

Liver enzymes are not directly involved in atrial fibrillation: a prospective cohort study

Running head: Atrial fibrillation and liver enzymes

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Abstract

Background Epidemiological evidence proposes the direct involvement of the liver enzymes in atrial fibrillation. These relationships are controversial and mechanistically unclear. As part of the British Regional Heart Study, we investigated whether change in liver enzymes over time associates with atrial fibrillation in men initially free of this heart condition.

Materials and Methods We prospectively investigated change (delta) in liver enzymes and new-onset atrial fibrillation in a representative sample of 1428 men aged 60-79 years.

Results Mean follow-up was 12.3 years, after which 108 new atrial fibrillation cases were identified. The liver enzymes did not differ at baseline or follow-up, except for gamma-glutamyl transferase which was higher at follow-up in men who developed atrial fibrillation compared to those who did not ($P<0.0001$). Change in GGT was greater in men who developed AF than those who did not (+6.12 vs. -2.60U/l, $P=0.036$). N-terminal pro-brain natriuretic peptide (baseline and follow-up, $P<0.0001$) and total bilirubin (follow-up only, $P<0.0001$) were also higher in men who developed atrial fibrillation while serum haemoglobin was similar at baseline and follow-up ($P\geq 0.74$). Atrial fibrillation was associated with change in gamma-glutamyl transferase (OR, 1.18; 95%CI, 1.01–1.37) after multiple adjustments and exclusions. However, after adjusting for baseline ($P=0.088$) or change ($P=0.40$) in N-terminal pro-brain natriuretic peptide, the association between atrial fibrillation and change in gamma-glutamyl transferase was lost.

Conclusion The direct relationship between atrial fibrillation and liver enzymes is absent and depends, at least in part, on the progression of heart failure as captured by N-terminal pro-brain natriuretic peptide.

Keywords: Liver enzymes, atrial fibrillation, heart failure

Introduction

The direct involvement of liver enzymes in cardiovascular disease, especially gamma-glutamyl transferase (GGT) [1], remains controversial [2,3]. Recent population-based evidence suggests that circulating liver enzymes also associates independently with atrial fibrillation (AF) [4-7]. The evidence on this finding is limited, inconsistent and mechanistically unclear. In the Framingham cohort, associations existed between AF risk and alanine transaminase (ALT) and aspartate transaminase (AST) [6], while in the Atherosclerosis Risk in Communities study, GGT but not ALT or AST predicted new-onset AF [7]. These studies [6,7] mostly accounted for baseline characteristics of participants initially free of AF, rendering it difficult to capture and account for the evolving pathophysiology. Heart failure contributes to atrial dysfunction and *vice versa* [8-11], but also increases central- and hepatic venous pressure leading to congestive hepatopathy, augmenting circulating GGT, bilirubin and alkaline phosphatase (ALP) levels [12-14]. However, the relationship between AF and liver enzymes could be more complex as the association also exists in the absence of clinically diagnosed heart failure [6]. Currently, no evidence is available on the change in liver enzymes over time and new-onset AF. With the incidence of AF projected to more than double by 2030 with significant economic implications [15], an understanding of the mechanisms at work has clinical importance. To investigate the relationship between new-onset AF and change in liver enzymes over time, we used prospective data from the British Regional Heart Study.

Materials and Methods

Reporting of this study conforms to STROBE statement [16].

Study population

The British Regional Heart Study is a prospective cohort study involving 7735 randomly selected men from 24 towns representing all major British regions (response rate, 78%) [17].

We followed the men since 1978–1980 when they were aged 40–59 years and re-examined them at ages 60–79 years (1998–2000) and 71–92 years (2010–2012). The first re-examination after 20 years included 4252 men (77% response rate) and the second after 32 years 1722 men (55% response rate). For the present investigation, we used the first re-examination as baseline (1998–2000) and the second (2010–2012) as the follow-up for outcome assessment. All participants gave informed written consent and could withdraw at any stage. The protocol complied with the Declaration of Helsinki of 1975 (as revised in 1983) and all relevant local research ethics committees provided ethical approval.

