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Published in:
Journal of Viral Hepatitis

DOI (link to publication from Publisher):
10.1111/jvh.13416

Publication date:
2021

Document Version
Accepted author manuscript, peer reviewed version

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Article type : Original Paper

Altered balance between collagen formation and degradation after successful direct-acting antiviral therapy of chronic hepatitis C

Running title: Collagen and hepatitis C antivirals

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Acknowledgements:

Conflict of interests:

IFV, DJL, MAK and NSG are full time employees of Nordic Bioscience, Herlev, Denmark. MAK and DJL own stocks in Nordic Bioscience, Herlev, Denmark. ALL is on advisory board for MSD, HG received grants from the NOVO Nordisk Foundation, ‘‘Savværksejer Jeppe Juhl og hustru Ovita Juhls mindelegat”, Abbvie, and Intercept, and is on advisory board of Ipsen and Novartis. TLL, CS, BT LHK, CHH, and PL have no conflicts of interest to declare.

Financial support:

The study was investigator-initiated and part of the study was sponsored by AbbVie Inc. The sponsor was not involved in collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the manuscript for publication.

Abstract (249 words)

The effect of direct-acting antiviral (DAA) therapy on extracellular matrix (ECM) turnover, a prominent feature of chronic hepatitis C (CHC), is unknown. ECM protein degradation and formation generate fragments reflecting the tissue turnover balance when quantified in the blood. PRO-C3 and PRO-C4 reflect type III and IV collagen formation; C3M and C4M are degradation markers of type III and IV. We aimed to assess the markers' dynamics with DAA therapy in CHC patients.

Plasma PRO-C3, PRO-C4, C3M, and C4M were assessed before, during, and up till one year after 12-24 weeks of DAA therapy in 77 CHC patients with advanced fibrosis (n=14) or cirrhosis (n=63). Liver stiffness was evaluated using transient elastography. PRO-C3, C3M, and C4M levels decreased significantly ($p<0.00001$) while PRO-C4 was unchanged ($p=0.20$) during the study period. There was a steep decrease in the PRO-C3/C3M ratio during DAA therapy and follow-up ($p<0.02$). The PRO-C4/C4M ratio was unchanged ($p>0.27$). The dynamics of the collagen markers behaved similarly between patients with advanced fibrosis and cirrhosis. However, the cirrhosis patients had >20% higher levels of C3M, PRO-C4, and C4M at all time points ($p<0.05$). The collagen markers correlated with liver stiffness at baseline and follow-up.Markers of type III and IV collagen formation and degradation decreased during and after successful DAA therapy in CHC patients with advanced liver disease, and associated with disease severity. These results indicate an altered balance between collagen formation and degradation after viral clearance suggesting favourable effects on liver fibrosis.

Keywords

Chronic hepatitis C, fibrosis, direct-acting antiviral therapy, collagen markers, macrophage activation.

Introduction

Direct-acting antiviral (DAA) therapy cure most patients with chronic hepatitis C (CHC) infection, even in those with advanced fibrosis or cirrhosis [1,2]. With successful viral eradication, there are several indices of fibrosis regression including decreases in fibrosis scores and non-invasive measures such as liver stiffness [3,4]. However, the evidence remains circumstantial and it is unclear what happens with the underlying structural liver disease and how and when fibrosis regresses or resolves after DAA therapy.

An approach with assessment of markers that directly evaluate the hepatic extracellular matrix (ECM) turnover could therefore prove important. During ECM turnover, protein fragments are generated, which reflect the formation and degradation of different types of collagen. PRO-C3 and PRO-C4 are markers of type III and IV collagen formation, whereas C3M and C4M reflect matrix metalloproteinases (MMP) degradation of type III and IV collagen, respectively [5-8]. The relationship between formation and degradation of the respective collagen markers is assumed to reflect the net production and therefore the deposition of the specific types of collagen in the liver. Type III collagen is a fibril-forming collagen produced mainly by activated fibroblasts as a central element of fibrosis development constituting the interstitial matrix. Type IV collagen on the other hand is a network-forming collagen mainly produced by epithelial cells embedded in the ECM as part of the basement membrane [9]. Especially in CHC patients with advanced fibrosis or cirrhosis, imbalanced ECM turnover is a prominent feature and reflects the stage of fibrosis [10]. ECM turnover has not been investigated prospectively in a DAA treated CHC patient cohort. In that context, specific macrophage activation markers, soluble (s)CD163 and soluble mannose receptor (sMR), should also be investigated in the CHC patients, as ECM turnover may also be affected by inflammation. sCD163 and sMR are elevated and associated with disease severity in chronic liver diseases and decrease with antiviral therapy [11-14].

Our aim was to investigate the dynamics of the collagen markers during and after DAA therapy in CHC patients with advanced fibrosis or cirrhosis. We hypothesised that the collagen markers would be elevated prior to DAA treatment followed by a decrease during and after treatment. In addition, we hypothesised the markers to be associated with initial fibrosis severity, liver stiffness and macrophage activation before and after DAA therapy.

Materials and methods

Design and patients

The CHC patients were included between September 2014 and December 2017 in two Danish, clinical, observational studies performed to assess effects of DAA therapy with either sofosbuvir-based DAA therapy or 3D combination therapy containing ombitasvir, paritaprevir, and ritonavir up till one year after therapy cessation. The inclusion criteria were CHC and advanced liver fibrosis (METAVIR≥F3 or liver stiffness ≥ 12kPa) or cirrhosis and planned initiation of DAA therapy and exclusion criteria were hepatitis B and/or HIV infection. For patients initiating sofosbuvir treatment, more details are described in the original study [11]. For patients initiating the 3D combination therapy, additional inclusion criteria were genotype 1 or 4 hepatitis C, Child-Pugh A cirrhosis, and age between 30-70 and exclusion criteria were Child-Pugh B or C cirrhosis, hepatitis B or HIV coinfection, expected survival below six months, planned liver transplantation or transjugular intrahepatic portosystemic shunt procedure within six months, current or expected pregnancy, breastfeeding, portal vein thrombosis, cancer, current alcohol use, and diabetes. The diagnosis of cirrhosis was based on either liver biopsy (n=14, out of a total of 26 biopsies), liver stiffness>15 kPa, or radiological, clinical, and/or biochemical signs of liver cirrhosis. In the present study, only patients with stored plasma samples available for analyses were included, i.e. 77 patients, 62 treated with sofosbuvir and 15 treated with 3D-therapy. The studies were conducted in accordance with the Helsinki declaration. The ethical Committee in the Central Denmark Region approved the studies (1-10-72-199-14 and 1-10-72-118-15) and the Danish Data Protection Agency was notified about the studies in accordance with Danish legislation (1-16-02-736-14 and 1-16-02-401-15). Informed consent forms were signed by all patients prior to inclusion in the studies.

Blood samples were obtained before initiation of therapy (n=77), at week 1 (n=35), 2 (n=46), 4 (n=46), and 8 (n=49) during treatment, at end of treatment (EOT) (n=59), at 12-weeks post-treatment (n=40) and one-year post-treatment (n=42). The reduced numbers at follow-up reflects logistic issues and/or little sample volume. All samples were stored at -80°C until batch analysis.

Blood parameters

All four collagen markers, PRO-C3, C3M, PRO-C4, and C4M, were quantified in plasma using competitive enzyme-linked immuno-sorbent assays (ELISA) at Nordic Bioscience, Herlev,

Denmark as previously described [5-8]. The macrophage activation markers, sCD163 and sMR, were quantified by in-house ELISAs as previously described [13,15].

Liver stiffness

Liver stiffness was assessed in the patients using transient elastography by FibroScan (Echosens, Paris, France) or by acoustic radiation force impulse (ARFI) technique as previously described [16,17].

Statistical methods

The dynamics of the collagen markers over time were analysed by multivariate repeated measurements analysis of variance (ANOVA) using mixed models; in some analyses taking baseline disease severity into account. Using this method, the paired design is taken into account as well as the missing values. When standard deviations and correlations between groups were unequal, this was considered in the models. In addition, model validation was performed by inspecting QQ-plots. Data were log-transformed when appropriate to obtain normality based on histograms and QQ-plots. Correlations between two continuous variables were performed using Spearman rank correlations. The data are reported as medians (back-transformed means) with 95% confidence intervals unless otherwise stated. P-values below 0.05 were considered statistically significant. The analyses were performed in Stata version 14.2.

