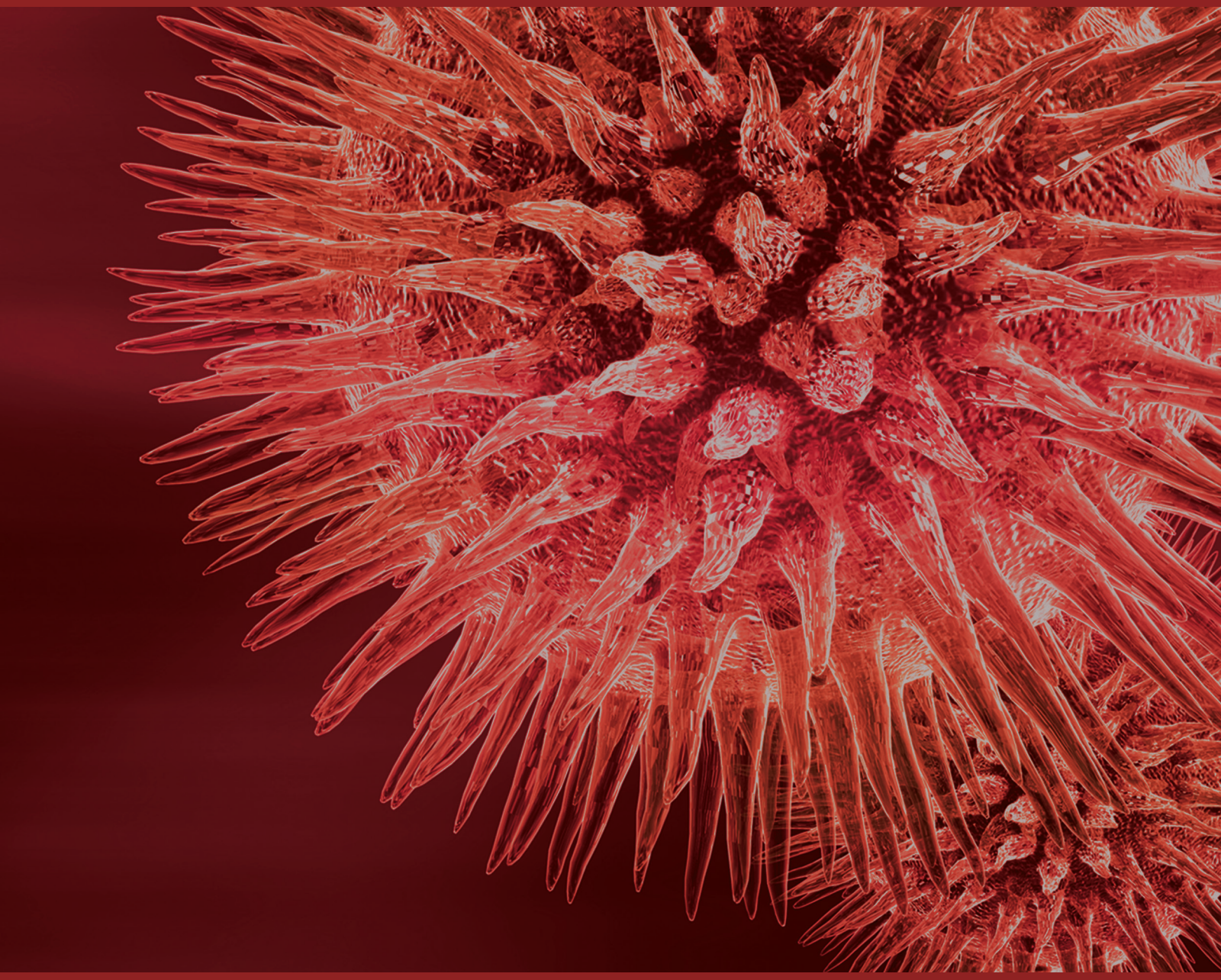
BioMed Research International

Biotechnological Drugs: The Breakthrough in Autoimmune Rheumatic Conditions

Guest Editors: Lorenzo Cavagna, Lesley-Ann Saketkoo, Andreas Schwarting, and Roberto Caporali



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Editorial

Biotechnological Drugs: The Breakthrough in Autoimmune Rheumatic Conditions

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Biotechnological drugs are a wide group of therapeutic agents obtained by means of genetic engineering methodologies. They act through the inhibition of specific cytokines/cells involved in the cascade of different pathological processes, leading to the modification of host immune response.

The main targets of these drugs are TNF-α, IL-6, IL-1, and B and T cells. In the latest years, according to the improved knowledge in the pathogenesis of several diseases, the use of these drugs is steadily increasing, and new agents have been marketed or are under development.

Autoimmune rheumatic diseases are the prototypical example of pathological conditions that may benefit from these therapies. Biotechnological drugs deeply improved patients' prognosis and quality of life in several rheumatic conditions, as, for example, rheumatoid arthritis (RA). Despite the large number of papers published and the large clinical experience obtained for some of these drugs, there are still many questions that should be clarified. This special issue is addressed to the analysis of some peculiar aspects of these drugs, in both clinic and research context.

