The University of Medicine and Pharmacy
"Carol Davila" Bucharest

THESIS

FROM TREAT TO TARGET CONCEPT (T2T) TO IMPLEMENTATION IN CLINICAL PRACTICE IN SPONDYLOARTHRITIS

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Bucharest

2023

CONTENT

Stage of knowledge

Biological treatment with anti-TNF-α agents has changed the way patients with ankylosing spondylitis (AS) or spondyloarthritis (SpA) are treated, having a favorable effect on both pain symptoms and radiographic progression, but also in increasing quality of life patients. The targeting of predictive agents for the favorable response to biological therapy and a good monitoring of patients under this treatment is a correct approach for a very good management of the disease. Extrapolated from rheumatoid arthritis (RA), the Treat to Target (T2T) concept in spondyloarthritis refers to the treatment of patients with this disease, with the main objective being remission in early forms of the disease or low activity in late forms of the disease. This presupposes a good knowledge of the pathogenesis of the disease and the natural history of the process that characterizes the disease, but also of the therapeutic options that exist and are currently accepted in the treatment of the disease. The structural damage involved in the disability is extremely important in the therapeutic decision.

As with other chronic diseases, a treatment targeting multidimensional approach in axial spondyloarthritis is attractive. The selection of the main risks to avoid (eg, structural progression versus cardiovascular disease) can be made at the individual level. Implementation of a T2T strategy in axial spondyloarthritis should benefit from experience in other chronic diseases (eg diabetes, hypertension) but also in other chronic inflammatory rheumatic diseases (eg RA).

If in the natural history of the disease, neither the inflammation nor the structural injury is inhibited, then the disability will manifest prominently in the clinical picture. If the inflammation is inhibited in the early stages, it will have a favorable response on the structural injury and both contribute to the reduction of disability. If in the late stages of the disease, both inflammation and structural damage are inhibited, the disability is evident through the old structural damage. Thus, the T2T concept should be implemented, where in the early stages it is necessary to inhibit inflammation and structural damage, both with a favorable effect on disability. There is an important need for this T2T concept to be implemented in clinical practice because it has been found that the correct treatment according to this concept greatly reduces the structural damage involved in disability, improving the quality of life and even productivity.

Through the topics proposed in this scientific paper and through the conclusions reported in the studies carried out, we want to clarify the hypotheses raised in the management of patients with SpA and the role of the microbiota in these diseases. At the same time, we want to see if the determination of these pathogens can predict how a patient with SpA will evolve and if the therapeutic scheme can be modified depending on the presence/absence of these microorganisms.

The purpose and objectives of the thesis

The main objective of the doctoral thesis is to highlight certain factors in achieving T2T in patients with spondyloarthritis and the correlation of these results with disease activity. The research aims to see if there are relationships between the intestinal microbiota and spondylarthritis and how it could help in optimizing the treatment of patients.

The follow-up of patients with AS and SpA is carried out through the three activity scores, respectively BASDAI, BASFI and ASDAS, along with inflammation markers (ESH, FBG, CRP), but also the clinical picture, all of which lead the doctor to an individualized therapeutic strategy for each individual patient.

The present work also aims to observe in patients with AS or SpA who have achieved remission or low disease activity according to T2T and in whom the tapering of the biological treatment could be instituted, if there are certain significant factors that could have led to this therapeutic approach and if there are certain correlations between them.

Another objective of the research was to establish certain correspondences between the intestinal microbiota determined in analyzes of blood, feces, urine, synovial fluid in these patients with SpA and a control group and to highlight whether patients with SpA present certain particularities of the microbiota that influence the strategy therapeutic. At the same time, we are looking for whether this microbiota is dependent on achieving T2T or not achieving remission/minimally active disease in SpA patients and whether a more aggressive treatment of these pathogens can lead to a new approach in this disease.

Material and methods

Over a period of 2 years (May 2015 - May 2017), we carried out the research activity that followed a total number of 425 patients with AS diagnosed according to the modified New York criteria from the "Sfânta Maria" Clinical Hospital, of which 120 patients were in biological treatment.

From the 120 AS patients under biological treatment, 27 patients were subsequently identified who were monitored between January 2016 and December 2017 in order to observe the role of the microbiota in triggering SpA and the characteristics of each individual germ involved in the etiopathogenesis of the disease .

Patients were diagnosed with ankylosing spondylitis according to the modified New York criteria (1987) or with SpA according to the ASAS criteria (2009), and biological therapy with anti-TNF-α was initiated according to the recommendations of the national treatment guidelines.

In patients eligible for the study, demographic data, clinical data and laboratory analyzes (ESR, CRP, FBG) were collected.

Disease activity was evaluated using known scores, namely BASDAI (Bath Ankylosing Spondylitis Disease Activity Index), BASFI (Bath Ankylosing Spondylitis Functional Index) and ASDAS (Ankylosing Spondylitis Disease Activity Score) calculated using CRP as an inflammation marker.

Microorganisms that may be involved in triggering SpA were searched for in the various samples collected from patients and controls using a combined approach based on conventional culture techniques and nucleic acid-based analyzes together with serological tests. Specimens submitted for microbiological investigation included stool (available from 25 patients and 26 controls), urine (available from 27 patients and 26 controls), synovial fluid (available from 3 patients) and serum (available from 27 patients and 26 witnesses).

SPSS v.20 for Windows (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. All statistical tests performed had a standard P value set at 0.05. Non-parametric tests ($\chi 2$ and Fisher's exact tests) were used to assess differences in test positivity between SpA patients and controls, and the Mann–Whitney U test was performed to assess age differences between these study groups. For the differences between the groups, the t-student test was used, and for the correlations, the Spearman and Pearson coefficients.

With the help of the ANOVA test, the subgroups in the study were compared, and the evaluation of the discriminating ability of the composite indices was carried out by ROC curves and standardized mean difference (SMD) analysis.

Written informed consent was obtained for each participant in the study, preserving the confidentiality of the collected data. Later, the collection of biological samples, urine, feces, synovial fluid was carried out to determine the analyzes necessary for the study.

The opinion of the Ethics Committee of the ″Sfânta Maria″ Clinical Hospital, the place of recruitment of patients with a favorable response to the conduct of the study, was requested.