Results

Patient and treatment characteristics

We included 77 patients; 14 (18%) with advanced liver fibrosis and 63 (82%) with cirrhosis. Baseline patient and treatment characteristics are presented in Table 1. In summary, the cirrhosis patients were older, had lower platelet counts, and had higher INR, alpha-fetoprotein, and liver stiffness compared with the patients with advanced liver fibrosis. Eight patients developed hepatocellular carcinoma (HCC) during follow-up; seven *de novo* and one recurrence HCC. Of the 77 included patients, 74 (96%) achieved a sustained virologic response (SVR) and data from all patients were analysed. Reasons for not achieving SVR were: reinfection with a different genotype (n=1), not initiating DAA therapy (n=1), and death (n=1).

Collagen formation and degradation during and after DAA therapy

During the study, the levels of PRO-C3, C3M, and C4M decreased significantly ($p<0.00001$), while the PRO-C4 level remained without significant changes ($p=0.20$). This was also the case in the subset of patients with all available samples at baseline, EOT, 12-weeks and one-year post-treatment (n=24). At baseline, the PRO-C3 level was 24.0 ng/mL (21.2-26.8) followed by a 22% decrease to 18.6 (16.4-20.9), at EOT ($p<0.0001$) and an additional 16% decrease to 15.6 (13.6-17.5) at one-year post-treatment ($p=0.01$) (Figure 1A). The baseline level of C3M was 9.3 ng/mL (8.6-10.0), and decreased with 6% (8.7 (8.0-9.4), $p=0.001$) from baseline to EOT with no additional change between EOT and one-year follow-up (8.5 (7.8-9.2), ($p=0.21$)) (Figure 1A). For PRO-C4, the baseline level was 158.2 ng/mL (142.0-174.4) and there was no change during or after therapy ($p=0.20$) (Figure 1A). At baseline, the level of C4M was 20.9 ng/mL (19.0-22.9) and decreased 10% during treatment (18.8 (17.0-20.6), $p<0.0001$). There was no additional change between EOT and one-year follow-up (18.9 (17.1-20.8), ($p=0.78$)) (Figure 1A).

When assessing the relationship between collagen formation and degradation, we observed a steep decrease of 16% in the PRO-C3/C3M ratio during DAA therapy ($p=0.01$), and a further 16% decrease during follow-up ($p=0.02$) (Figure 1B). There was no change in the PRO-C4/C4M ratio during DAA therapy or follow-up ($p>0.27$), even though the level at one-year follow-up tended to be increased compared with baseline ($p=0.07$) (Figure 1B).

Associations with cirrhosis

To account for the effect of disease severity at baseline, we separated the patients into two groups with either advanced fibrosis or cirrhosis. The collagen markers showed similar dynamics in both patient groups for each collagen marker (Figure 2). However, the cirrhosis patients had 20% higher C3M levels at all time points compared with the patients with advanced fibrosis (p=0.04), and approximately 25% higher levels of PRO-C4 and C4M (p<0.04). The difference in PRO-C3 between the patients with advanced fibrosis and the patients with cirrhosis was not significant (p=0.15).

Associations with liver stiffness

At baseline, there were significant correlations between C3M, PRO-C4, and C4M and liver stiffness (r>0.39, p<0.001), whereas PRO-C3 did not correlate with liver stiffness (r=0.23, p=0.1) (Table 2). At one-year follow-up, PRO-C3, C3M and PRO-C4 correlated well with liver stiffness (r>0.47, p<0.01), while C4M did not (r=0.23, p=0.21) (Table 2). There were no associations between the collagen markers and liver stiffness by ARFI-scans (data not shown).

High and low fibrogenesis activity

To investigate the effects of high or low fibrogenesis activity, we separated the patients into those with baseline PRO-C3 above 20 ng/mL (high activity, n=46, 61%) or below 20 g/mL (low activity, n=29, 38%) based on a previous study [18]. The patients with highly active fibrogenesis had higher levels of sCD163 (7.2 vs. 5.7, p=0.04) and slightly higher levels of INR at baseline (1.2 vs. 1.1, p=0.02). Patients with high fibrogenesis activity at baseline decreased more in PRO-C3 during treatment, while the dynamic of PRO-C3 was similar after EOT for patients with high as well as for patients with low fibrogenesis activity (p=0.12) (Figure 3A). For C3M, the dynamics differed (p=0.02), but there were no differences between the two groups at any specific time points (Figure 3B). Patients with high fibrogenesis activity tended to have higher levels of PRO-C4 at baseline, during treatment and at follow-up (p=0.08) (Figure 3C). The C4M level at baseline was approximately 25% higher in patients with high fibrogenesis activity, and this high level was sustained during treatment and at follow-up (p=0.02) (Figure 3D). Patients with high fibrogenesis activity were more likely to have an improved liver stiffness response (Figure 4). Those patients were among the 50% of patients with the largest decrease in liver stiffness at one-year follow-up (p=0.04). In addition, the decrease in liver stiffness from baseline to one-year follow-up also tended to be more distinct in the patients with high fibrogenesis activity (34% vs. 21%, p=0.08).

Associations with HCC

The PRO-C3 level at EOT was significantly higher in patients who developed HCC compared with those who did not (28.3 (13.3-60.5) vs. 18.2 (16.1-20.7), p=0.04) and at 12 weeks post-treatment (34.1 (9.3-124.5) vs. 17.9 (15.9-20.2), p=0.007). There were no differences in PRO-C3 levels between the two groups at baseline or at one-year follow-up (p>0.60). The C3M level tended to be higher at 12 weeks post-treatment in patients who developed HCC post-treatment compared with those who did not (11.0 (7.4-16.5) vs. 8.3 (7.5-9.2), (p=0.06). However, there were no differences between the two groups at baseline, at end of treatment, or at one-year follow-up (p>0.21). The PRO-C4 and C4M levels were comparable between patients with and without HCC at all timepoints (p>0.28).

Associations between blood and clinical parameters

The collagen markers were significantly correlated with the two macrophage activation markers, sCD163 and sMR at baseline and during follow-up. At baseline and EOT, all the collagen markers correlated with sCD163 and sMR (r>0.27, p<0.02). At one-year follow-up, all correlation with sMR disappeared, whereas the correlations with sCD163 remained (r>0.39, p<0.02) (Table 3).

In addition, all the collagen markers correlated with the model of end-stage liver disease (MELD)-score at baseline (r>0.24, p<0.04), this was not the case during follow-up.

The collagen markers were not affected by the presence of type 2 diabetes (n=14) and the dynamics of the collagen markers were not different between patients with and without diabetes and the levels were similar at baseline, EOT, and after treatment.

Discussion

The main findings of the present study were that PRO-C3, C3M and C4M levels decreased significantly in CHC patients during DAA therapy. PRO-C3 continued to decrease during the one-year follow-up after treatment. Further, we demonstrated a decrease in the PRO-C3/C3M ratio suggesting an altered balance in favour of collagen degradation over formation during anti-viral therapy. Cirrhotic patients had higher levels of C3M, PRO-C4, and C4M; however, the dynamics of the collagen markers were similar between patients with cirrhosis and patients with advanced fibrosis. Interestingly, the collagen markers correlated with liver stiffness at multiple time points. Further, PRO-C3 was elevated at EOT and 12 weeks post-treatment in patients who developed HCC. Therefore, the collagen markers may potentially be used as biomarkers for disease severity and treatment response.

The results are strengthened by the prospective design, whereas the major limitation pertains to the lack of liver biopsies for fibrosis assessment. However, the use of liver stiffness as a liver fibrosis predictor is well-established in the literature and studies demonstrated good concordance between liver stiffness and histological fibrosis stages [19,20]. In addition, this is supported by the correlation between liver stiffness and the collagen markers. Another limitation pertains to the uncomplete dataset during follow-up, but as the missing data are due mainly to logistic reasons, we have no reason to suspect a systematic loss to follow-up owing to for example disease severity. In addition, we performed statistical analyses taking missing data into account. Further, we performed the analyses in the subset of patients with all available samples at baseline, EOT, and 12-weeks and one-year post-treatment and found similar results.

Much research concerning fibrosis markers in CHC have been conducted, however, there is still an unmet need for better disease specific markers, especially after therapy where no markers have been robustly validated. Most fibrosis markers are indirect markers, whereas the collagen markers in our study are direct markers of ECM turnover and thus reflect formation and degradation of collagen specifically. Additionally, they were not affected by the presence of diabetes. Of importance is the evaluation of the two different types of collagen and it should be kept in mind that they originate from different cells, and are different in structure and function [21]. PRO-C3 levels have previously been shown to be elevated in CHC patients with liver fibrosis and to a similar extent as we observed prior to treatment [22,23]. In addition, PRO-C3 predicts fibrosis

progression in CHC patients [18,24] and lower C4M levels are associated with survival in female patients with decompensated cirrhosis [25]. In addition, a model combining PRO-C3 and C4M with age, BMI, and sex was predictive of fibrosis presence in biopsies from CHC patients [26]. Compared with the previous studies, the present study is the first to evaluate the collagen markers in a prospective set-up with CHC DAA therapy.