F. Atzeni et al. evaluated in a real life study the effects of anti-TNF-α drugs on RA related disability, suggesting that benefits from these therapies may be relevant also in patients with long-standing diseases. Interestingly, results showed that the improvement in disability was marked during the first year of anti-TNF therapy, with a subsequent slower but significant recovery over the subsequent four years.

The article by L. Cavagna and W. J. Taylor is focused on gout: this disease is the typical example of how, in few years, technologic advances improved therapeutic approach. New and old biologics on pipeline are extensively reviewed, in order to help clinicians in their daily activity.

E. G. Favalli et al. managed an interesting review addressed to RA therapeutic approach: they demonstrate that, with respect to treatments available, up to now only a limited number of head-to-head randomized controlled trials are available, with subsequent limitations in the optimal use of different drugs available in daily practice. However, the authors provided some preliminary but encouraging suggestions on how to deal with the complexity of the available therapeutic armamentarium.

M. Schulz et al. assessed the differences in TNF-α regulation in patients with ankylosing spondylitis (AS) and RA being treated with infliximab and etanercept. Results suggested that drug metabolism between these diseases is different and they are a further input for the pathogenetic studies in both conditions.

The paper of F. Genre et al. deeply analysed the role of adipokines in the assessment of cardiovascular risk of AS, particularly in anti-TNF-α treated patients. By considering the impact of cardiovascular mortality in the setting, authors' suggestions are an important impulse for further studies on these interesting molecules.

Finally, Y. Hu et al. first analysed the role of a peculiar neuronal nicotinic acetylcholine receptor, named α7nAChR,

and of its partial agonist GTS-21 in the experimental model of collagen induced arthritis (CIA), identifying another interesting and potential target of treatment in RA.

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Andreas Schwarting
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Research Article

Effects of Anti-TNF Alpha Drugs on Disability in Patients with Rheumatoid Arthritis: Long-Term Real-Life Data from the Lorhen Registry

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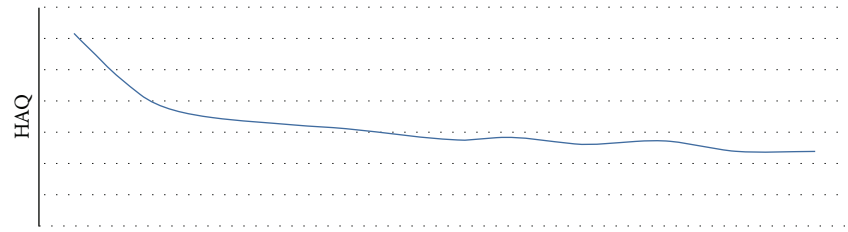
This study involving 1033 patients with RA confirms the effectiveness of etanercept, adalimumab, and infliximab in reducing RA-related disability even in patients with a history of highly active and longstanding RA. Moreover, we found that the improvement in disability was biphasic, with a marked improvement during the first year of anti-TNF therapy, followed by slower but significant recovery over the subsequent four years.

1. Introduction

Rheumatoid arthritis- (RA-) related disability is one of the major problems faced by clinicians and patients: it reduces working capacity [1], affects the personal relationships and lifestyles of patients and their relatives [2], and increases the direct and indirect costs of the disease [3]. The wide range of factors that may give rise to patient disability include disease activity, joint damage [4], articular pain [5], and comorbidities [6, 7]. However, despite the established impact of disability in RA, the current treatment guidelines are driven by evaluations of disease activity based on composite scores such as the 28-joint disease activity score (DAS28). Introduced in 1995 [8], the DAS28 has a cut-off value of <2.6 defining RA remission [9] but does not include a disability assessment. Moreover, real-life practice clearly shows that multiple joints can remain swollen or tender, and that pain can persist even when patients meet the remission cut-off score [10]. It is interesting to note that a recent large-scale observational study found disparities between the reduction in disease activity as expressed by DAS28 scores and the progression of disability [11]. The recently published ACR/EULAR remission criteria are also affected by these limitations [12]. The fact that the available disease activity scores do not necessarily correlate with structural remission or disability therefore suggests that there is a need for additional means of evaluation and a more detailed consideration of the quality of remission [13].

This is particularly important because the therapeutic approach to RA has greatly improved as a result of its earlier diagnosis and treatment [14, 15] and the availability of bio(techno)logical drugs such as anti-TNFα agents [16]. The European League Against Rheumatism (EULAR) recommendations stress the well-timed use of anti-TNF agents in the case of the premature failure of traditional disease modifying antirheumatic drugs (DMARDs) [17].

