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# Complement proteins in axial spondyloarthritis: associations with disease activity and TNFi treatment response in a multicentre RCT

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## Abstract

**Objectives:** To investigate lectin pathway proteins (LPPs) and complement activation marker C3dg as biomarkers for disease activity and treatment response in a multicentre, randomized controlled trial of axial spondyloarthritis (axSpA) patients initiating TNF inhibitor (TNFi) therapy.

**Methods:** Serum samples from 108 patients with active radiographic axSpA from the CONSUL study, collected before and after 12 weeks of TNFi therapy, were measured using immunoassays for LPPs (MBL, CL-L1, M-, L-, and H-ficolin, MASP-1, -2, and -3, MAp44) and the complement activation marker C3dg.

**Results:** After 12 weeks of TNFi therapy, serum levels of LPPs L-ficolin, M-ficolin, and MASP-2 decreased, while MASP-3 increased after adjustment for baseline CRP. Baseline L-ficolin levels correlated positively with baseline ASDAS-CRP and BASFI. C3dg correlated positively with ASDAS-CRP. Conversely, MASP-1 and MAp44 correlated negatively with ASDAS-CRP. Assessed by univariate logistic regression, C3dg and MASP-1 were associated with treatment response of clinically important improvement (ΔASDAS-CRP $\geq$1.1) and inactive disease (ASDAS-CRP $<$1.3) at week 12, respectively. Only C3dg remained significant in a multivariate regression analysis.

**Conclusion:** In this study, complement LPPs L-ficolin, M-ficolin, and MASP-2 levels decrease following initiation of TNFi therapy, whereas alternative pathway critical component MASP-3 increases. Baseline L-ficolin and C3dg correlated positively with ASDAS-CRP, potentially by CRP influence. Nevertheless, baseline C3dg levels were associated with treatment response (ASDAS-CRP $<$1.3) at week 12. Our results provide important perspectives on the inflammatory processes in axSpA, shedding light on the involvement of the complement system related to disease activity, treatment response, and potentially to prognosis.

## Lay Summary

**What does this mean for patients?**

Axial spondyloarthritis is a condition that causes pain and stiffness in the spine, significantly affecting quality of life. In recent years, the introduction of various biological therapies has improved care for people with this disease. However, there is still much to learn about why some patients respond well to treatments while others do not. With more treatment options available, there is a need for better methods to assess disease activity and predict which treatments will work best for each patient. This could potentially reduce the time to effective treatment and improve overall prognosis. Our study focused on a part of the immune system called the complement system, involving 108 patients at high risk of severe disease progression. We found significant changes in complement protein levels after starting biological therapy. Moreover, we discovered that complement activation before treatment predicted a favourable clinical response after 12 weeks of therapy. These findings suggest that the complement system plays a role in axial spondyloarthritis. Measuring complement proteins could help doctors choose the best treatments for people with this condition, leading to more personalized and effective care.

**Keywords:** axial spondyloarthritis, biomarker, tumour necrosis inhibitor therapy, complement activation, lectin pathway.

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**Key messages**

- In axSpA patients prior to TNFi treatment, elevated levels of L-ficolin and C3dg correlated significantly with disease activity as measured by ASDAS-CRP.
- Baseline C3dg levels were predictive of a significant response to TNF inhibitor (TNFi) therapy by week 12, as demonstrated by multivariate analysis.
- Complement protein biomarkers might guide treatment decisions for axSpA patients among the growing therapeutic repertoire.

## Introduction

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease affecting an estimated 0.3–1.4% of the population, varying according to ethnicity and geographical location [1, 2]. In recent decades, significant advancements have been achieved in therapeutics [3]. These advancements have led to unmet clinical needs for monitoring disease activity and ideally predicting individual treatment responses in axSpA patients.

Studies have explored complement proteins as potential diagnostic biomarkers in axSpA [4–6]. It has been demonstrated that levels of complement proteins such as L-ficolin are elevated in axSpA patients compared with healthy blood donors [4] or chronic low back pain (cLBP) patients [5], and that levels of complement activation marker C3dg are elevated in newly diagnosed axSpA patients compared with cLBP patients [7]. Elevated levels of the terminal complement component, that is, soluble membrane attack complex (MAC), have also been observed prior to tumour necrosis inhibitor (TNFi) therapy in axSpA patients [8]. Additionally, complement involvement in axSpA pathogenesis has been supported by observations in animal models, where complement inhibition mitigates structural spinal damage associated with the disease [9] and prevents chronicity of peripheral arthritis [10].

The complement system is a cornerstone of innate immunity, orchestrated in a complex, tightly controlled cascade. It serves pivotal functions in the first line of host defence, homeostasis, and maturation of specific cells, e.g. B cells [11, 12]. The complement system can be activated via three distinct pathways, specifically the classical, lectin, and alternative pathways. All three activating pathways converge in a common terminal pathway. Essentially, all functions of the complement system are based on a biological principle of pattern recognition. Pattern recognition molecules (PRMs), such as mannan-binding lectin (MBL), collectin LK (CL-LK), or ficolins (M-ficolin, L-ficolin, and H-ficolin, also termed ficolin-1, ficolin-2 and ficolin-3, respectively), recognize molecular patterns on foreign pathogens, e.g. bacteria, or altered self-structures, e.g. apoptotic cells, often referred to as pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), respectively. When a PRM binds a compatible structure, this leads to the activation of adherent enzymes MBL-associated protease 1 (MASP-1) and MASP-2, which causes downstream complement activation [13]. This results in the release of anaphylatoxins (C3a and C5a), opsonization for phagocytosis through deposition of C3b, e.g. on surfaces of intruding bacteria or apoptotic cells, and direct clearing of the complement-activating agent through the assembling of the MAC and subsequent lysis [12].

