Association of serum autotaxin levels with liver fibrosis in patients pretreatment and posttreatment with chronic hepatitis C

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Title: Association of Serum Autotaxin Levels with Liver Fibrosis in Patients Pre- and Post-Treatment with Chronic Hepatitis C

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Abstract

Background & Aim: The evaluation of liver fibrosis in patients with chronic hepatitis C virus (HCV) infection is important as it is a risk factor for hepatocellular carcinoma. In the recent years, autotaxin (ATX) has been established as a new non-invasive biomarker to predict liver fibrosis. However, antiviral treatment has been reported to decrease serum ATX, but it is unclear whether post-treatment ATX levels reflect liver fibrosis. In the present study, we analyzed the correlation between ATX and liver fibrosis in pre- and post-treatment patients with HCV infection.

Methods: We used 199 samples from 136 patients with HCV infection who had undergone hepatic biopsy before and/or after antiviral treatment at Osaka City University Hospital. Post-treatment patients included 38 interferon (IFN)-treated patients and 80 IFN-free direct-acting antiviral-treated patients; all patients achieved a sustained virological response (SVR). Serum ATX levels were determined by enzyme immunoassay with an AIA-2000 analyzer.

Results: Serum ATX levels were largely correlated with liver fibrosis stage in patients with HCV infection before and after antiviral treatment. The measured values decreased even in similar liver fibrosis stages after treatment. The area under the receiver operating characteristic curve for the ability of ATX to diagnose above F2 stage before treatment was 0.81 (both male and female) and that after achieving SVR was 0.71 (male) and 0.72 (female).

Conclusions: Serum ATX levels were correlated with histological liver fibrosis stage after achieving SVR. However, we should establish separate cutoff values before and after antiviral therapy.

Keywords: Autotaxin, Chronic hepatitis C virus, Liver fibrosis stage, Serum liver fibrosis marker, Sustained virological response

Introduction

Patients infected with hepatitis C virus (HCV) develop chronic hepatitis and 20-30% of them progress to liver cirrhosis and/or hepatocellular carcinoma (HCC) [1]. In the recent years, most patients could achieve sustained virological response (SVR) by interferon (IFN)-free direct-acting antiviral agent (DAA) therapy [2-4]. However, HCC develops in patients with liver fibrosis regardless of achieving SVR. Therefore, the evaluation of liver fibrosis stage is needed even after antiviral treatment. Hepatic biopsy is the gold standard by which to evaluate liver fibrosis, but it is invasive [5]. In addition, it has problems of sampling error and inter-observer disparity. Therefore, serum liver fibrosis markers are being paid more attention as non-invasive biomarkers. Platelet counts are known to associate with liver fibrosis stage. The FIB-4 index and aspartate aminotransferase (AST)-to-platelet ratio (APRI) are markers that can be calculated by basic laboratory data [6,7]. In contrast, these fibrosis markers are influenced by various factors, as these are calculated by age, platelet counts, AST, and alanine aminotransferase (ALT).

Autotaxin (ATX) was discovered as an autocrine motility-stimulating protein in the conditioned medium from A2058 human melanoma cell cultures [8]. ATX has lysophospholipase D activity to generate lysophosphatidic acid (LPA) from lysophospholipids in the blood [9]. LPA is involved in such physiological roles as cell migration, cell proliferation, neurogenesis, and angiogenesis [10]. LPA also stimulates the proliferation and contractility of hepatic stellate cells. Recent studies have revealed that serum ATX levels correlate with liver fibrosis stage in patients with HCV [11,12], chronic hepatitis B virus (HBV) [13], non-alcoholic fatty liver disease (NAFLD) [14], and primary biliary cholangitis (PBC) [15,16]. Because ATX is metabolized by liver sinusoidal endothelial cells, reduced metabolism by liver fibrosis and/or damage leads to an increase in serum ATX [17]. A sex difference in ATX has been

reported, where the reference value of females is higher than that of males [18]. In Japan, ATX has been used in clinical practice since 2018, and the cutoff values for predicting above the F2 stage are set at 0.91 mg/L (male) and 1.27 mg/L (female), and in predicting liver cirrhosis at 1.69 mg/L (male) and 2.12 mg/L (female). On the other hand, IFN-free DAA therapy has been reported to decrease serum ATX levels [19-21]. There is no data on the relationship between post-treatment histological liver fibrosis stage and ATX, and it is unclear whether serum ATX levels are useful in assessing liver fibrosis after achieving SVR.

In the present study, we revealed an association of serum ATX levels with liver fibrosis stage in patients with HCV before and after antiviral treatment.

Methods

Subjects

The classification of patients in this study is shown in Figure 1. We used 199 samples from 136 patients with HCV infection who had undergone hepatic biopsy before and/or after antiviral therapy at Osaka City University Hospital between 1999 and 2019. We diagnosed chronic hepatitis C based on the presence of serum HCV antibody and detectable HCV RNA by real-time PCR. There were 81 samples collected before antiviral treatment and 118 samples collected after achieving SVR as a result of antiviral therapy. Paired biopsies before and after antiviral therapy were performed in 63 patients. Serum samples were collected from these 63 patients both before and after treatment. The therapy regimens of patients collected after treatment were as follows: IFN-based therapy (n = 38), sofosbuvir + ledipasvir (n = 33), sofosbuvir + ribavirin (n = 21), asunaprevir + daclatasvir (n = 19), elbasvir + grazoprevir (n = 5), and ombitasvir + paritaprevir + ritonavir (n = 2). The median period from the end of treatment to sera collection was 15 months. Blood samples

were obtained within six months before or after hepatic biopsy. This study was conducted according to the principals of the Declaration of Helsinki and was approved by the Ethics Committee of the Osaka City University Graduate School of Medicine (approval number: 4097).

Measurement of serum ATX levels

Serum samples were stored at −80℃ until testing. Before measuring ATX, serum samples were thawed at room temperature and centrifuged at 3,500 g for 5 min. Serum ATX levels were measured by a two-site enzyme immunoassay with an AIA-2000 analyzer (Tosoh Co.; Tokyo, Japan).

Laboratory data

Platelet counts and routine biochemical tests such as AST and ALT were analyzed by standard procedures. Laboratory data were obtained by medical records. We used laboratory data within seven days before and after taking the blood. The FIB-4 index and APRI were calculated according to the published formulae (FIB-4 index, (age [years] × AST [U/L]) / (platelet count [10^9/L] × ALT [U/L]$^{1/2}$; APRI, (AST [U/L] / 40 [U/L]) × (100 / platelet count [10^9/L])) [6,7].

Hepatic biopsy evaluation

Hepatic biopsy was performed with a 16- or 18-gauge Tru-Cut needle (Merit Medical; South Jordan, UT) under ultrasound guidance. We evaluated hepatic fibrosis stage according to the New Inuyama Classification [22]. The stage of fibrosis was classified from F0 to F4 as follows: F0 = no fibrosis, F1 = portal expansion of fibrosis, F2 = bridging fibrosis (portal-portal or portal-central linkage), F3 = bridging fibrosis with lobular distortion (disorganization), and F4 = cirrhosis.

Statistical analysis

All statistical analyses and data visualizations were performed by R software (version 3.5.3; R Foundation for Statistical Computing, Vienna, Austria). Comparisons of serum ATX levels for each fibrosis stage were performed by the Steel–Dwass test. Comparisons of serum ATX levels before and after antiviral therapy were analyzed by the Wilcoxon signed-rank test. The predictive capabilities of liver fibrosis stage were analyzed by the area under the receiver operating characteristic (ROC) curve (AUC). Cutoff values were identified by the Youden index. A *p*-value of less than 0.05 was considered statistically significant.

Results

Serum ATX levels and other fibrosis markers pre- and post-treatment

The clinical characteristics of patients with HCV before and after antiviral therapy are summarized in Table 1. First, we revealed that serum ATX levels correlated with liver fibrosis stage in patients with HCV before treatment (Figure 2A). Median values of ATX in patients with F1, F2, F3, and F4 stage fibrosis were 1.05, 1.23, 1.71, and 2.74 mg/L (male) and 1.11, 1.86, 1.44, and 2.41 mg/L (female), respectively (Figures 2B, C). Platelet counts, the FIB-4 index, and APRI were also correlated with liver fibrosis (Supplemental Figure 1). Second, we analyzed post-treatment HCV patients. We also revealed that serum ATX levels reflected liver fibrosis stage even after antiviral therapy (Figure 3A). There was no significant difference between male patients of each fibrosis stage, but there was a correlation (Figure 3B). Median ATX values in patients with F1, F2, F3, and F4 stage fibrosis were 0.80, 0.95, 1.02, and 1.22 mg/L (male), and 1.21, 1.56, 1.39, and 1.83 mg/L (female), respectively (Figures 3B, C). Other liver fibrosis markers also similarly correlated with liver

fibrosis stage (Supplemental Figure 2).

Change in the serum ATX levels between pre- and post-treatment

We selected the 63 patients who had undergone hepatic biopsy both before and after antiviral treatment and analyzed the change in serum ATX levels among therapy. Serum ATX levels after antiviral treatment decreased than that before treatment (Figure 4A). Next, we divided patients into 3 groups according to the alteration of liver fibrosis stage as follows: improved (n = 17), sustained (n = 31), and exacerbated groups (n = 15). After achieving SVR, serum ATX levels decreased in patients whose liver fibrosis stage improved (median value; 1.39 mg/L vs. 1.09 mg/L, Figure 4B) and was sustained (1.24 mg/L vs. 1.16 mg/L, Figure 4C). Despite worsening liver fibrosis, serum ATX levels tended to decrease, but without statistical significance (1.37 mg/L vs. 1.31 mg/L, Figure 4D). Also, we analyzed whether IFN-based therapy and IFN-free DAA therapy differed in the changes in ATX. Of the 63 patients who underwent paired biopsy, 36 patients received IFN-based therapy (improved, 10; sustained, 16; exacerbated, 10), and 27 patients received DAA therapy (improved, 7; sustained, 15; exacerbated, 5). There was no difference in the rate of ATX change before and after antiviral treatment between IFN-based therapy and DAA therapy (−11.99% vs. −10.42%, Supplemental Figure 3).

Predictive capabilities of liver fibrosis markers in pre- and post-treatment

We analyzed the predictive capabilities for fibrosis above F2 stage of serum ATX levels, platelet counts, the FIB-4 index, and APRI. ATX had the highest AUC among the other fibrosis markers in male patients before treatment (0.81, Figure 5A). The AUC of female patients was equal to that of male patients (0.81, Figure 5B). In contrast, the diagnostic performance of each fibrosis marker decreased after

achieving SVR. However, ATX had a higher AUC than platelet counts, and it was equal to APRI (male, 0.71; female, 0.72; Figures 5C, D). In pre-treatment, cutoff values for predicting fibrosis above F2 stage in male and female patients were 1.21 mg/L (sensitivity: 0.75, specificity: 0.9) and 1.11 mg/L (sensitivity: 1.0, specificity: 0.53), respectively. On the other hand, after achieving SVR, cutoff values for male patients was 0.76 mg/L (sensitivity: 0.90, specificity: 0.47) and 1.32 mg/L (sensitivity: 0.76, specificity: 0.73) in female patients, respectively. We also compared AUCs between serum ATX levels and liver fibrosis markers in predicting each liver fibrosis stage before and after antiviral treatment (Table 2). Before treatment, ATX had the highest AUC compared to platelet counts, the FIB-4 index, and APRI (≥ F2; 0.81, ≥ F3; 0.89, = F4; 0.89) in male patients. ATX in female patients also had a high diagnostic performance (≥ F2; 0.81, ≥ F3; 0.77, = F4; 0.80). However, the FIB-4 index and APRI had higher AUCs than ATX after achieving SVR. The diagnostic performance for predicting above F3 stage (FIB-4, 0.76; APRI, 0.73) and F4 stage (FIB-4, 0.76; APRI, 0.76) was superior, especially in male patients. On the other hand, we also found that ATX in female patients had the highest AUC compared to other fibrosis markers only for predicting F4 stage after treatment (AUC = 0.86).

