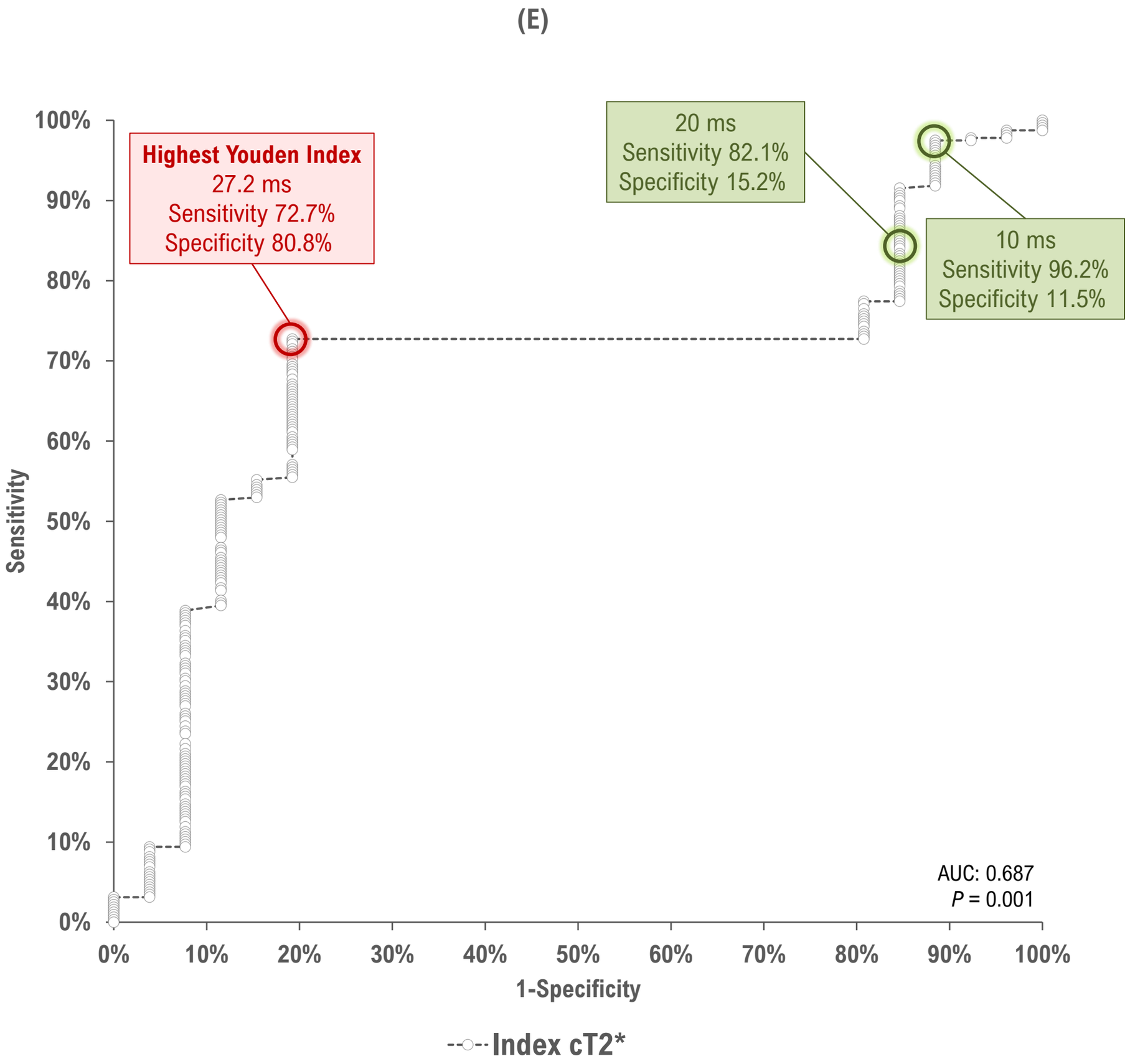
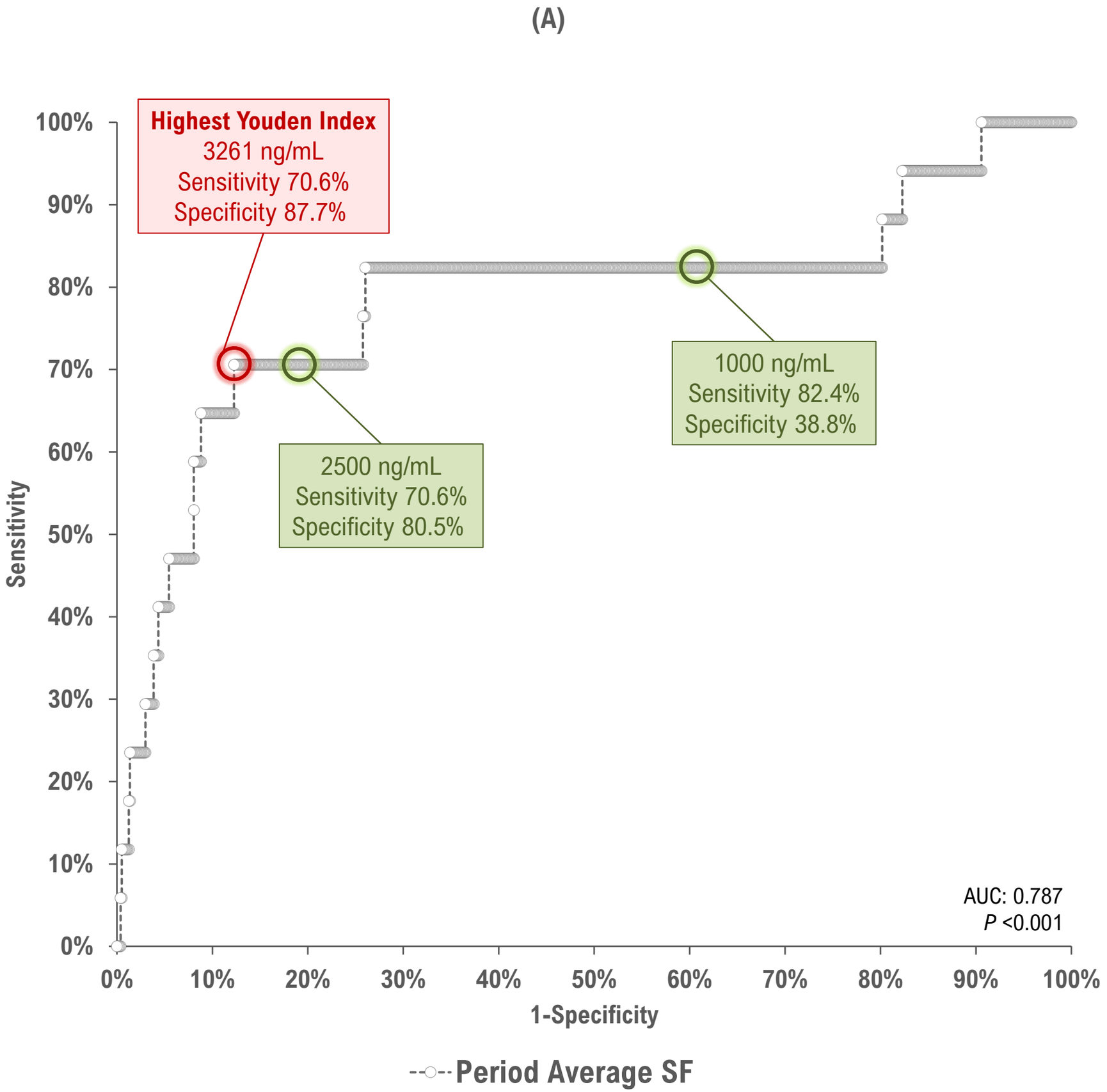