In axSpA, elevated CRP and inflammation visualized on MRI are well-established predictors of treatment response [14, 15]. CRP is a known activator of the complement cascade, primarily through the classical pathway by interacting with C1q. Additionally, CRP can form complexes with immunoglobulins, such as IgG and IgM, which are effective activators of the classical pathway [16]. However, previous studies have shown that approximately 50% of patients with axSpA do not display elevated CRP levels [17]. Thus, CRP cannot be reliably used as a marker of disease activity in a large proportion of the patients. This fuels an ongoing exploration for alternative biomarkers beyond CRP to track axSpA disease activity. Furthermore, the expanding array of therapeutics, including targeted synthetic or biologic DMARDs (ts/bDMARDs), holds promise for tailoring treatments to individual patients. However, the realization of personalized medicine hinges on a profound understanding of the underlying inflammatory mechanisms, leaving much to be unveiled in axSpA pathogenesis.

The current study aimed to investigate complement lectin pathway proteins (LPPs) and complement activation (C3dg) as biomarkers of disease activity in radiographic axSpA (r-axSpA) patients with a high risk of radiographic progression before and after 12 weeks of TNFi therapy. Furthermore, to investigate baseline levels of LPPs and complement activation as predictors of treatment response by week 12.

## Materials and methods

### Materials

In total, 108 patients with r-axSpA were included from the multicentre randomized controlled trial (RCT) COmparison of the effect of treatment with Nonsteroidal anti-inflammatory drugs added to anti-TNF therapy versus anti-TNF therapy alone on progression of StrUctural damage in the spine over 2 years in patients with ankyLosing spondylitis (CONSUL), recruited from 16 centres in Berlin, Germany [18, 19]. The patients included in the present analysis are depicted in Fig. 1.

All included patients had active disease (BASDAI ≥4) and at least one risk factor for radiographic spinal progression (CRP >5 mg/L or existing ≥1 syndesmophyte(s)) at inclusion and were treated with TNFi, i.e. golimumab 50 mg subcutaneously every 4 weeks. Patients were allowed to have previously received bDMARD treatment if no history of primary non-response to TNFi was documented and if the previous bDMARD treatment was discontinued at least 12 weeks prior to the baseline visit. Patients were allowed to receive NSAIDs or conventional synthetic DMARDs (csDMARDs), such as methotrexate or sulfasalazine, if the dose had been stable for at least 2 weeks prior to the baseline visit.

For the presented project, the clinical evaluations and assessment of disease activity, including BASDAI and Axial Spondyloarthritis Disease Activity Score (ASDAS-CRP, hereafter referred to as ASDAS), Assessment of SpondyloArthritis International Society Health Index (ASAS-HI), and functional

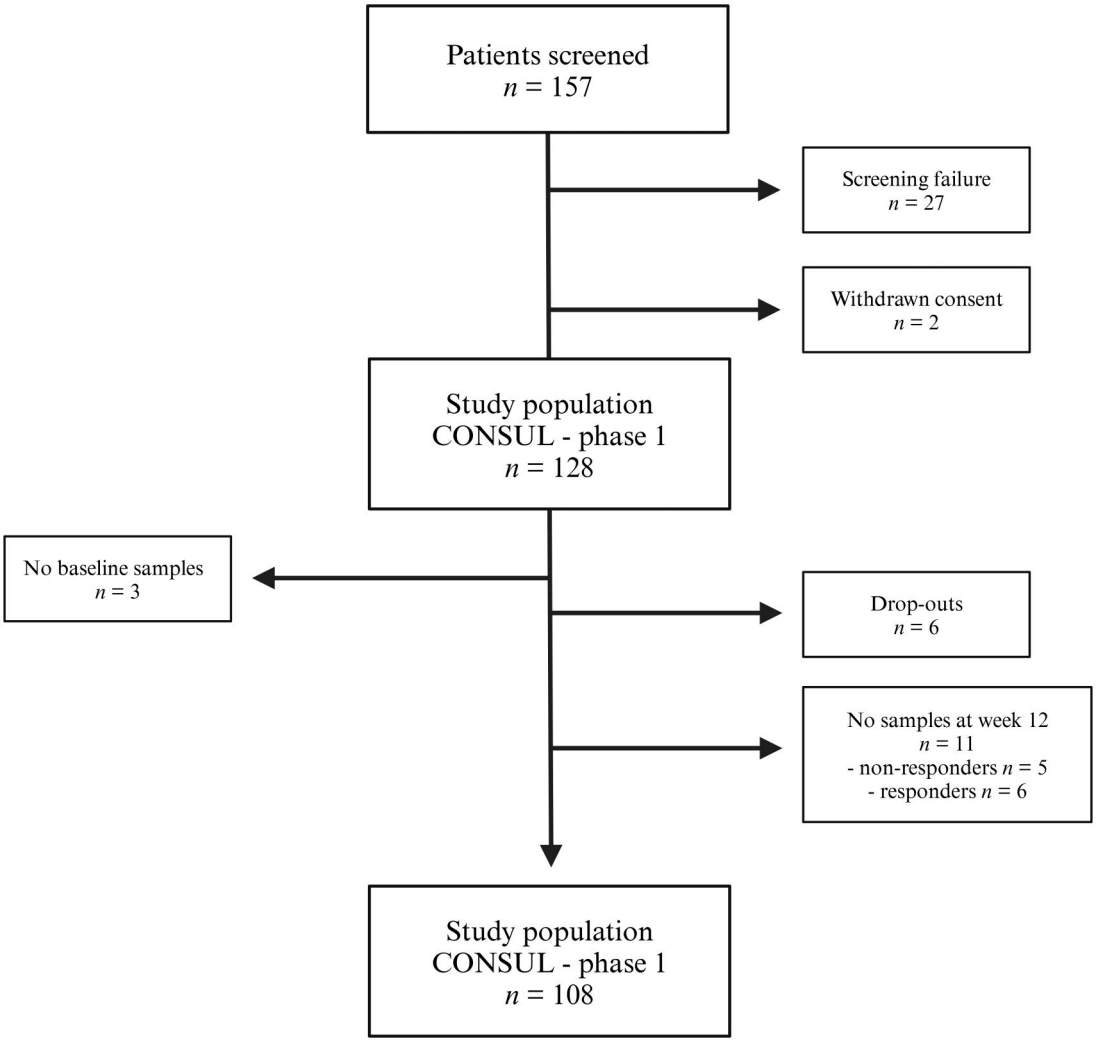


**Figure 1.** Flowchart of the included study population

impairment, i.e. BASFI, performed at baseline and after 12 weeks of treatment with golimumab were utilized. Additionally, optional serum samples collected at baseline and week 12 were analysed alongside these assessments.