Before treatment, the PRO-C3/C3M ratio was high suggesting that the formation of ECM exceeds degradation and the ECM turnover therefore is tipped towards fibrosis formation. During treatment, when the virally-induced liver damage ceases, the PRO-C3 level and PRO-C3/C3M ratio decreases. This could reflect both inflammatory resolution and fibrosis regression. The concomitant decrease in the macrophage activation markers during treatment is in favour of inflammatory resolution. In addition, the correlation between the collagen markers and macrophage activation markers indicates that macrophage activation is associated with activation of fibroblasts, since fibroblasts are the main collagen producing cells in fibrosis [27]. This is also supported by the higher level of sCD163 at baseline in the patients with highly active fibrogenesis. There was a prolonged effect on PRO-C3 after treatment, where the level continued to decrease, which may reflect fibrosis regression. This suggests that after DAA therapy, ECM turnover is reversely tipped towards fibrosis resolution. The combination of significant associations between the collagen markers and liver stiffness with the increased levels of PRO-C4, C3M, and C4M in the cirrhosis patients indicate that the collagen markers reflect the actual fibrosis stage. The fact that the PRO-C3 level was not increased in the patients with cirrhosis could be due to the severity stages being close to each other and the limited number of patients with advanced liver fibrosis. Together, these findings combined with the previous establishment of especially PRO-C3 as a liver fibrosis marker indicate the relevance of assessing ECM turnover also after DAA therapy as a potential fibrosis assessment. The results are additionally supported by several studies assessing portal hypertension after DAA. These studies indicate that at least at long term follow-up after DAA therapy portal hypertension is ameliorated correlating with fibrosis regression and with the potential of decreasing the risk of hepatic decompensation [28-30].

The PRO-C4/C4M ratio tended to be increased at one-year follow-up compared with baseline, which could indicate that basement membrane formation may increase after viral clearance potentially reflecting the structural support for cell regeneration as part of the repair process.

Interestingly, PRO-C3 did not correlate with liver stiffness at baseline, whereas C3M and the type IV markers did, but at one-year follow-up there was a good correlation between PRO-C3 and liver stiffness. These findings could indicate high activity at baseline and more stable fibrosis at follow-up. When separating patients into those with high and low fibrogenesis activity defined as above or below an initial PRO-C3 level of 20 ng/ml, several differences were found. The patients with high activity had more pronounced decreases in PRO-C3 and C3M over the entire study period compared with the patients with low activity. In addition, the patients with highly active fibrogenesis were more likely to have achieved an improved liver stiffness response after treatment. These results could suggest that high fibrogenesis activity may predict a better liver response as a result of viral clearance.

HCC development is dependent on the amount of collagen in the liver [31,32], and we observed increased PRO-C3 levels at EOT and 12-weeks follow-up in patients who developed HCC with the reservation that only eight patients developed HCC. In perspective, these results may suggest PRO-C3 as marker of HCC development, but require a larger and prospective study to further address this question. In addition, we speculate that the decreasing levels of the collagen markers will continue to decrease as long as active fibrosis regression occurs. However, this requires a study with longer follow-up, which is also warranted to validate our findings. Advantageously, such a study would also include more patients with advanced fibrosis and preferably also patients with lower stage fibrosis.

In conclusion, the plasma concentrations of the applied markers, for type III and IV collagen formation and degradation, decrease during and after successful DAA therapy in CHC patients with advanced liver disease. These markers are correlated with liver stiffness at baseline and during follow-up. The results indicate an altered balance between collagen formation and degradation after viral clearance suggestive of a favourable effect on liver fibrosis and with implications as biomarkers for disease severity and treatment response.

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Tables

Table 1. Baseline patient and treatment characteristics

	Advanced liver fibrosis (n=14)	**Cirrhosis (n=63)**
	Numbers (%)	
Gender (male)	7 (50)	44 (70)
Diabetes	1 (7)	13 (21)
Alcohol (none/occa./previous†)	9 (64) / - / 5 (36)	28 (44) / 1 (2) / 34 (54)
Genotype		
1	2 (14)	29 (46)
2	-	2 (3)
3	11 (79)	31 (49)
4	-	1 (2)
6	1 (7)	-
Ethnicity (Caucasian)	13 (93)	59 (94)
DAA (sofosbuvir/3D)	14 (100) / 0 (0)	48 (76) / 15 (24)
Child-Pugh Class (A/B)	-	53 (84) / 7 (11)
Previous HCC	-	3 (5)
	Median (95% CI)	
Age (Years)	49 (41-61)	57 (54-59)*
BMI (kg/m^2)	26 (21-31)	25 (24-27)
MELD score	7 (7-9)	8 (7-8)
Liver stiffness		
FibroScan (kPa) (n=53)	11 (8-15)	25 (21-36)*
ARFI-scan (m/s) (n=24)	1.6 (1.5-2.0)	2.0 (1.8-2.2)*
ALT (IU/L)	98 (27-169)	76 (58-91)
Bilirubin (umol/L)	8 (7-9)	10 (8-13)
Albumin (g/L)	36 (34-40)	36 (35-38)
Creatinine	75 (58-91)	70 (63-73)
Platelets (*10^9/L)	181 (122-228)	136 (119-162)*
INR	1.1 (1.0-1.2)	1.2 (1.1-1.2)*

AFP (x10^3 IU/L)	2 (1-4)	5 (4-8)*

†none, no history of excessive alcohol consumption; occa., occasional alcohol use; previous, previous over intake. DAA, direct-acting antiviral. 3D, combination DAA therapy consisting of ombitasvir, paritaprevir, ritonavir, and potentially dasabuvir. HCC, hepatocellular carcinoma. CI, confidence interval. BMI, body mass index. MELD, model of end-stage liver disease. ALT, alanine aminotransferase. INR, international normalized ratio. * indicates $p<0.05$, when comparing advanced liver fibrosis patients with cirrhosis patients.

Table 2. Associations between the collagen markers and liver stiffness by FibroScan.

	Baseline		EOT		12-weeks		1-year	
	rho	p-value	rho	p-value	rho	p-value	rho	p-value
PRO-C3	0.23	*0.1*	0.3	*0.07*	0.23	*0.23*	0.53	0.002
C3M	0.52	0.0001	0.47	0.004	0.24	*0.23*	0.47	0.01
PRO-C4	0.43	0.001	0.34	0.04	0.09	*0.66*	0.47	0.01
C4M	0.39	0.004	0.04	*0.8*	-0.02	*0.92*	0.23	*0.21*

EOT, end of treatment. 12-weeks, 12 weeks after treatment cessation. 1-year, one year after treatment cessation. Italics indicate $p>0.05$.

Table 3. Associations between the collagen markers and the macrophage activation markers

		Baseline		EOT		12-weeks		1-year	
		rho	p-value	rho	p-value	rho	p-value	rho	p-value
PRO-C3	sCD163	0.35	0.002	0.45	0.0003	0.35	0.04	0.49	0.004
	sMR	0.27	0.02	0.4	0.002	0.3	*0.08*	0.15	*0.36*
C3M	sCD163	0.52	<0.00001	0.51	<0.00001	0.46	0.005	0.39	0.02
	sMR	0.43	<0.00001	0.44	0.0005	0.42	0.01	0.21	*0.18*
PRO-C4	sCD163	0.49	<0.00001	0.5	0.0001	0.41	0.01	0.53	0.001
	sMR	0.43	0.0001	0.48	0.0001	0.34	0.04	0.26	*0.1*
C4M	sCD163	0.35	0.002	0.33	0.01	0.31	*0.07*	0.4	0.02
	sMR	0.41	0.0002	0.34	0.01	0.11	*0.53*	0.25	*0.12*

EOT, end of treatment. 12-weeks, 12 weeks after treatment cessation. 1-year, one year after treatment cessation. Italics indicate $p>0.05$.