Discussion

Previous studies revealed that ATX is a useful biomarker in predicting liver fibrosis stage [12-15]. In the present study, we analyzed patients before treatment and after achieving SVR. First, the present study showed that serum ATX levels correlated with liver fibrosis stage in patients with HCV infection before treatment. Moreover, the predictive potential of ATX for liver fibrosis stage was higher compared to platelet counts, the FIB-4 index, and APRI in male patients. ATX in female patients also had a high diagnostic capacity in predicting hepatic fibrosis. In particular, the diagnostic

ability of liver cirrhosis was superior and may be useful to identify high-risk patients for HCC before antiviral therapy.

Second, we also found that serum ATX levels largely correlated with liver fibrosis stage after achieving SVR. On the other hand, the measured values after antiviral treatment decreased compared to before treatment. We revealed that the rate of ATX reduction before and after antiviral treatment in patients whose liver fibrosis stage improved tended to be higher than that in patients whose fibrosis became exacerbated, but without statistical significance (median values; −26.97% vs. −7.47%, $p = 0.08$, Supplemental Figure 4). We suggested that the rate of ATX change predicts the improvement of the fibrosis stage, but it is necessary to evaluate a larger sample size. According to a previous study, IFN-free DAA therapy has been reported to decrease serum ATX levels four weeks after the start of treatment [19]. Because liver fibrosis does not improve in such a short period [23], serum ATX levels are expected to reflect not only liver fibrosis but also hepatitis activity and/or the presence of HCV. Yamazaki et al. [12] reported that serum ATX levels were weakly correlated with serum ALT levels (r = 0.329). We also demonstrated a weak correlation between ATX and ALT (r = 0.231, $p = 0.001$). In addition, we have illustrated the clinical characteristics of patients whose liver fibrosis were improved or sustained but serum ATX levels were not decreased (group A, B) as well as those of patients whose liver fibrosis was exacerbated but serum ATX levels were decreased (group C) in Supplemental Table 1. Of the 63 patients in whom we performed paired biopsy, only 4 patients did not have decreased ALT levels following treatment, but 3 of those patients were included in group B (No. 9, 10, 13, Supplemental Table 1). Also, in patients with improved or sustained fibrosis, 5 patients (of 13 patients) in the ATX-elevated group and only 2 patients (of 35 patients) in the ATX-decreased group had post-treatment ALT levels of > 30 U/L. We suggested that ATX reduction may

be inhibited in patients with residual liver inflammation. On the other hand, the patients in whom liver fibrosis was exacerbated, pretreatment ALT levels in the ATX-decreased group were higher than those in the ATX-elevated group (median value; 117 U/L vs. 23 U/L, $p = 0.03$). Patients in the ATX-decreased group may have strong hepatic inflammation, increasing pretreatment ATX levels. Therefore, serum ATX levels were suggested to reflect not only liver fibrosis but also liver inflammation. In fact, we saw no change in serum ATX levels in patients with treatment failure (data not shown). This is similar to *Wisteria floribunda agglutinin*-positive (WFA^+) Mac-2-binding protein (M2BPGi), which is a glycomarker for predicting liver fibrosis [24].

Moreover, the present study showed that the diagnostic ability of ATX in predicting liver fibrosis stage decreased after achieving SVR. From the results in Table 2, ATX was expected to be superior as a liver fibrosis marker before treatment. However, the FIB-4 index and APRI had better predictive performance after antiviral therapy. We revealed that serum ATX levels varied even in patients with similar liver fibrosis stage before and after antiviral therapy. We suspect that serum ATX levels are influenced by factors other than liver fibrosis. The reference values of serum ATX in females are significantly higher than in males [18]. In addition, ATX has been reported to be elevated in pregnant women [25], and may be affected by sexual hormones and female reproductive organs. A previous study has also shown that serum ATX levels increased in patients with follicular lymphoma [26]. On the other hand, it has been reported that there are few changes in ATX in kidney disease, heart disease, and diabetes [27]. It is also unaffected by meals [27]. It is important that the factors affecting serum ATX levels are identified to make accurate clinical decisions.

In the recent years, IFN-free DAA therapy could achieve SVR in most patients with HCV infection. However, HCC develops in patients with SVR and

advanced hepatic fibrosis. It is important to monitor HCC after SVR to establish predictive markers for hepatic fibrosis. Our data showed that ATX is associated with histological hepatic fibrosis both before and after antiviral therapy. We also showed that cutoff values for predicting above the F2 stage differed before and after antiviral therapy. A similar liver fibrosis marker, M2BPGi, has been suggested as a reliable serum marker for liver carcinogenesis [24,28]. ATX plays a role in converting lysophosphatidylcholine to LPA, which is involved in physiological roles. Interestingly, the ATX-LPA pathway has been reported to associate with the development of HCC [29,30]. ATX might be a more reliable marker for the development of HCC. Further research is needed on the relationship between ATX and SVR-subsequent carcinogenesis.

A limitation of this study is the small number of patients, especially those with advanced liver fibrosis. There were only 3 male patients with F3 fibrosis after treatment and 5 with F4 fibrosis. The lower ATX of F3 stage than that of F2 stage in female may be due to that. The need to analyze by sex makes it more difficult to collect a proper number of samples. We also could not analyze M2BPGi, type IV collagen 7S, or hyaluronic acid, which are established liver fibrosis markers. However, this is the first report to reveal the association of ATX with histological liver fibrosis stage after achieving SVR.

In conclusion, we revealed that serum ATX levels were correlated with histological liver fibrosis stage before and after antiviral therapy. Also, our findings indicate that the diagnostic capability of ATX for predicting liver fibrosis differs before and after antiviral therapy. We should establish separate cutoff values before and after treatment. Although several non-invasive liver fibrosis markers have been reported, we need to consider the characteristics of each marker.

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Table 1. Clinical characteristics of patients with HCV infection pre- and post-treatment

	Pre	Post
Male/Female	38/43	55/63
Age	61 (52-66)	68 (59-73)
Therapy regimen (A/B/C/D/E/F)†	—	38/33/21/19/5/2
Post-treament period (month)	—	15 (6-37)
F0/F1/F2/F3/F4	1/26/24/11/19	4/39/47/14/14
ATX (mg/L)	1.44 (1.11-2.17)	1.17 (0.90-1.44)
PLT ($\times 10^4/\mu L$)	13.8 (10.7-17.5)	17.5 (13.2-21.3)
AST (U/L)	49 (34-82)	23 (19-32)
ALT (U/L)	49 (29-94)	19 (13-28)
FIB-4 index	3.12 (1.83-4.66)	1.97 (1.59-3.07)
APRI	0.91 (0.50-1.62)	0.36 (0.25-0.53)

Data are presented as median and interquartile range.

† A: interferon-based therapy, B: sofosbuvir + ledipasvir, C: sofosbuvir + ribavirin, D: asunaprevir + daclatasvir, E: elbasvir + grazoprevir, F: ombitasvir + paritaprevir + ritonavir

HCV; hepatitis C virus, ATX; autotaxin, PLT; platelet counts, AST; aspartate aminotransferase, ALT; alanine aminotransferase, APRI; AST to platelet ratio

Table 2. Comparisons of AUCs between serum ATX levels and other fibrosis markers in predicting fibrosis stage before and after antiviral therapy

			≥F2	≥F3	F4
Before treatment	Male	ATX	0.81	0.89	0.89
		PLT	0.7	0.88	0.86
		FIB-4	0.72	0.84	0.84
		APRI	0.63	0.73	0.76
	Female	ATX	0.81	0.77	0.8
		PLT	0.73	0.68	0.6
		FIB-4	0.86	0.73	0.67
		APRI	0.8	0.73	0.7
After treatment	Male	ATX	0.71	0.68	0.67
		PLT	0.64	0.64	0.63
		FIB-4	0.68	0.76	0.76
		APRI	0.72	0.73	0.76
	Female	ATX	0.72	0.65	0.86
		PLT	0.6	0.63	0.67
		FIB-4	0.67	0.67	0.72
		APRI	0.73	0.76	0.78

AUC; area under the curve, ATX; autotaxin, PLT; platelet counts, APRI; asperate aminotransferase to platelet ratio

Figure 1. Classification of patients

Figure 2. Correlation between serum ATX levels and liver fibrosis stage before antiviral treatment

Data from all patients (A), male patients (B), and female patients (C) are shown. The number of samples for each fibrosis stage is as follows: F0/F1/F2/F3/F4 = 1/26/24/11/19 (all), 1/9/12/6/10 (male), 0/17/12/5/9 (female). Boxes represent the interquartile range of the data. The horizontal lines in the boxes indicate the median values. The vertical lines connect the nearest values of 1.5 times the interquartile range from the quartile point. The dots indicate outliers. ***: $p < 0.001$, **: $p < 0.01$.

Figure 3. Correlation between serum ATX levels and liver fibrosis stage after achieving SVR

Data from all patients (A), male patients (B), and female patients (C) are shown. The number of samples for each fibrosis stage is as follows: F0/F1/F2/F3/F4 = 4/39/47/14/14 (all), 1/16/30/3/5 (male), 3/23/17/11/9 (female). Boxes represent the interquartile range of the data. The horizontal lines in the boxes indicate the median values. The vertical lines connect the nearest values of 1.5 times the interquartile range from the quartile point. The dots indicate outliers. *: $p < 0.05$.

Figure 4. Change in serum ATX levels after antiviral treatment for each alteration of liver fibrosis

The figures show the change in serum ATX levels in all patients (A), the patients whose liver fibrosis stage improved (n = 17) (B), sustained (n = 31) (C), and became exacerbated (n = 15) (D). Boxes represent the interquartile range of the data. The horizontal lines in the boxes indicate the median values. The vertical lines connect the nearest values of 1.5 times the interquartile range from the quartile point. pre: pre-treatment, post: post-treatment. ***: $p < 0.001$, *: $p < 0.05$, NS: not significant.

Figure 5. Receiver operating characteristic curves for predicting above F2 stage

The data in male patients before treatment (A), female patients before treatment (B), male patients after treatment (C), and female patients after treatment (D) are shown. The numbers at the bottom right are the area under the curve of each liver fibrosis marker.

Supplemental Table 1. Clinical characteristics in patients whose fibrosis and ATX changed opposite direction.