## Methods

Blood samples were collected from study participants before and after 12 weeks of treatment with golimumab and handled according to a standardized protocol; samples were centrifuged in a Thermo Fisher Scientific Megafuge 1.0R at 3000 rpm using a rotor with a radius of 15 cm for 10 min at 4°C and frozen at −80°C after collection. Each sample was thawed and diluted one in four in Tris-buffered saline (TBS; 10 mM Tris, 145 mM NaCl, pH 7.4) before measurements of complement LPPs (MBL, H-ficolin, L-ficolin, M-ficolin, CL-L1, MASP-1, MASP-2, MASP-3, and MAp44) and C3dg. Time-resolved immunofluorometric assays (TRIFMAs) were applied to measure all proteins (except for L-ficolin, which was assessed using a commercially available ELISA kit (Hycult Biotechnology, Uden, the Netherlands). Detailed explanations of the specific custom-developed assays and procedures have been previously described [20–28]. Sample dilution and loading on microtiter plates were automated using a pipetting robot (JANUS, PerkinElmer, Hamburg, Germany). All analyses were performed in duplicate, and measurements were repeated if the duplicate analyses' coefficient of variation (CV) was above 15%. Inter-assay CV based on internal controls was also determined for each protein (all CVs were below 15%). Protein measurements were conducted independently, blinded to the clinical study data, ensuring an unbiased approach throughout the process.

## Statistics

Patient demographics were reported as median values with interquartile range (IQR) for continuous variables and by numbers with percentages for categorical variables due to non-Gaussian distributions. The Mann–Whitney *U* test assessed statistical comparisons for continuous variables, while the chi-square test assessed categorical variables. Serum concentrations of complement LPPs and complement activation, i.e. C3dg, at baseline and week 12 were evaluated by paired *t*-tests. Subsequent covariance analysis was performed after adjusting for baseline CRP levels. Spearman's correlation assessed levels of complement LPPs and clinical measurements of BASDAI, ASDAS, ASAS-HI, and BASFI. Correlations were also assessed for patients with baseline CRP within the normal range (≤5 mg/L).

Univariate logistic regression analyses assessed the achievement of clinical treatment responses at week 12, determined by a reduction of BASDAI ≥2, a reduction of ASDAS ≥1.1, and achievement of inactive disease, defined as ASDAS <1.3. Multivariate logistic regression analyses assessed predictors of a reduction of ASDAS ≥1.1 and accomplishment of ASDAS <1.3, as only significant observations regarding complement LPPs were observed for these measurements in the univariate analyses.

### Ethics

The written approval of the central independent ethics committee (ethics committee of the Federal State Berlin), of the German federal authority (Paul-Ehrlich-Institut, Langen, Germany), and the local ethics committees of the participating study centres was obtained. Participants gave informed written consent to participate in the study before any study-related procedures.

### Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

## Results

The demographics of the study population are shown in Table 1. Briefly, the median age was 38 years, and 71% were male. The majority was HLA-B27 positive (86%), and approximately one in four had previously received bDMARD treatment (discontinued at least 12 weeks preceding study enrolment). None of the patients received csDMARD at the time of inclusion. The median BMI was 26, and 38% were current smokers. The median time since diagnosis in the entire study population was 3 years, and the median time since the onset of symptoms was 12 years. Peripheral arthritis at any time was reported in 46% of the study population, and extra musculoskeletal manifestations (EMMs), namely psoriasis (PsO) and uveitis, were reported in 19% and 30%, respectively. None of the patients had inflammatory bowel disease. The median CRP level was 9.1 mg/L, and 69% presented elevated CRP values (>5 mg/L). The median modified stoke ankylosing spondylitis spinal score (mSASSS) was 4.3, the median number of syndesmophytes in the study population was 1.6, and 48% had at least one syndesmophyte at baseline per the aforementioned inclusion criteria. The patients showed a median ASDAS, BASDAI, and BASFI of 3.6, 6.2, and 5.2, respectively, reflecting a high disease activity. Regarding clinical characteristics, only the prevalence of PsO and time since diagnosis differed significantly in bDMARD-naïve and bDMARD-experienced patients (Supplementary Table S1, available at *Rheumatology Advances in Practice* online).

**Table 1.** Baseline demographics of the investigated patient population from the CONSUL RCT ($n = 108$)

| | |
|---|---|
| Age, median (IQR) | 38 (31–49) |
| Male, *n* (%) | 77 (71) |
| HLA-B27 positive, *n* (%) | 89 (86)[b] |
| Smokers, *n* (%) | 41 (38)[c] |
| BMI, median (IQR) | 26 (23–30) |
| NSAID intake score, median (IQR) | 100 (50–100) |
| Use of NSAID, *n* (%) | 99 (92) |
| Previous treatment with bDMARD, *n* (%) | 26 (24) |
| Time since diagnosis in years, median (IQR) | 3.0 (0.3–9.0) |
| Years since onset of symptoms, median (IQR) | 12 (6.0–21.0) |
| Arthritis ever, *n* (%) | 49 (46)[d] |
| Psoriasis ever, *n* (%) | 20 (19)[c] |
| Uveitis ever, *n* (%) | 32 (30)[d] |
| CRP, mg/L (IQR) | 9.1 (4.1–20.0) |
| Elevated CRP (>5 mg/L), *n* (%) | 75 (69) |
| mSASSS, median (IQR) | 4.3 (0.3–18)[e] |
| Syndesmophytes present, median (IQR) | 1.6 (0.0–6.3)[e] |
| Presence of ≥ 1 syndesmophyte(s)[a], *n* (%) | 43 (48)[e] |
| ASDAS-CRP, median (IQR) | 3.6 (3.1–4.1) |
| BASDAI, median (IQR) | 6.2 (5.2–7.0) |
| BASFI, median (IQR) | 5.2 (4.0–6.4) |