Figure legends

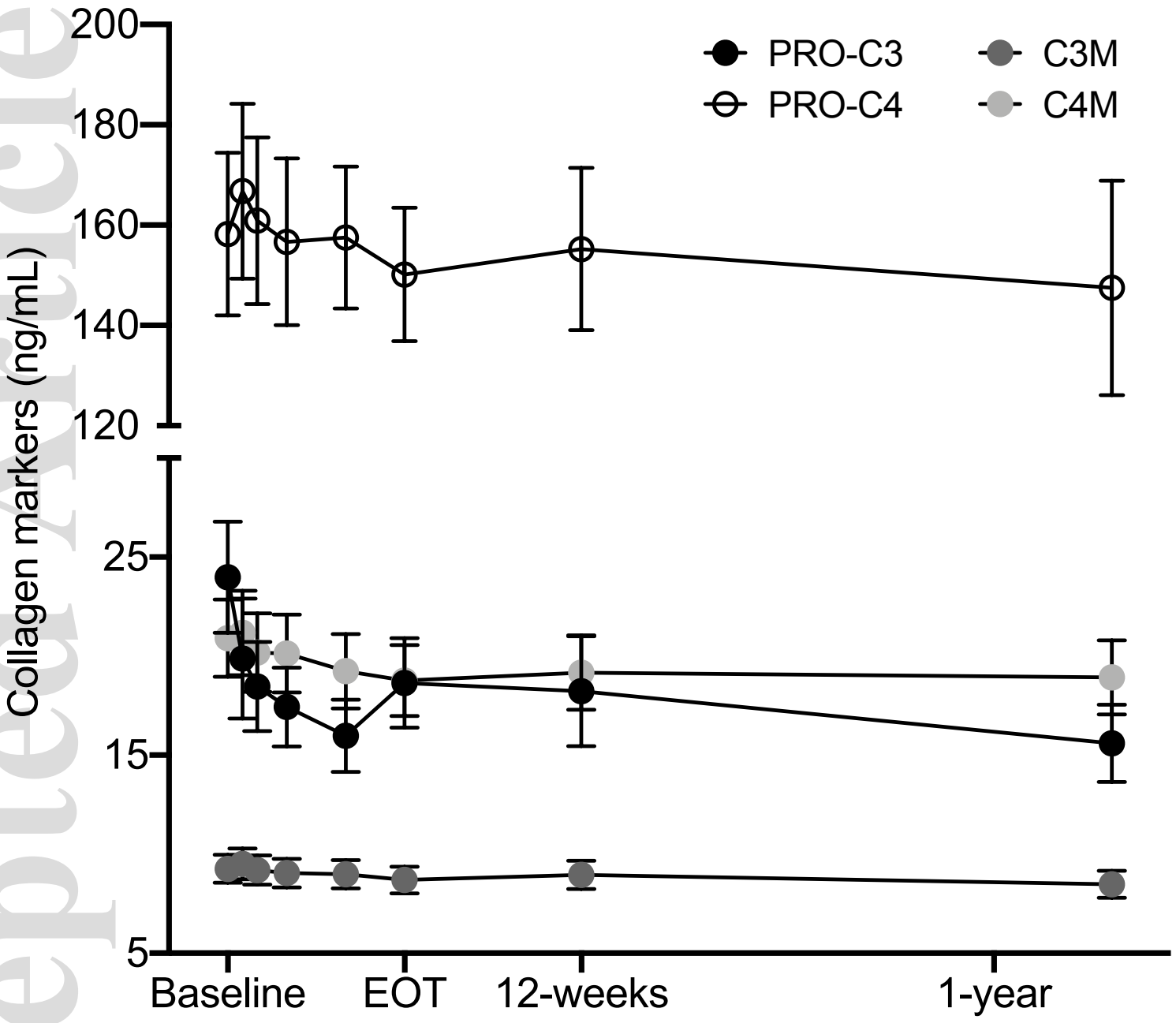
Figure 1. Dynamics of the collagen markers during and after DAA therapy. A) PRO-C3, C3M, PRO-C4, and C4M. B) PRO-C3/C3M ratio and PRO-C4/C4M ratio

Figure 2. Dynamics of the collagen markers separated according to baseline disease severity in cirrhosis patients and patients with advanced liver fibrosis (- cirrhosis). A) PRO-C3, B) C3M, C) PRO-C4, and D) C4M.

Figure 3. Dynamics of the collagen markers separated according to baseline level of PRO-C3. A PRO-C3 level > 20 ng/mL is defined as high fibrogenesis activity and <20 ng/mL as low fibrogenesis activity. A) PRO-C3, B) C3M, C) PRO-C4, and D) C4M.

Figure 4. The change in liver stiffness according to baseline level of PRO-C3. A PRO-C3 level > 20 ng/mL is defined as high fibrogenesis activity and <20 ng/mL as low fibrogenesis activity.

A



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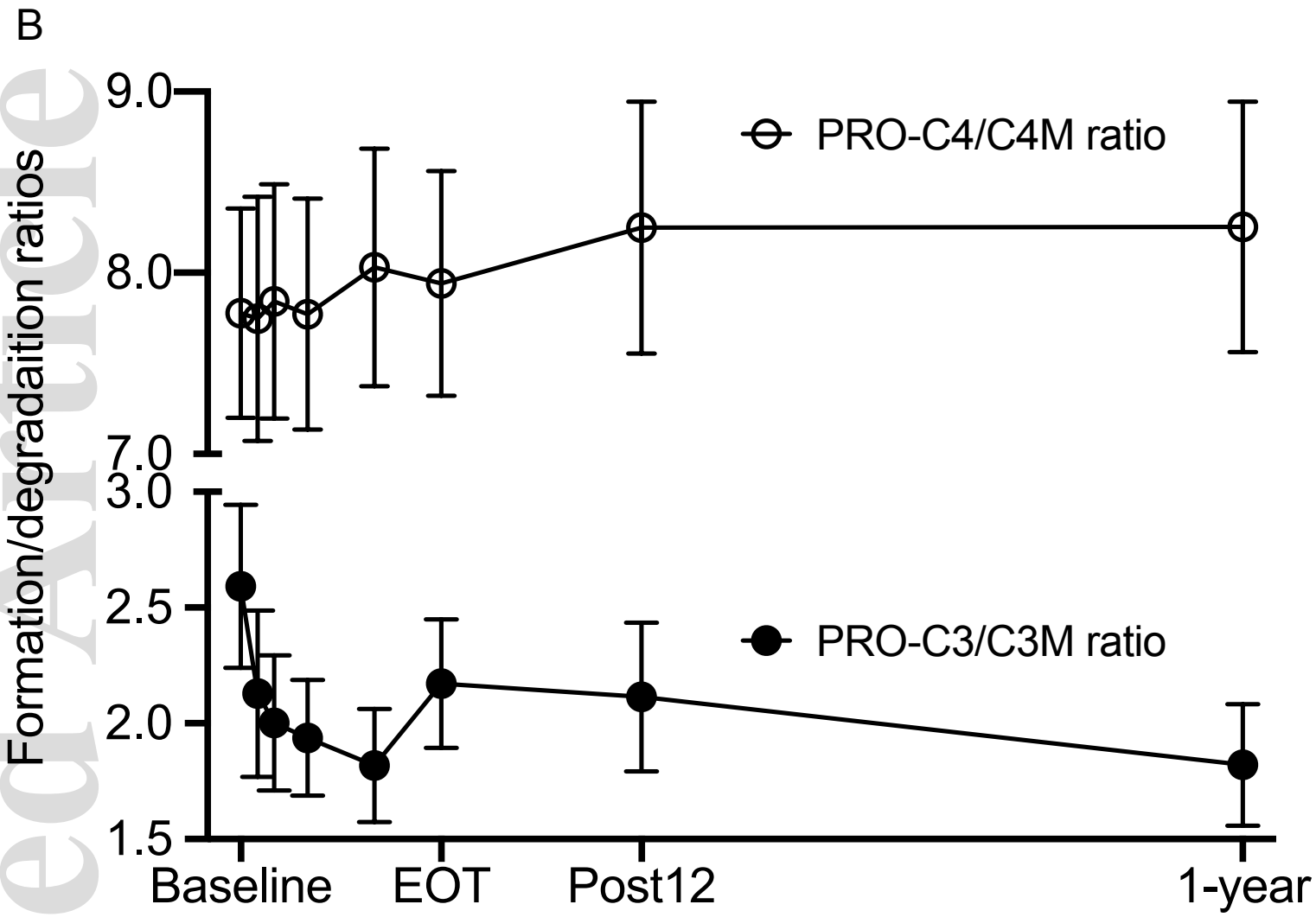