Participant examinations and exclusions

The re-examinations included completion of a questionnaire on lifestyle and medical history, a physical examination, a 12-lead electrocardiogram (ECG), and collection of fasting blood samples using the Sarstedt Monovette System for biochemical analysis.

Electrocardiograms were recorded using a Siemens Sicard 460 and transmitted to the University of Glasgow ECG core lab for analysis using the University of Glasgow ECG interpretation program [18] with an additional model for automated Minnesota Coding [19]. All reports were carefully reviewed and edited wherever necessary. Prevalent heart failure at baseline and follow-up was based on a doctor-confirmed diagnosis of heart failure from medical records and verified by review of available clinical information from primary and secondary care records.

Blood samples were frozen and stored at -20°C on the day of collection and transferred in batches for storage at -70°C until analysis, which was carried out after no more than one freeze-thaw cycle. Details of measurement and classification methods for smoking status, alcohol intake, blood pressure, and blood lipids in this cohort have previously been described [20–22]. C-reactive protein (CRP) was assayed by ultrasensitive nephelometry (Dade

Behring, Milton Keynes, United Kingdom) and plasma glucose by a glucose oxidase method using a Falcor-600 automated analyzer. Hepatic enzymes including GGT, AST, ALT and ALP, as well as serum albumin, total protein and total bilirubin, were measured using a Hitachi 747 automated analyzer. The normal laboratory range was 8 to 61 U/l for GGT, <37 U/l for AST, <41 U/l for ALT and 35-129 U/l for ALP. The measurement of baseline and follow-up liver enzymes followed the same enzymatic calorimetric assay principle [23-25]. N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was measured using the Elecsys 2010 electrochemiluminescence method (Roche Diagnostics, Burgess Hill, UK).

Statistical analysis

For database management and statistical analysis, we used SAS software, version 9.4 (SAS Institute Inc., Cary, NC). The distributions of GGT, ALT, AST, ALP, blood glucose, NT-proBNP, and C-reactive protein were normalized by logarithmic transformation. We compared means and proportions by the standard normal z-test and the χ^2 statistic, while longitudinal changes in means and proportions were assessed by the PROC MIXED procedure and McNemar's test, respectively. Statistical significance was set at a level of 0.05 on 2-sided tests. We analyzed associations with AF using logistic regression analysis to calculate odds ratios (OR) standardized to 1 standard deviation (SD) and 95% confidence intervals (CI), while allowing for covariates and potential confounders. We applied the forward stepwise procedure with *P* values for explanatory variables to enter and stay in the model set at 0.05. Covariates included in the model were respective baseline liver enzymes, baseline age, body mass index, systolic blood pressure, total cholesterol, serum glucose, physical activity, smoking, drinking, use of anti-hypertensive medication, and follow-up time. Physical activity used in the regression models was classified into inactive, occasional, light, moderate and vigorous; smoking into never, former and current smokers; and drinking into none, occasional, light, moderate, and heavy drinkers. We performed sensitivity analysis

by additionally, but separately, adjusting for baseline C-reactive protein, ALT, AST, ALT/AST ratio, bilirubin, NT-proBNP and their respective change (Δ). We also repeated our analysis after excluding participants with GGT outside its reference range (>61 U/l), and those who had a previous myocardial infarction or heart failure at baseline or follow-up. Lastly, since NT-proBNP levels above 900 pg/ml in people aged 50-75 years is indicative of heart failure [26,27], we repeated our analysis in participants who had NT-proBNP levels below 900 pg/ml at both baseline and follow-up.