[a] Determined by three calibrated readers blinded for clinical data.
[b] Data available on $n = 105$.
[c] Data available on $n = 107$.
[d] Data available on $n = 106$.
[e] Data available on $n = 89$.
CONSUL: COmparison of the effect of treatment with Nonsteroidal anti-inflammatory drugs added to anti-tumour necrosis factor a therapy versus anti-tumour necrosis factor a therapy alone on progression of StrUctural damage in the spine over 2 years in patients with ankyLosing spondylitis; RCT: randomized controlled trial; IQR: interquartile range; bDMARD: biological DMARD; mSASSS: modified stoke ankylosing spondylitis spinal score; ASDAS: Axial Spondyloarthritis Disease Activity Score.

### Changes in LPPs between baseline and week 12

Complement LPP serum levels of L-ficolin, M-ficolin, MBL, MAp44, and complement activation, i.e. C3dg, decreased significantly after 12 weeks of treatment with TNFi, as shown in Fig. 2. In contrast, serum levels of CL-L1 and MASP-3 increased significantly. No significant raw differences were observed for the remaining complement proteins (Supplementary Fig. S1, available at *Rheumatology Advances in Practice* online). CRP levels also decreased significantly following TNFi (Fig. 2). After adjustment for baseline CRP, changes in L-ficolin, M-ficolin, and MASP-3 remained significant (Supplementary Table S2, available at *Rheumatology Advances in Practice* online). Contrary to the findings of the unadjusted analysis, MAp44 was observed to increase following treatment with TNFi. Additionally, serum levels of MASP-1 increased marginally, and serum levels of MASP-2 decreased after adjustment for baseline CRP.

### Correlation of complement protein levels with clinical data

Baseline serum levels of L-ficolin correlated positively with baseline ASDAS and BASFI, with Spearman rho 0.290 and 0.302, respectively (both *P*-values $< 0.05$) (Fig. 3A). Baseline M-ficolin also correlated positively, albeit weakly, with baseline ASDAS, Spearman rho 0.193 ($P < 0.05$). Baseline complement activation marker C3dg correlated positively with ASDAS, Spearman rho 0.328 ($P < 0.05$). In contrast, baseline MASP-1 and MAp44 correlated negatively with ASDAS (Spearman rho −0.204 and −0.218, respectively).

However, baseline L-ficolin, M-ficolin, and C3dg also correlated positively with baseline CRP, whereas no significant correlations were found between CRP and MASP-1 or MAp44. In patients with CRP within the normal range (≤5 mg/L), baseline MASP-3 correlated negatively with ASDAS and BASDAI (Spearman rho −0.418 and −0.401, respectively) but not with CRP. Again, C3dg levels correlated positively with CRP (Spearman rho 0.365) (Supplementary Fig. S2, available at *Rheumatology Advances in Practice* online).

At week 12, serum levels of complement activation marker C3dg correlated positively with disease activity, resembling the correlation observed between CRP and disease activity

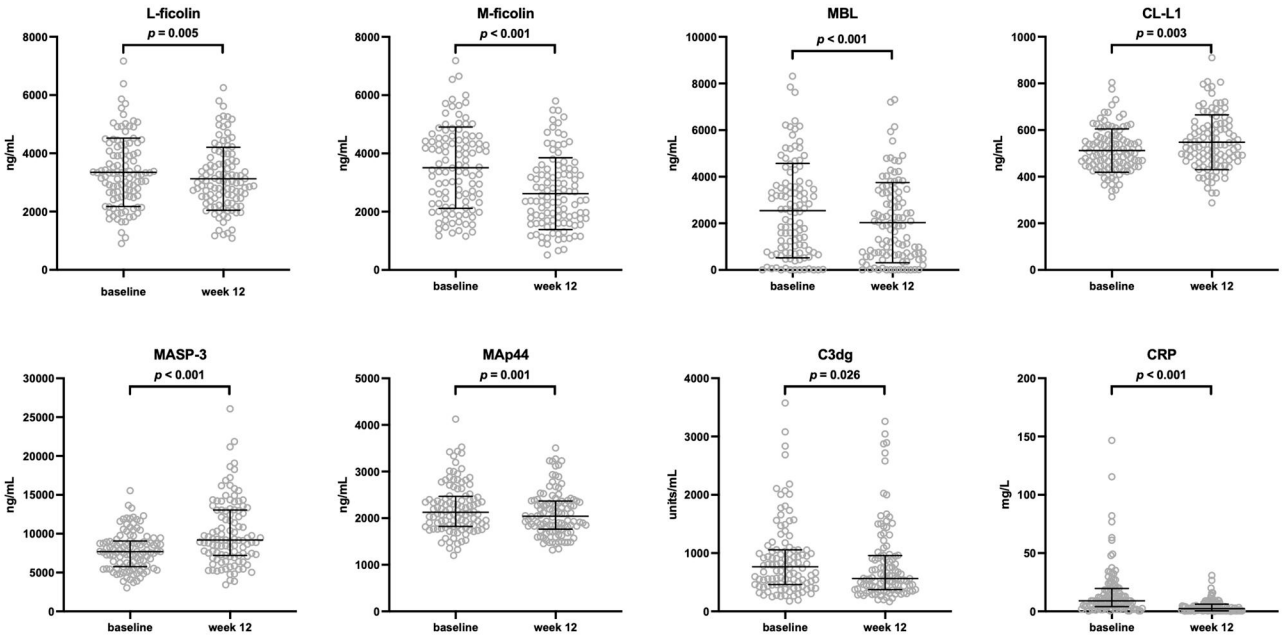


**Figure 2.** Differences in lectin pathway protein serum levels and CRP levels were compared by paired *t*-tests. Bars indicate median and IQR or mean and standard deviation (L-ficolin, M-ficolin, CL-L1 and MBL). *P*-values are indicated in graphs. IQR: interquartile range

