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Original Article

Factors Associated with the Development of Anti-drug Antibodies to TNFi and the Consequences for Axial Spondyloarthritis: A Two-year Follow-up Study

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Abstract

Objective: To evaluate the development of anti-drug antibodies (ADAb) against tumor necrosis factor inhibitors (TNFi) therapy during a 2-year period and search the factors linked to patients with axial spondyloarthritis (axSpA).

Methods: Biologic-naive patients with axSpA were included in this observational study. Serum drug levels and ADAb were measured at weeks 12, 24, 52, and 104 of treatment by enzyme-linked immunosorbent assay (ELISA). The development of ADAb and factors related to ADAb over time were investigated using generalized estimating equations (GEE).

Results: A total of 180 patients with axSpA (116 male, mean (±SD) 45.6 (±11.9) years) who started TNFi treatment (etanercept (32.2%), adalimumab (27.2%), golimumab (20.6%), infliximab (20%)) were included. In the etanercept treatment group, only 1 patient had ADAb at 12 weeks and 24 weeks. Anti-drug antibodies against TNFi drugs were present in the adalimumab group in 32.7% of patients and in the infliximab group in 21.2% of patients at 12 weeks, and the proportion of ADAb-positive patients were found to be stable throughout the follow-up for adalimumab- and infliximab-treated patients. In the golimumab group, one patient had ADAb against golimumab at 12 weeks and the proportion of ADAb-positive patients increased throughout follow-up.

In longitudinal analysis, baseline age, TNFi type, longitudinal Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and ASDAS-CRP scores, serum C-eeactive protein (CRP) levels, presence of adverse events and treatment discontinuation were associated with the presence of ADAb.

Conclusion: The development of ADAb against TNFi therapy is associated with younger age, high disease activity, the development of adverse events and more common treatment discontinuation in patients with axSpA during 2-year follow-up.

Keywords: Axial spondyloarthritis, anti-drug antibody, tumor necrosis factor inhibitors

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Introduction

Axial spondyloarthritis (axSpA) is a chronic inflammatory rheumatic disease affecting sacroiliac joints and the spine, as well as peripheral joints and entheses. Tumor necrosis factor inhibitors (TNFi) are widely used in patients with persistently high disease activity, despite non-steroidal anti-inflammatory drugs (NSAIDs).[1,2] Currently, 5 TNFi (monoclonal antibody-based proteins: adalimumab (ADA), infliximab (INF), golimumab (GOL), certolizumab (CERT), and fusion protein: etanercept (ETN)) are approved for patients diagnosed with axSpA.

Treatment with TNFi demonstrates efficacy in alleviating back pain, maintaining as well as preserving and enhancing spinal flexibility, and decreasing the peripheral and extra-musculoskeletal manifestations in most patients with axSpA.[1] Nonsteroidal anti-inflammatory drugs (NSAIDs) are recommended as the initial treatment for patients with axial spondyloarthritis experiencing pain and/or stiffness.[1,2] Tumor necrosis factor inhibitors (TNFi) treatment is recommended for active axial disease despite NSAID usage.[1,2] Although the majority of patients have a good response to TNFi treatment, some patients with axial spondyloarthritis

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fail to respond or lose responsiveness during therapy (secondary non-response), and some of them have adverse events (AEs) limiting the use of this therapy.[3-6]

The development of anti-drug antibodies (ADAb) may be considered a reason for mostly secondary non-response or some adverse events. To date, there are studies concerning immunogenicity and clinical non-responses in patients with axSpA with contradictory results. In some of those studies, the presence of ADAb was found to be related to the TNFi type, absence of HLA-B27, older age, higher body mass index (BMI) scores, longer disease duration, and co-medications, in addition to higher disease activity.[6-12] Moreover, the detection of anti-infliximab antibodies has been associated with infusion-related reactions.[8] Although some studies indicate a correlation between ADAb and high disease activity, a recent large-scale study found no association between the presence of ADAb and disease activity.[13] However, there is a lack of data regarding the longitudinal course of the development of ADAb in patients with axSpA.