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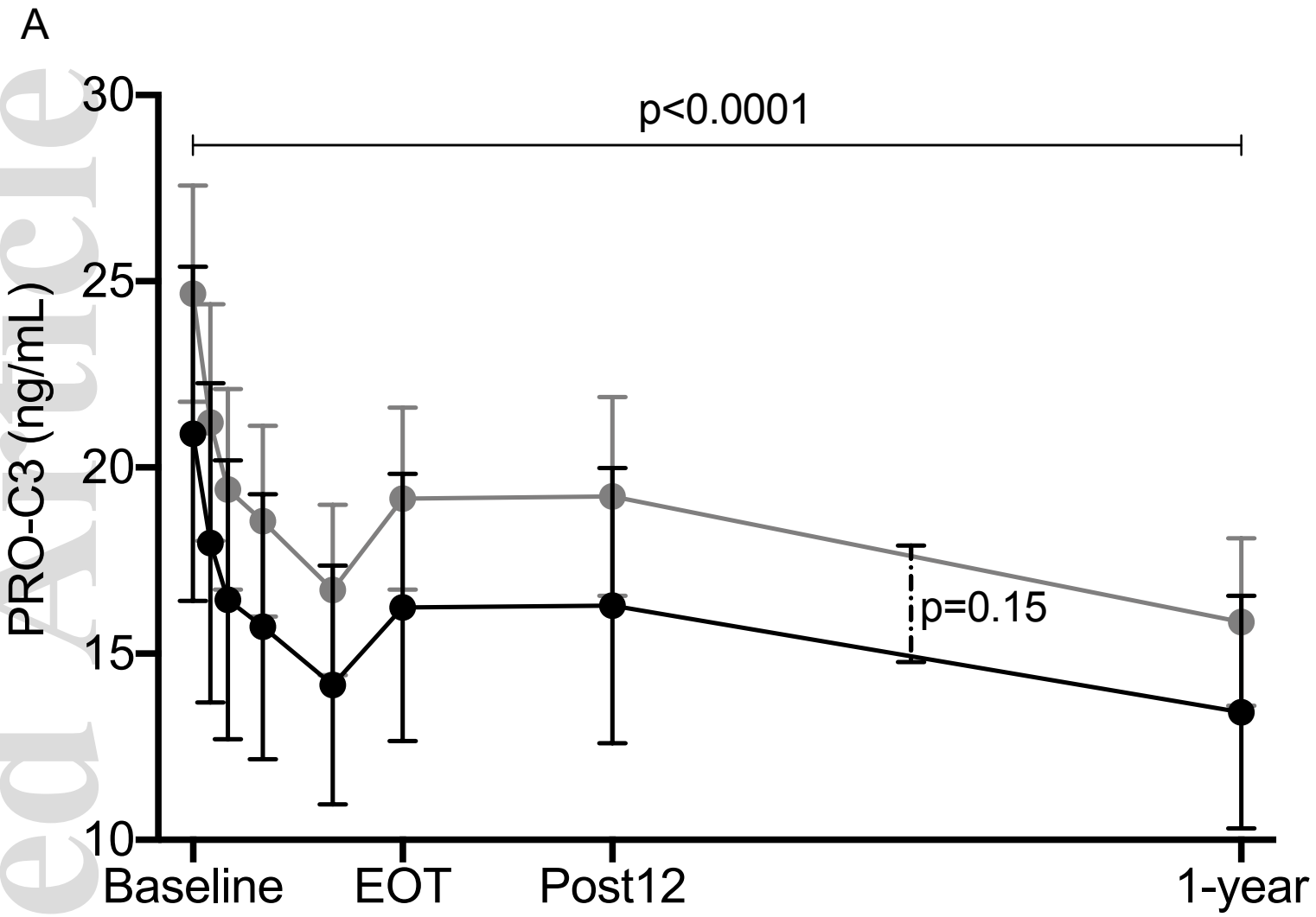

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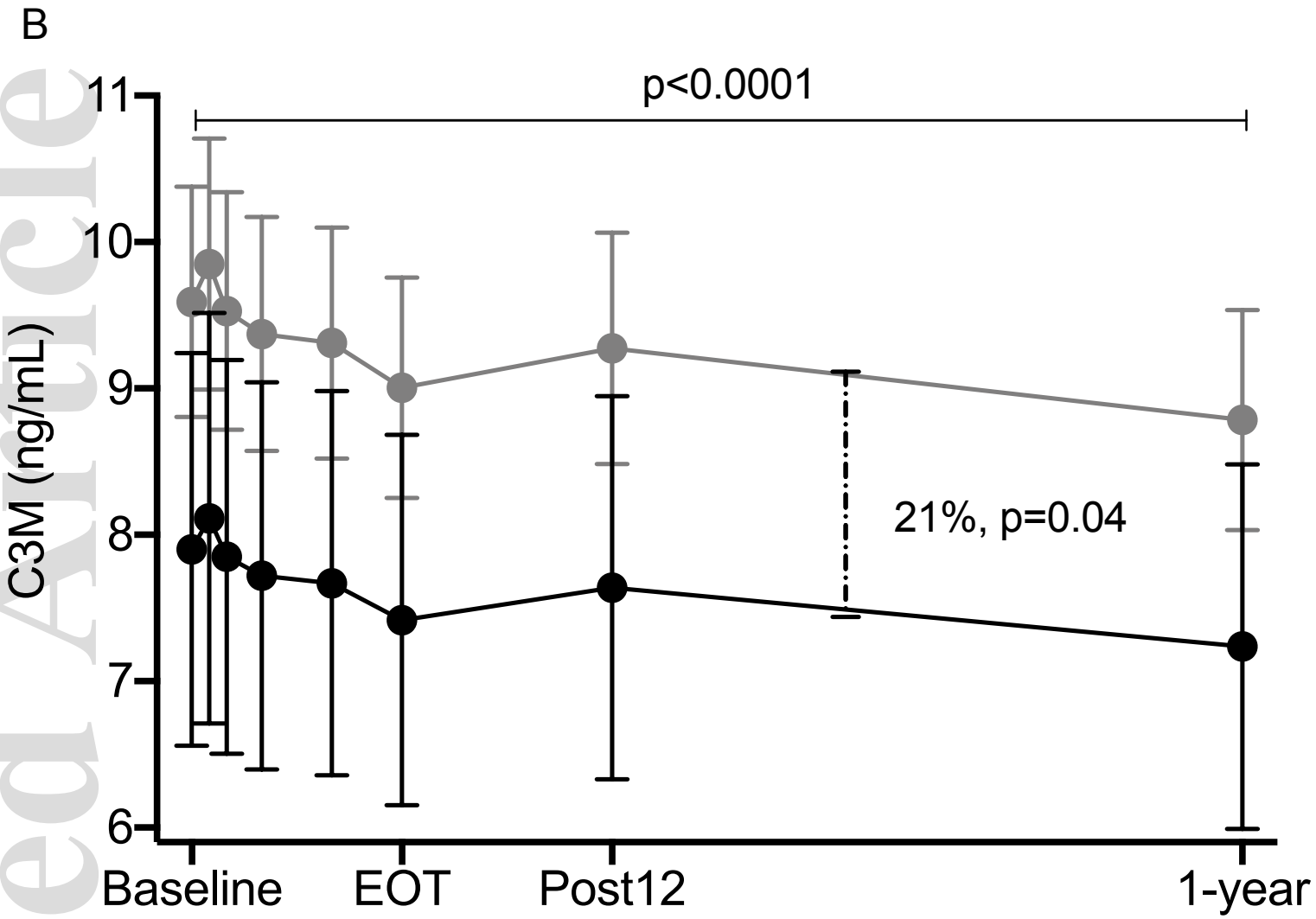