Results

Participant characteristics

We based our study on 1722 men who took part in both the first and second follow-up examinations. We excluded 256 participants without blood measurements at baseline or follow-up, 20 with no electrocardiogram measurements at follow-up, and 18 with AF at baseline, leaving 1428 participants for inclusion in the analysis. After a mean follow-up of 12.3 years (5th to 95th percentile interval, 12.1–12.9 years), 108 new cases of AF were identified. The baseline characteristics of participants who developed or did not develop AF are presented in Table 1 and follow-up characteristics in Supplementary Table 1. Circulating levels of AST, ALT and ALP at baseline ($P \geq 0.083$) and follow-up ($P \geq 0.054$) did not differ between men who did or did not develop AF (Supplementary Figure 1). GGT was also similar in both groups at baseline ($P=0.93$), however GGT at follow-up ($P < 0.0001$, Figure 1A) and change (Δ) in GGT from baseline to follow-up (+6.12 vs. -2.60 U/l, $P=0.036$) were higher in the men with AF at follow-up. In addition, both NT-proBNP ($P < 0.0001$, Figure 1B) and total bilirubin ($P < 0.0001$, Figure 1C) at follow-up were higher in the AF group and increased more markedly from baseline to follow-up (both $P < 0.0001$), while mean haemoglobin did not differ between the groups at both baseline ($P=0.82$) and follow-up ($P=0.74$, Figure 1D).

Self-reported alcohol intake did not differ between the groups at baseline ($P=0.55$) or follow-up ($P=0.62$) and intake declined from baseline to follow-up in both the AF ($P=0.028$) and non-AF groups ($P<0.0001$; data not shown). Similarly, the AST/ALT ratio as a measure of alcoholic liver damage did not differ between the groups at baseline ($P=0.18$) or follow-up ($P=0.21$) and decreased in both groups from baseline to follow-up (with AF, $P<0.0001$; without AF, $P<0.0001$; data not shown).

Univariate and multivariate analyses

In univariate analysis in the total group (Supplementary Table 2), Δ NT-proBNP correlated with Δ GGT ($r=0.14$, $P<0.0001$), Δ total bilirubin ($r=0.13$, $P<0.0001$) and Δ ALP ($r=0.10$, $P=0.0001$), but not with Δ ALT ($r=0.01$, $P=0.61$) or Δ AST ($r=0.04$, $P=0.17$).

In univariate logistic regression analysis (Table 2), none of the liver enzymes at baseline, or the change in AST, ALT or ALP was associated with AF. However a 1-SD increase in Δ GGT was associated with a 21.7% (95% CI, 5.2–40.6%; $P=0.008$) increased odds of developing AF. After adjusting for baseline GGT and other covariates and potential confounders (Table 2), the above association between AF and Δ GGT remained significant (OR, 1.18; 95% CI, 1.01–1.37; $P=0.032$). Associations with AST, ALT and ALP ($P\geq 0.14$) or their change ($P\geq 0.73$) remained non-significant.

Sensitivity analysis

Additional adjustments (Table 3) for baseline C-reactive protein as a marker of inflammation ($P=0.018$), ALT ($P=0.032$), AST ($P=0.031$), the AST/ALT ratio to account for alcoholic liver disease ($P=0.032$), ALP ($P=0.032$) as well as their respective change from baseline to follow-up ($P\leq 0.032$), did not appreciably affect our findings. Our results also remained significant after excluding those with GGT levels outside the reference range (>61 U/l, $P=0.0004$), those with a previous myocardial infarction ($P=0.032$), and even after excluding 74 participants

with prevalent and incident heart failure ($P=0.015$). However, when accounting for baseline ($P=0.088$) or change in NT-proBNP ($P=0.40$) as a clinical measure of heart failure and left ventricular systolic- and diastolic dysfunction, the relationship between AF risk and Δ GGT was no longer present. When adjusting for Δ total bilirubin, statistical significance was also lost ($P=0.13$). Entering both Δ NT-proBNP and Δ total bilirubin in the model confirmed the absence of an independent relationship between AF and Δ GGT ($P=0.62$). Lastly, by repeating our analysis in participants with NT-proBNP levels below 900 pg/ml at both baseline and follow-up, the relationship between AF and Δ GGT was absent (OR, 0.86; 95% CI, 0.55–1.33; $P=0.50$).