Hence, the objective of this current study was to assess the occurrence of ADAb against TNFi and track its progression longitudinally for up to 2 years in patients diagnosed with axSpA. Additionally, the study aimed to identify factors linked to the development of ADAb in patients undergoing their first TNFi therapy.

Materials and Methods

Study Design and Patient Population

This study was a 104-week prospective, multicenter, observational study. Axial spondyloarthritis (AxSpA) patients prescribed their first TNFi treatment at the time of enrollment or in the past 2-week period were included in the study. All patients diagnosed with axSpA were aged 18 years or older and met the Assessment of Spondyloarthritis International Society (ASAS) classification criteria for axSpA.[14] Written informed consent was obtained from all participants involved in the study.

This study was approved by the local ethics committee of Izmir Katip Celebi University (approval number: 104/2014) as well as the central ethics committee of Medical Devices Agency (approval number: 2014-PMS-21).

TNFi treatment was initiated following the ASAS/European League Against Rheumatism (EULAR) guidelines as well as local national agencies.[2,15] The selection of TNFi type was determined at the discretion of the local physician. The biosimilars and certolizumab were not approved at the beginning of the study. Therefore, ETN (50 mg /week), ADA (40 mg/2 week), GOL (50 mg/month), and INF (5 mg/kg/6 week) were used as TNFi treatments in patients diagnosed with axSpA in the present study.

Data Collection

Socio-demographic features, disease-related characteristics (including the presence of extra-musculoskeletal and peripheral involvement), and disease activity measurements were registered. Throughout the follow-up period (at 12, 24, 52, and 104 weeks), information regarding axSpA-related characteristics, co-medications, and disease activity measurements, in addition to serum samples for TNFi drug level and ADAbs, were re-collected at each visit. We computed the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Ankylosing Spondylitis Disease Activity Score (ASDAS)-CRP using a structured form as part of the assessment. The presence of adverse events, according to patient reports, and the cessation of treatment were also noted at each visit.

Measurement of Serum Drug and Anti-drug Antibody Concentrations

Baseline and follow-up blood samples were taken just before the administration of TNFi, and all patients were fasting when blood samples were taken.

Main Points

- The development of anti-drug antibodies against tumor necrosis factor inhibitors (TNFi) is linked to elevated disease activity, along with an increased risk of adverse events and treatment discontinuation in patients with axial spondyloarthritis.
- In contrast to monoclonal TNFi, etanercept appeared to exhibit lower immunogenicity.
- Serum trough drug levels may be lower in patients who develop anti-drug antibodies, particularly with monoclonal TNFi.

In this study, serum trough levels of ADA (Cat No 50802300DV), ADAb against ADA (Cat No 509023300DV), serum trough level of GOL (Cat No 520023300DV), ADAb against GOL (Cat No 52102330000), serum trough level of ETN (Cat No 511023300DV), ADAb against ETN (Cat No 51202300DV), serum trough level of IFX (Cat No 50602300DV) and ADAb against IFX (Cat No 50702300DV) were measured by the enzyme-linked immunosorbent assay (ELISA) method using commercial kits (Grifols Promonitor).

Each sample was diluted as specified in the protocols and assayed (Supplementary Table 1).

We performed readings using a Varioscan Flash Multi Mod Reader (Thermo Scientific, Massachusetts, USA). The analyses of the measurements were carried out using the Promonitor My Assays Program suggested by the kit provider. The intra-assay and inter-assay coefficient of variability (CV) for the tests were given in Supplementary Table 2.

Statistical Analysis

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software version 13.0 (Chicago, IL, USA). The distribution of continuous variables was examined using visual methods such as histograms and probability plots, as well as analytical techniques like the Kolmogorov–Smirnov test. Since most continuous variables did not follow a normal distribution, their values were presented as medians and interquartile ranges (IQR), while categorical variables were expressed as numbers and percentages. The Mann–Whitney *U* test was employed to compare non-normally distributed variables between groups, and the Chi-square test or Fisher's exact test was used for the comparison of categorical data.

As the age, education level, disease activity measurements, serum CRP levels, duration of symptoms, and delay in diagnosis did not follow normal distribution the Kruskal–Wallis test was conducted to compare these parameters among treatment groups.