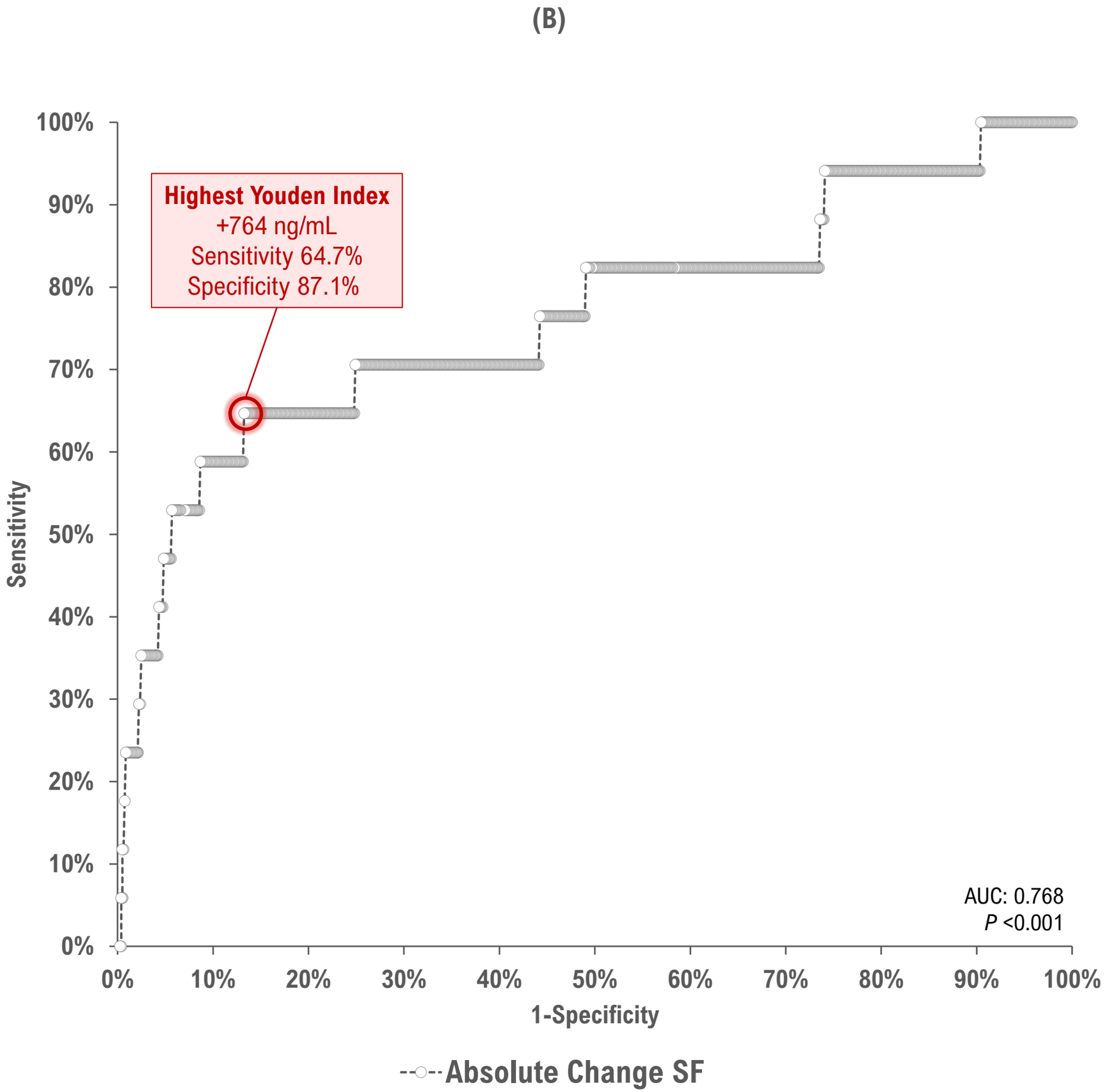
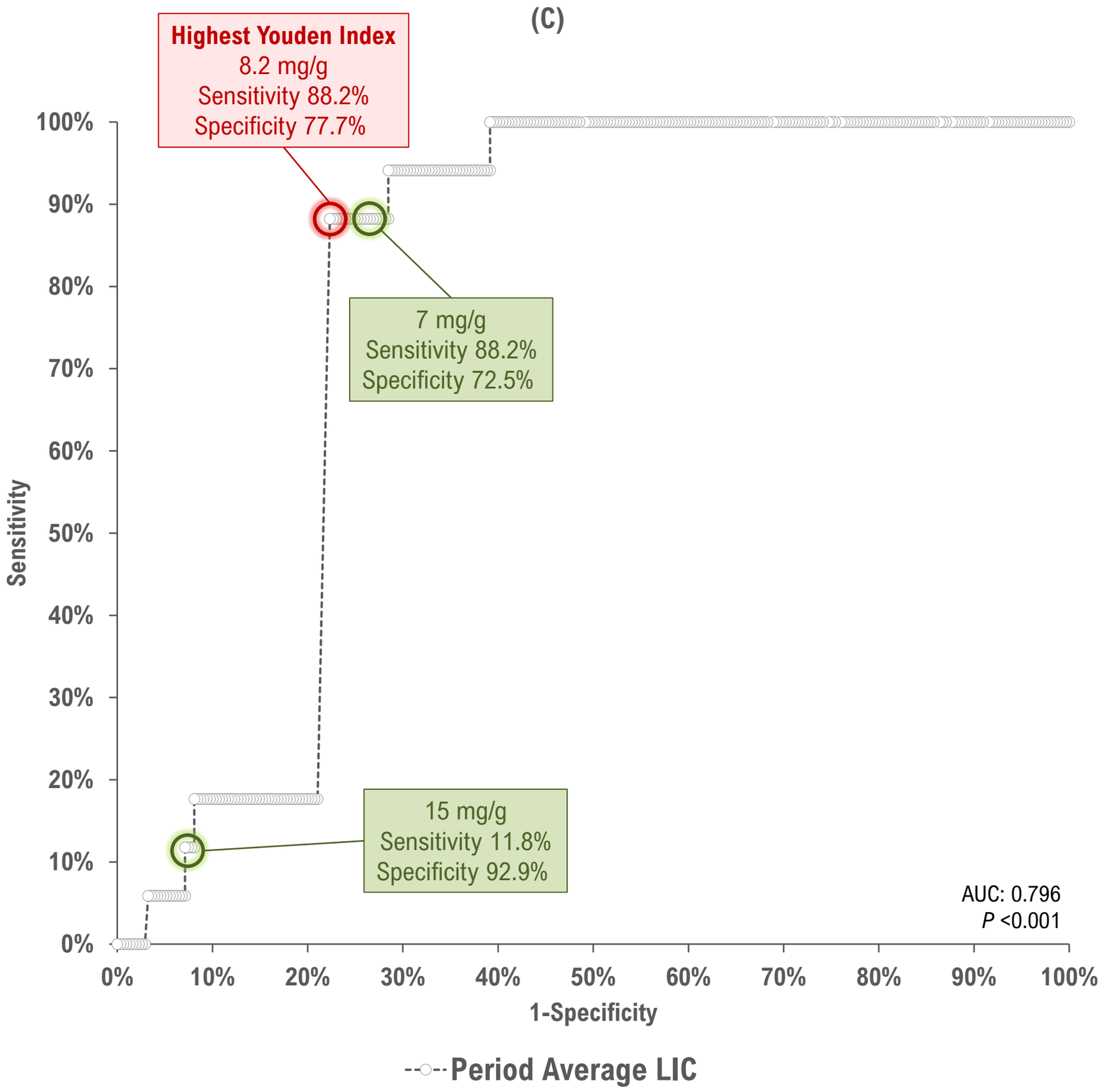