	No.	Age	Sex	Therapy	pre Fibrosis	post Fibrosis	pre ATX (mg/L)	post ATX (mg/L)	pre AST (U/L)	post AST (U/L)	pre ALT (U/L)	post ALT (U/L)
improved (group A)	1	68	F	IFN	F3	F2	1.44	1.56	50	43	69	31
	2	42	F	IFN	F1	F0	0.79	0.87	15	13	17	9
sustained (group B)	3	59	F	DAA	F1	F1	0.94	0.94	34	20	31	11
	4	66	M	DAA	F2	F2	1.02	1.03	28	17	18	12
	5	46	M	DAA	F2	F2	1.23	1.28	56	47	42	40
	6	72	F	DAA	F1	F1	2.44	2.58	166	25	201	18
	7	53	F	IFN	F1	F1	1.11	1.19	16	13	12	7
	8	61	M	IFN	F1	F1	0.59	0.63	25	21	33	19
	9	41	F	IFN	F1	F1	0.72	0.87	34	96	53	125
	10	59	F	IFN	F2	F2	1.29	1.71	24	36	15	35
	11	47	F	IFN	F1	F1	0.97	1.31	19	13	18	8
	12	52	F	DAA	F1	F1	0.82	1.14	87	16	197	14
	13	38	M	IFN	F2	F2	0.53	0.76	39	53	50	50
exacerbated (group C)	14	63	M	IFN	F2	F3	1.45	1.02	103	19	151	15
	15	52	F	IFN	F2	F3	1.85	1.39	111	44	195	71
	16	68	F	IFN	F2	F4	2.35	1.84	75	35	73	30
	17	72	M	DAA	F1	F2	2.07	1.64	101	25	113	15
	18	75	F	DAA	F2	F3	1.71	1.40	30	19	27	16
	19	54	M	IFN	F1	F2	1.21	1.00	341	38	122	61
	20	53	F	IFN	F2	F3	1.13	1.00	37	26	25	15
	21	61	F	IFN	F2	F3	1.37	1.26	39	29	45	25
	22	54	M	IFN	F1	F2	1.21	1.13	341	35	122	21
	23	52	F	IFN	F2	F4	1.85	1.79	111	27	195	39

ATX; autotaxin, AST; aspartate aminotransferase, ALT; alanine aminotransferase, IFN; interferon, DAA; direct-acting antiviral agent

Supplemental Figure 1. Correlation between serum liver fibrosis markers and liver fibrosis stage before antiviral treatment

The number of samples for each fibrosis stage is as follows: F0/F1/F2/F3/F4 = 1/26/24/11/19. Boxes represent the interquartile range of the data. The horizontal lines in the boxes indicate the median values. The vertical lines connect the nearest values of 1.5 times the interquartile range from the quartile point. The dots indicate outliers. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$.

Supplemental Figure 2. Correlation between serum liver fibrosis markers and liver fibrosis stage after achieving SVR.

The number of samples for each fibrosis stage is as follows: F0/F1/F2/F3/F4 = 4/39/47/14/14. Boxes represent the interquartile range of the data. The horizontal lines in the boxes indicate the median values. The vertical lines connect the nearest values of 1.5 times the interquartile range from the quartile point. The dots indicate outliers. **: $p < 0.01$, *: $p < 0.05$.

Supplemental Figure 3. Comparisons of rate of ATX change between IFN-based and DAA therapy.

Boxes represent the interquartile range of the data. The horizontal lines in the boxes indicate the median values. The vertical lines connect the nearest values of 1.5 times the interquartile range from the quartile point. The dots indicate outliers. NS: not significant.

Supplemental Figure 4. Association between changes in liver fibrosis stage and rate of ATX change.

Boxes represent the interquartile range of the data. The horizontal lines in the boxes indicate the median values. The vertical lines connect the nearest values of 1.5 times the interquartile range from the quartile point. The dots indicate outliers.

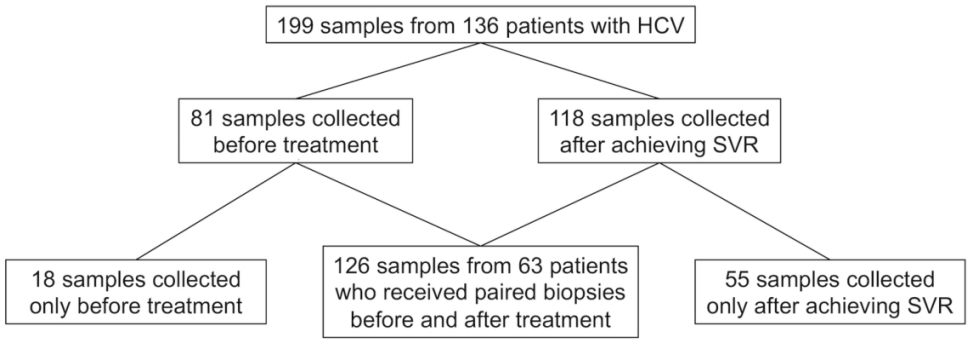

Figure 1

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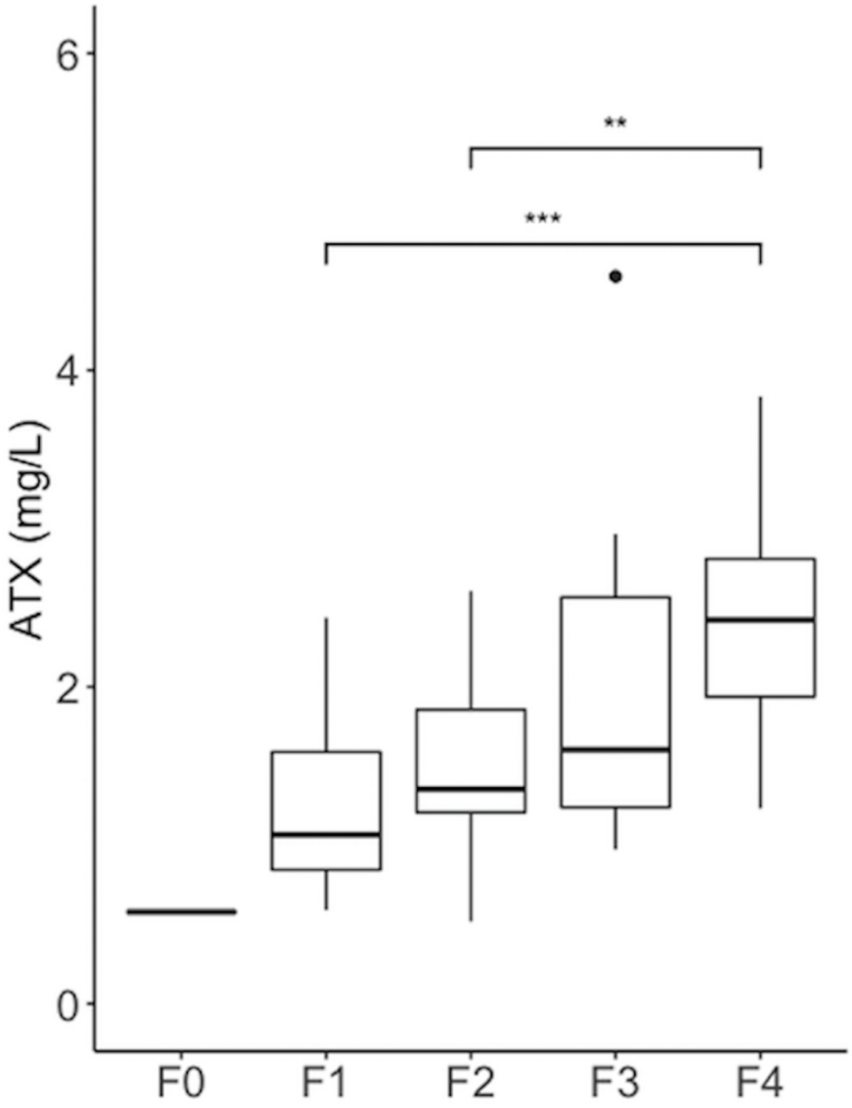

Figure 2A

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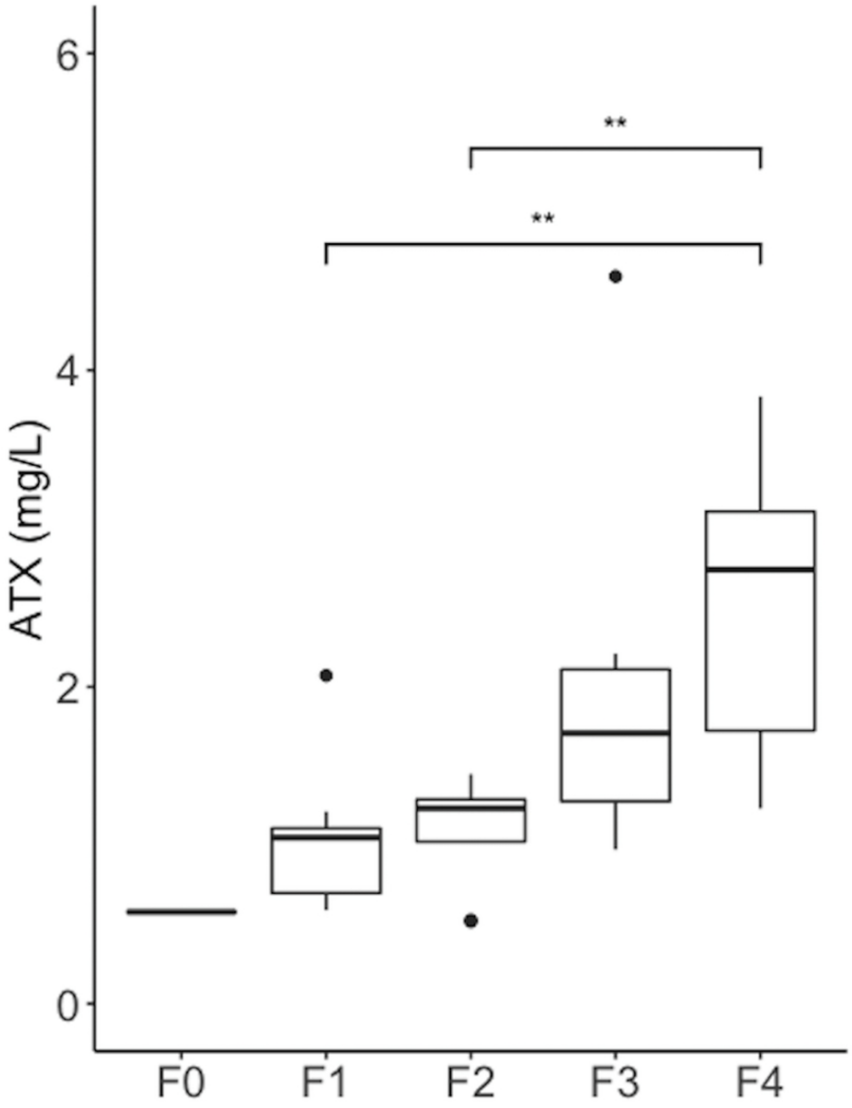