Friedman tests were executed to examine whether there was a significant alteration in disease activity measurements, considering the violations of parametric test assumptions (non-normal distribution). The significance of pairwise differences was assessed using the Wilcoxon signed-rank test, with the Bonferroni correction applied to adjust for multiple comparisons.

The correlation coefficients and their significance were estimated by using the Spearman test while investigating the associations between non-normally distributed serum drug levels and disease activity parameters.

The presence of ADAbs over time was explored using generalized estimating equations (GEE). The GEE procedure facilitates the analysis of repeated measurements in longitudinal studies, making it appropriate for assessing longitudinal relationships involving continuous and dichotomous outcome variables (dependent). It accommodates several time-dependent and independent covariates and allows the use of unequal numbers of repeated measurement data, even when they deviate from normality.[16,17] We analyzed "the presence of serum ADAb (as a dichotomous variable)" as the dependent variable at visits during 102 weeks of follow-up. Socio-demographic characteristics, axSpA-related features, co-medications, and disease activity measurements were analyzed as independent variables, and we ran univariate and multivariate GEE models with all potential explanatory variables.

Results

In total, 180 patients diagnosed with axSpA, with 74.5% diagnosed with AS, were included in this study during the period of March 2015 to February 2018. The mean (SD) age was 45.6 (11.9) years, 66.1% of patients were male, and the median disease duration was 5 years (range 0-43). The baseline demographic and disease-related characteristics of the patients are outlined in Table 1. Of those patients, 58 (32.2%) used ETN, 49 (27.2%) ADA, 37 (20.6%) GOL, and 36 (20%) INF. Baseline characteristics, disease-related features, and activity assessments did not differ significantly among treatment subgroups (Table 1). In the

Table 1. Baseline Demographic and Disease Characteristics

	Total (n = 180)	ETN (n = 58)	ADA (n = 49)	GOL (n = 37)	INF (n = 36)	*P*
Age, years mean (SD)	45.6 (11.9)	44.9 (12)	42.2 (10.7)	44.6 (11.1)	46.2 (11.0)	.37
Sex, male, %	66.1	65.5	61.2	73	66.7	.72
HLA-B27 positive, n (%)	43 (72)/60	12 (80)/15	12 (63)/19	8 (67)/12	78.6	.64
Diagnosis, %						
r-axSpA	74.5	67	72	87	79	.46
nr-axSpA	25.5	33	28	13	21	
Education level, median (IQR 25-75) years	8 (8)	8 (4-12)	11.5 (4-15.5)	12 (4.5-12)	6.5 (4-10)	.11
Peripheral arthritis, %	21 (20)/103	4 (14)/28	7 (22)/32	3 (21.4)/14	7 (24) /29	.81
Enthesitis, n (%)	8 (8) /103	2 (7)/28	5 (16)/32	0/14	1 (3) /29	.20
Anterior uveitis, n (%)	1 (1) /103	0	0	0/14	1 (3) /29	.46
Psoriasis, n (%)	3 (3) /103	1 (4)/28	1 (3)/32	0/14	1 (3) /29	.91
IBD, n (%)	5 (5)/92	1 (4)/25	1 (4)/26	0/14	3 (11) /27	.44
Current csDMARD use, n (%)	22 (23) / 94	7 (29)/24	6 (22)/27	3 (21.4)/14	6 (21) /29	.89
Current sulfasalazine use, n (%)	20 (22)/93	6 (26)/23	5 (19)/27	3 (21.4)/14	6 (21) /29	.75
Current MTX use, n (%)	5 (5)/94	2 (8)/24	1 (4)/27	1 (7.1)/14	1 (3) /29	.93

ADA, adalimumab; csDMARD, conventional disease-modifying antirheumatic drug; ETN, etanercept; GOL, golimumab; HLA-B27, Human leucocyte antigen B27; IBD, Inflammatory bowel disease; INF: infliximab, m NY modifiye Newyork classification; MTX: methotrexate.

2-year follow-up, 33 patients (6 in ETN, 9 in ADA, 13 in INF, and 5 in the GOL treatment group) stopped their first TNFi with a median of 52 weeks, and 76 (42.2%) patients had 135 adverse events related to TNFi treatment, according to treating physicians.