Discussion

We investigated in a representative sample of older British men initially free of AF, whether change in liver enzymes over 12.3 years were associated with new-onset AF. While no evidence existed for baseline liver enzymes, initial findings indicated a seemingly robust association between AF and change in GGT after multiple adjustments and exclusions including participants with prevalent and incident heart failure. However, this relationship was dependent on the progression of heart failure as represented by change in NT-proBNP.

The concept of cardiovascular events, and more recently AF relating to liver enzymes has become a matter of debate and remains mechanistically unclear [1-7]. Sinner et al. [6] followed 3,744 participants (mean age, 65 years) free of AF and heart failure at baseline and found a respective 19% and 12% increased risk of developing new-onset AF per one standard deviation increase in baseline ALT and AST. The authors confirmed these results after multiple adjustments including interim heart failure as a dichotomous variable, but not for AST after adjusting for C-reactive protein as a marker of systemic inflammation [6]. They provided no descriptive data on interim heart failure and incidence was likely higher in the AF group that was older by a mean of 6.8 years [6]. In our study population of similar age,

no such evidence existed for the liver transaminases, even in univariate analysis and including participants with incident heart failure. Alonso et al. provided the first prospective evidence for linking new-onset AF with GGT, but were unable to confirm a relationship with ALT or AST in 9,333 men and women aged 53-75 years [7]. This study involved extreme-case statistical analysis by quintiles of baseline GGT. The authors did include baseline NT-proBNP and C-reactive protein in the quintile analysis, and confirmed the results after excluding participants with prevalent heart failure at baseline. However, there were no indication whether NT-proBNP was included in the continuous analysis when mentioning a 20% increased risk of AF per doubling of GGT [7].

The above studies were limited in their ability to capture the pathophysiological progression of disease by accounting only for baseline covariates and potential confounders. We were able to show in those who developed AF, that a greater increase in GGT was paralleled by NT-proBNP and bilirubin as respective sensitive markers of progressive heart failure [28] and congestive hepatopathy [29]. In heart failure patients, elevated liver function tests are a consequence of either backward failure, increasing central venous pressure; or forward failure with attenuated cardiac output and consequent liver perfusion [12-14,30]. Decreased liver perfusion elevates the transaminases due to hepatocellular damage [14,29,31], while increased central venous pressure transmits to the hepatic veins and results in elevations in GGT, bilirubin and ALP [32,33]. In our study, GGT and bilirubin, which did not differ at baseline between the groups, were higher in those with AF at follow-up, while ALP was borderline higher in the AF group at baseline and follow-up. ALP is normally elevated in more extreme cholestatic conditions [34] and it is reasonable to assume that the effects of hepatic congestion would firstly be seen for GGT and bilirubin before the impediment to bile flow. Van Deursen et al. [14] reported that abnormalities in all liver function tests were associated with invasively-measured central venous pressure.

Importantly, higher central venous pressure was predominantly related to GGT and bilirubin [14]. From our results, it seems more likely that the backward failure–central venous pressure mechanism could explain our findings, especially since the liver transaminases did not differ between those with and without AF. In addition, the positive univariate associations in the total group of NT-proBNP with Δ GGT, Δ ALP and Δ total bilirubin, but not with Δ ALT or Δ AST, at least in part, supports this pathway.

The link between AF and Δ GGT was dependent on Δ NT-proBNP, even in the absence of diagnosed heart failure, therefore capturing the confounding effect of heart failure at subclinical level. Supporting this was the absence of the AF- Δ GGT link when repeating our analysis in participants who consistently had NT-proBNP levels below 900 ng/ml (above which is indicative of heart failure for people aged 50-75 years) [26,27]. Heart failure and AF often coincide with causal pathways in both directions [8-11]. This is at least partly supported by our results confirming the known [35,36] relationship between NT-proBNP and AF (OR, 1.53; 95% CI, 1.24-1.88; $P < 0.0001$) in fully adjusted models which also included Δ GGT and Δ total bilirubin. On balance, the link between the failing heart and the liver described above also seems evident with the disappearance of the association between AF and GGT after adjusting for Δ total bilirubin as both GGT and bilirubin are elevated in congestive hepatopathy [12-14]. Taken together, no direct relationship seems apparent between AF and the liver enzymes, which, even in the absence of prevalent and incident heart failure, seems due to progression of heart failure leading to both AF and hepatic congestion.