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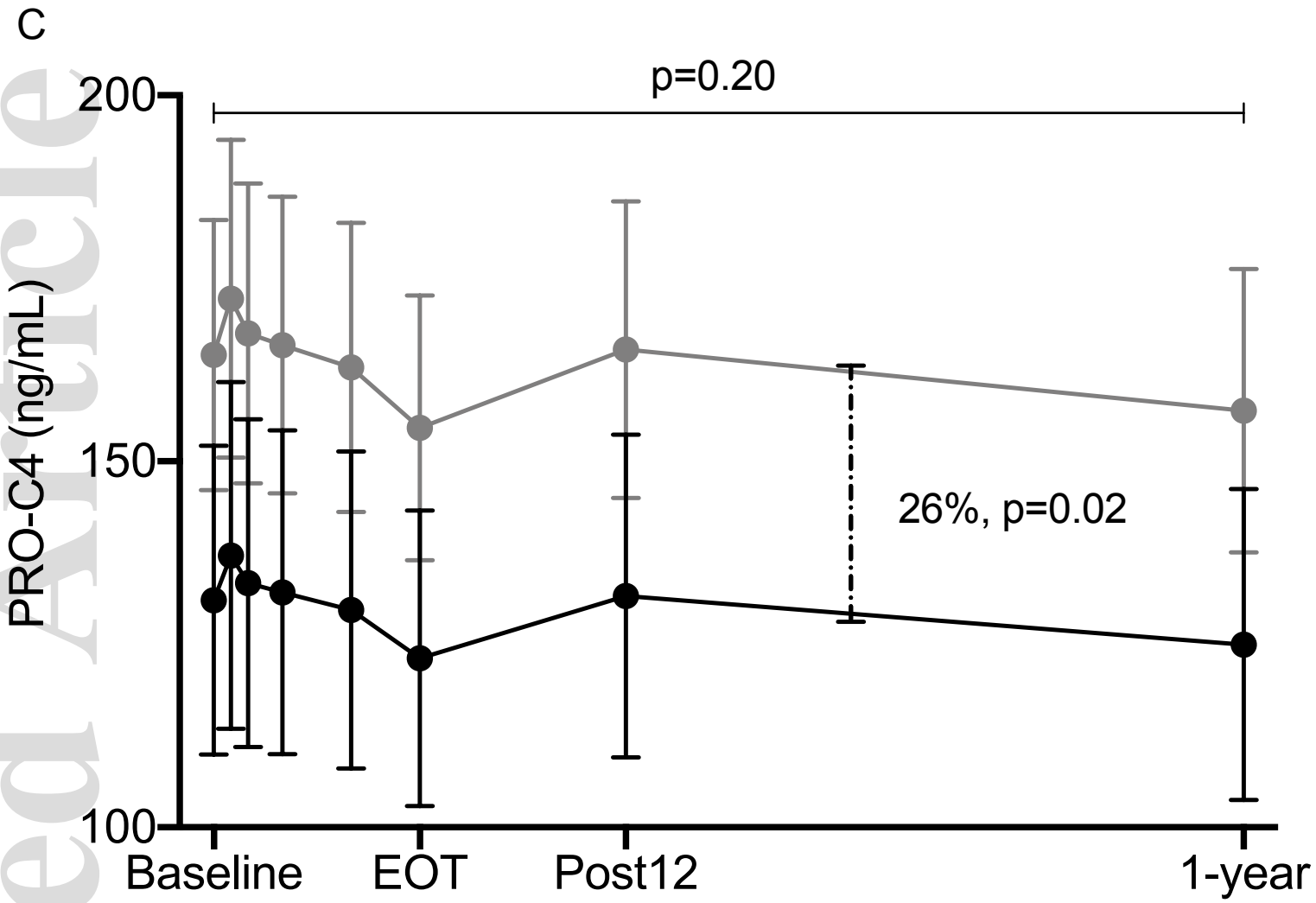

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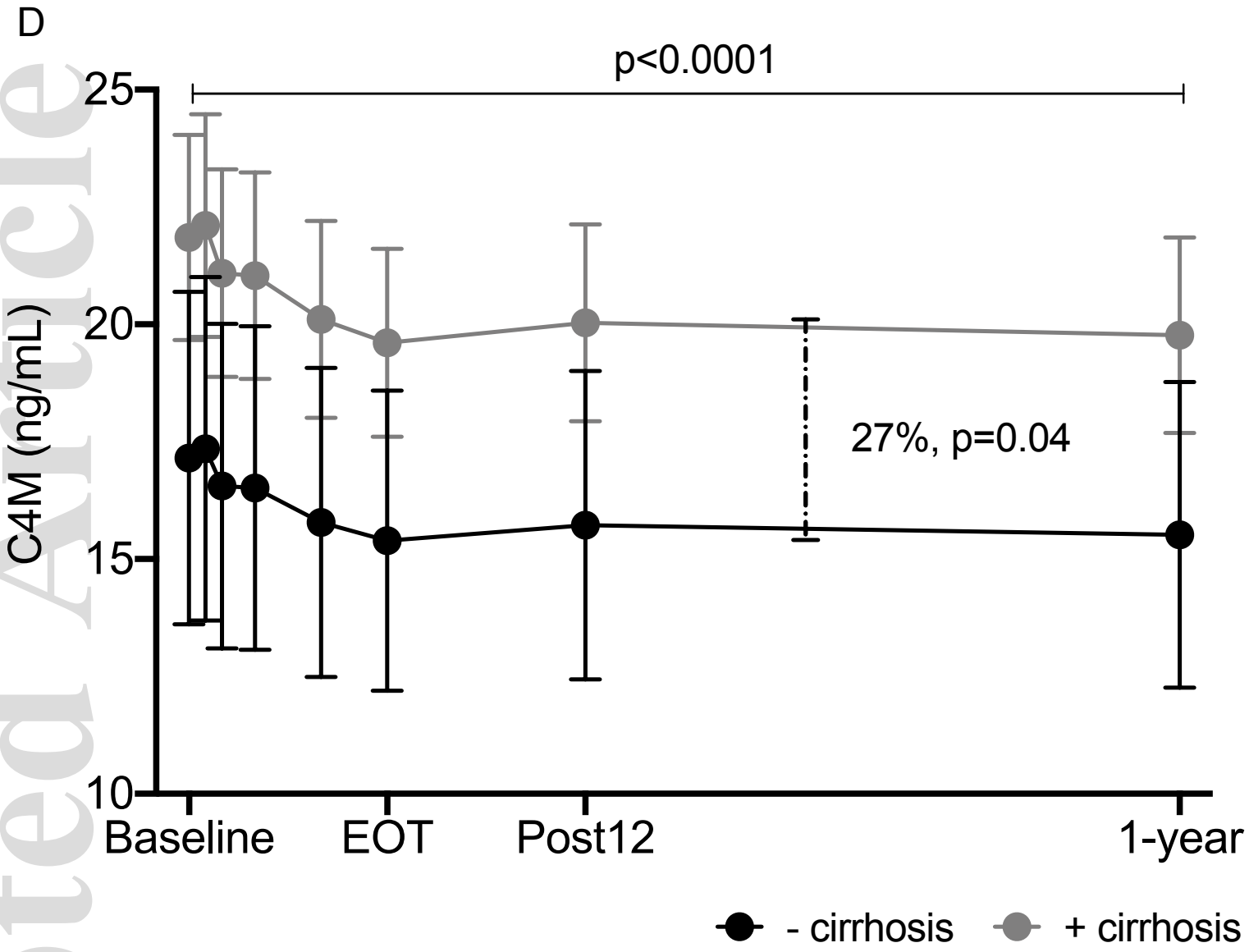

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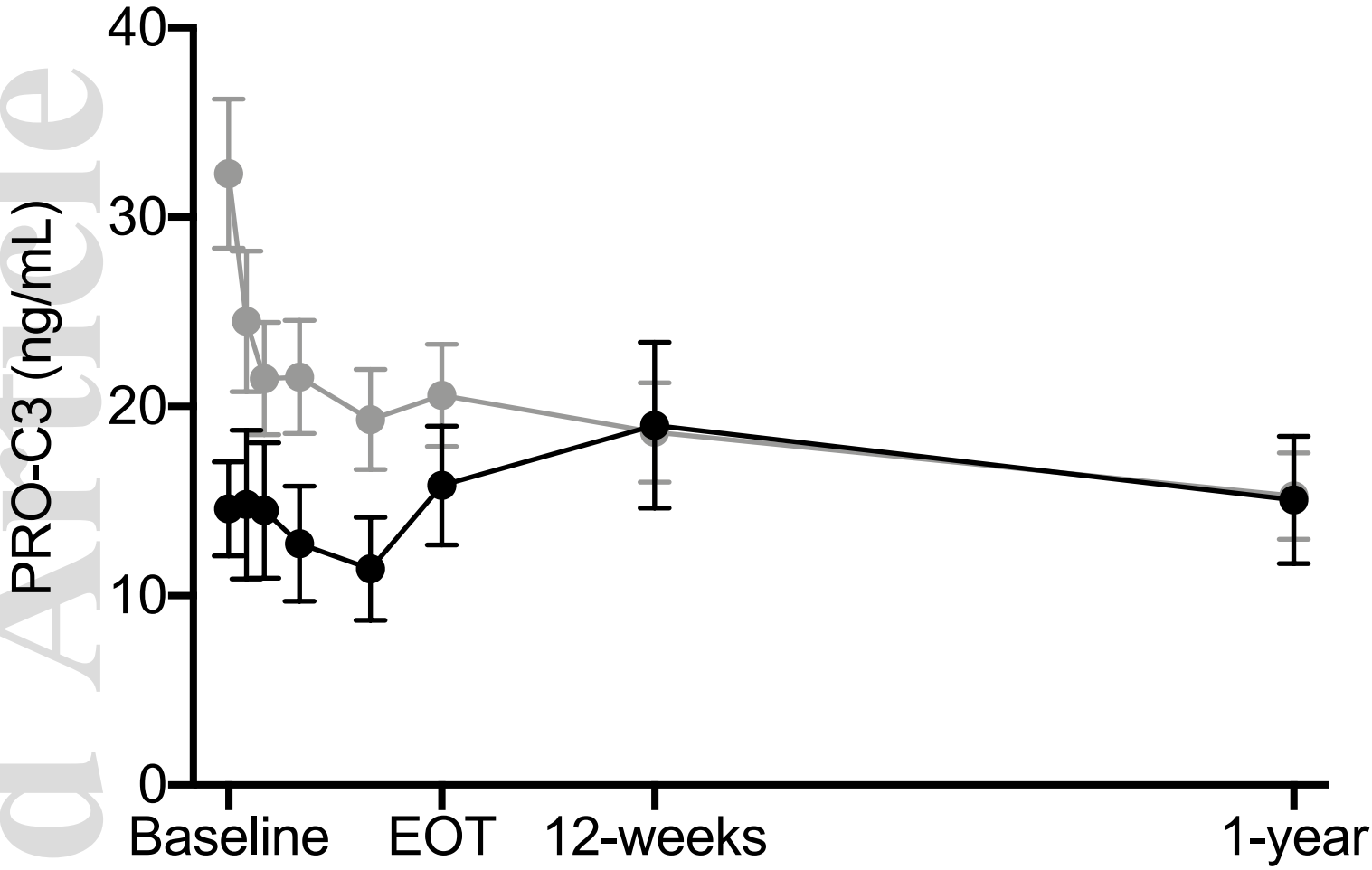