Figure 2B

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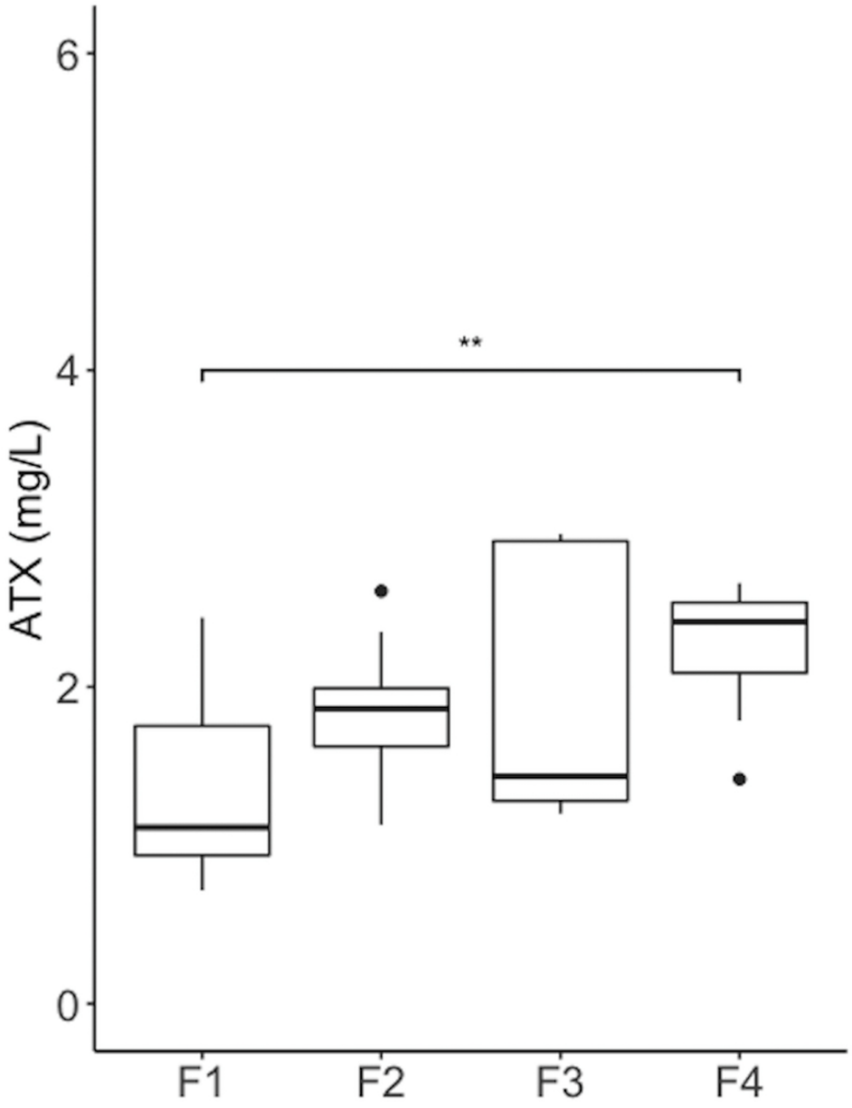

Figure 2C

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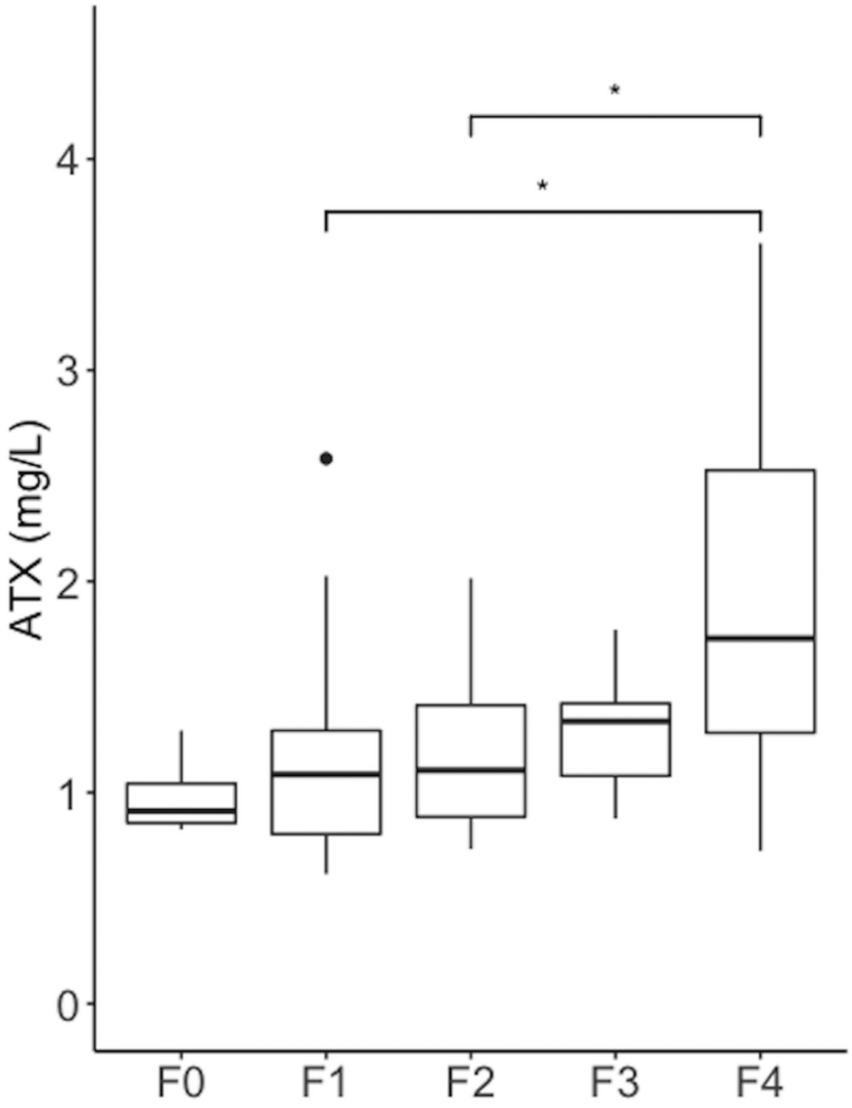

Figure 3A

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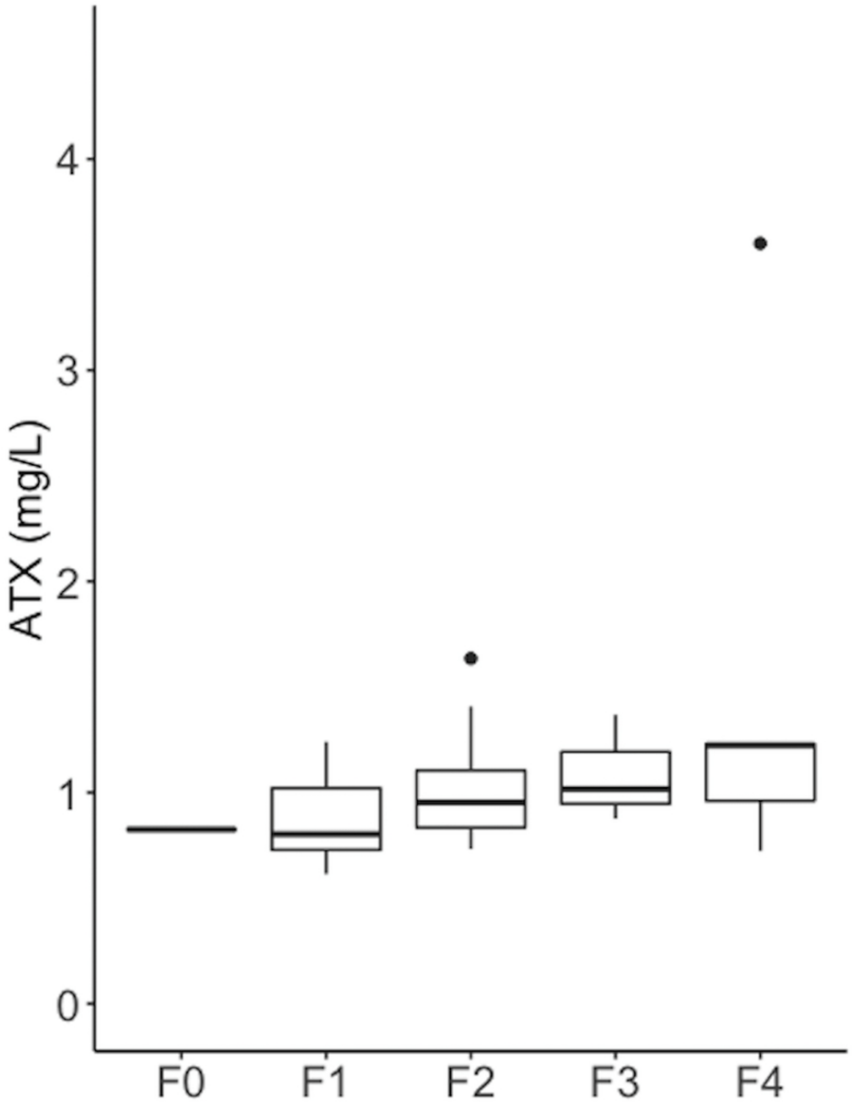

Figure 3B

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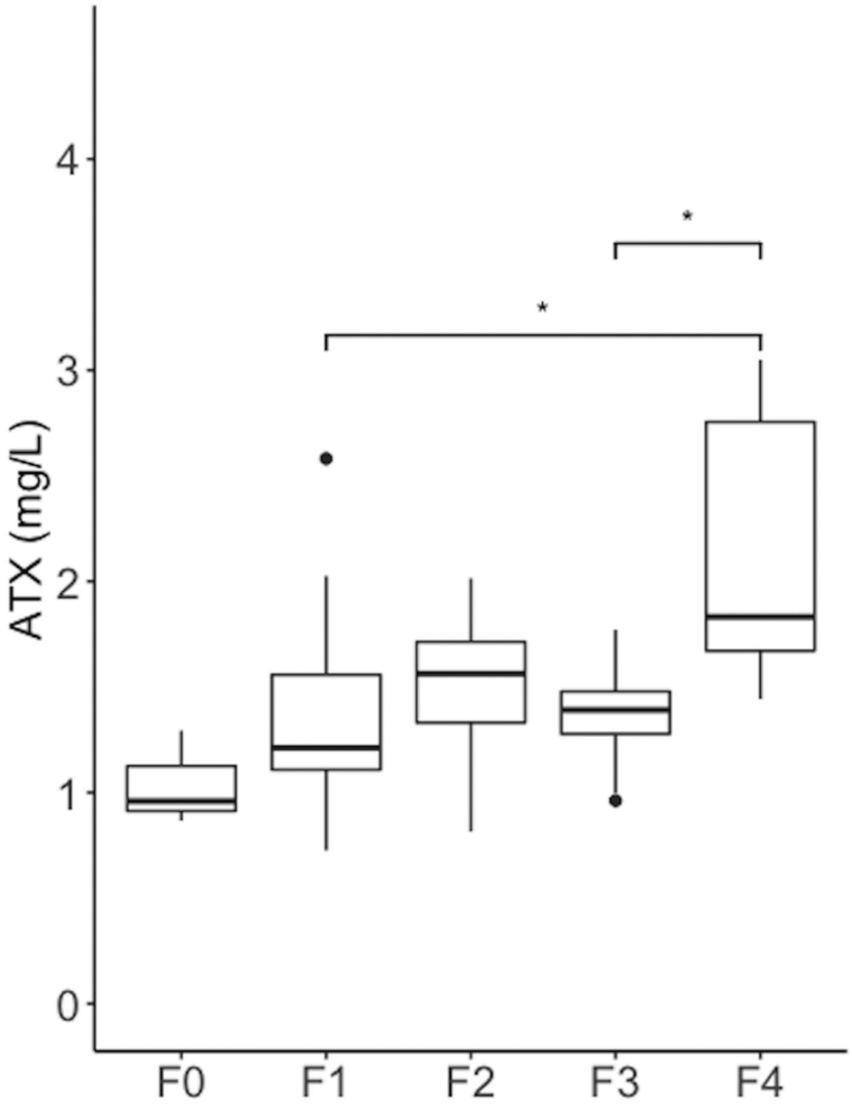