The current study must be interpreted within the context of its potential limitations. Although our cohort is socially representative of older white British men, our results may not be generalizable to younger populations, women and other ethnicities. We investigated overall AF only and therefore cannot comment on the associations of the liver enzymes and AF type (i.e., valvular, paroxysmal, persistent, or permanent). The increase in total bilirubin

from baseline to follow-up could also be due to augmented bilirubin production (increased haemolysis) [37], however this seems unlikely since haemoglobin levels did not differ between those with and without AF at both baseline and follow-up. Although the same enzymatic calorimetric assay principle was used to determine the liver enzyme levels at baseline and follow-up, the storage time difference of samples could have affected the results. However, a similar trend in GGT levels could be seen in participants who passed away between after 1998-2000 (not included in the present study) with GGT being higher in those with AF compared to those without (Supplementary Figure 2).

In conclusion, the direct relationship between atrial fibrillation and liver enzymes is absent and depends, at least in part, on the progression of heart failure as captured by NT-proBNP.

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Conflict of Interest

None declared

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Legend

Figure 1. Comparison of gamma-glutamyl transferase, N-terminal prohormone of brain natriuretic peptide (NT-proBNP), total bilirubin and haemoglobin at baseline and follow-up between those with and without atrial fibrillation at follow-up. AF, atrial fibrillation.

Table 1 Baseline characteristics of men with and without atrial fibrillation (AF) at follow-up

	Without AF (n=1320)	With AF (n=108)	P
Age (years)	66.1 ± 4.6	67.9 ± 5.2	0.001
Body mass index (kg/m ²)	26.6 ± 3.3	27.2 ± 3.3	0.10
Systolic blood pressure (mmHg)	145.5 ± 21.8	149.8 ± 24.7	0.12
Diastolic blood pressure (mmHg)	85.0 ± 10.2	85.3 ± 10.1	0.21
Gamma-glutamyl transferase (U/l)	27.8 (13.0-80.0)	27.9 (13.0-94.0)	0.93
Aspartate transaminase (U/l)	23.5 (16.0-35.0)	22.9 (16.0-38.0)	0.36
Alanine transaminase (U/l)	16.7 (9.0-33.5)	15.6 (8.0-34.0)	0.12
Alkaline phosphatase (U/l)	78.3 (51.0-119.5)	81.4 (56.0-117.0)	0.083
ALT:AST	1.48 ± 0.50	1.54 ± 0.54	0.18
Total bilirubin (µmol/l)	9.11 (4.00-17.00)	9.70 (5.00-18.00)	0.22
Total protein (g/l)	76.6 ± 4.3	76.4 ± 4.5	0.53
Serum albumin (g/l)	44.5 ± 2.6	43.7 ± 2.7	0.004
Blood glucose (mmol/l)	5.74 (4.70-7.40)	5.73 (4.80-7.10)	0.60
Total cholesterol (mmol/l)	6.09 ± 1.06	6.17 ± 1.09	0.46
C-reactive protein (mg/l)	1.31 (0.23-8.10)	1.73 (0.33-12.2)	0.013
NT-proBNP (pg/ml)	63.3 (12.0-344.0)	116.2 (26.0-699.0)	<0.0001
Current smoking n, (%)	97 (7.4)	7 (6.5)	0.70
Drinking n, (%)			0.55
None	91 (7.0)	6 (5.7)	
Occasional	334 (25.5)	25 (23.6)	
Light	625 (47.8)	51 (48.1)	
Moderate	230 (17.6)	19 (17.9)	
Heavy	26 (2.0)	5 (4.7)	
Physically inactive n, (%)	66 (5.0)	6 (5.6)	0.80
Previous myocardial infarction] n, (%)	117 (8.9)	10 (9.3)	0.70
Heart failure n, (%)	11 (0.8)	1 (0.9)	0.86
Anti-hypertensive meds n, (%)	305 (23.4)	33 (30.6)	0.092