Disease Activity Parameters during Follow-up

All disease activity measurements (BASDAI, ASDAS-CRP, and serum CRP levels) exhibited a significant decrease in patients who initiated TNFi by week 12, and these measurements remained stable throughout the follow-up period.

The Prevalence of Anti-drug Antibodies and Tumor Necrosis Factor Inhibitor Levels during Follow-up

In total, 180 patients had serum samples examined at 12 weeks, 154 at 24 weeks, 121 at 52 weeks, and 73 at 104 weeks. In the ETN treatment group, only 1 patient had ADAb at 12 weeks and 24 weeks, and he stopped ETN treatment at 12 weeks, but none of them had ADAb at 52 or 104 weeks. ADAb against the TNFi drug was present in the ADA treatment group in 32.7% of patients and in the INF group in 21.2% of patients at 12 weeks. The proportion of ADAb-positive patients was found to be stable throughout the follow-up for ADA- and INF-treated patients. In the GOL treatment group, one patient had ADAb against GOL at 12 weeks, and the proportion of ADAb-positive patients increased throughout follow-up (Table 2).

Serum median drug levels and the proportion of patients with detectable serum drug levels were summarized in Table 2. We analyzed the relationship between serum drug levels (ETN, ADA, GOL, and INF levels separately) and disease activity measurements, which included BASDAI, ASDAS-CRP, and serum CRP levels. Negative correlations were only found between serum GOL levels and serum CRP levels and ASDAS-CRP at 52 and 104 weeks ($P = .02$, $P = .03$ at 52. weeks, $P < .001$, $P = .01$ at 104 weeks, respectively).

In addition, we analyzed the correlation between serum TNFi levels (ADA, GOL, and INF levels separately) and ADAb titers and found a significant negative correlation between ADAb titers and serum drug levels in patients undergoing treatment with ADA and INF but no significant correlations were identified between serum GOL levels and ADAb titers during follow-up visits (Supplementary Table 3). We did not analyze serum ETN levels and ADAb titers because of the low number of patients who had detectable ADAb titers against ETN during the follow-up period.

Factors Associated with Anti-drug Antibodies

In the entire axSpA cohort, disease activity measurements, including BASDAI, ASDAS-CRP, and serum CRP level, were statistically higher in the ADAb positive group than the ADAb negative group at the 12th and 24th weeks (Table 3). At the 52nd and 104th weeks, disease activity was observed to be higher in the ADAb-positive group; however, this difference did not reach statistical significance (Table 3). At 12 weeks of follow-up, the frequency of adverse events was statistically higher in the ADAb-positive group (60% vs. 30%) but there were no significant differences observed during the remainder of the follow-up period (Table 3). In all follow-up visits, the rate of treatment discontinuation was statistically higher in the ADAb-positive group than in the ADAb-negative group (Table 3).

Using GEE for the entire axSpA cohort, we longitudinally assessed factors/covariates associated with the development of serum ADAb (as a dichotomous variable). Univariate longitudinal analysis revealed that the development of ADAb was significantly associated with TNFi type (B:–2.00, 95% CI: (–4.09-0.09); $P = .06$ for ETN), age (B:–0.03, 95% CI: (–0.06-–0.001); $P = .04$), BASDAI (B:0.03, 95% CI: (0.01-0.04); $P < .001$), ASDAS (B:0.64, 95% CI: (0.41-0.87); $P < .001$), serum CRP level (B:0.02, 95% CI: (0.002-0.03); $P = .026$), the presence of adverse events (B:–0.54, 95% CI: (–1.098-0.011); $P = .06$), and treatment discontinuation (B:1.81, 95% CI: (0.96-2.67); $P < .001$). However, there was no relationship between the presence of serum ADAb and methotrexate (MTX) or conventional synthetic DMARD (csDMARD) use (MTX and/or sulphasalazine use) ($P = .55$ and $P = .16$, respectively). Hence, we constructed 2 multivariate models to examine the associated

Table 2. Serum Trough Drug Levels, Percentages of Patients with Detectable Serum Drug Level and ADAb in Follow-up