Figure 3C

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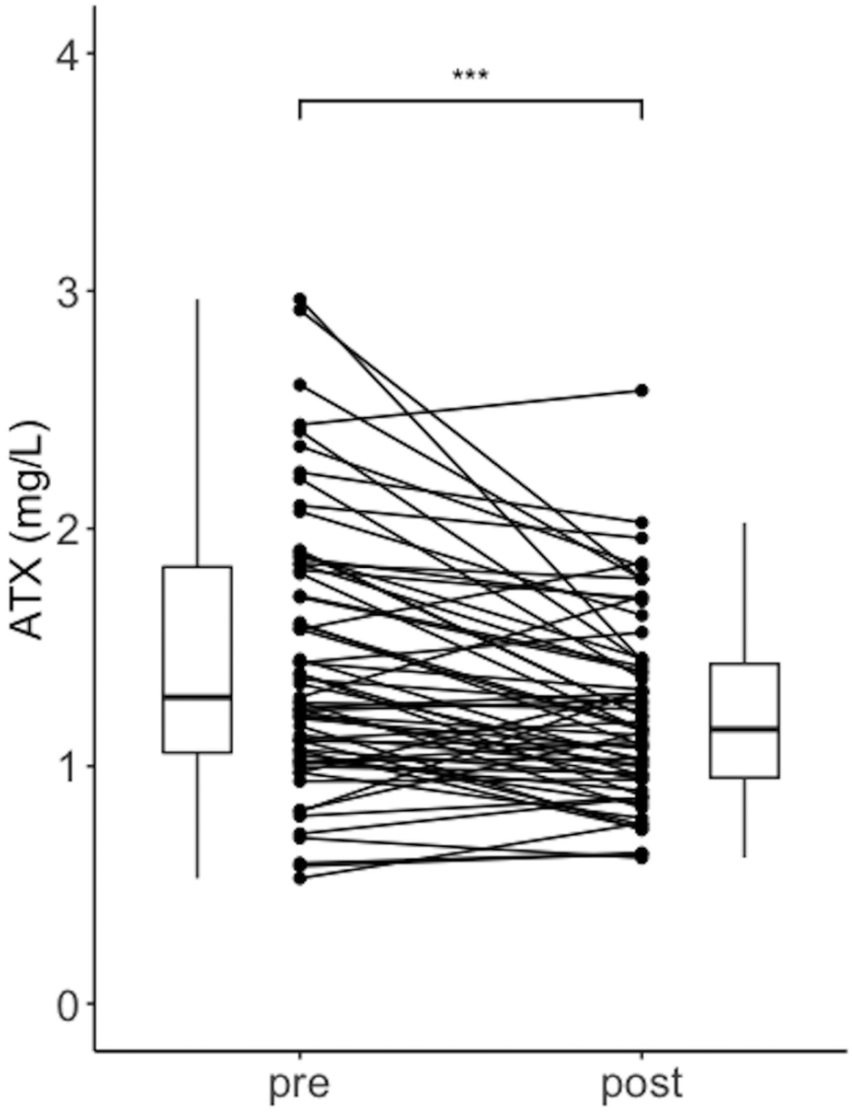

Figure 4A

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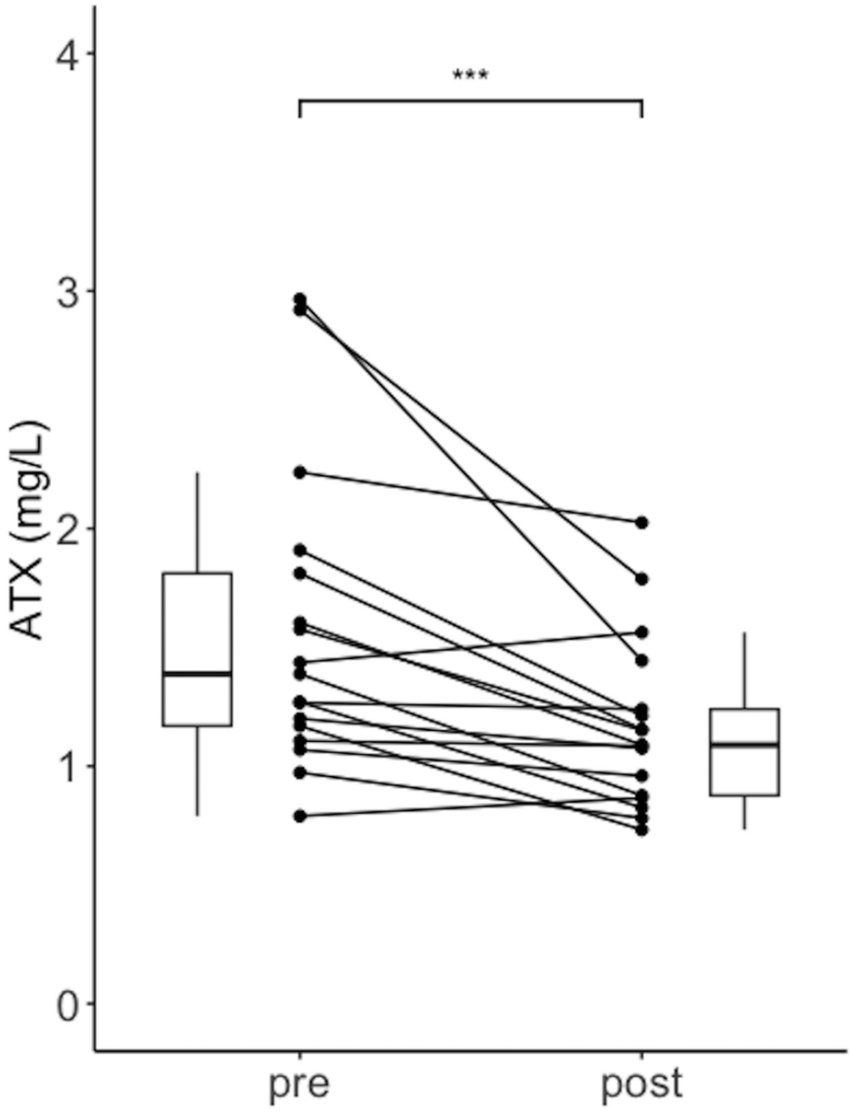

Figure 4B

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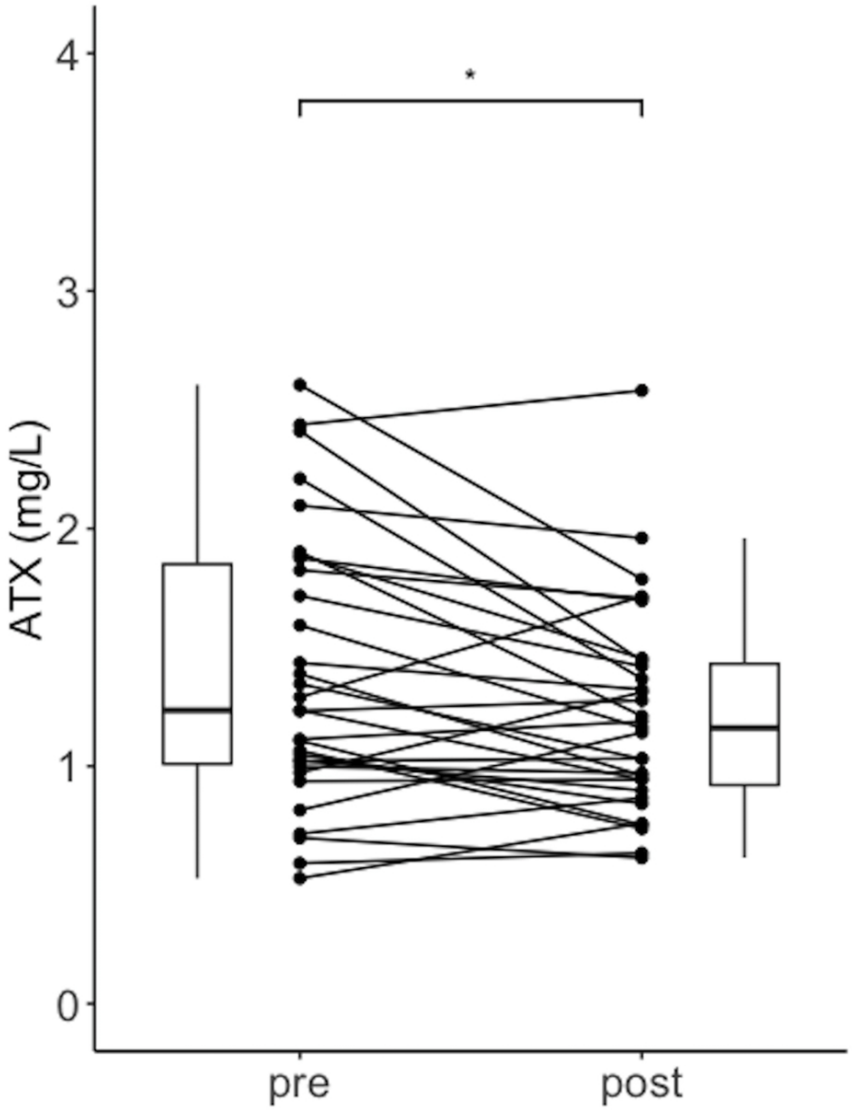

Figure 4C

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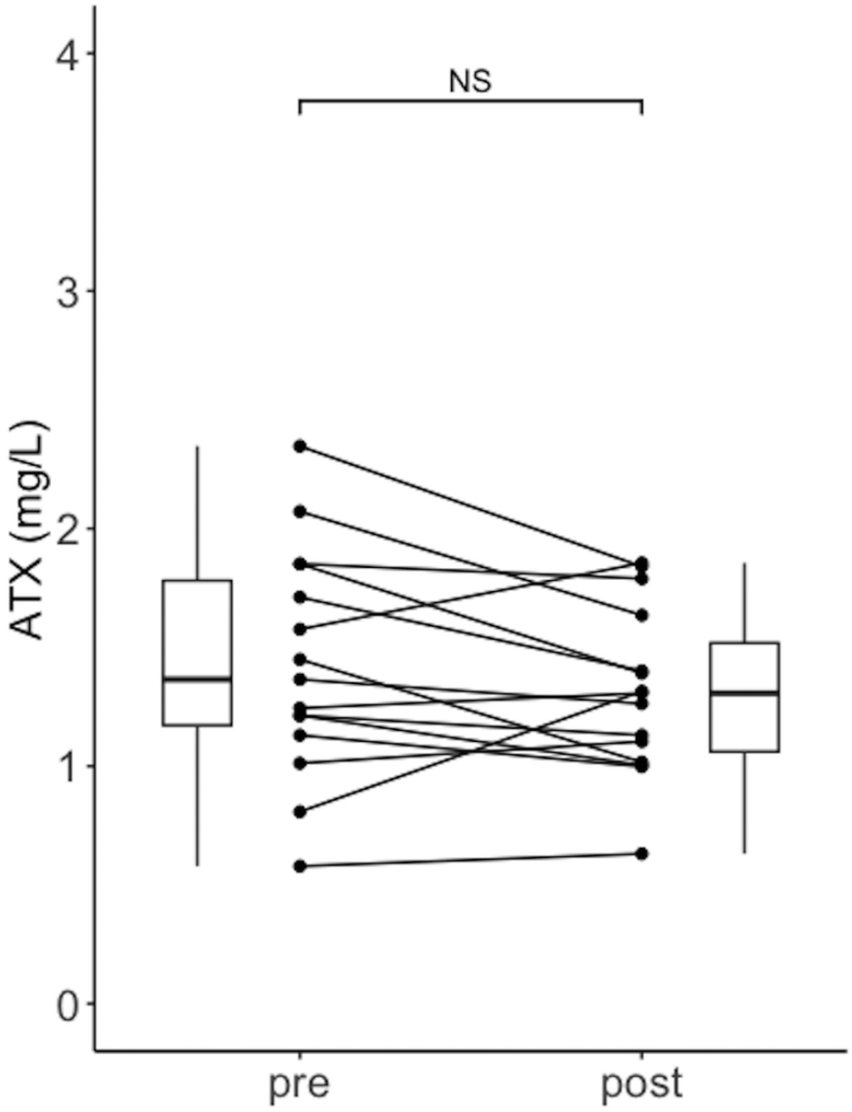