The three studies evaluated persistence on biological treatment in patients with ankylosing spondylitis but also the existence of probable agents in ankylosing spondylitis, significant factors of tapering in ankylosing spondylitis but also the importance of complex bacteriological and serological analysis in patients with spondylitis.

Results

Batch characteristics:

A total of 120 patients met the inclusion criteria in the study (Table 1). The sample was predominantly male with a percentage of 85.8%, with an average age of 44.5 years. Regarding the disease, it had an average duration of 12.1 years, half of the cases presented peripheral manifestations (47.5%) and almost all patients were HLA-B27 carriers (95.8%). 118 patients (98.3%) were treated with prescribed NSAIDs and 59 patients (49.2%) were treated with sulfasalazine, knowing that 57 of the patients (47.5%) had peripheral manifestations that justified this treatment.

Table 1. General characteristics of the group (n = 120)

1. ***Caracteristici demografice***		**2.** ***Fenotipul bolii***	
Vârsta (a)	44.5 ± 11.6	Durata bolii (a)	12.1 ± 8.4
Sex masculin	103 (85.8%)	Artrite periferice	57 (47.5%)
IMC (kg/m^2)	25.2 ± 2.7	Coxită	8 (6.7%)
Obezitate	5 (4.2%)	Uveită	32 (26.7%)

Mediu de proveniență	88 (73.3%)	BII	4 (3.3%)
Nivel educație	9 (7.5%)	Psoriazis	0 (0.0%)
3. *Actitvitatea bolii*		HLA-B27	115 (95.8%)
VSH (mm/h)	23 ± 14	**4. *Tratament***	
PCR (mg/L)	4.28 (0.4-142)	AINS	118 (98.3%)
Fibrinogen (mg/dL)	387 (45-789)	Sulfasalazină	59 (49.2%)
BASDAI	1.0 (0-9.0)	Tratament anti-TNFα	120 (100%)
BASFI	2.0 (0-9)		
$ASDAS_{CRP}$	1.30 (0.64-5.54)		

$ASDAS_{PCR}$ = Ankylosing Spondylitis Disease Activity Score using CRP; BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; BASFI = Bath Ankylosing Spondylitis Functional Index; IMC = Indice de masă corporală; PCR = Proteina C-reactivă; VSH = Viteza de sedimentare a hematiilor; FBG = fibrinogen; HLA = antigen leucocitar uman; BII = boală inflamatorie intestinală; AINS = antiiflamatoare non-steroidiene; a = ani

Among the 120 patients treated with anti-TNFα agents (Figure 1), 24 patients (20%) switched to the second anti-TNFα agent, 7 patients (5.8%) received the third anti-TNFα agent and 4 patients (3.3%) performed a fourth therapeutic switch; it should be noted that 96 patients (80%) are persistent on the same biological treatment since inclusion in the study. (figure 1).

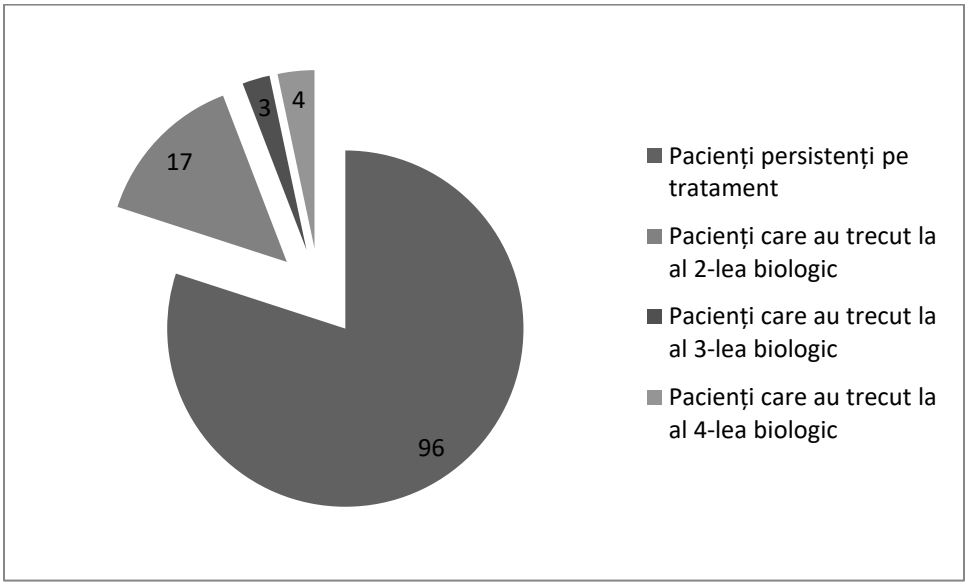


Figure 1. Distribution of patients who performed therapeutic switch and persistent patients on treatment

Among the 24 patients who made a therapeutic switch, 42% switched to etanercept, 38% to adalimumab, 17% to golimumab and 4% to infliximab (Figure 2).

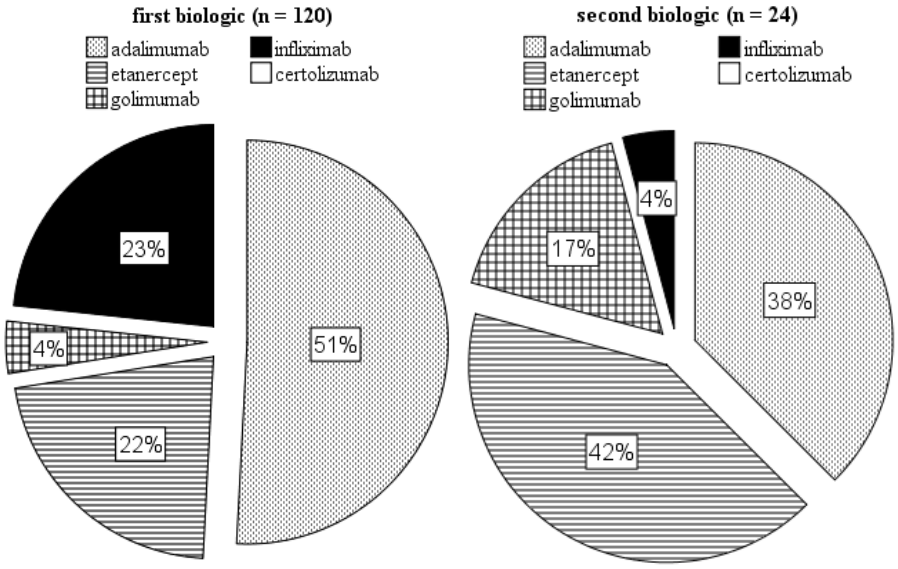


Figure 2. The percentage of patients with anti-TNFα treatment at baseline (left) and the percentage of patients after the first therapeutic switch (right).