(Fig. 3B). This included a positive correlation of C3dg with ASDAS, BASDAI, ASAS-HI, and BASFI (Spearman rho 0.236–0.386, all *P*-values $< 0.05$). Akin to the correlations observed at baseline, L-ficolin and M-ficolin again correlated positively with CRP. In patients with CRP within the normal range ($\leq$5 mg/L), no significant correlations with ASDAS, BASDAI, ASAS-HI, or BASFI were observed for either complement proteins or CRP at follow-up (Supplementary Fig. S2, available at *Rheumatology Advances in Practice* online).

### Predictors of treatment response

Assessment of treatment efficacy in clinical practice is often performed by a dichotomous approach to the various validated disease activity composite scores, e.g. clinically significant improvement is defined as a reduction in ASDAS $\geq$1.1. This dichotomous approach was also applied in the current study, where univariate logistic regression assessed the predictive value of continuous baseline LPP and complement activation levels and clinical outcomes at week 12 by (i) reduction in BASDAI $\geq$2, (ii) reduction in ASDAS $\geq$1.1, and (iii) achievement of inactive disease, i.e. ASDAS $<$1.3 (Table 2). Baseline serum levels of MASP-1 were significantly associated with clinically important improvement (reduction in ASDAS $\geq$1.1), whereas baseline serum levels of C3dg were associated with achievement of inactive disease (ASDAS $<$1.3). The differences in baseline serum levels of MASP-1 and C3dg are illustrated in Fig. 4.

In corresponding multivariate logistic regression analyses of clinically important improvement (reduction in ASDAS $\geq$1.1) and achievement of inactive disease (ASDAS $<$1.3) performed with backward selection (initial models are shown in Supplementary Table S3, available at *Rheumatology Advances in Practice* online), only C3dg remained significant in predicting inactive disease at week 12 (Table 2).

## Discussion

This study investigates the dynamics of complement LPP serum levels and complement activation marker C3dg before and after 12 weeks of TNFi therapy in a longitudinal cohort of 108 patients with active r-axSpA. The primary aim was to explore complement proteins as disease activity biomarkers and treatment response predictors within a clinically relevant time frame.

After adjustment for baseline CRP, we observed decreasing levels of L-ficolin, M-ficolin, and complement lectin pathway-activating enzyme MASP-2 following TNFi therapy. In contrast, MASP-3 levels rose, accompanied by slight increases in MASP-1 and MAp44 levels. Notably, baseline complement proteins L-ficolin and M-ficolin and total complement activation marker C3dg correlated positively with disease activity, whereas C3dg additionally correlated with disease activity and functional impairment at follow-up. In the 33 patients with CRP $\leq$5 mg/L, baseline MASP-3 correlated negatively with ASDAS and BASDAI. Baseline C3dg levels further predicted achievement of inactive disease by week 12 in a multivariate regression analysis.

Assessing disease activity is crucial in clinical practice. The absence of reliable biomarkers complicates objective evaluation in many axSpA patients when assessed by validated composite scores like ASDAS, BASDAI, and ASAS-HI since only ASDAS encompasses objective measurements like CRP [29–31].

The positive correlations between baseline serum levels of L-ficolin, M-ficolin, and complement activation marker C3dg with ASDAS are akin to our recent findings in newly diagnosed treatment-naïve axSpA patients, where L-ficolin and C3dg correlated positively with ASDAS and BASFI [7]. As illustrated in Supplementary Fig. S3, available at *Rheumatology Advances in Practice* online, specific LPPs are known to be upregulated during inflammation. However, their relations with pentraxins like CRP remain poorly understood. Previous studies have not disclosed that L-ficolin or