Figure 4D

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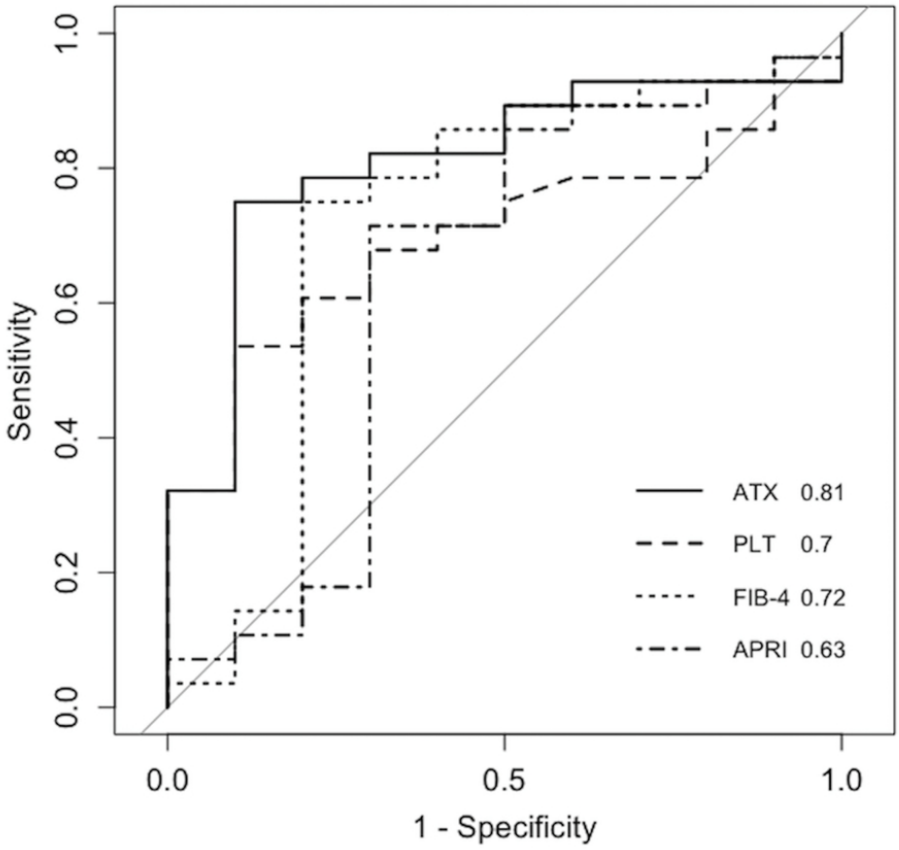

Figure 5A

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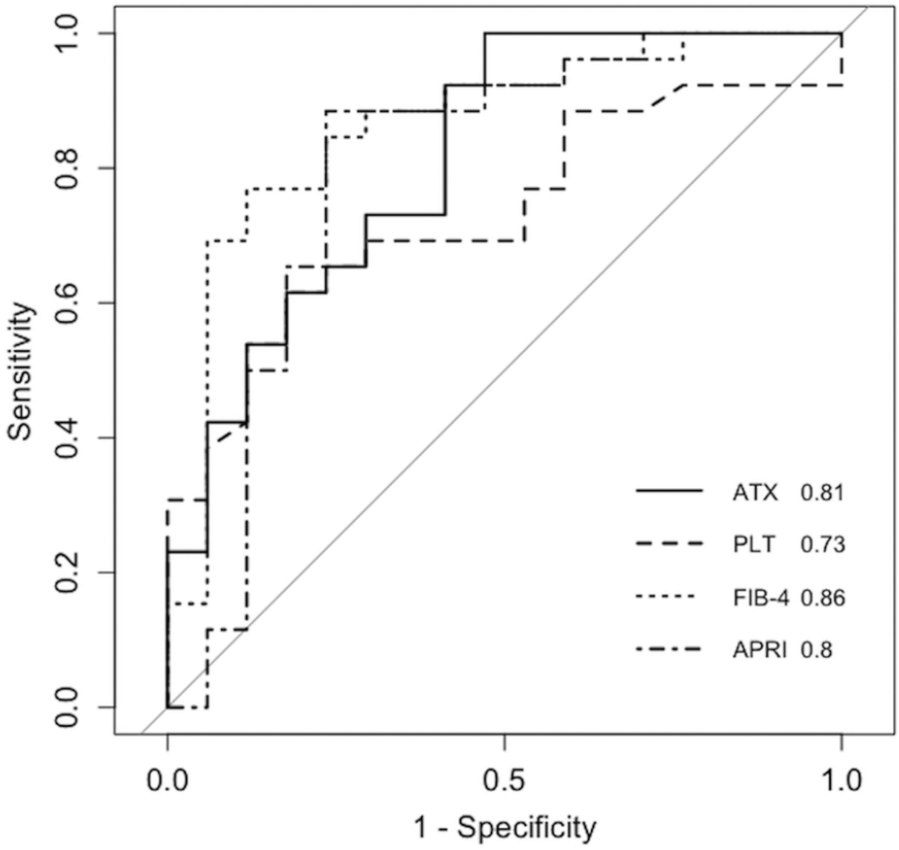

Figure 5B

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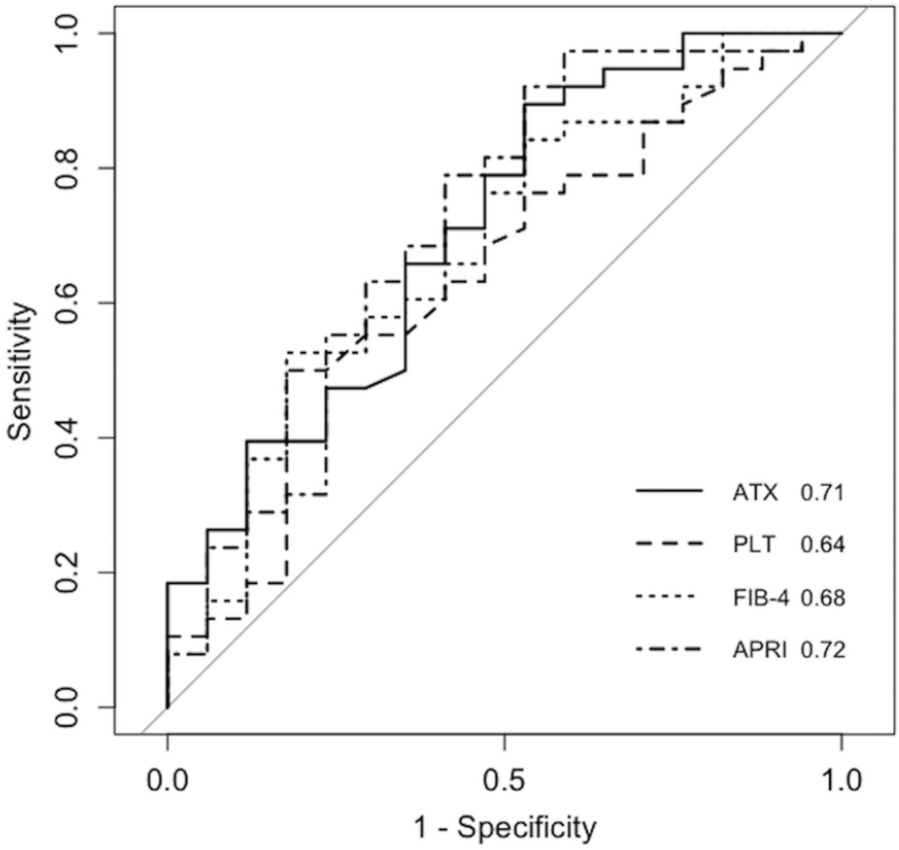

Figure 5C

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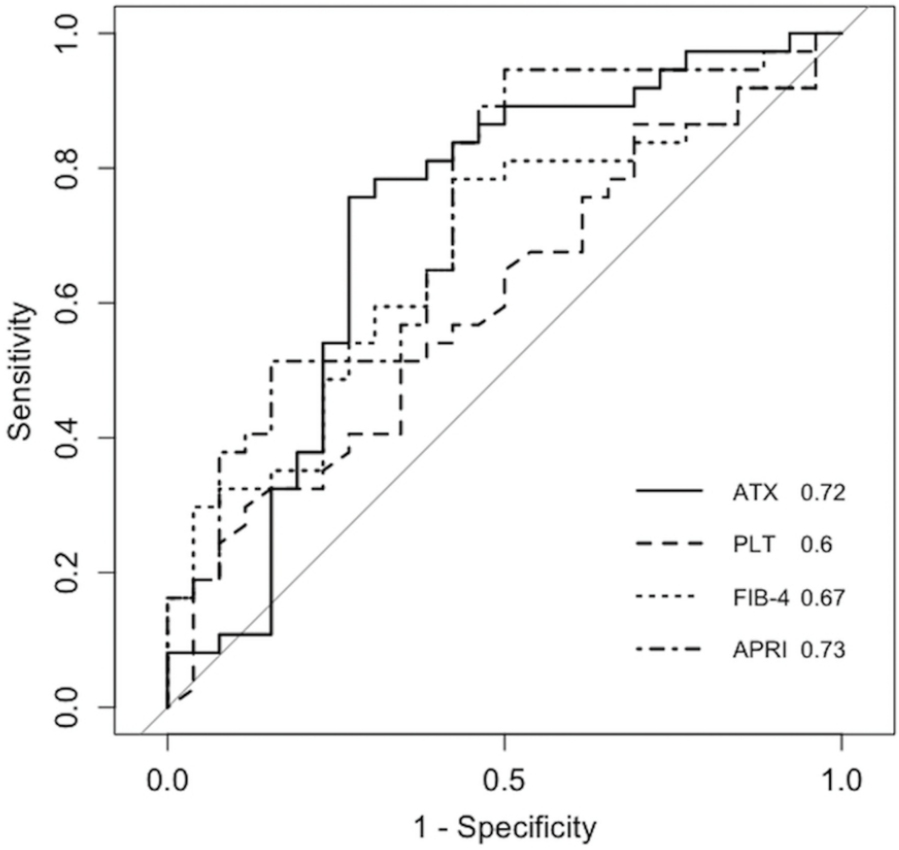