The percentage of patients who were initially on adalimumab decreased from 51% to 38%, the percentage of patients who were on infliximab decreased from 23% to 4%, and the percentage of patients on etanercept from 22% to 42% and on golimumab increased from to 4% to 17%.

Patients persistent on treatment versus patients who made a therapeutic switch

By dividing the study sample into two subgroups, patients who changed their first anti-TNFα agent and patients who did not until the end of the observation period, two significant differences emerged (Table 2): compared to persistent patients on treatment, patients who switched had higher disease activity indices (BASFI, ASDAS-CRP) and a significantly higher prevalence of uveitis.

Table 2. Comparisons of variables at baseline in patients persistent on treatment, and in patients who made a therapeutic switch (n = 120).

	Pacienți persistenți pe tratament (n = 96)	*Pacienți care au făcut switch (n = 24)*	*p*
Vârsta (a)	44 (18)	42 (13)	0.346[#]
Durata bolii (a)	9 (11)	11 (7)	0.207[#]
IMC (kg/m^2)	25.2 (4.1)	23.9 (4.5)	0.192[#]
VSH (mm/h)	14 (18)	15 (29)	0.238[#]
Fibrinogen (mg/dL)	354 (132)	394 (180)	0.698[#]
PCR (mg/L)	3.4 (7.3)	6.4 (11.9)	0.177[#]
BASDAI	1.0 (1.2)	4.2 (1.7)	0.086[#]
BASFI	2.0 (2.0)	2.7 (1.3)	*0.009*[#]
$ASDAS_{CRP}$	1.20 (0.75)	2.3 (0.64)	*0.025*[#]
Mediu de proveniență (%)	85.4%	66.7%	0.409[*]
Obezitate (%)	4.2%	4.2%	0.739[*]
Artrite periferice (%)	47.9%	45.8%	0.855[*]
Uveită (%)	22.9%	41.7%	*0.043*[*]
Inflamaţie (cresterea VSH, CRP, FBG) (%)	47.9%	66.7%	0.100[*]
HLA-B27 (%)	94.8%	100%	0.582[&]
AINS (%)	97.9%	100%	0.639[&]
Sulfasalazină (%)	47.9%	54.2%	0.584[*]

Test statistic: # – Mann Whitney U test; & – Fisher's exact test; * – χ^2 test;

$ASDAS_{PCR}$ = Ankylosing Spondylitis Disease Activity Score using CRP; BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; BASFI = Bath Ankylosing Spondylitis Functional Index; IMC = Indice de masă corporală; PCR = Proteina C-reactivă; VSH = Viteza de sedimentare a hematiilor; FBG = fibrinogen; HLA = antigen leucocitar uman; BII = boala inflamatorie intestinală; AINS = antiiflamatoare non-steroidiene; a = ani

The therapeutic switch performed in patients with inactive disease (BASDAI<4, ASDAS<2.1), was applied to those with an adverse reaction to treatment (local injection reactions) or to patients who presented extra-articular manifestations under a biological agent and was considered appropriate change of therapy to another biological agent. An aspect worth noting is the fact that the number of patients who presented "de novo" uveitis episodes during the evolution of the disease is double in the subgroup of patients who performed a therapeutic switch versus the group of patients persistent on treatment (41.7% versus 22.9% patients), this being the real reason for the change in treatment.

Persistence on treatment:

The median duration of treatment (time from initiation to discontinuation) of the first biologic was 6.0 (1.0–15.0) years. At the end of the observation period (after a median of 7 years of observation), 96 patients (80.0%) were still being treated with the first anti-TNFα agent, with a Kaplan-Meir survival estimate of 11.5 (10, 1-12.9) years (standard error 0.75). Several indicators of disease activity correlated significantly and negatively with the treatment time of the first biologic, namely PCR ($r = -0.219$, $p = 0.016$), BASDAI ($r = -0.394$, $p < 0.001$), BASFI ($r = -0.381$, $p <0.001$) and ASDAS-CRP ($r = -0.380$, $p <0.001$). Compared to rural patients, urban patients had a higher treatment persistence to the first anti-TNFα agent, namely 6.0 (1.0-15.0) years versus 4.5 (2.0 -12.0) years ($p = 0.044$), probably due to easier periodic access to monitoring controls and probably to differences in social status.

Survival on the first anti-TNFα agent differed according to baseline ASDAS-CRP value (Figure 3): patients with inactive disease had the longest survival on treatment with the first biologic, while patients with very active disease had shortest survival per treatment.