(E)
Highest Youden Index
27.2 ms
Sensitivity 72.7%
Specificity 80.8%
20 ms
Sensitivity 82.1%
Specificity 15.2%
10 ms
Sensitivity 96.2%
Specificity 11.5%
AUC: 0.687
P = 0.001
Sensitivity
1-Specificity
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Index cT2*

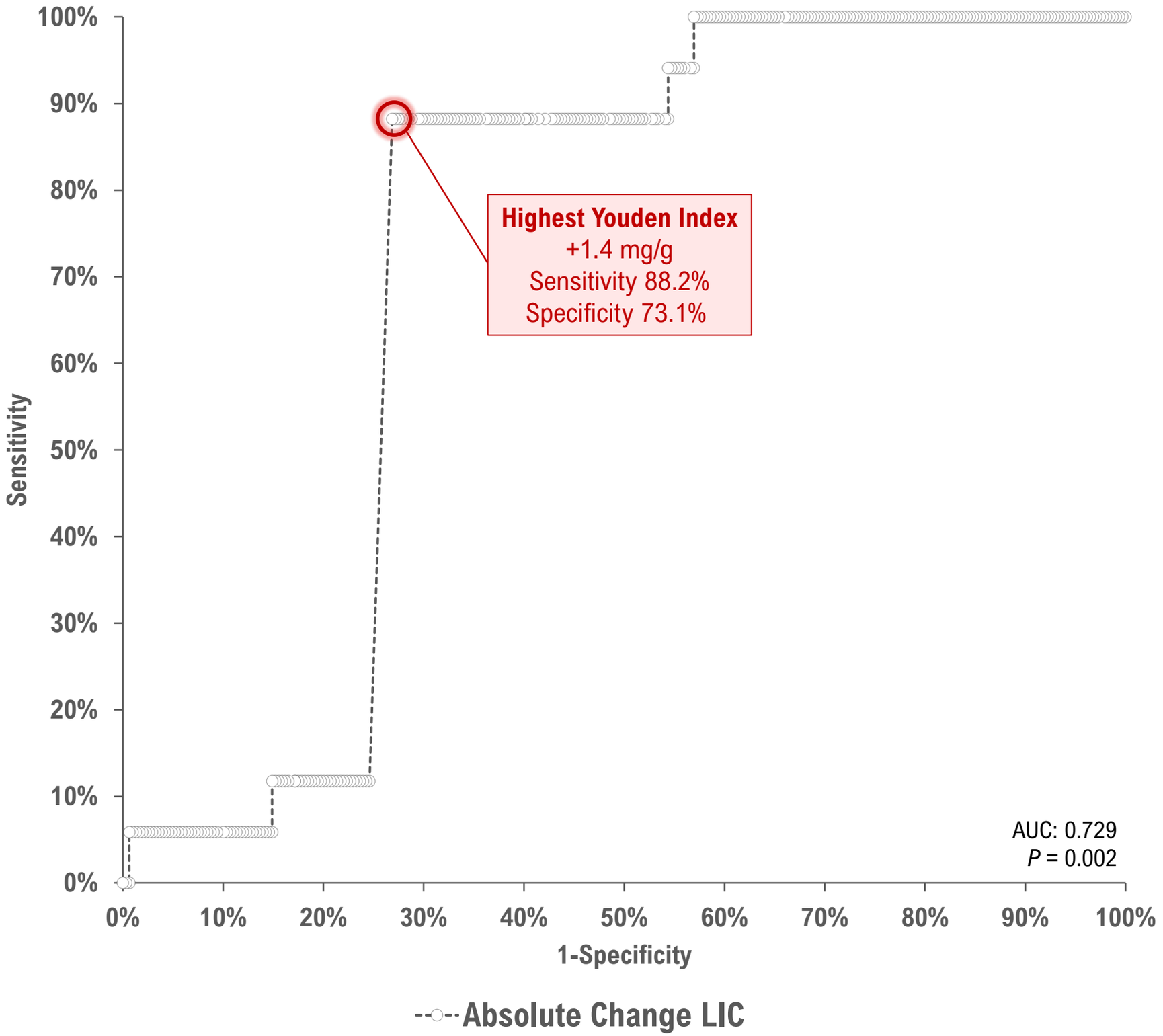
Figure 2


(A)
Highest Youden Index
3261 ng/mL
Sensitivity 70.6%
Specificity 87.7%
2500 ng/mL
Sensitivity 70.6%
Specificity 80.5%
1000 ng/mL
Sensitivity 82.4%
Specificity 38.8%
AUC: 0.787
P <0.001
Sensitivity
1-Specificity
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Period Average SF


(B)
Highest Youden Index
+764 ng/mL
Sensitivity 64.7%
Specificity 87.1%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Sensitivity
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
1-Specificity
AUC: 0.768
P <0.001
Absolute Change SF


(C)
Highest Youden Index
8.2 mg/g
Sensitivity 88.2%
Specificity 77.7%
7 mg/g
Sensitivity 88.2%
Specificity 72.5%
15 mg/g
Sensitivity 11.8%
Specificity 92.9%
AUC: 0.796
P <0.001
Sensitivity
1-Specificity
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Period Average LIC

(D)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Sensitivity
Highest Youden Index
+1.4 mg/g
Sensitivity 88.2%
Specificity 73.1%
AUC: 0.729
P = 0.002
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
1-Specificity
Absolute Change LIC

(E)