Figure 5D

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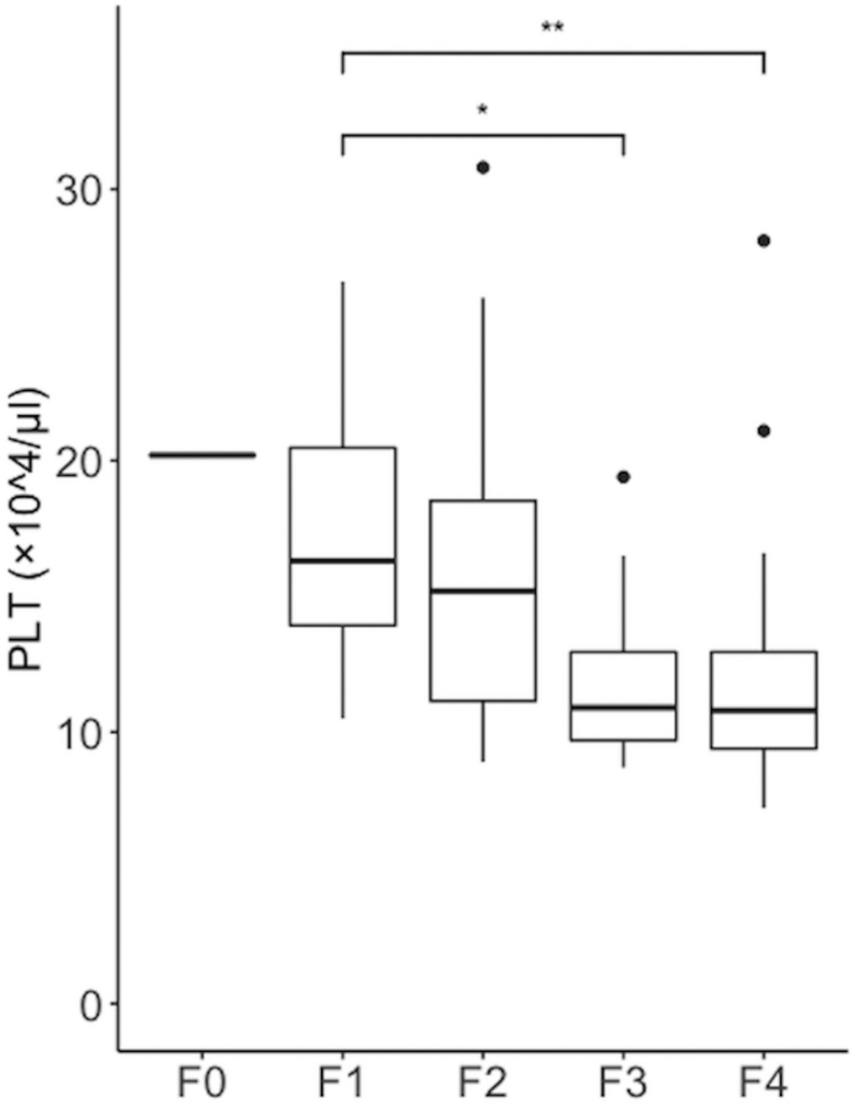
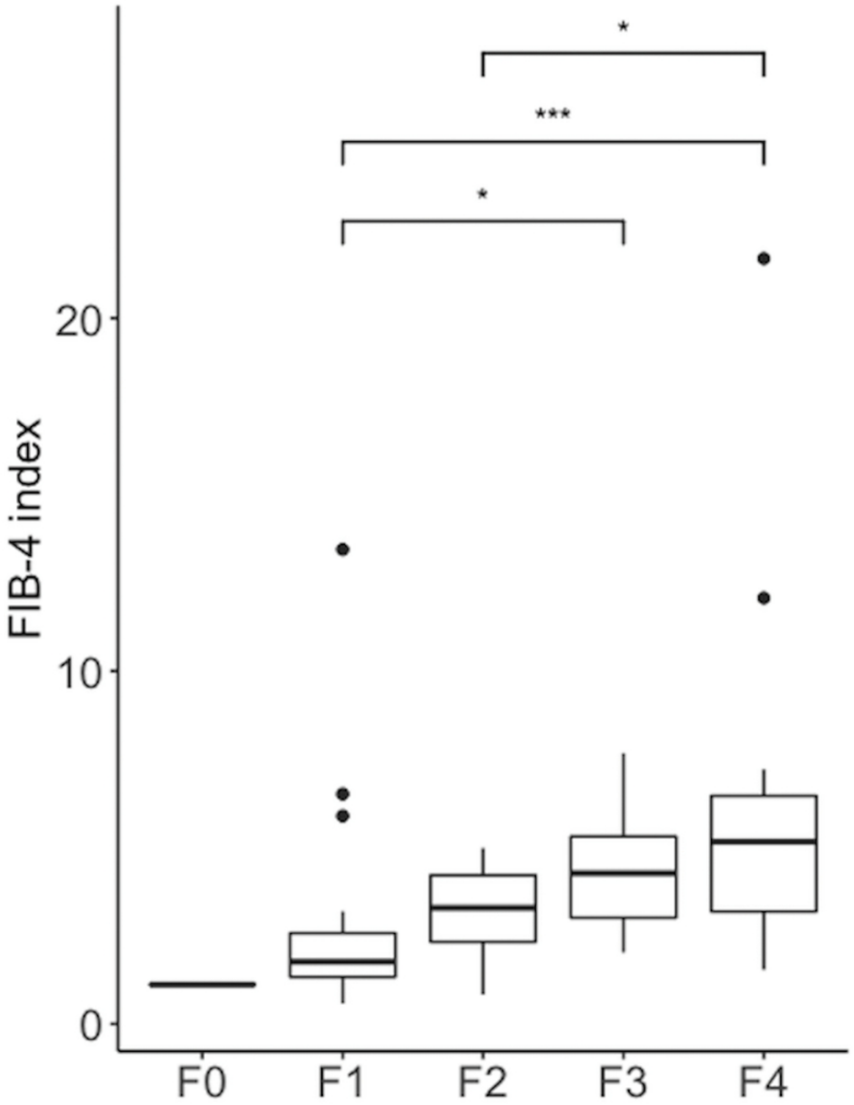


Supplemental Figure 1A

80x107mm (300 x 300 DPI)



Supplemental Figure 1B

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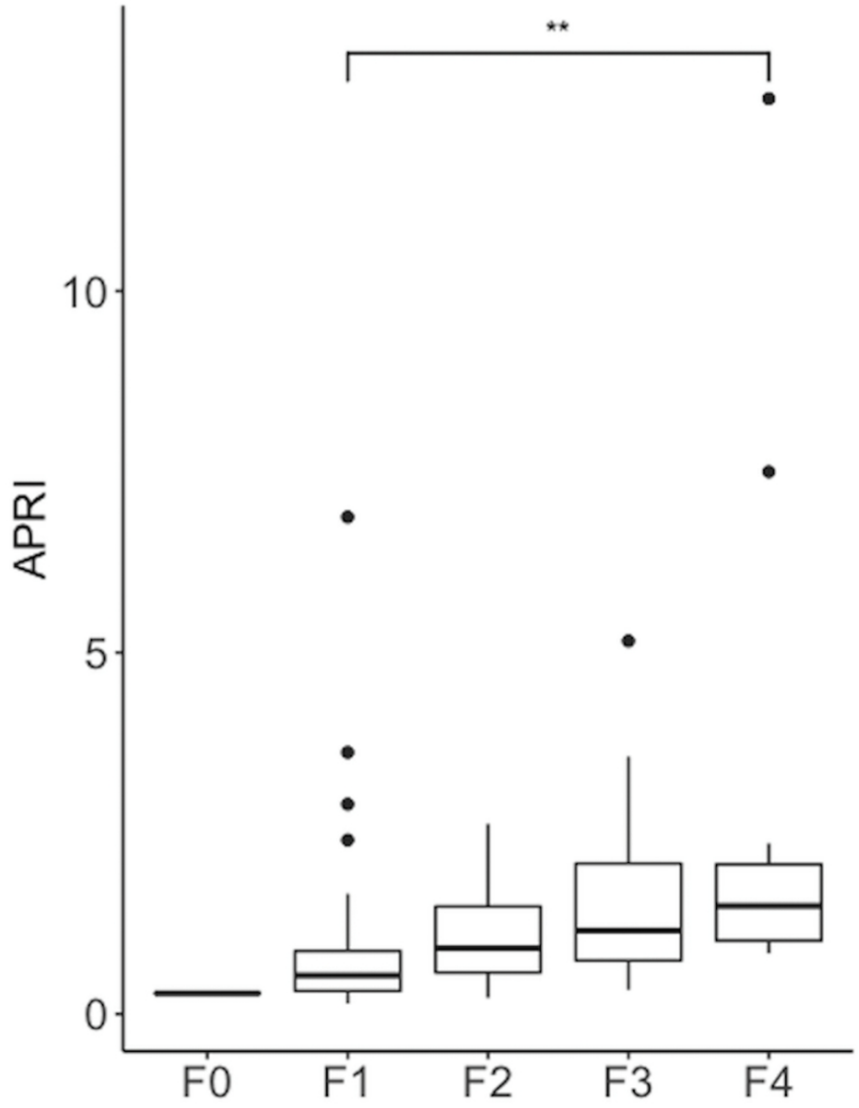

Supplemental Figure 1C

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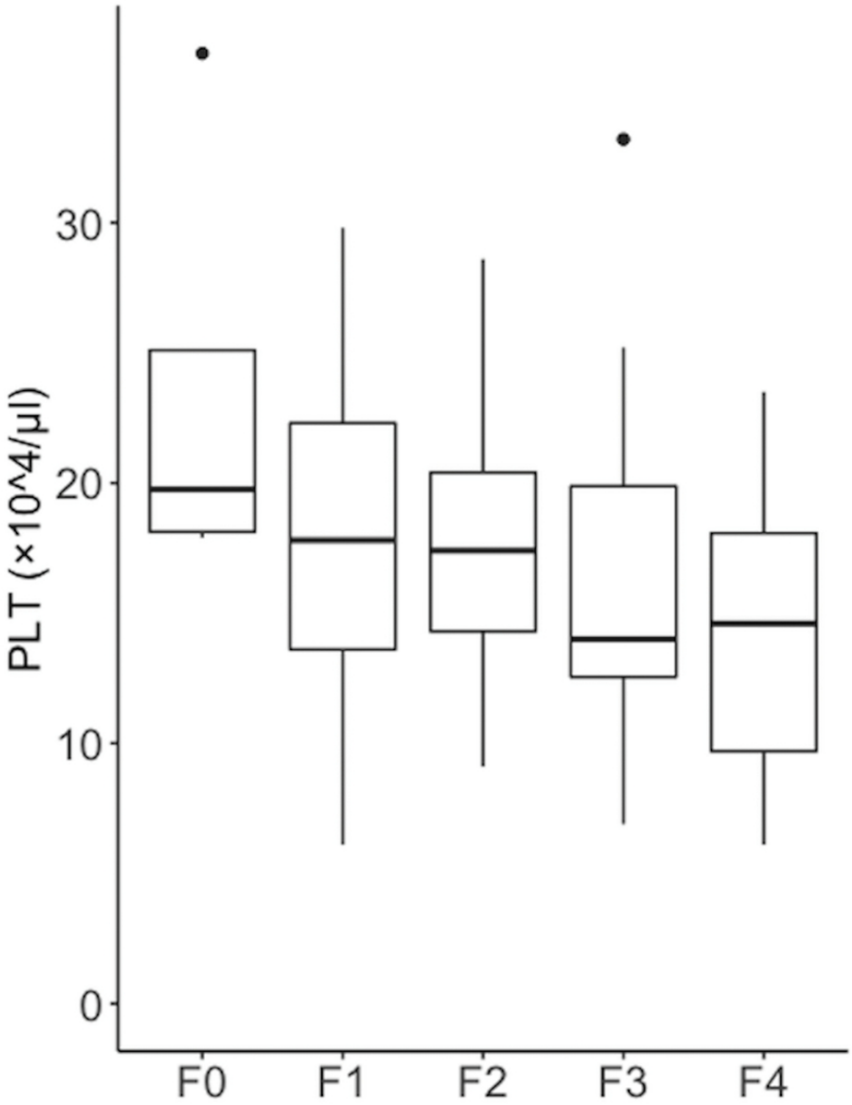