Values are number of subjects (%), arithmetic mean ± SD, or geometric mean (5th to 95th percentile interval). ALT, alanine transaminase; AST, aspartate transaminase; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

Table 2 Associations between atrial fibrillation and baseline liver enzyme concentrations and changes in liver enzyme concentrations between baseline and follow-up

Main independent variables	Atrial fibrillation (Odds Ratio, 95%CI)		
	Unadjusted	+Age	Primary model
Baseline γ -glutamyl transferase (U/l)	1.01 (0.83 – 1.23)	1.04 (0.85 – 1.26)	0.95 (0.77 – 1.18)
Δ γ -glutamyl transferase (U/l)	1.22 (1.05 – 1.41)†	1.21 (1.04 – 1.40)*	1.18 (1.01 – 1.37)*
Baseline aspartate transaminase (U/l)	0.89 (0.72 – 1.09)	0.92 (0.75 – 1.12)	0.89 (0.72 – 1.10)
Δ Aspartate transaminase (U/l) (U/l)	1.09 (0.87 – 1.37)	1.08 (0.86 – 1.35)	0.97 (0.74 – 1.26)
Baseline alanine transaminase (U/l)	0.84 (0.69 – 1.03)	0.91 (0.74 – 1.12)	0.85 (0.69 – 1.06)
Δ Alanine transaminase (U/l)	1.05 (0.85 – 1.28)	1.05 (0.85 – 1.30)	1.00 (0.77 – 1.29)
Baseline alkaline phosphatase (U/l)	0.57 (0.16 – 2.06)	1.13 (0.93 – 1.36)	1.11 (0.92 – 1.35)
Δ Alkaline phosphatase (U/l)	1.06 (0.88 – 1.26)	1.01 (0.85 – 1.20)	1.00 (0.84 – 1.20)

Primary model included baseline gamma-glutamyl transferase, age, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, serum glucose, physical activity, smoking, drinking, use of anti-hypertensive medication, and follow-up time. *, $P < 0.05$; †, $P < 0.01$. Δ denotes delta or change from baseline to follow-up.

Table 3 Sensitivity analysis for the association between atrial fibrillation and gamma-glutamyl transferase

Atrial fibrillation		
Δ Gamma-glutamyl transferase (U/l)	OR (95%CI)	P
Primary model	1.18 (1.01 – 1.37)	0.032
+ baseline CRP	1.20 (1.03 – 1.40)	0.018
+ ΔCRP	1.20 (1.03 – 1.39)	0.020
+ baseline ALT	1.18 (1.01 – 1.37)	0.032
+ ΔALT	1.18 (1.01 – 1.37)	0.032
+ baseline AST	1.18 (1.01 – 1.37)	0.032
+ ΔAST	1.18 (1.02 – 1.38)	0.031
+baseline ALP	1.18 (1.01 – 1.37)	0.032
+ ΔALP	1.18 (1.01 – 1.37)	0.032
+ AST/ALT ratio	1.18 (1.01 – 1.37)	0.032
+ ΔAST/ALT ratio	1.18 (1.02 – 1.38)	0.031
+ exclude GGT >61 U/l	1.44 (1.18 – 1.76)	0.0004
+ exclude previous MI	1.18 (1.01 – 1.37)	0.032
+ exclude HF	1.21 (1.04 – 1.42)	0.015
+ Baseline bilirubin	1.18 (1.01 – 1.37)	0.032
+ Δbilirubin	1.13 (0.97 – 1.32)	0.13
+ Baseline NT-proBNP	1.15 (0.98 – 1.34)	0.088
+ ΔNT-proBNP	1.08 (0.91 – 1.28)	0.40
+ ΔNT-proBNP + Δ bilirubin	1.05 (0.88 – 1.25)	0.62

The primary model included baseline gamma-glutamyl transferase, age, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, serum glucose, physical activity, smoking, drinking, use of anti-hypertensive medication, and follow-up time. ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; HF, heart failure; MI, myocardial infarction; NT-proBNP, N-terminal prohormone of brain natriuretic peptide. Δ denotes delta or change from baseline to follow-up.