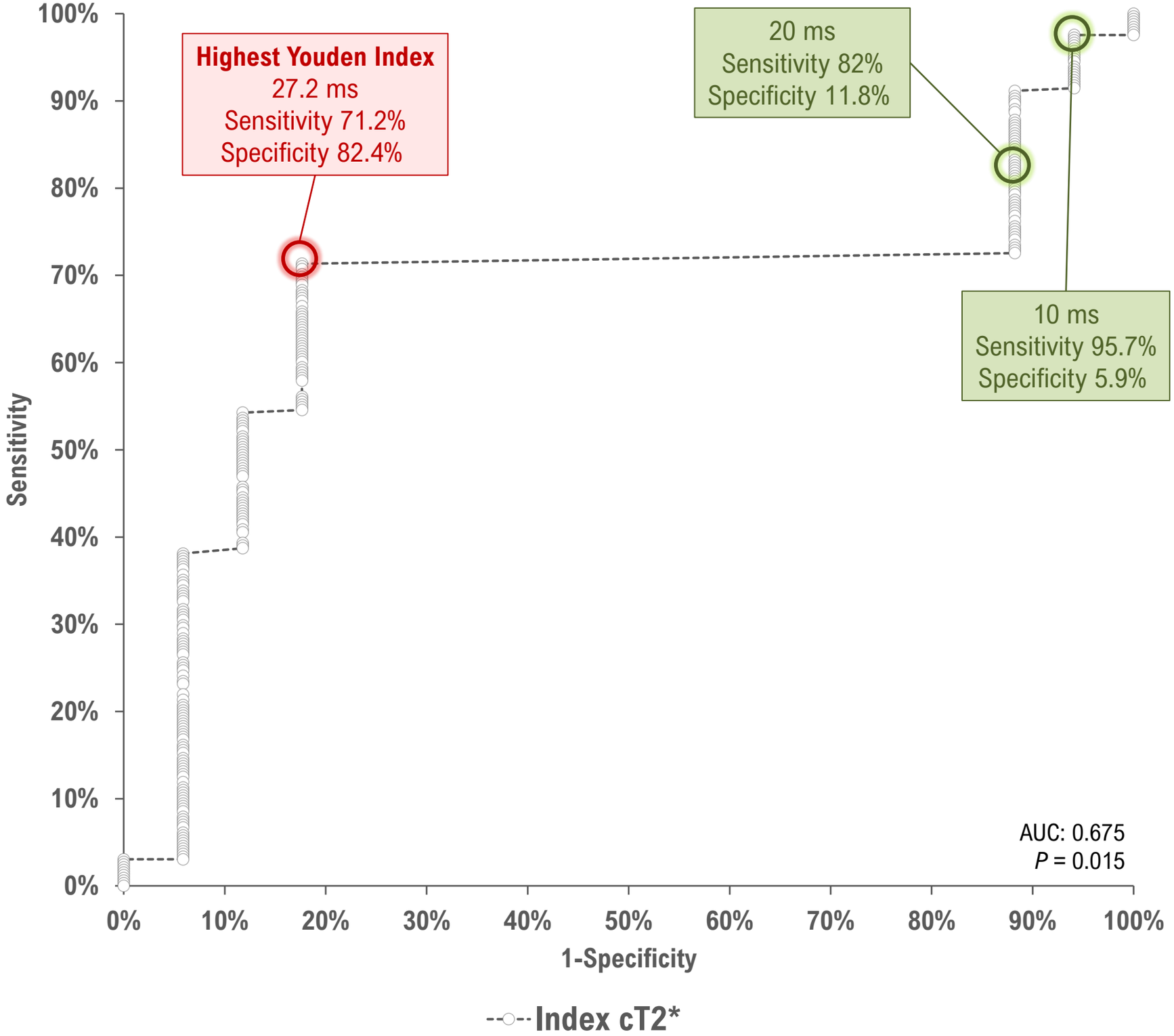
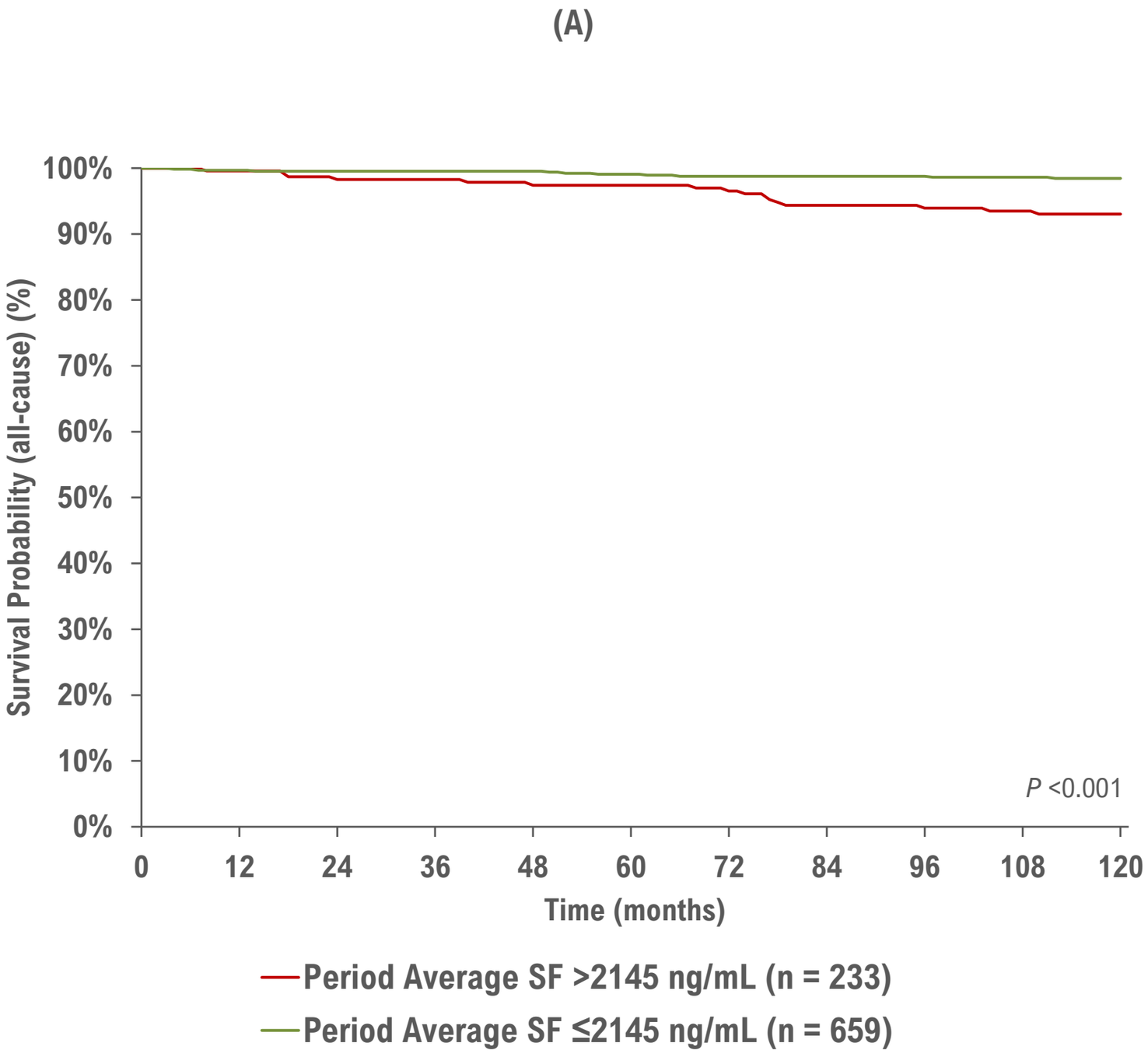
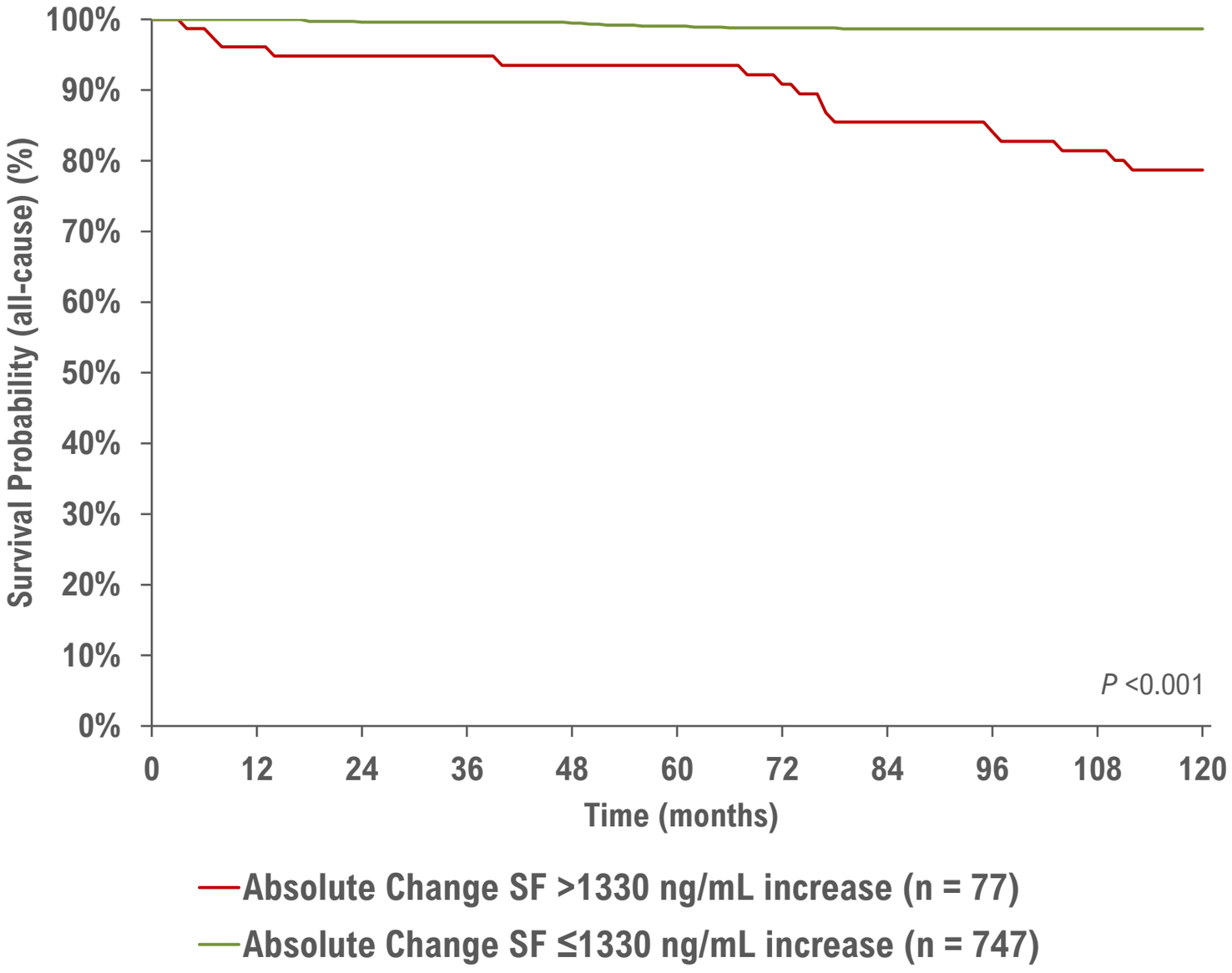

Highest Youden Index
27.2 ms
Sensitivity 71.2%
Specificity 82.4%
20 ms
Sensitivity 82%
Specificity 11.8%
10 ms
Sensitivity 95.7%
Specificity 5.9%
AUC: 0.675
P = 0.015
Sensitivity
1-Specificity
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Index cT2*