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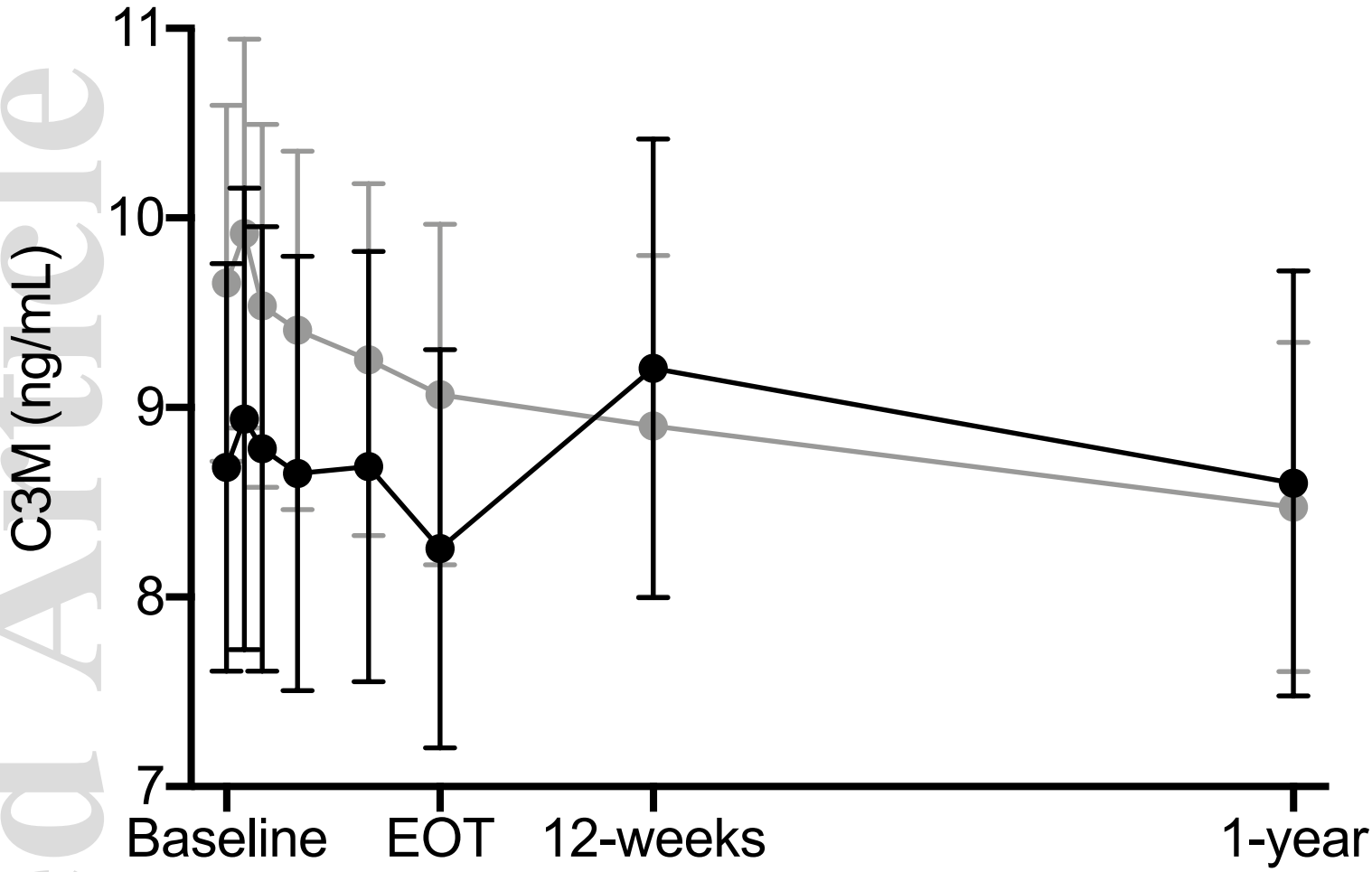

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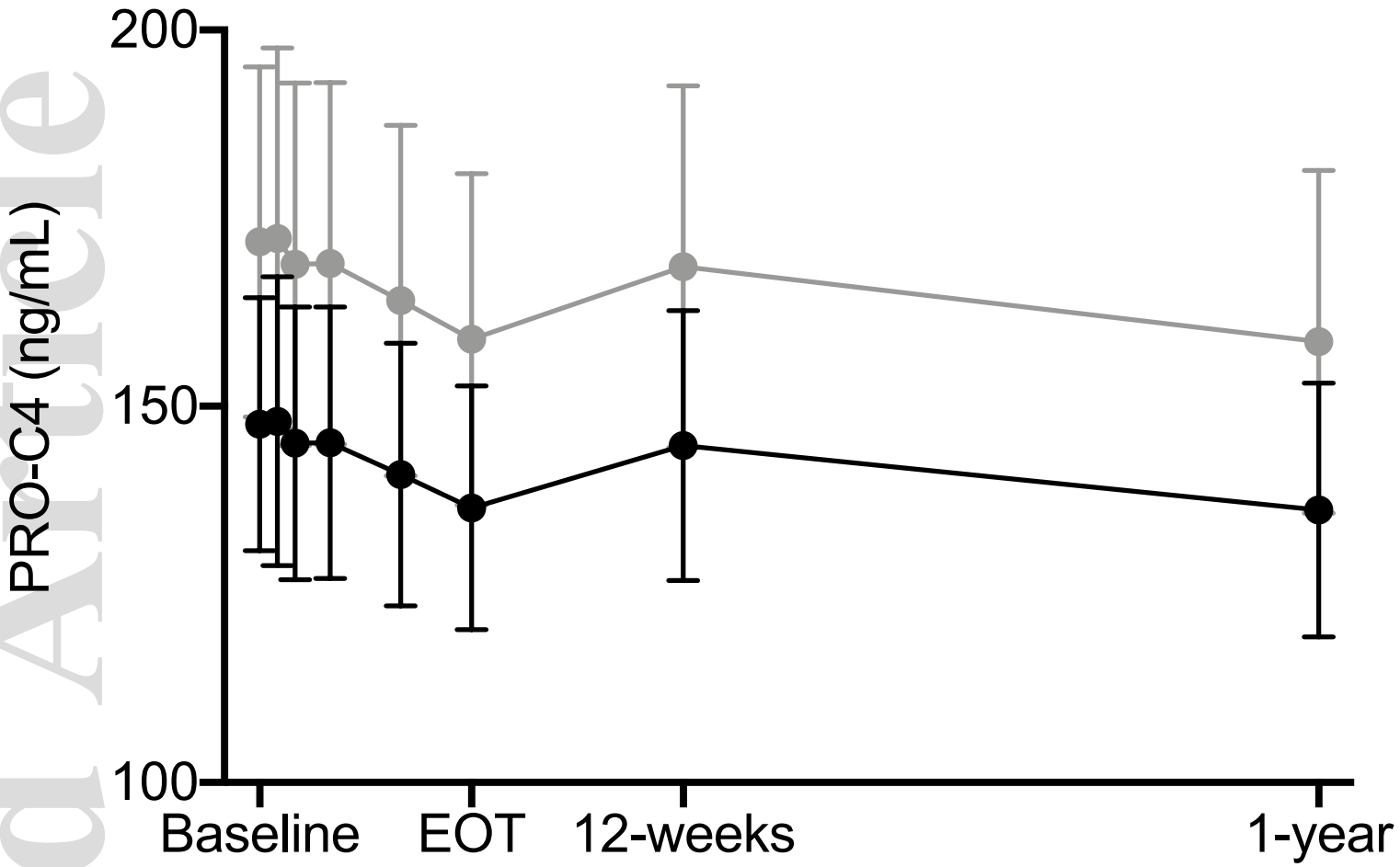