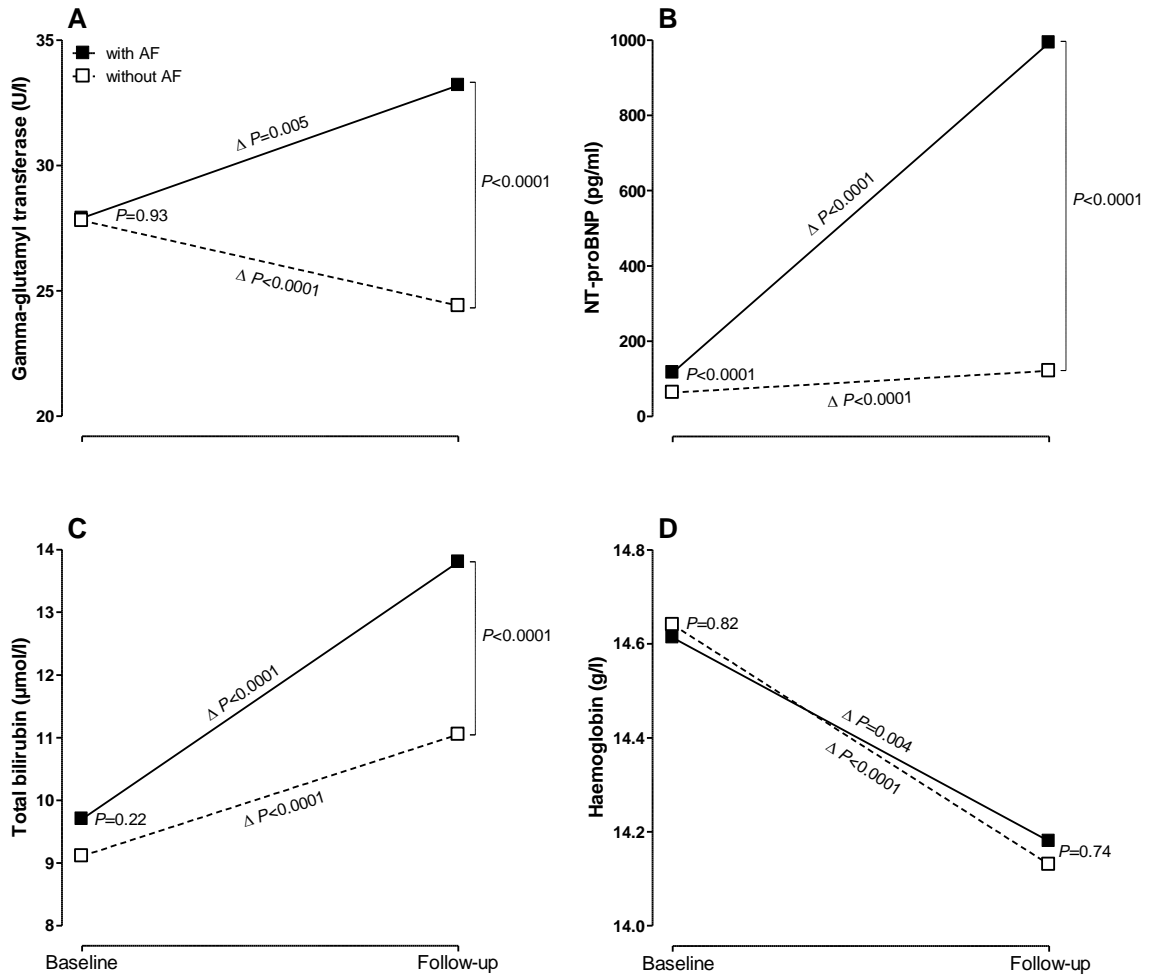


Figure 1. Comparison of gamma-glutamyl transferase, N-terminal prohormone of brain natriuretic peptide (NT-proBNP), total bilirubin and haemoglobin at baseline and follow-up between those with and without atrial fibrillation at follow-up. AF, atrial fibrillation.