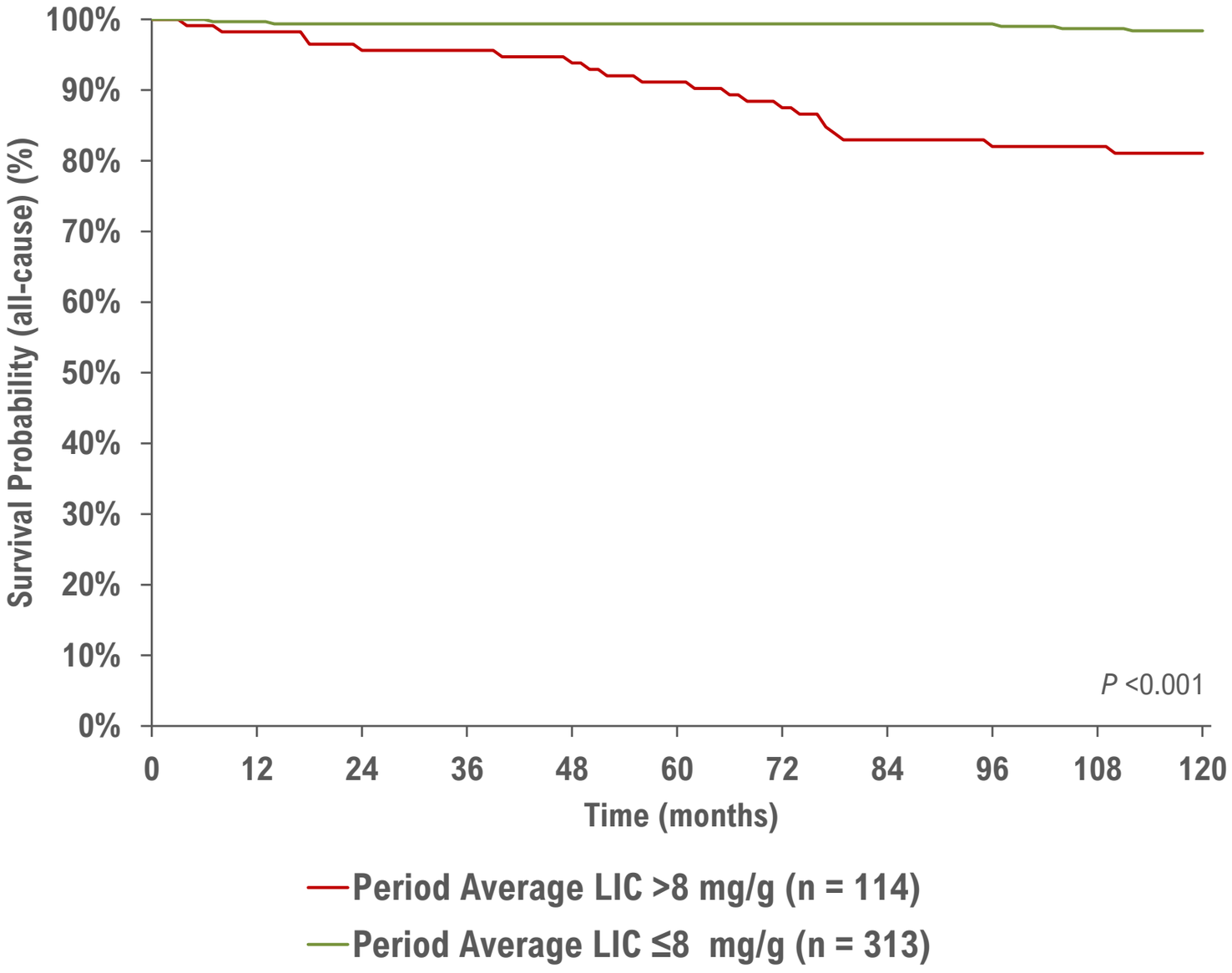
Figure 3


(A)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival Probability (all-cause) (%)
P <0.001
0
12
24
36
48
60
72
84
96
108
120
Time (months)
Period Average SF >2145 ng/mL (n = 233)
Period Average SF ≤2145 ng/mL (n = 659)

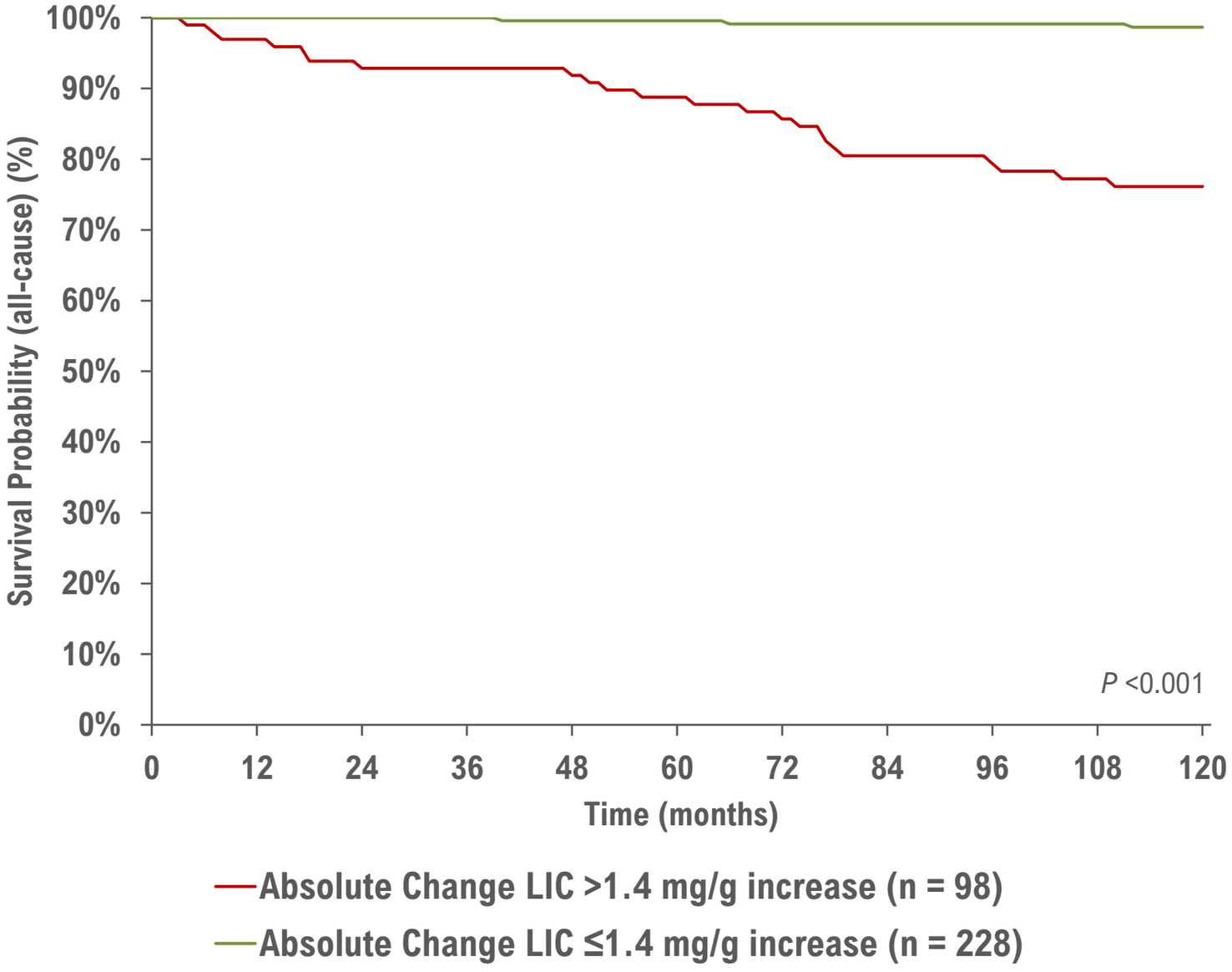
(B)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival Probability (all-cause) (%)
P <0.001
0
12
24
36
48
60
72
84
96
108
120
Time (months)
Absolute Change SF >1330 ng/mL increase (n = 77)
Absolute Change SF ≤1330 ng/mL increase (n = 747)

(C)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival Probability (all-cause) (%)
P <0.001
0
12
24
36
48
60
72
84
96
108
120
Time (months)
Period Average LIC >8 mg/g (n = 114)
Period Average LIC ≤8 mg/g (n = 313)