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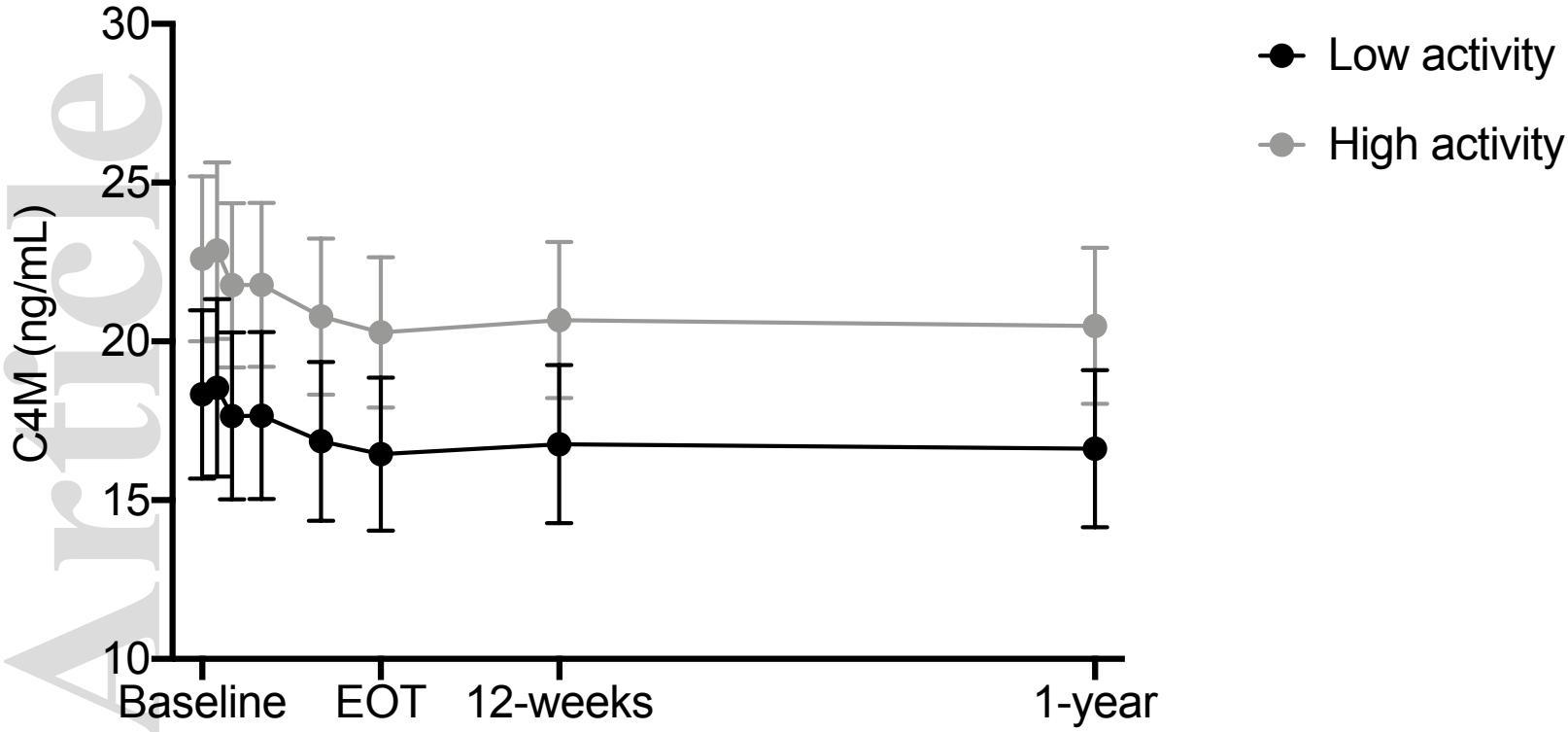

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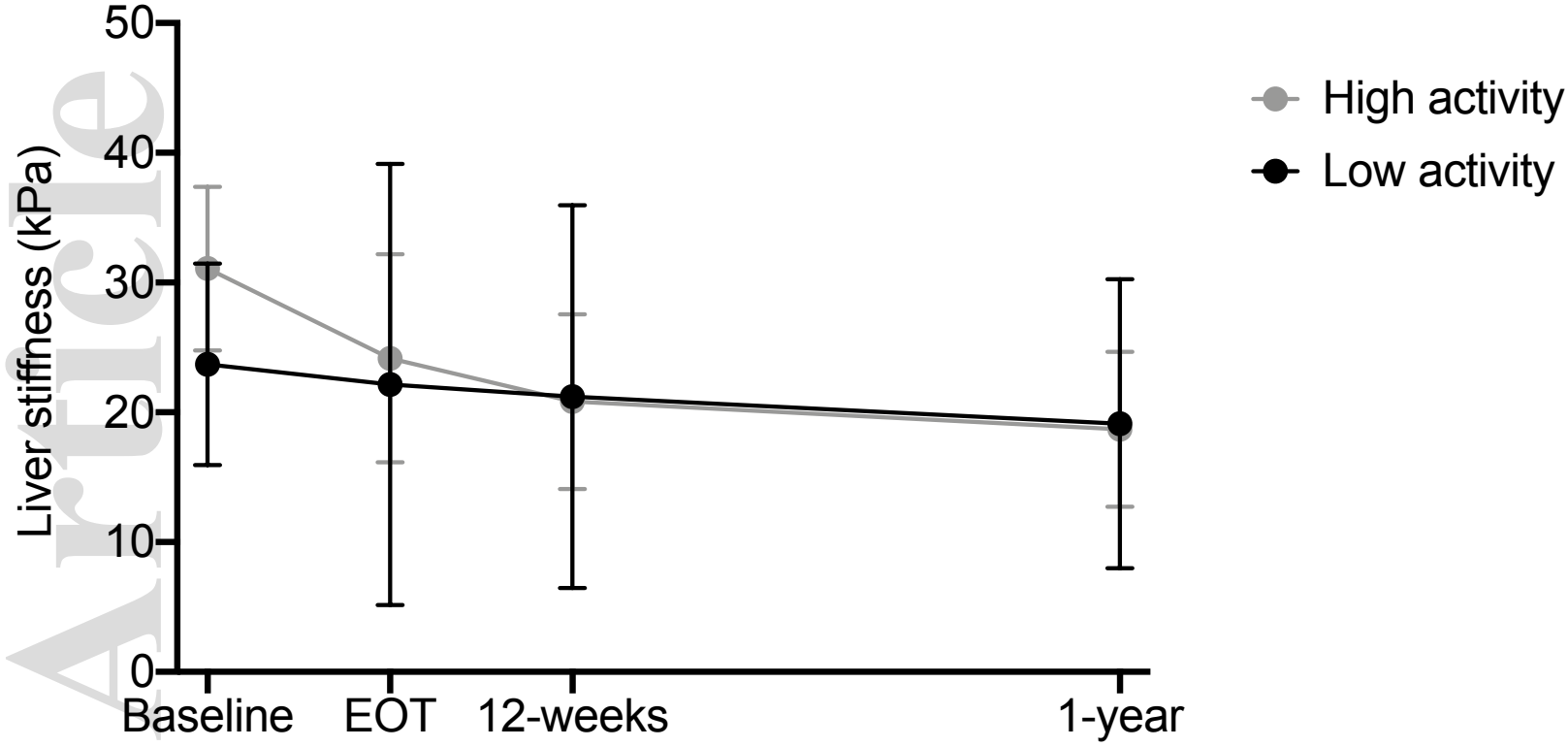

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