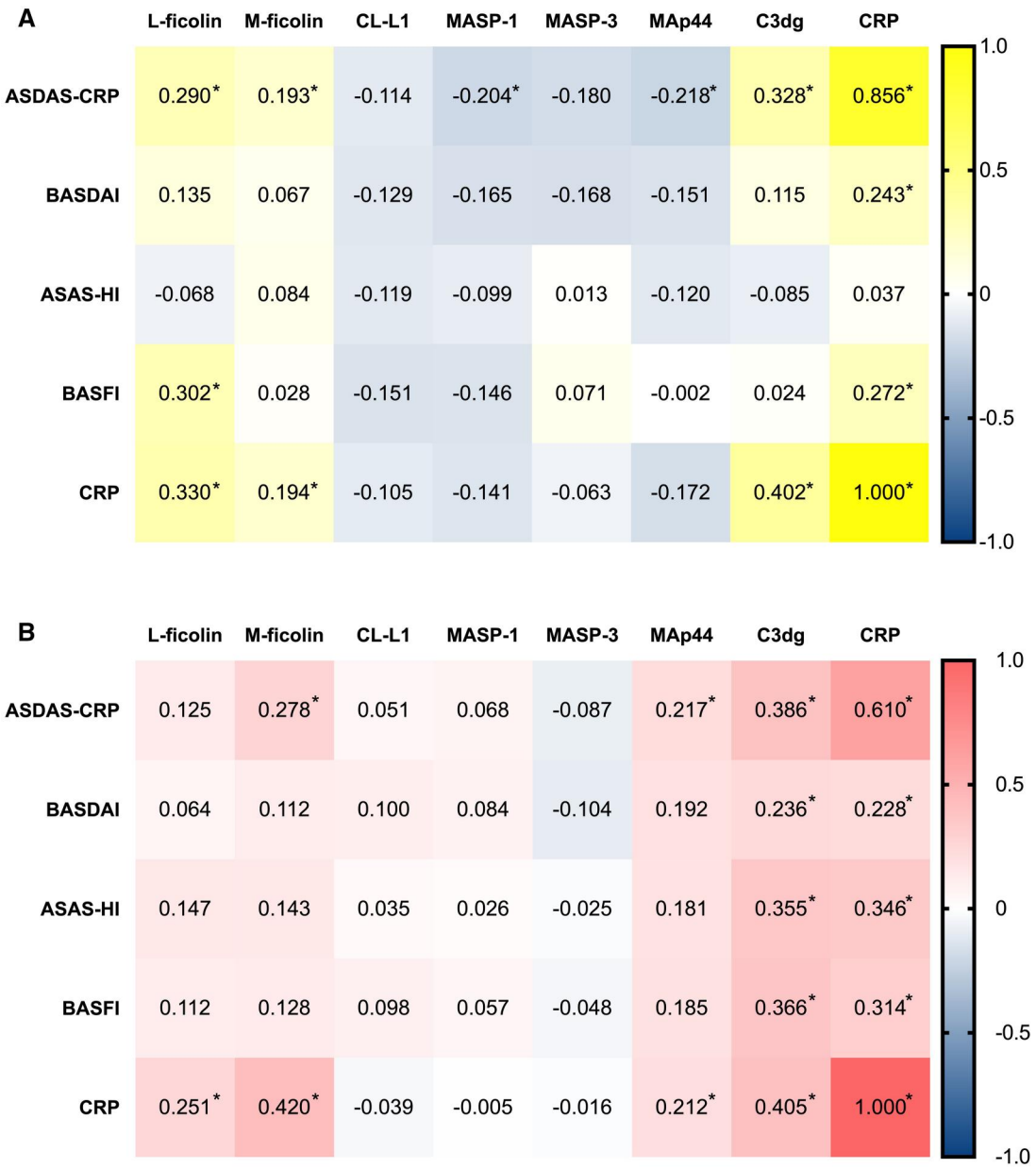


**Figure 3.** Heatmaps. (A) Heatmap over correlations between baseline protein levels and baseline disease activity or functional impairment. Data available on $n = 108$. (B) Heatmap over correlations between protein levels and disease activity or functional impairment at week 12. Data available on $n = 105$. Numbers are Spearman rho, and asterisk marks correspond to *P*-value <0.05. ASDAS: Axial Spondyloarthritis Disease Activity Score; ASAS-HI: Assessment of SpondyloArthritis International Society Health Index

M-ficolin act as classic acute phase reactants like CRP [32]. Still, M-ficolin levels correlate with CRP in certain other diseases [33], potentially due to its origin in monocytes and granulocytes since such cell types often increase during the acute phase response [22]. L-ficolin, like CRP, is synthesized in the liver; however, many other proteins also share this origin. The precise mechanisms that govern the potential biological interactions between L-ficolin and CRP have yet to be elucidated. Notably, L-ficolin and M-ficolin levels decrease following TNFi, aligning with previous observations in axSpA patients [5]. Elevated levels of L-ficolin and M-ficolin have also been observed in a clinical cohort of 23 newly diagnosed axSpA patients compared with 119 cLBP individuals [5], indicating that axSpA patients might display elevated levels of those PRMs, which decrease after the initiation of TNFi.

The low levels of MASP-3 observed in newly diagnosed treatment-naïve axSpA patients compared with cLBP individuals [7] also support our findings that MASP-3 levels increase following TNFi therapy, which has also been observed among responders to TNFi therapy in Crohn's disease [34]. Like MASP-1 and MAp44, MASP-3 originates from the *MASP1* gene [24]. Given that our current study revealed significant, albeit varying, increases in MASP-1, MASP-3, and MAp44 following initiation of TNFi therapy after adjusting for CRP, this could indicate increased synthesis following TNFi or decreased consumption due to lower inflammatory burden. However, there is limited knowledge regarding the