(D)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival Probability (all-cause) (%)
P <0.001
0
12
24
36
48
60
72
84
96
108
120
Time (months)
Absolute Change LIC >1.4 mg/g increase (n = 98)
Absolute Change LIC ≤1.4 mg/g increase (n = 228)

(E)

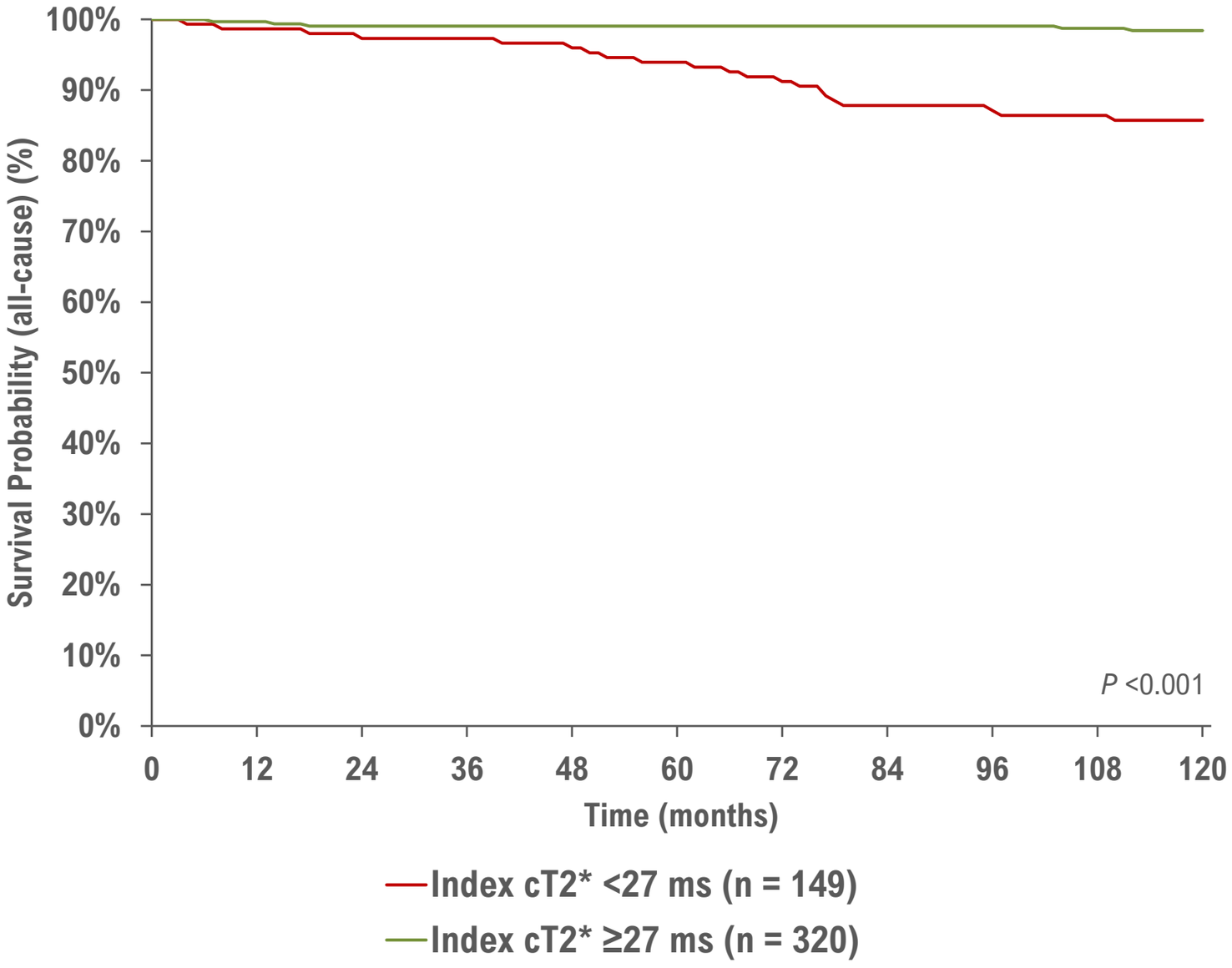
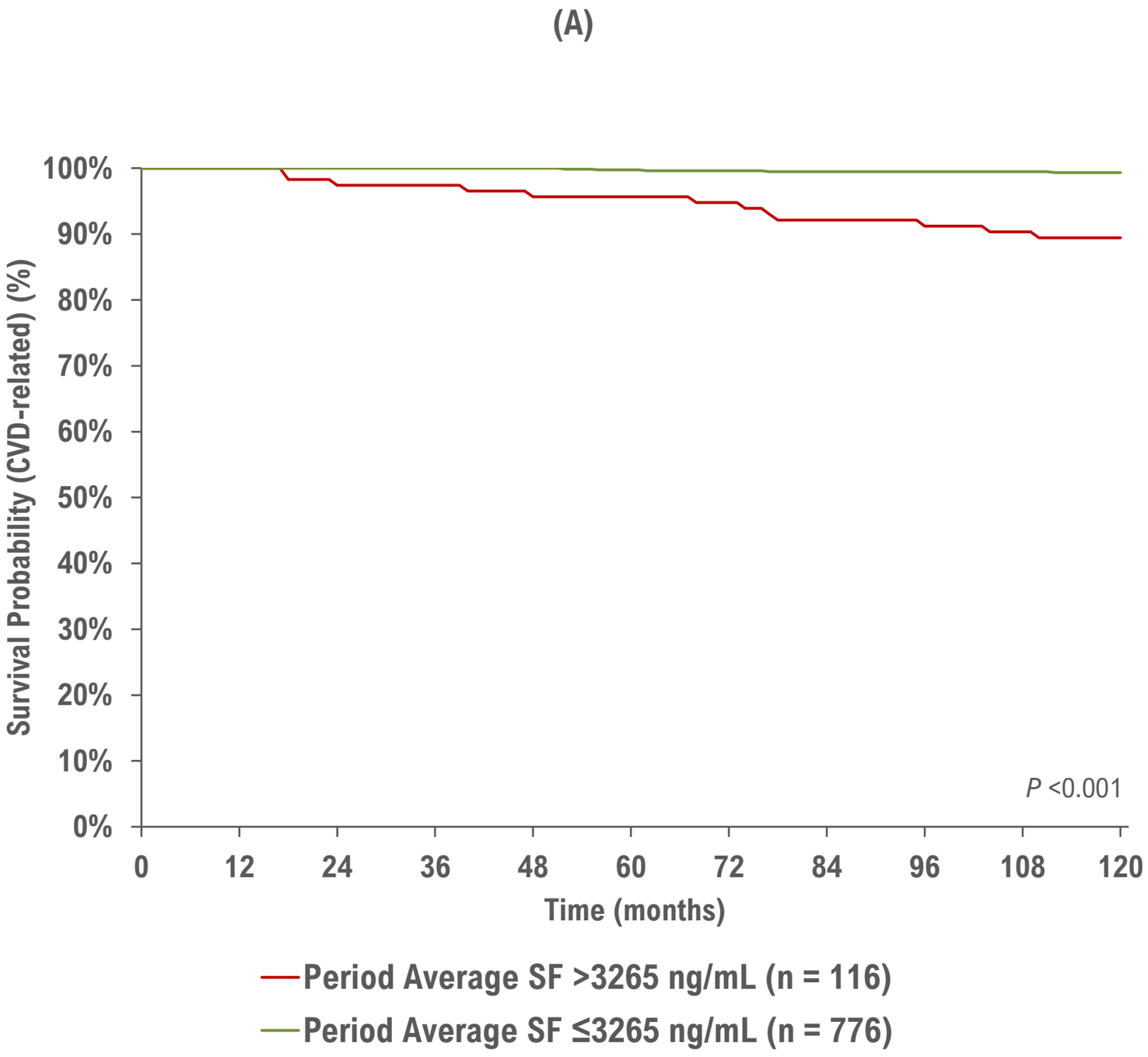
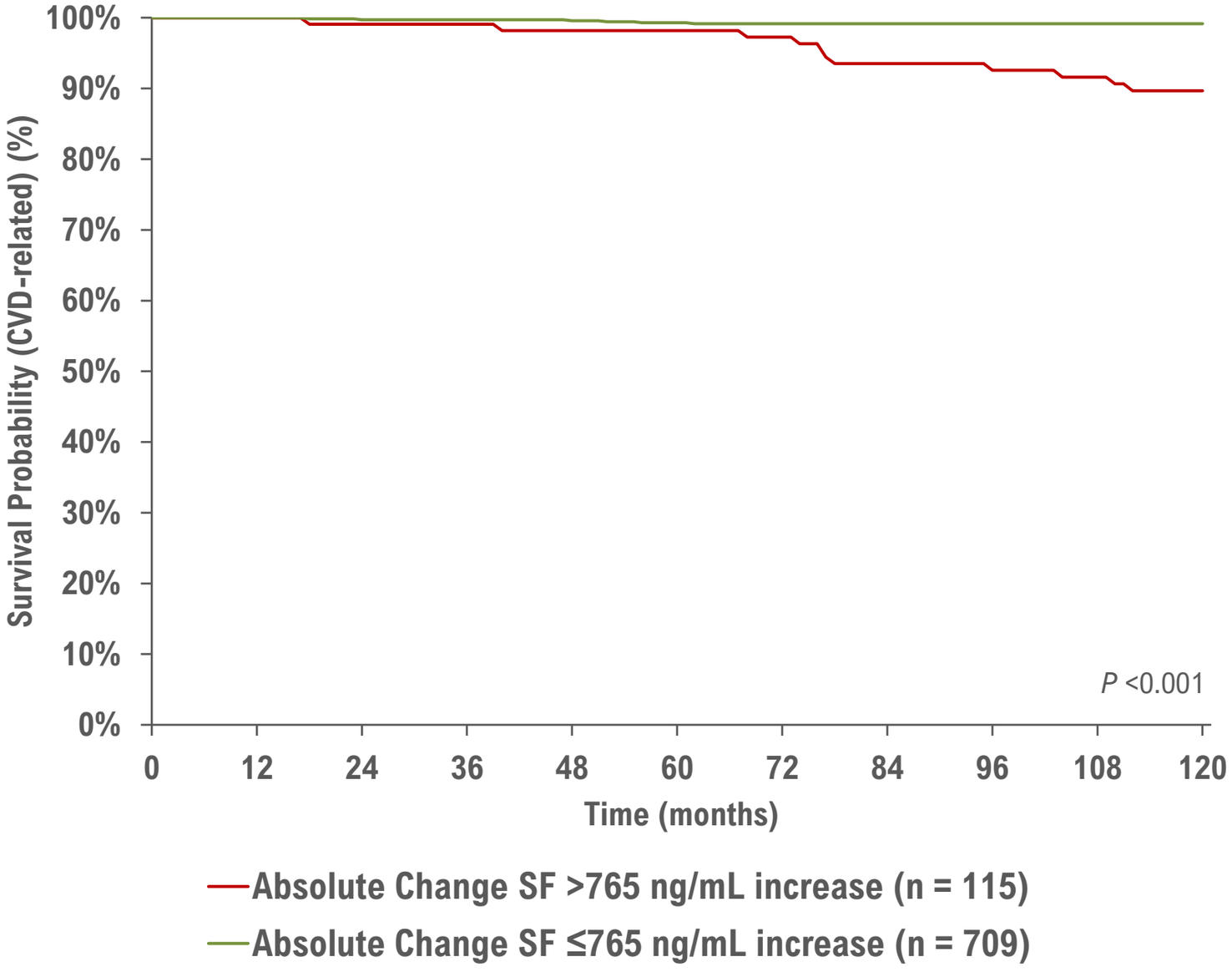