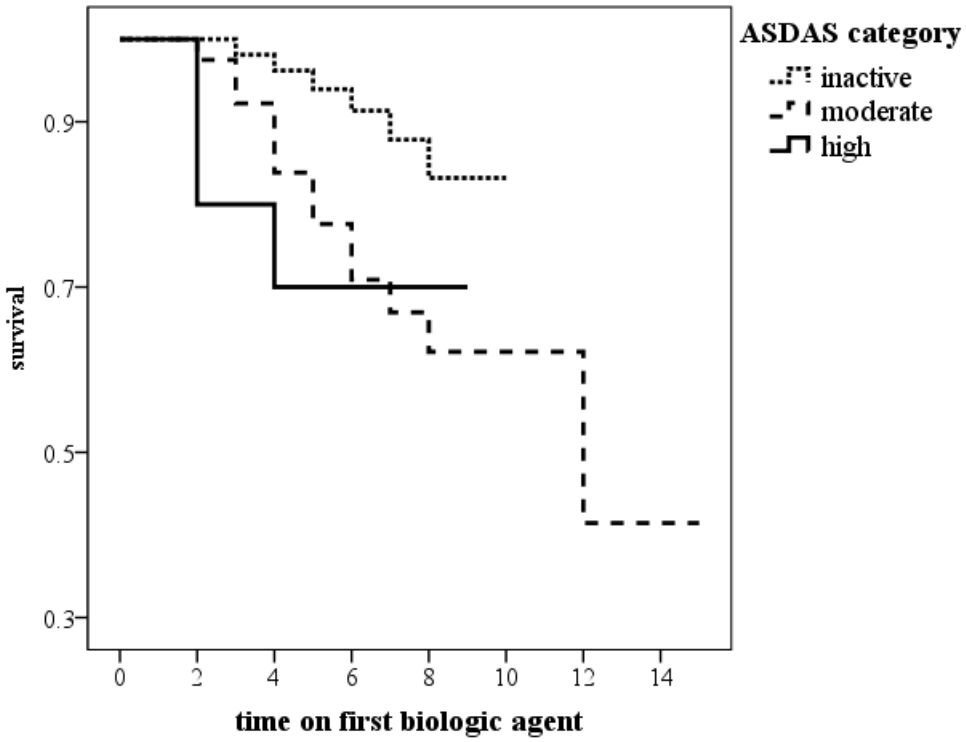


Figure 3. Kaplan-Meier time course of treatment to switch to first biological treatment by ASDASCRP categories: 9.3 (8.8-9.8; se = 0.25) years for inactive disease, 10.6 (8.8-12.5; se = 0.94) years for moderate disease and 7.1 (5.7-8.5; se = 0.74) years for highly active disease (Mantel-Cox $\chi2(2) = 10.3$, $p = 0.006$; Breslow $\chi2(2) = 13.2$, $p = 0.001$; Tarone-Ware $\chi2(2) = 12.1$, $p = 0.002$).

The shorter survival on biological treatment until the first switch in patients with highly active disease compared to patients with moderately active or inactive disease is explained by the fact that the first category of patients could present more aggressive forms of the disease that required the selection of another anti-TNF agent .

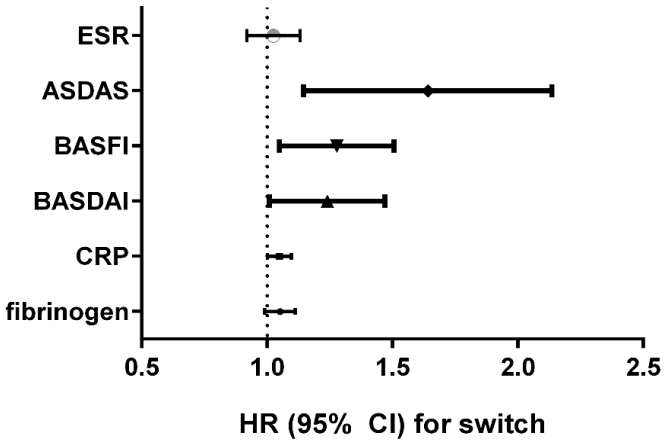
From this point of view, it should be mentioned that a correct selection of patients with AS who would initiate biological therapy is fundamental in persistence on the same biological treatment.

Predictors of therapeutic switch:

Multivariate Cox regression analysis showed that baseline markers of acute phase reactants (PCR, FBG) and baseline disease activity indices (BASDAI, BASFI, ASDAS-CRP) were able to predict switching to the first anti-TNFα agent in the study sample (Table 3), while extraspinal (peripheral arthritis) and extraarticular (uveitis) manifestations were not significant predictors of switch.

Table 3. Predictors of the switch

model	*HR (95% CI)*	*p*
Artrite	0.883 (0.387-2.015)	0.768
Uveite	1.948 (0.841-4.509)	0.120
VSH	1.012 (0.997-1.028)	0.110
Fibrinogen	1.011 (1.003-1.021)	*0.047*
PCR	1.019 (1.007-1.031)	*0.002*
BASDAI	1.226 (1.017-1.477)	*0.032*
BASFI	1.264 (1.057-1.513)	*0.010*
$ASDAS_{CRP}$	1.592 (1.173-2.159)	*0.003*



- $ASDAS_{CRP}$ = Ankylosing Spondylitis Disease Activity Score using CRP; BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; BASFI = Bath Ankylosing Spondylitis Functional Index; BMI = body mass index; CI = interval de incredere; PCR = Proteina C-reactivă ; VSH = viteza de sedimentare a hematiilor; HLA = antigen uman leucocitar; HR = hazard ratio.

Initial biological treatment:

More than half of the sample was treated with adalimumab as the first-choice biologic agent, followed by infliximab, etanercept, and golimumab (Figure 4).

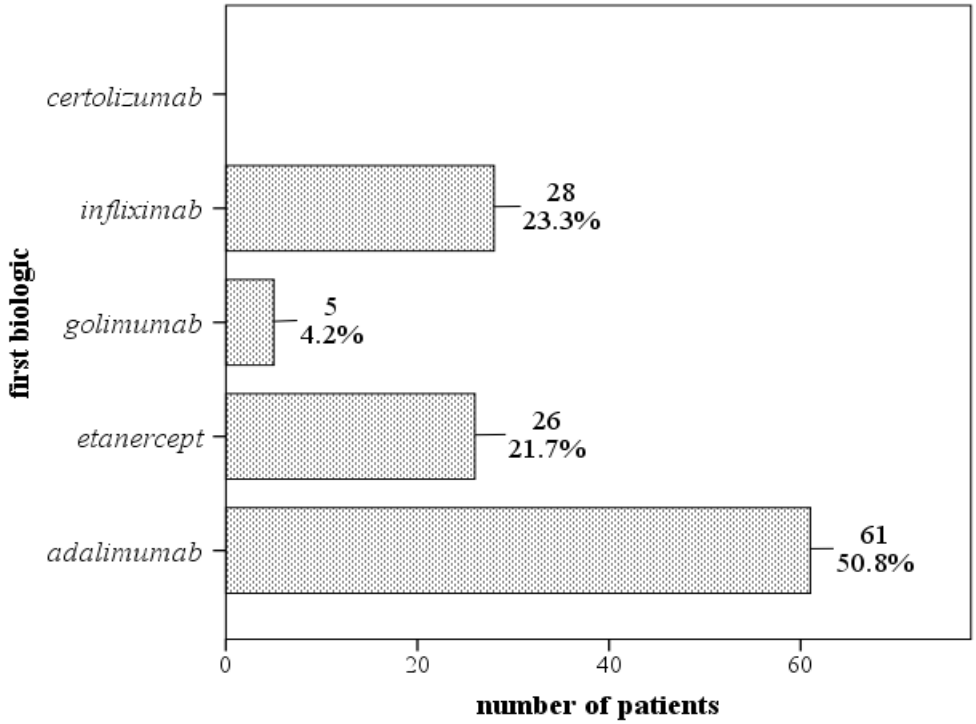


Figure 4. Distribution of the first biological treatment in patients with AS (n = 120).