Supplemental Figure 2A

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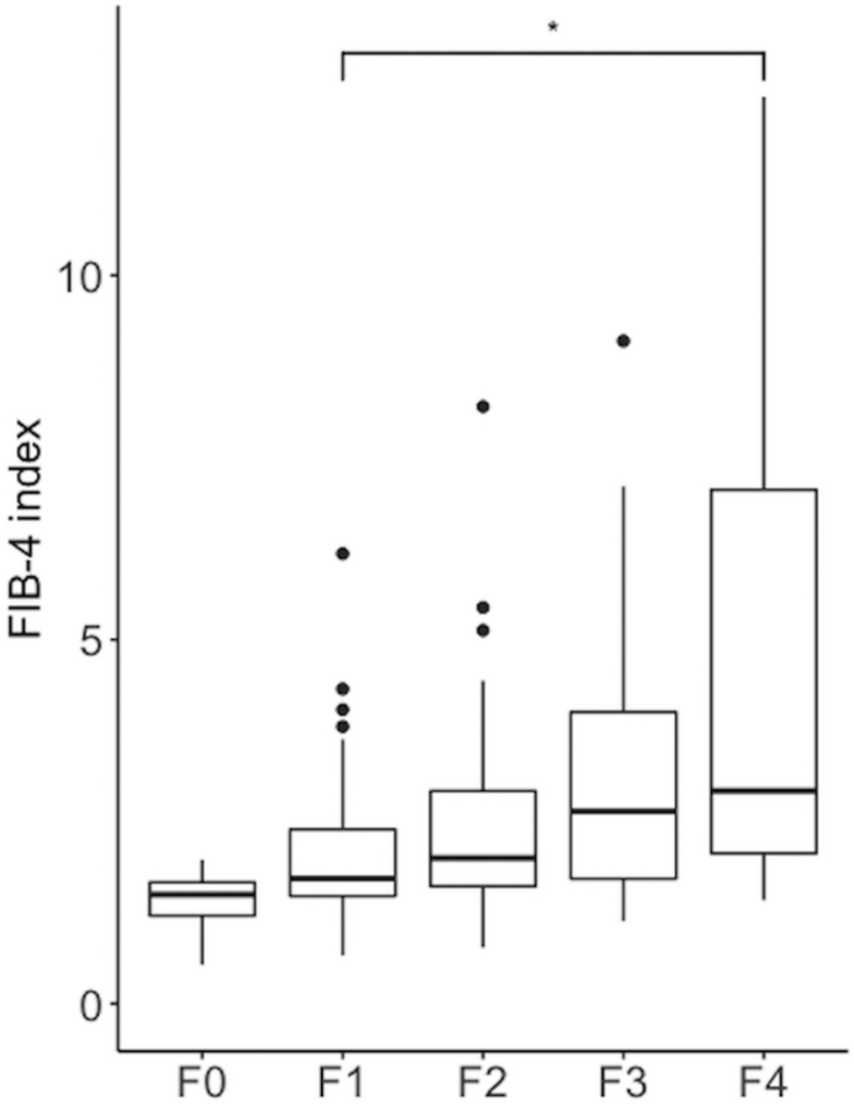

Supplemental Figure 2B

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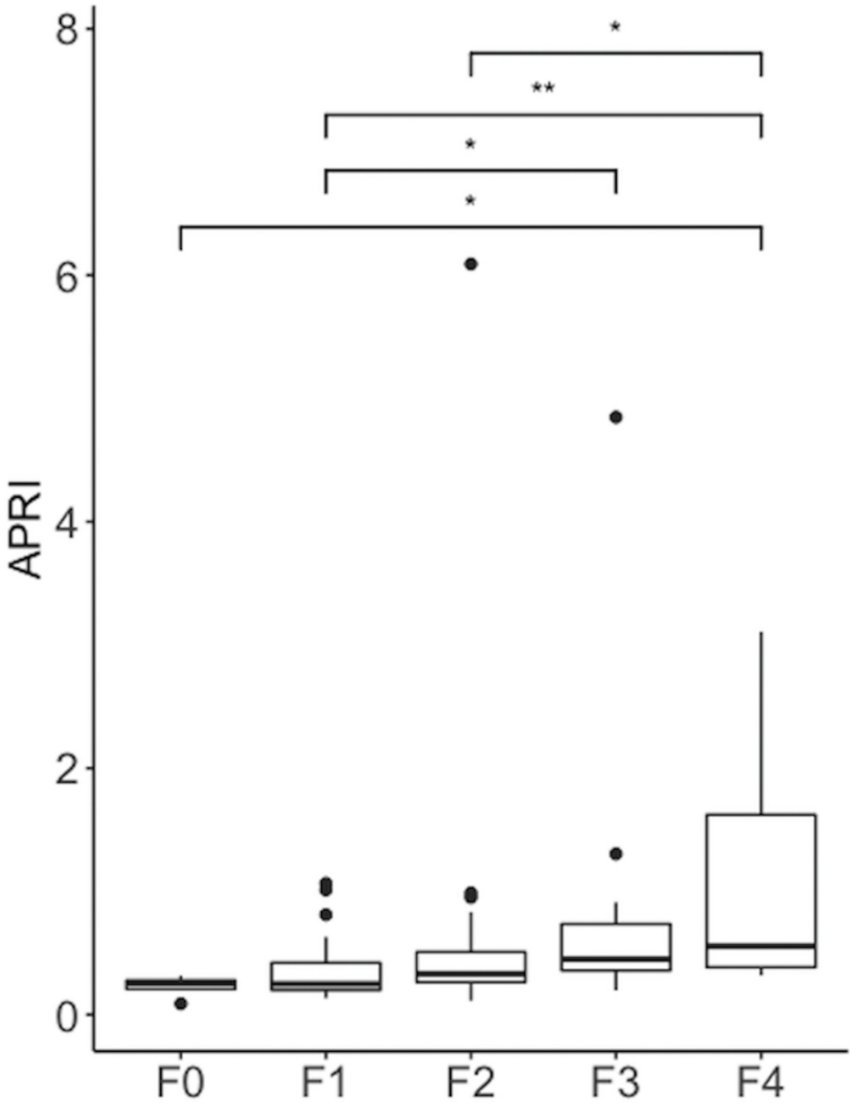

Supplemental Figure 2C

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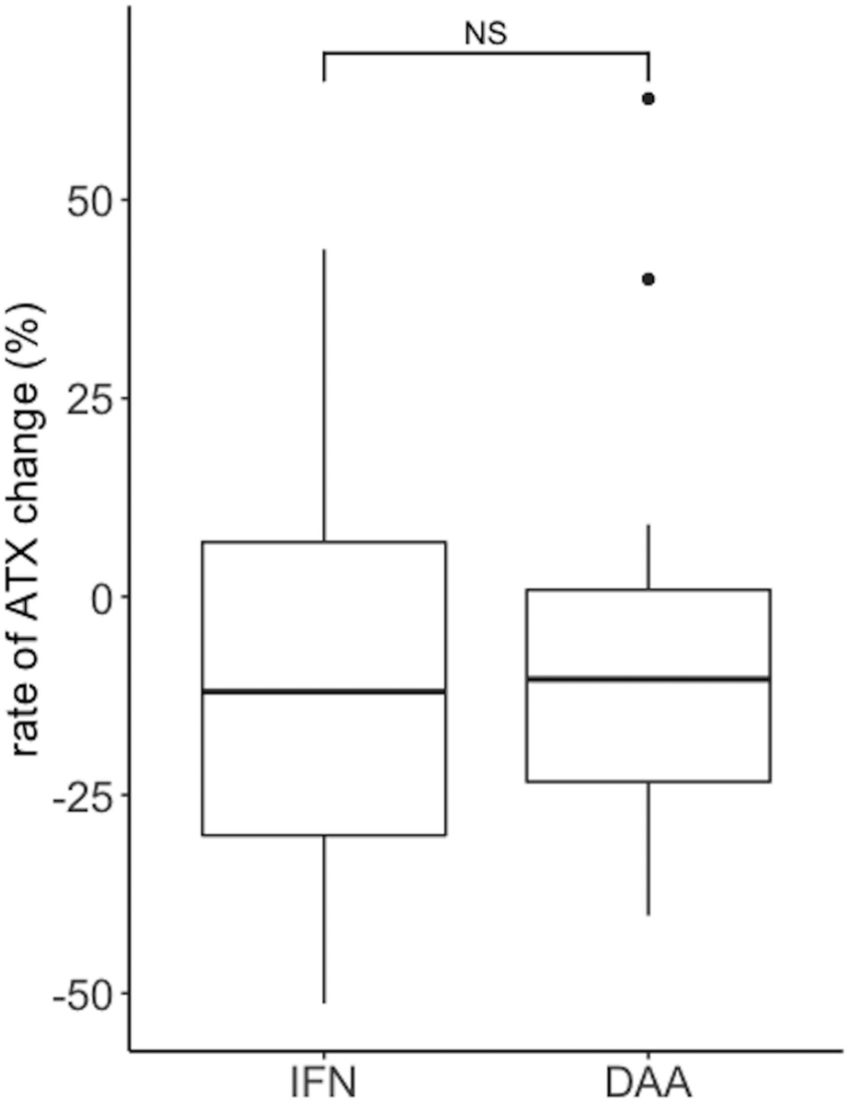

Supplemental Figure 3

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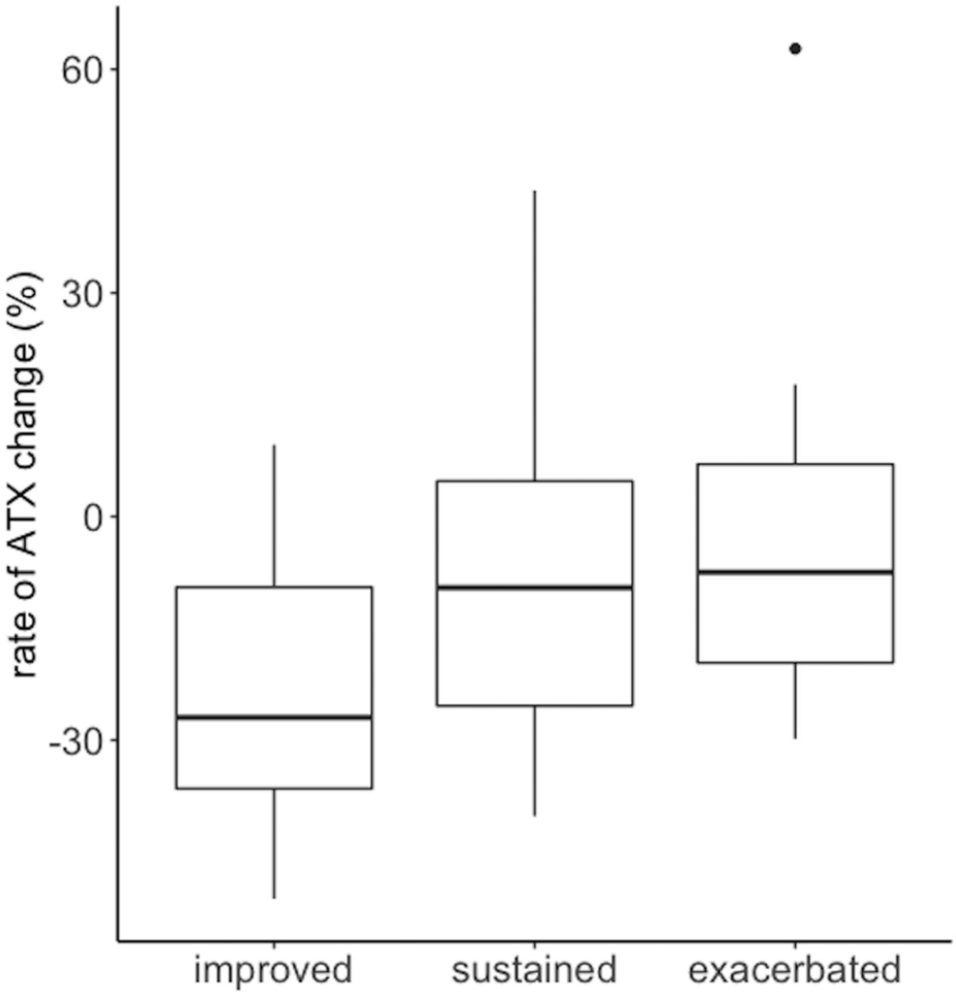

Supplemental Figure 4

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