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival Probability (all-cause) (%)
P <0.001
0
12
24
36
48
60
72
84
96
108
120
Time (months)
Index cT2* <27 ms (n = 149)
Index cT2* ≥27 ms (n = 320)

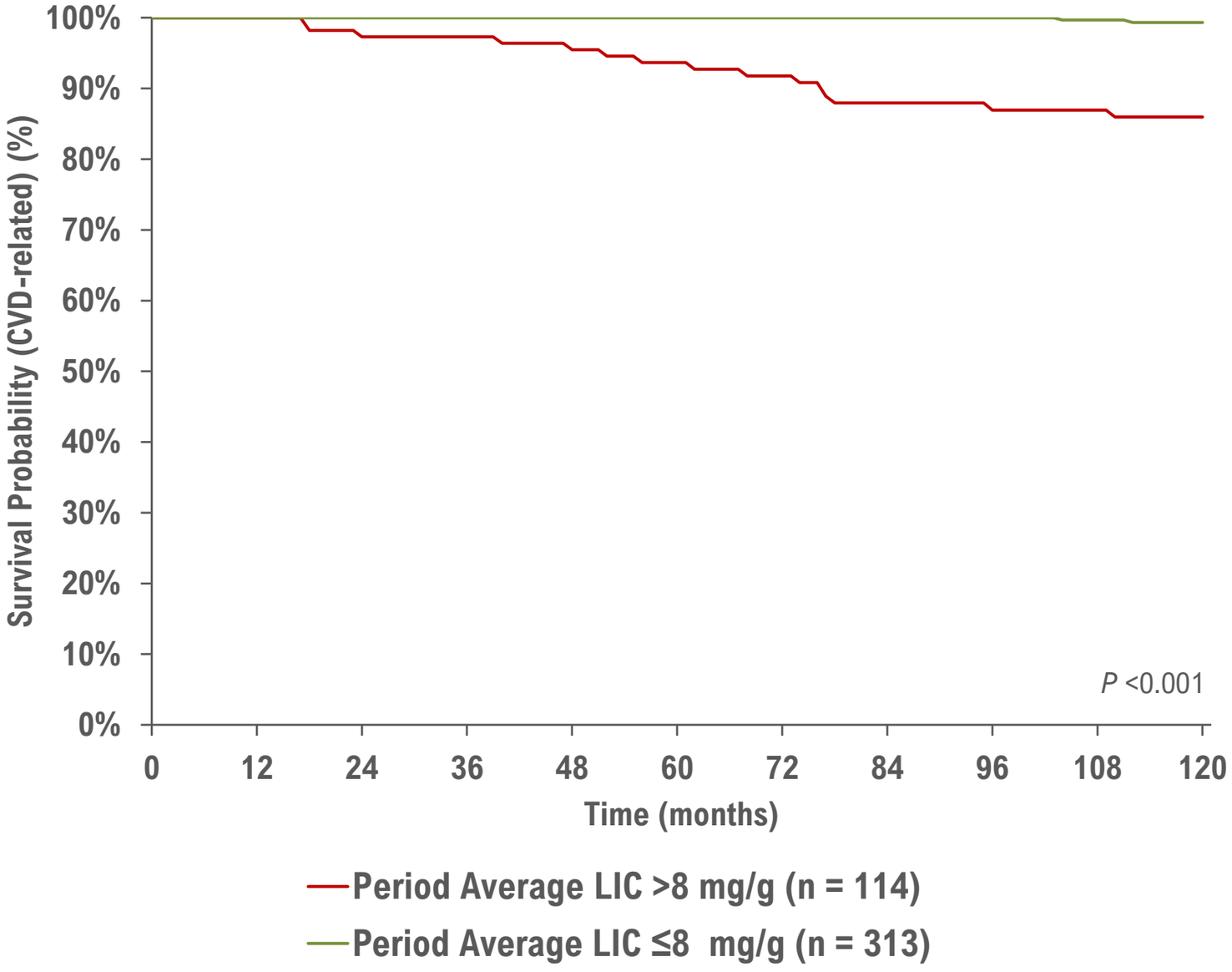
Figure 4


(A)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival Probability (CVD-related) (%)
P <0.001
0
12
24
36
48
60
72
84
96
108
120
Time (months)
Period Average SF >3265 ng/mL (n = 116)
Period Average SF ≤3265 ng/mL (n = 776)