**Table 2.** Logistic regression of baseline lectin pathway protein serum levels and treatment response at week 12

| | Δ BASDAI ≥2 | | | Clinically important improvement Δ ASDAS ≥1.1 | | | Inactive disease ASDAS <1.3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | OR | 95% CI | *P* | OR | 95% CI | *P* | OR | 95% CI | *P* |
| Univariate model | | | | | | | | | |
| L-ficolin | 0.99989 | 0.99929, 1.00049 | 0.727 | 1.00022 | 0.99977, 1.00066 | 0.339 | 0.99977 | 0.99940, 1.00014 | 0.231 |
| M-ficolin | 1.00033 | 0.99977, 1.00090 | 0.251 | 1.00015 | 0.99979, 1.00051 | 0.410 | 1.00013 | 0.99984, 1.00043 | 0.378 |
| H-ficolin | 0.99998 | 0.99991, 1.00005 | 0.592 | 1.00000 | 0.99995, 1.00005 | 0.878 | 0.99998 | 0.99994, 1.00003 | 0.411 |
| CL-L1 | 0.99891 | 0.99137, 1.00650 | 0.777 | 1.00019 | 0.99493, 1.00547 | 0.945 | 1.00133 | 0.99693, 1.00574 | 0.555 |
| MBL | 1.00013 | 0.99974, 1.00052 | 0.514 | 1.00004 | 0.99979, 1.00028 | 0.779 | 0.99993 | 0.99972, 1.00014 | 0.511 |
| MASP-1 | 0.99997 | 0.99984, 1.00010 | 0.648 | 0.99988 | 0.99978, 0.99998 | **0.015** | 0.99997 | 0.99988, 1.00006 | 0.551 |
| MASP-2 | 1.00036 | 0.99800, 1.00272 | 0.765 | 0.99959 | 0.99826, 1.00091 | 0.539 | 0.99972 | 0.99846, 1.00098 | 0.665 |
| MASP-3 | 1.00022 | 0.99988, 1.00057 | 0.204 | 0.99997 | 0.99977, 1.00017 | 0.789 | 0.99985 | 0.99966, 1.00003 | 0.099 |
| MAp44 | 1.00108 | 0.99938, 1.00278 | 0.212 | 0.99985 | 0.99896, 1.00074 | 0.741 | 0.99950 | 0.99868, 1.00032 | 0.232 |
| C3dg | 0.99998 | 0.99885, 1.00110 | 0.970 | 1.00020 | 0.99938, 1.00103 | 0.627 | 0.99905 | 0.99816, 0.99994 | **0.037** |
| Age (years) | 0.81226 | 0.70699, 0.93321 | **0.003** | 0.91127 | 0.86392, 0.96121 | **0.001** | 0.95010 | 0.91123, 0.99063 | **0.016** |
| Sex = male | 0.35616 | 0.08301, 1.52812 | 0.165 | 0.52381 | 0.18930, 1.44942 | 0.213 | 0.57358 | 0.21732, 1.51388 | 0.262 |
| HLA-B27 = positive | 0.95122 | 0.10578, 8.55369 | 0.964 | 1.33571 | 0.27045, 6.59682 | 0.722 | 0.38312 | 0.07952, 1.84583 | 0.232 |
| Previous bDMARD use | 0.28571 | 0.06604, 1.23602 | 0.094 | 0.92308 | 0.29836, 2.85584 | 0.890 | 0.33498 | 0.10476, 1.07107 | 0.065 |
| Symptom duration (years) | 0.97073 | 0.90043, 1.04652 | 0.439 | 0.94157 | 0.89583, 0.98964 | **0.018** | 0.95151 | 0.90718, 0.99800 | **0.041** |
| Smoking | 1.05555 | 0.23839, 4.67385 | 0.943 | 2.75510 | 0.84331, 9.00097 | 0.093 | 1.53333 | 0.66153, 3.55405 | 0.319 |
| BMI | 0.87033 | 0.76004, 0.99661 | **0.045** | 0.89023 | 0.80946, 0.97906 | **0.017** | 0.87985 | 0.79974, 0.96799 | **0.009** |
| CRP (mg/L) | 1.035559 | 0.96280, 1.11381 | 0.347 | 1.09179 | 1.014264 1.175231 | **0.019** | 1.01780 | 0.99830, 1.03767 | 0.074 |
| NSAID intake score at baseline | 1.012806 | 0.99405, 1.0319 | 0.182 | 1.01503 | 1.001879 1.028342 | **0.025** | 1.00407 | 0.99319, 1.01507 | 0.465 |
| Multivariate model | | | | | | | | | |
| Age | | | | 0.90054 | 0.84753, 0.95687 | **0.001** | | | |
| CRP (mg/L) | | | | 1.11341 | 1.02382, 1.21084 | **0.012** | | | |
| Symptom duration (years) | | | | | | | 0.94742 | 0.90163, 0.99553 | **0.033** |
| C3dg | | | | | | | 0.99905 | 0.99814, 0.99996 | **0.040** |

Lectin pathway protein levels and CRP were analysed as continuous variables. Multivariate logistic regression analyses were performed using backward selection, and the initial model is shown in Supplementary Table S3, available at *Rheumatology Advances in Practice* online. ΔBASDAI data were available on $n = 107$. ΔASDAS-CRP data were available on $n = 105$. *P*-values <0.05 are marked in bold.
ASDAS: Axial Spondyloarthritis Disease Activity Score; OR: odds ratio; MBL: mannan-binding lectin; MASP: MBL-associated protease; bDMARD: biological DMARD.

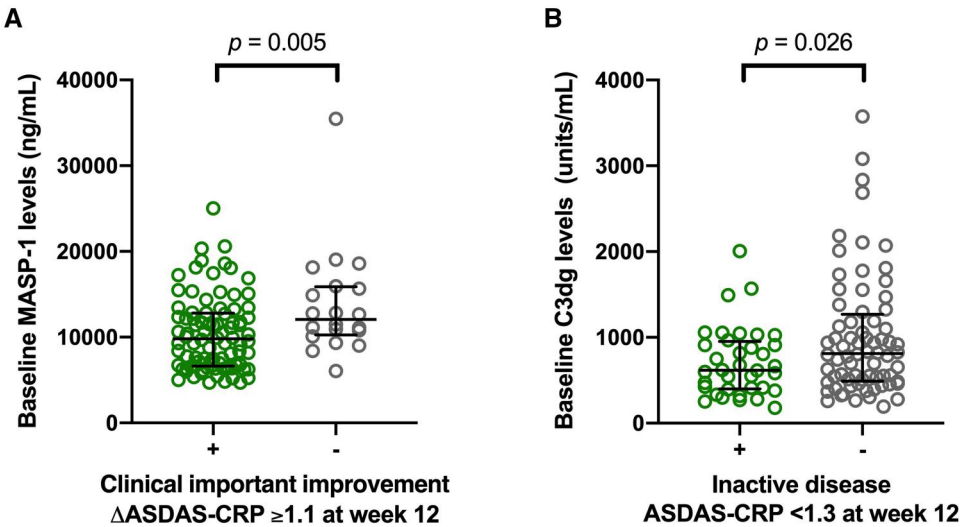


**Figure 4.** Baseline MASP-1 and C3dg serum levels. (A) Baseline MASP-1 serum levels in patients grouped according to achievement of clinically important improvement (reduction of ASDAS-CRP ≥1.1 at week 12). Groups were compared by *t*-test. Bars indicate the median and IQR, and *P*-value is indicated in the graph. (B) Baseline C3dg serum levels in patients grouped according to achievement of inactive disease (ASDAS-CRP <1.3) at week 12. Groups were compared by *t*-test. Bars indicate the median and IQR, and *P*-value is indicated in the graph. MASP-1: MBL-associated protease 1; ASDAS: Axial Spondyloarthritis Disease Activity Score; IQR: interquartile range

regulatory mechanisms involved in *MASP1* gene variant synthesis, and previous studies have not consistently shown increases in all *MASP1* gene products following TNFi therapy [5, 34]. Notably, MASP-1 is crucial for lectin pathway activation, while MASP-3 functions in the alternative pathway, often seen as an amplification loop of complement activation.