	12 week	24 week	52 week	104 week
Etanercept				
Serum ETN level, median (IQR) (ug/mL)	3.12 (4.83)/54	3.14 (3.89)/48	2.11 (3.34)/36	1.25 (1.11)/17
Patients with detectable ETN, %	98.1/54	97.9/48	100/36	100/17
Patients with ADAb, %	1.9/53	2.1/49	0 /34	0/18
Adalimumab				
Serum ADA level, median (IQR) (ug/mL)	4.59 (8.46)	2.67 (8.82)/44	5.37 (10.83)/37	2.50 (9.10)/22
Detectable ADA, %	81.6	84.1/44	78.4/37	52.4/22
ADAb, %	32.7	30.2/43	24.3/37	18.2/22
Golimumab				
Serum GOL level, median (IQR) (ug/mL)	0.64 (0.90)/36	0.31 (1.65)/31	0.66 (1.20)/23	0.55 (2.3)/11
Detectable GOL, %	100/36	100/31	100/23	95.8/11
ADAb, %	2.8/36	3.2/31	17.4/23	27.3/11
Infliximab				
Serum INF level, median (IQR) (ug/mL)	2.61 (6.40)/33	0.58 (4.54)/32	1.36 (3.40)/28	0.58 (4.40)/24
Detectable INF, %	100/33	93.5/32	95.7/28	81.8/24
ADAb, %	21.2/33	34.4/30	30.8/24	26.1/21

ADA, adalimumab; ADAb, anti-drug antibody; ETN, etanercept; GOL, golimuma; INF, infliximab; IQR, interquartile ranges.

Table 3. Relationship Between the Presence of Ant-drug Antibodies and Clinical Characteristics

	12 week			24 week			52 week			104 week		
	ADAb+ (n = 25)	ADAb - (n = 147)	*P*	ADAb+ (n = 26)	ADAb - (n = 128)	*P*	ADAb+ (n = 21)	ADAb - (n = 100)	*P*	ADAb+ (n = 13)	ADAb - (n = 60)	*P*
BASDAI, median (IQR)	4.5 (2.9)	2.7 (2.1)	< .001	3.0 (3)	2.3 (2.1)	.006	2.3 (2.7)	1.8 (1.6)	.28	2.4 (2.7)	1.6 (2)	.05
ASDAS-CRP, median (IQR)	3.1 (1.7)	1.8 (1.4)	< .001	2.4 (1.7)	1.7 (1.5)	< .001	1.9 (1)	1.4 (1.2)	.08	1.9 (1.6)	1.6 (1.4)	.09
Serum CRP level (mg/L), median (IQR)	6.9 (15)	2.5 (7)	.006	7.3 (17)	2.6 (6.9)	.001	4.0 (10.3)	2.8 (5)	.49	4.0 (15)	2.0 (6)	.18
Adverse event, %	60	30.3	.03	43.1	36.8	.79	37.3	52.9	.25	50.0	37.3	.42
Treatment discountinuation, %	12.5	1.3	.02	21.1	4.6	.04	33.3	6.4	.007	27.3	5.9	.03

ADAb, anti-drug antibody; ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score-CRP; BASDAI, Bath AS Disease Activity Index; CRP, serum C reactive protein; IQR, interquartile ranges.

factors/covariates linked to the presence of ADAb over time. One model incorporated ASDAS-CRP, while the other included BASDAI and serum CRP levels as disease activity measures. Our findings indicated that the presence of ADAb was significantly associated with age, TNFi type, BASDAI, serum CRP level, ASDAS-CRP, treatment discontinuation, and the occurrence of adverse events (Table 4).

Discussion

The findings from this 2-year follow-up study demonstrate that the emergence of ADAb against TNFi is correlated with elevated disease activity, along with the occurrence of adverse events and treatment discontinuation in patients diagnosed with axSpA. However, there were no correlations between co-medications (MTX or sulphasalazine) and the presence of ADAb. In contrast with monoclonal TNFi, ETN seemed to be less immunogenic in the present study. Additionally, the serum trough drug levels might be lower in patients who developed ADAb with at least monoclonal TNFi.