From the first biologic, an equal percentage of patients (40.8%) were either tapered or maintained on the initial dose, with the remainder either switched to another biologic (16.7%) or lost to follow-up (1.7%). Tapering was implemented by joint physician-patient decision in case of persistent remission (defined as ASDAS $\leq$ 1.3 and normal values of ESR and/or CRP on two consecutive assessments, at least 6 months apart). Apart from a period of TNF administration of less than 2 years, which did not allow achieving persistent remission from a time perspective, physicians were motivated to maintain their AS patients on the initial dosage for the following reasons: the long duration of the disease and the aggressive status of the disease; persistence of extra-articular manifestations (for example, uveitis); shared decision based on patient choice.

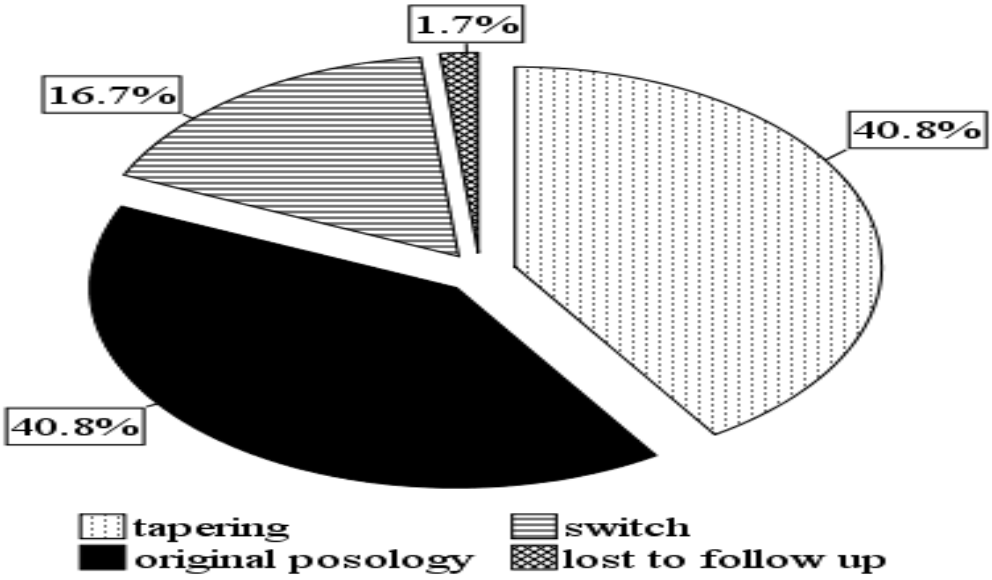


Figure 5. End-point of the first biological agent

Causes of switch included: 5 primary non-responders (25%), 12 secondary non-responders (60%), 2 cases of uveitis during biologic therapy (10%), and 1 case of inflammatory bowel disease during biologic therapy (5%). It should be noted that there were no cases that completely stopped the biological treatment during monitoring.

After excluding the two cases lost to follow-up (n = 2), the sample was divided according to the end-point of the first biological agent (figure 5), respectively tapering (n = 49), initial dose (n = 49) and switch (n = 20). Comparing these three subgroups, significant differences were observed across inflammatory markers and disease activity scores. The post hoc analysis revealed that these differences were generated by comparing the "tapering" subgroup with the "initial doses" subgroup and respectively by comparing "tapering" with "switch" (table 4).

Table 4. Comparison of inflammatory markers and disease activity scores between subgroup endpoints of the first biological agent (n = 118).

	Tapering *(n = 49)*	**Doza initiala** *(n = 49)*	**Switch** *(n = 20)*	**p**
VSH(mm/h)	11 (11)	17 (29)*	18 (31)*	0.023
FBG(mg/dL)	324 (101)	400 (154)[#]	400 (217)[#]	0.001
PCR(mg/L)	3.3 (4.6)	6.8 (17.3)[&]	10.5 (50.9)[&]	0.048
BASDAI	0.80 (0.80)	1.15 (2.00)[§]	2.10 (2.50)[§]	0.022
BASFI	1.1 (1.0)	2.2 (2.8)[¶]	3.1 (1.7)[¶]	< 0.001
ASDAS$_{PCR}$	1.20 (0.46)	1.30 (1.83)[‡]	1.71 (1.16)[‡]	0.005

ASDAS$_{PCR}$ = Ankylosing Spondylitis Disease Activity Score using PCR; BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; BASFI = Bath Ankylosing Spondylitis Functional Index; PCR = Proteina C-reactivă; VSH = viteza de sedimentare a hematiilor; FBG = fibrinogen;

Predictors of tapering:

Because inflammatory markers and activity indices produced significant differences between study subgroups, they were included in logistic regression models to predict tapering (table 5), obtaining significant results: both inflammatory markers and AS activity scores reduce significantly the chances of tapering to the first biological agent.

Table 5. Binary logistic regression model for tapering

OR	*P*	*95% CI*
0.962	0.004	0.938 – 0.988
0.992	0.001	0.988 – 0.997
0.936	0.014	0.887 – 0.986
0.565	0.005	0.377 – 0.845
0.399	< 0.001	0.253 – 0.627
0.335	0.003	0.165 – 0.683

ASDAS$_{PCR}$ = Ankylosing Spondylitis Disease Activity Score using PCR; BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; BASFI = Bath Ankylosing

Spondylitis Functional Index; PCR = Proteina C-reactivă; VSH = viteza de sedimentare a hematiilor; FBG = fibrinogen; OR = odds ratio, CI- Interval de încredere

The causes of switching to the first biological agent were studied between the 3 subgroups. The prevalence of uveitis was significantly lower in the tapering subgroup compared to the initial dose subgroup (Figure 6).