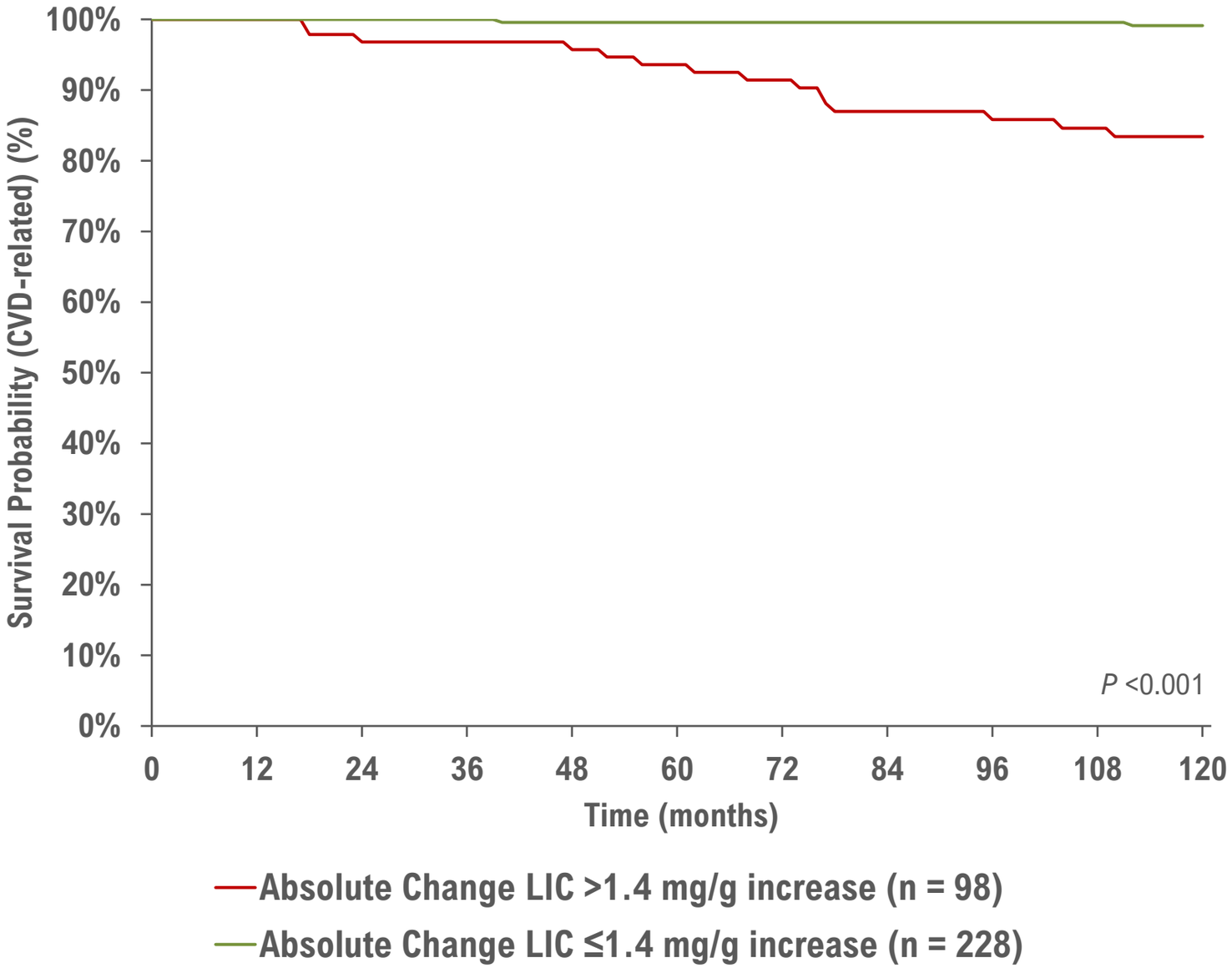
(B)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival Probability (CVD-related) (%)
P <0.001
0
12
24
36
48
60
72
84
96
108
120
Time (months)
Absolute Change SF >765 ng/mL increase (n = 115)
Absolute Change SF ≤765 ng/mL increase (n = 709)

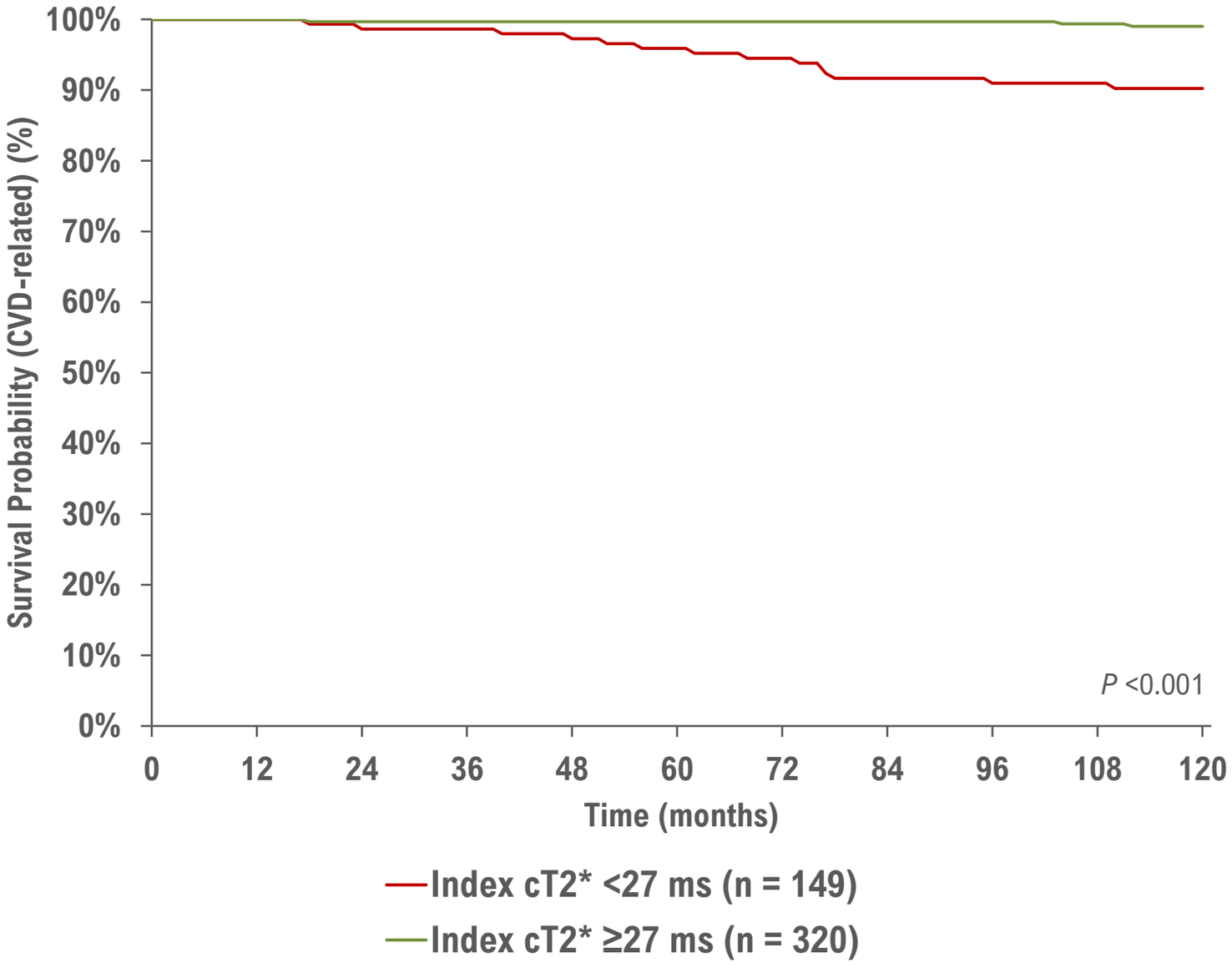
(C)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival Probability (CVD-related) (%)
P <0.001
0
12
24
36
48
60
72
84
96
108
120
Time (months)
Period Average LIC >8 mg/g (n = 114)
Period Average LIC ≤8 mg/g (n = 313)

(D)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival Probability (CVD-related) (%)
P <0.001
0
12
24
36
48
60
72
84
96
108
120
Time (months)
Absolute Change LIC >1.4 mg/g increase (n = 98)
Absolute Change LIC ≤1.4 mg/g increase (n = 228)

(E)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Survival Probability (CVD-related) (%)
P <0.001
0
12
24
36
48
60
72
84
96
108
120
Time (months)
Index cT2* <27 ms (n = 149)
Index cT2* ≥27 ms (n = 320)