In our study, we longitudinally evaluated the factors/covariates associated with the development of serum ADAb over a 2-year follow-up period. The presence of ADAb was related to younger age, active disease, more frequent adverse events, and treatment discontinuation. Some reports have suggested an association between disease activity and the presence of ADAb in patients treated with TNFi, but the majority of these studies were cross-sectional analyses.[7,10,18] Only one study specifically investigated the longitudinal association between disease activity and the presence of ADAb. This study reported that serum drug levels were statistically lower in patients with ADAb against ADA and were associated with disease activity.[11] While there were studies examining the relationship between immunogenicity and disease activity, there is limited data available specifically on the development of ADAb and treatment discontinuation because there is inadequate longitudinal data and also follow-up periods were not long enough.[19] In a 1-year follow-up study, ADAb against ADA or INF was associated with early discontinuation of treatments in patients with AS. In a multicenter randomized trial, ADAb-negative patients received ADA treatment for longer in contrast to ADAb-positive patients (mean 98.6 vs. 56.9 weeks, $P = .015$).[19] In our study, we analyzed the association by comparing ADAb positive and negative patients in addition to analyzing treatment discontinuation and ADAb at each visit longitudinally. De Vries et al[8] found that anti-infliximab antibodies were related to infusion reactions. Adverse events were related to the development of ADAb in our study, but we did not analyze the types of adverse events such as infections or allergic reactions, so the relationship between allergic reactions and immunogenicity could not be determined.

In our study, about 30% of patients in ADA and 20% of patients in INF treatment groups had ADAb at 12 weeks, and the percentages of ADAb-positive patients were stable during follow-up. As expected, ETN was less immunogenic, and only 1 patient had ADAb. Interestingly, only 2% of patients in the GOL treatment group had ADAb in the third month, but the rate of ADAb-positive patients increased up to 27% in the 2-year follow-up. In previous studies, the percentage of patients who developed ADAb against ADA, INF, and ETN was similar to our findings.[9,12] Although ADAb was found in 3% of patients who were using GOL in a recent cross-sectional study, there was no data about the development of ADAb against GOL in patients with axSpA

Table 4. Factors Associated with Development of Anti-drug Antibodies

	Model 1			Model 2		
	B	95% CI	*P*	B	95% CI	*P*
Age years	- 0.061	- 0.109– - 0.012	***.015***	- 0.058	- 0.107– - 0.010	***.018***
TNFi treatment						
ETN	- 1.981	- 4.369– - 0.134	*.104*	- 2.475	- 4.791– - 0.076	***.036***
ADA	1.438	- 0.002–0.407	*.073*	1.275	- 0.119– - 0.160	*.064*
INF	1.550	3.010–3.102	*.050*	1.255	2.666–2.629	*.073*
GOL	0[a]			0[a]		
Presence of adverse event, no	- 0.824	- 1.451– - 0198	***.010***	- 0.835	- 1.461– - 0.208	***.009***
TNF treatment discontinuation	1.289	0.043–2.534	***.043***	1.248	- 0.075–2.571	*.065*
BASDAI	0.035	0.015–0.055	***.001***			
CRP	0.020	- 0.035–0.005	***.008***			
ASDAS-CRP				0.852	0.466–1.238	<***0.001***

[a]Longitudinal comparison of the explanatory variable.
ADA, adalimumab; ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score-CRP; BASDAI, Bath AS Disease Activity Index; CRP, serum C reactive protein; ETN, etanercept; GOL, golimumab; INF, infliximab; TNFi, tumor necrosis factor inhibitor.

during follow-up.[13] As a result of our findings, GOL is associated with the development of ADAb which may be clinically significant during long-term use.

Previous studies have shown a relationship between serum drug levels and disease activity measurements in patients with spondyloarthritis.[10-12] However, we did not observe significant correlations between them in our study. This lack of correlation is likely attributed to the necessity for some patients in our study to switch treatments, rendering serum drug levels unreliable. It's worth noting that many studies demonstrating a relationship between serum drug levels and disease activity are cross-sectional in nature, with patients categorized based on serum drug levels.