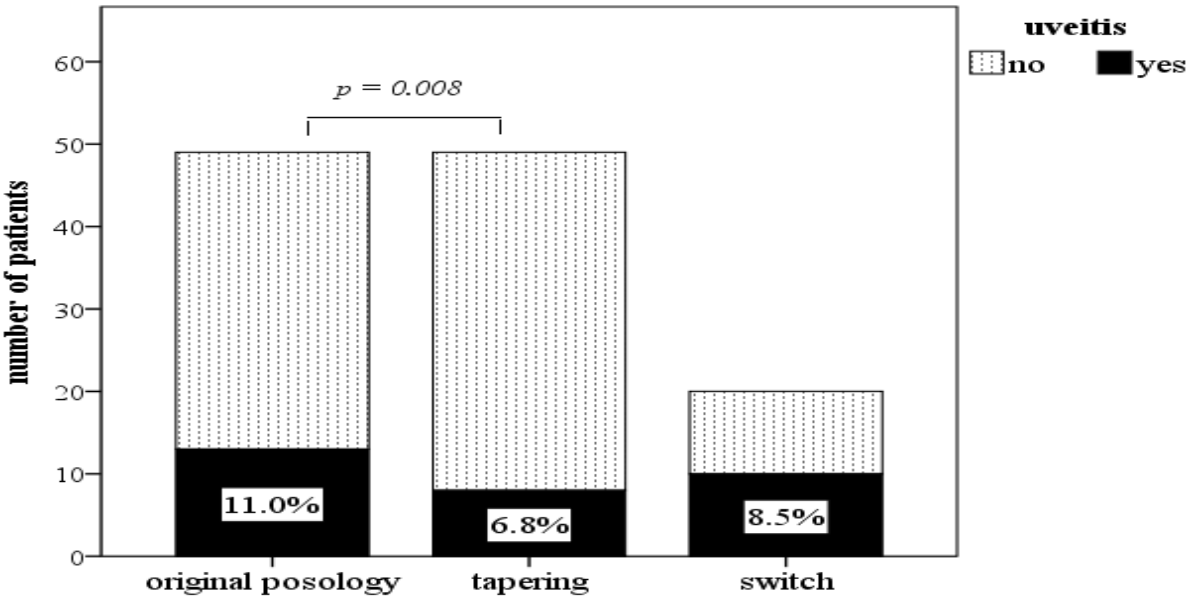


Figure 6. Prevalence of uveitis in patients with tapering, initial doses and switch (p = 0.016 for overall comparison using $\chi 2$ test, p = 0.008 for post hoc comparison between tapering and initial dose).

Thus, of the 120 patients enrolled in the study, 49 did tapering, 49 stayed on the initial dose, 20 switched, and 2 were lost from the records at the end of the study.

Tapering was done after a period of 12 or 18 months, a decision made by mutual agreement with the patient. The tapering consisted in increasing the interval between administrations, respectively: 20 patients switched to Adalimumab at 3 weeks, 6 patients

switched to Adalimumab at 4 weeks, 10 patients switched to Etanercept at 10 days, 5 patients switched to Infliximab biosimilar (Remsima) at 10 weeks, 3 patients switched to original Infliximab (Remicade) at 10 weeks and 5 patients to original Infliximab (Remicade) at 12 weeks. It should be mentioned that out of all the patients who did tapering, none of them returned to the initial dosage, all of them being kept in a state of sustained inactivity of the disease.

Of all the patients who made a therapeutic switch, 5 were primary non-responders (2 from Etanercept switched to Adalimumab, 1 from biosimilar Infliximab / Inflectra switched to Adalimumab and 2 from Infliximab switched to Etanercept) . It should be noted that from the category of all patients on biological therapy, only 2 patients developed de novo uveitis under biological treatment, which is why it was decided to switch therapeutic from Infliximab to Adalimumab, one patient presented inflammatory bowel disease, which is why the therapeutic switch from original Infliximab/Remicade to Etanercept was decided) and 12 patients were secondary non-responders (some of them even suffering several switches throughout the study – from original Infliximab/Remicade to Golimumab - 2, Etanercept-Adalimumab- 4, Remicade- Etanercept-3, Adalimumab-Etanercept-Simponi-Remicade-1, Adalimumab-Etanercept-Simponi-Cimzia-1, Adalimumab-Etanercept-Remicade.

The last study looked at the involvement of the patients' intestinal microbiota and whether certain infectious trigger factors can modify their genetic predisposition to the development of SpA.

Demographic data and clinicopathological data. Demographic parameters (age, sex) and HLA-B27 antigen presence in patients and controls, as well as disease duration, CRP values and disease activity scores in SpA patients are listed in Table 6. Of the 27 SpA patients (24 male and 3 female), 24 (88.9%) were HLA-B27-positive.

Bacterial stool culture. With one exception, stool specimens recovered from SpA patients were negative for traditional intestinal pathogens, including Shigella spp., Salmonella spp., Yersinia spp., and Campylobacter spp. The only stool specimen identified as positive contained Salmonella enterica serovar Rissen. As expected, E. coli was the predominant aerobic species of enterobacteria isolated from stools in the two study groups. Specifically, a practical culture of E coli was obtained from 23 of the patients (88%) and 25 controls

(96%). The remaining 4 patients had stool cultures dominated by K. pneumoniae (3 patients) or Citrobacter spp. (1 patient), while in the control group, one subject was positive for Citrobacter spp.

DNA-based stool analysis. Phylotyping showed that the Escherichia coli strains isolated from the available stool specimens belonged to several phylogroups. However, the major proportion of strains colonizing the intestine of patients and controls belonged to phylogenetic group A. The majority of subjects (17 patients and 19 controls) was colonized by E. coli strains representative of only one phylogenetic group, which was mainly A, but the simultaneous presence of two phylogroups was also observed. While phylogroup A predominated in the intestinal microflora of patients and controls, differences were observed in the prevalence of the other phylogroups (Table 7). Of note, phylogroup B1 and C strains were exclusively isolated from patients.