The distinct pattern of the LPP levels in untreated axSpA patients and the dynamics following TNFi, i.e. decreasing levels of L-ficolin, M-ficolin, and MASP-2, along with an increase in MASP-3, suggests that TNFi therapy lowers inflammation through lectin pathway activation, reducing amplification of the alternative pathway and thus decreasing the use of MASP-3. However, these hypotheses remain unconfirmed.

Of the composite scores for disease activity, ASDAS has been shown to predict long-term radiographic changes after 12 years of follow-up [35]. However, the composite scores do not predict treatment response within a clinically relevant time frame, typically after 12 weeks of treatment. Identifying potential predictors of treatment response in individual patients is pertinent due to the growing range of therapeutic options, including TNFi, interleukin-17 inhibitors (IL-17i), and Janus kinase inhibitors (JAKi). Given that treatment response is often gauged by a dichotomous approach, univariate logistic regression analyses assessed various clinical responses in the current study. The analyses showed baseline MASP-1 levels to predict achievement of a clinically important improvement (reduction of ASDAS $\geq$1.1) at week 12 and baseline C3dg levels to predict achievement of inactive disease (ASDAS $<$1.3) at week 12. In corresponding multivariate analyses, including age, HLA-B27 positivity, biological sex, symptom duration, and CRP, followed by stepwise backward selection, only baseline C3dg levels and symptom duration were significant predictors of inactive disease (ASDAS $<$1.3).

MASP-1 acts as the initial enzymatic component in the complement lectin pathway activation by activating zymogen MASP-2, thereby driving further downstream complement activation, including the generation of complement activation marker C3dg [36]. C3dg originates from activation through the lectin pathway as well as the classical and alternative pathways. However, the corresponding findings of baseline MASP-1 and C3dg levels with treatment response link the observed complement activation with the lectin pathway from a biological perspective.

A significant strength of our study includes the use of a multicentre longitudinal RCT cohort of well-characterized r-axSpA patients, including both bDMARD-naïve and bDMARD-experienced patients, characterized by high disease activity and high risk of radiographic progression. The well-characterized patients and specific EMMs are essential in axSpA, as treatment efficacy data indicate varying disease mechanisms across different manifestations, making EMMs highly relevant for assessing the external validity of biomarkers. Furthermore, our work represents the most extensive investigation of combined measurements of complement activation (i.e. C3dg) and LPPs in patients with axSpA in a clinically relevant time frame before and following 12 weeks of TNFi therapy according to a pre-specified protocol [18]. As the study was exploratory, adjustments for multiple *t*-tests were not implemented. While controlling for multiple comparisons is crucial in confirmatory studies to reduce false positives, overly strict corrections in exploratory research could hinder the discovery of potential findings [37]. Given that blood samples were provided optionally, samples were unavailable for 20 patients; however, this was evenly distributed between responders and non-responders (Fig. 1). A noteworthy limitation is the high prevalence of patients with elevated CRP ($>$5 μg/L), constituting approximately 75% in our study. This is important for extrapolating the results because nearly half of axSpA patients typically do not exhibit elevated CRP levels [17]. The unmet clinical need for objective disease activity biomarkers is particularly pronounced in axSpA patients with CRP within the normal range. Nevertheless, patients with elevated CRP face an increased risk of radiographic progression, often necessitating more intensified treatments (e.g. ts/bDMARDs) to control inflammation and decrease disease activity, and may benefit from future stratified treatment approaches. Despite only 33 patients having CRP $\leq$5 mg/L, we observed significant negative correlations between MASP-3, ASDAS, and BASDAI. This requires further analyses in other cohorts of patients with CRP within the normal range. Furthermore, measurements of complement proteins were performed on serum samples, which is not ideal for measurements of L-ficolin [38]. However, we have previously tested the specific set-up and found correlations of $R^2 = 0.6454$, $P < 0.001$, with levels found in EDTA plasma [7].

In summary, this study explores the complement system in a cohort of r-axSpA patients experiencing a high disease burden, initiating treatment with TNFi within a longitudinal RCT. The dynamics of complement protein levels, their correlations with disease activity, and the prediction of inactive disease suggest they might serve as a proxy measurement for the inflammatory burden in r-axSpA. Thus, the complement system might be involved in the pathogenesis, potentially contributing to the chronic radiographic changes observed in axSpA. This speculation finds support in animal models, where inhibition of the complement system led to reduced structural damage in r-axSpA [9, 10], though this remains to be assessed in humans.

Future investigations into complement activation and its associations with disease course, i.e. radiographic changes, are necessary to deepen our understanding of the underlying pathogenesis in axSpA. Such knowledge could facilitate and potentially guide pre-treatment stratification of patients with axSpA, utilizing the expanding therapeutic repertoire of b/tsDMARDs.

## Supplementary material

Supplementary material is available at *Rheumatology Advances in Practice* online.

## Data availability

Data is available upon reasonable request.

## Contribution statement

C.M., A.T., A.G.L., S.T., D.P., and F.P. conceptualized the study. B.M., V.R.R., M.T., M.P., J.S., J.L., D.P., and F.P. contributed to acquiring and handling the data. C.M. conducted laboratory experiments to measure lectin pathway proteins and complement activation. C.M., A.T., A.G.L.,

S.T., and F.P. handled data analysis, statistical evaluations, and manuscript writing. C.M., S.T., D.P., and J.S. obtained the grants for the study. All authors revised and approved the final version of the manuscript. F.P. acts as the guarantor of this work.

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