While Gallelo et al[12] demonstrated that younger age was associated with a reduced risk of immunogenicity in patients treated with ADA, we found that younger patients in our study had a higher risk of developing ADAb. However, they did not analyze patients treated with ETN and GOL, which might have a lower immunogenic effect. Therefore, these findings may be related to the number of patients or their study population.

Additionally, we did not find a correlation between csDMARD or MTX use and the presence of ADAb. This may be related to our study population because, in our study, only 23.5% of patients were using csDMARDs and 5.3% of them were using MTX.

Our study has some limitations. Firstly, we analyzed patients based on their baseline treatment, even though some patients underwent treatment changes during the follow-up period. Secondly, we did not assess the relationship between the development of ADAb and body mass index. Moreover, no data was collected regarding the specific types of adverse events experienced by the patients.

The main strengths of the present study include the extended follow-up period and the inclusion of a patient population comprising individuals with axSpA treated with both monoclonal TNFi and fusion proteins. This study is the first to demonstrate that, in addition to TNFi type, treatment discontinuation, adverse events, younger age, and disease activity may be associated with the development of ADAb against TNFi in patients with both radiographic and non-radiographic axSpA. Furthermore, we used generalized estimating equations (GEE) for longitudinal analysis, which can be used for evaluating relationships with both continuous and dichotomous outcome variables, as well as multiple time-dependent and independent covariates.

In conclusion, this study is the first to comprehensively evaluate the development of ADAb and related factors in all diagnostic subgroups of axSpA in a 2-year prospective, multicenter longitudinal study. Our findings revealed a notably higher rate of ADAb positivity detected in the third month among patients treated with INF or ADA. However, the development of ADAb against GOL may be delayed during follow-up and could be associated with responsiveness after prolonged treatment. Furthermore, our results underscored that ETN seems to provoke less immunogenicity compared to the monoclonal antibody TNFi. The presence of ADAb may be associated not only with challenges in disease activity control but also with the occurrence of adverse events and treatment discontinuation during follow-up. Furthermore, younger patients may be at a higher risk of developing ADAb.

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Informed Consent: Written informed consent was obtained from all participants who agreed to take part in the study.

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Supplementary Table 1. Dilution Ratios for Assays

Assay Name	Dilution Ratio
ADA	1 : 200
GOL	1 : 40
ETN	1 : 50
IFX	1 : 200
anti-ADA	1 : 2
antiGOL	1 : 2
anti-ETN	1 : 2
anti-IFX	1 : 2

ADA, adalimumab; ETN, etanercept; GOL, golimumab; INF, infliximab.

Supplementary Table 2. Performance Characteristics of the Assays

Assay Name	Intra-assay CV (%)	Inter-assays CV (%)	Linearity	Linearity Lower Limit of Quantitation (LLoQ)
ADA	7	7	3.9-60 ng/mL	0.4 μg/mL
GOL	12.7	13.1	0.1-8 μg/mL	0.036 μg/mL
ETN	9.8	7.6	3.1-172ng/mL	3.1 ng/mL
IFX	8.4	8.4	1.7-54 ng/mL	0.3 μg/mL
anti-ADA	6.6	6.6	8.5-196 AU/mL	3.7 AU/mL.
anti-GOL	12	6	28-1008 AU/mL	56 UA/mL
anti-ETN	8	8	61-337 AU/mL	61 AU/mL
anti-IFX	10	8	2-139 AU/mL	2 AU/mL

ADA, adalimumab; ADAb, anti-drug antibody; CV, Coefficient of variability; ETN, etanercept; GOL, golimumab; INF, infliximab; LLoQ, linearity lower limit of quantitation.

Supplementary Table 3. Correlation between Serum Drug Levels and ADAb

	12 Week		24 Week		52 Week		104 Week	
	Rho	*P*	Rho	*P*	Rho	*P*	Rho	*P*
ADA	- 0.67	< .001	- 0.75	< .001	- 0.62	< .001	- 0.56	< .001
GOL		.98		.14		.65		.37
INF	- 0.58	.001	- 0.70	< .001	- 0.66	< .001	- 0.68	.001

ADA, adalimumab; GOL, golimumab; INF, infliximab.