PCR-based multiplex panels were used as an alternative to culture experiments and confirmed stool specimens that tested positive in cultures for Salmonella. In addition, they identified patients with positive samples for Clostridium perfringens (n = 6), Clostridium difficile toxin B (n = 1), Aeromonas spp. (n = 1), and non-O157:H7 VTEC (n = 2). The simultaneous presence of Clostridium difficile B toxins and Aeromonas spp. was also identified in one of the SpA patients. Of note, the patient with the predominant culture of Citrobacter spp. was also identified as positive for VTEC and Clostridium perfringens by PCR. In addition, 2 patients tested positive for Clostridium perfringens. Regarding the control group, PCR identified VTEC in 4 subjects, Salmonella spp.in 2, Aeromonas spp.in 1 and Clostridium difficile toxin B in 1 subject (respectively, some subjects tested positive for multiple strains) .

Urine analysis. Urine samples were negative for enterobacteria, enterococci, and staphylococci in most patients and controls. Only 2 urine samples from SpA patients were positive in culture. Specifically, one specimen yielded Escherichia coli belonging to phylogenetic group B2 and another containing Pseudomonas aeruginosa. Among the control group, one subject was identified with significant bacteriuria with Escherichia coli of phylogenetic group B2. Of note, the SpA patient and the control subject carried the same phylogenetic group of Escherichia coli in their stool. Chlamydia trachomatis DNA in the urine of neither patients nor controls was identified using the real-time PCR method.

Table 6. Clinicopathological characteristics of control and SpA groups.

Parametrii	SpA (n=27)	Controls (n=26)	P-value
Vârsta (ani)	46.07±14.37 (34-68)	60.9±7.69 (54-77)	<0.001[a]
Sex masculin	24 (88.9)	12 (46.2)	0.002[b]
HLA-B27	24 (88.9)	2 (7.7)	<0.001[b]
Durata bolii (ani)	5.77±6.58 (1-33)	-	-
CRP (mg/l)	12.52±10.24 (1.8-32)	-	-
BASDAI	3.35±1.7 (1.1-7.0)	-	-
BASFI	3.3±1.96 (1.0-7.2)	-	-
ASDAS$_{CRP}$2.	18±1.27 (0.9-5.2)	-	-

Valoarea aP determinată prin testul U de la Mann-Whitney sau testul x2. Limita superioară a valorilor normale pentru CRP este de 5 mg / l. Valorile sunt exprimate ca medie ± deviație standard (interval) sau n (%). ASDAS-CRP, scorul de activitate al bolii de spondilită anchilozantă utilizând CRP; CRP, proteină C reactivă; BASDAI, Indice de activitate al spondilitei anchilozante; BASFI, indicele funcțional al spondilitei anchilozante ; HLA, antigen de leucocite uman; SpA, spondiloartrită.

Table 7. Escherichia coli phylogenetic groups identified in stool samples from the two groups using the revised Clermont protocol based on multiplex polymerase chain reaction.

	SpA	Control
Filogrup	(n=25)	(n=26)
A	12	15
B1	1	0
B2	2	0
C	1	0
E	0	1
F	1	3
A+B1	4	0

A+B2	0	2
A+d	1	1
A+E	1	2
A+F	2	2

SpA, Spondilartrite

Synovial fluid analysis. Synovial fluid samples recovered from 3 SpA patients were negative on culture and 16S ribosomal RNA PCR analysis.

Serum analysis. Antibodies against bacteria were detected in 22 (81%) of the SpA patients, with only one exception regarding the HLA-B27 phenotype. The prevalence of anti-bacterial antibodies detected in serum was highest for anti-Yersinia antibodies (16 patients, 67%), followed by anti-Klebsiella antibodies (11 patients, 46%), anti-Salmonella antibodies (5 patients, 21% anti-Campylobacter antibodies (5 patients, 21%) and anti-Chlamydia antibodies (4 patients, 17%; Table III).

Antibodies against the bacteria were identified in a similar number of subjects in the control groups. Specifically, 21 controls (81%) developed anti-bacterial antibodies, of which 12 developed antibodies against a single species (eg Yersinia, Klebsiella, Salmonella) and 9 against multiple species. The prevalence of anti-bacterial antibodies detected was highest for anti-Klebsiella antibodies (14 controls, 54%), followed by anti-Yersinia antibodies (11 controls, 42%), anti-Salmonella antibodies - chlamydia antibodies (2 controls, 8 %) and anti-Campylobacter antibodies (1 control, 4%; Table 8).

According to the co-occurrence of antibodies against different species, an increased number of serological profiles were identified, which comprised between 1 and 4 antibody categories depending on their bacterial trigger (Table 9). A total of 12 serological profiles were identified among patients and 5 serological profiles among controls. More than half of the subjects in the control group (14/26 subjects) tested positive for antibodies directed against one species of enterobacteria (eg, Klebsiella, Yersinia, or Salmonella) and the majority (19/26 subjects) had antibodies against no more of two categories of microbes. In contrast, less than one-third of SpA patients (8/27 subjects) developed antibodies against a single species of enterobacteria, namely Yersinia or Klebsiella, while the rest showed a variety of serological profiles with antibodies against up to four categories of microbes.

Table 8. Distribution by bacterial trigger specificity and Ig class of antimicrobial antibodies detected in sera of SpA patients and control subjects.

Tipul de agent patogen / anticorpi	SpA (n=27)	Control(n=26)	P-value
Klebsiella	11 (40.7)	21 (80.8)	0.003[a]
Anti-K21	4 (11.1)	14 (80.8)	
Anti-K36	1 (3.7)	0	
Anti-K50	6 (22.2)	0	
Yersinia	22 (81.5)	11 (42.3)	0.006[a]
IgA	15 (55.6)	5 (19.2)	
IgG	7 (25.9)	6 (23.1)	
Salmonella	8 (29.6)	5 (19.2)	0.296[a]
IgA	5 (18.5)	3 (11.5)	
IgG	3 (11.1)	2 (7.7)	
Campylobacter	7 (25.9)	1 (3.9)	0.024[b]
IgA	4 (14.8)	1 (3.9)	
IgG	3 (11.1)	0 (0.0)	
Chlamydia	5 (18.5)	2 (7.7)	0.248[b]
IgA	2 (7.4)	1 (3.9)	
IgG	3 (11.1)	1 (3.9)	

Valoarea aP determinată de testul $\chi 2$ sau testul exact al bFisher. Valorile sunt exprimate ca n (%). SpA, spondiloartrita; IgG, imunoglobulina G.

Table 9. Serologic profiles of spondyloarthritis patients and control subjects based on specific antibodies against target pathogens.

Profilul serologic (specii specificitatea anticorpilor)	Pacienţi	Control
K	2	-
Y, K	3	3
Camp, K	1	0
Chl, K	1	0
Y, Camp	1	0
Y, Chl	0	1

Y, Sal	1	1
Chl, Sal	1	0
Y, Camp, K	1	0
Y, Chl, K	1	0
Y, Sal, K	0	1
Y, Chl, Camp	1	0
Y, Camp, Chl, K	0	1
Y , Sal, Camp, K	1	0
Y, Sal, Chl, K	1	0

Y, *Yersinia enterocolitica*; Camp, *Campylobacter*; Chl, *Chlamydia trachomatis*; K, *Klebsiellapneumoniae*; Sal, *Salmonella.*

The three most complex serological profiles detected in the present study included a combination of anti-enterobacterial antibodies with anti-Campylobacter or anti-Chlamydia antibodies and the profiles obtained for SpA patients differed from those of the control group. These were identified in 3 patients and one control subject respectively. Of note, regarding anti-Yersinia antibodies, the IgA class was obviously better represented in patients than in controls.

Certain results associated with immunological and microbiological status are noted. For example, of the 3 patients who were initially positive for anti-Yersinia antibodies, the 2 who showed an HLA-B27 phenotype were retested for anti-Yersinia antibodies after an interval of 6 months. In one patient, the antibody titer increased, in another, the titer was unchanged, and in the third patient, antibodies with new specificities were detected (ie, anti-Klebsiella pneumoniae and anti-Campylobacter antibodies in the second serum sample) with the simultaneous isolation of Klebsiella pneumoniae from feces. The first case was an HLA-B27 positive patient with a disease duration of 30 years and a clinical diagnosis of SpA associated with uveitis. Progressively increasing anti-Yersinia IgA antibody titer was observed in the three sera collected at 6-month intervals during the study as follows (optical density at 450 nm): 0.980 (first sample), 1.103 (second sample) and 2.287 third sample). Anti-Salmonella antibodies were detected mainly in the serum of HLA-B27 positive patients in whom other antibodies were simultaneously detected. One of the 5 patients positive for anti-Salmonella antibodies (IgA and IgG),

who was also positive for Yersinia antibodies and anti-Campylobacter IgA, had a concomitant extraintestinal complication (uveitis). Enterobacteriaceae were dominant in the intestinal microbiota of this patient with E. coli derived from phylogenetic group B2. The patient had a history of urinary tract infections. A second sampling of feces from this dominant group Klebsiella pneumoniae and phylogenetic group B2 of E. coli detected in culture, but sterile urine cultures. For this patient, anti-Yersinia antibodies were identified in serum and synovial fluid samples. The concomitant presence of anti-Yersinia antibodies in serum and synovial fluid was also observed for the other 2 patients who provided the two types of samples (serum and synovial fluid) for laboratory analysis.

Among the 16 anti-Yersinia positive patients, only 2 were detected positive for ATPO and two for ATG antibodies. In one of these patients with an increase in anti-Yersinia antibody titer 1 year after the first sampling, the first sample was positive for ATPO and ATG and the second sample remained positive . This patient was diagnosed with SpA 17 years before being enrolled in the present study. In the control group (n = 26) 2 of the 11 subjects who were positive for anti-Yersinia antibodies were concurrently positive for ATPO antibodies.

The presence of antibacterial antibodies in patients with different levels of disease activity (assessed by ASDAS-CRP, BASFI and BASDAI scores) is shown in table 14. The number of subjects was not sufficient for statistical evaluation (Table 10).

Table 10. The number of patients with spondylotrophitis with positivity / negativity of antibacterial antibodies according to the categories of disease activity scores.

	BASDAI		BASFI		ASDAS$_{CRP}$	
ANTICORPI/STATUS	4-6	>6	4-6	>6	1.3-2.1	>2.1
IgA						
Positive	2	1	5	2	4	6
Negative	1	1	1	1	0	2
IgG						
Positive	2	3	4	3	5	5

Negative	1	1	2	2	1	3

Ig, imunoglobulină; ASDAS-CRP, scorul de activitate al bolii utilizând CRP; CRP, proteină C reactivă; BASDAI, Indice de activitate al spondilitei anchilozante; BASFI, Indexul funcțional al spondilitei anchilozante .

Conclusions

1. AS patients in Romania on anti-TNFα agents show a high rate of treatment persistence.

2. Patients who required switch have significantly higher disease activity indices at baseline, which, together with acute phase reactants also at baseline, can significantly predict switching to the first anti-TNFα agent.

3. Patients under treatment with anti-TNFα agents remained either on the first biological, or changed one, two or three biological treatments to reach the therapeutic target according to T2T.

4. Compared with treatment persisters, switch patients had higher disease activity indices (BASFI, ASDAS-CRP) and a significantly higher prevalence of uveitis.

5. Compared to rural patients, urban patients had a higher treatment persistence with the first anti-TNFα agent.

6. Baseline markers of acute phase reactants (PCR, FBG) and disease activity indices at baseline (BASDAI, BASFI, ASDAS-CRP) were able to predict switching to the first anti-TNFα agent in the study sample, while extraspinal manifestations (peripheral arthritis) and extra-articular (uveitis) were not significant predictors of switch.

7. Tapering was done after a period of 12 or 18 months, a decision made by mutual agreement with the patient.

8. Reaching the therapeutic target allowed a spacing of the biological in several steps, different depending on the type of biological.

9. The reasons for therapeutic switch were either primary or secondary non-responsiveness, or the appearance of extraspinal manifestations (uveitis, inflammatory bowel disease) under biological therapy.

10. SpA patients had a significantly higher prevalence of phylogenetic group B1 Escherichia coli in stool cultures.

11. SpA patients have a higher prevalence of anti-Yersinia antibodies in serum and a higher prevalence of anti-Campylobacter antibodies in serum.

12. Anti-Yersinia IgA antibodies were significantly better represented in SpA patients than in control subjects.

13. In addition, there were more patients positive for anti-bacterial serum antibodies in the higher SpA disease activity categories compared to the inactive disease group.

14. In certain SpA patients with high clinical scores, thyroid-specific autoantibodies have also been identified.

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