The Health Assessment Questionnaire (HAQ) is the most widely used index of disability in RA: it is sensitive, effective, reliable, cheap and rapid to administer, reflects the patients' point of view, and correlates well with measures of chronic



	Baseline	6th month	12th month	18th month	24th month	30th month	36th month	42nd month	48th month	54th month	60th month
HAQ	1.42	1.01	0.89	0.85	0.81	0.76	0.77	0.73	0.75	0.68	0.69

Figure 1

inflammation [18]. If an HAQ score is <0.5 during a year, RA treatment can be considered very effective, but this is true of only 38% of the patients with a DAS28 score of <2.6, and 56% of those with the HAQ a simple disease activity index (SDAI) of <3.3 [18]. In addition, HAQ is related to working capacity [19], the need for specialist examinations [20], and the *quoad vitam* prognosis [21], and is also an appropriate means of summarising outcomes and the direct and indirect costs of the disease [22].

The primary aims of this study were to define the long-term effects of anti-TNFα drugs (etanercept, adalimumab, and infliximab) on disability in patients with early or long-standing RA and evaluate whether an improvement in HAQ scores correlates with an improvement in DAS28 scores. The secondary aims included identifying the baseline factors associated with disability, evaluating the kinetics of drug-induced improvements in disability, and indirectly observing whether there are differences in functional responses to the three anti-TNF drugs.

2. Materials and Methods

The source of the data used in this study was the online Lombardy Rheumatology Network (LORHEN) registry, which contains the clinical history and demographic data of all patients satisfying the 1987 revised American College of Rheumatology (ACR) criteria for RA [23] attending four Rheumatology Centres in Lombardy (Spedali Civili in Brescia, Ospedale L. Sacco and Istituto G. Pini in Milan, and Policlinico San Matteo in Pavia) since 1999 who have been treated with bio(techno)logical drugs until last year. The registry has been previously used as a source for other scientific publications [24, 25]. The inclusion criteria were beginning first-line bio(techno)logical treatment with an anti-TNF agent (infliximab, adalimumab, or etanercept) and at least six months of followup. The data were collected at baseline and then every six months until a maximum followup of 60 months (end of collection: March 2013) and included the number of swollen and tender joints (out of 28 joints), laboratory findings (rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPAs), C-reactive protein (CRP) levels, the erythrocyte sedimentation rate (ESR)), and DAS28 and HAQ scores [26].

The enrolled patients were stratified on the basis of different variables: age at the time of beginning anti-TNFα therapy (≥65 versus <65 years); gender (males versus females); RF (seronegative versus <3 times the upper normal limit of 42 IU/mL (low titre) versus ≥3 times the upper normal limit (medium/high titre)); disease duration at baseline (<3, 3–5, 5–10, 10–15, or ≥15 years); disease activity at baseline assessed on the basis of DAS28 scores (<2.6 = remission; 2.6–3.2 = low disease activity; 3.3–5.0 = moderate disease activity; ≥5.1 = high disease activity); baseline HAQ scores (<0.5 = no disability; 0.5–1 = mild disability; 1-2 = moderate disability; ≥2 = severe disability) [27]; the concurrent use of DMARDs and steroids (yes versus no); the anti-TNF agents used (infliximab, etanercept, and adalimumab); and the number of comorbidities (1, 2, 3, or ≥4), including all comorbidities that have a potential impact on patient disability such as cardiovascular and lung involvement, peripheral neuropathy, type 2 diabetes mellitus, dyslipidemia, thyroid illness, and osteoporosis.

The improvement in disability was considered clinically significant if it was more than the minimally important difference (MID; HAQ > 0.22) [28].

3. Statistical Analysis

The differences between the anti-TNF agents were analysed on the basis of the data related to all LORHEN patients with at least a 6-month HAQ score using the Kruskal-Wallis nonparametric test for continuous variables (mean values and standard deviations) and the chi-squared test for categorical variables (absolute numbers and percentages). The changes from baseline were analysed using Wilcoxon's signed-rank test. The multivariate analyses were made using stepwise logistic regression models, with the response variable being defined as a >0.5 decrease in HAQ scores after one and five years. All of the analyses were made using SAS version 9.2 (SAS Institute, Inc., Cary, NC), and a P value of 0.05 or less was considered statistically significant.

All of the statistical analyses excluded patients with missing data.

4. Results

The LORHEN registry includes 1381 patients satisfying the 1987 revised ACR criteria for RA [24]. We considered only

those receiving infliximab, adalimumab, or etanercept as a first-line bio(techno)logical drug.

The final study population consisted of 1033 patients (847 females, 186 males) whose baseline clinical and demographic characteristics are shown in Tables 1 and 2. At the end of the study, 42% of the patients were still receiving an anti-TNFα agent (Figure 3). Disability as assessed on the basis of their HAQ scores significantly decreased in all cases (ΔHAQ −0.78; $P < 0.05$): at the end of the followup, the HAQ score was 0.69 (mild disability) as against 1.42 at baseline (moderate disability). Furthermore, 193/904 patients (21.35%) had no disability (HAQ < 0.5) after one year and 123/344 (35.75%) after five years of followup.

The change in HAQ scores over time had a biphasic course (Figure 1), with a rapid clinical improvement in the first year (ΔHAQ 0-1: −0.53; $P < 0.05$), followed by a further slower improvement that did not become clinically or statistically significant until the end of the followup (ΔHAQ 1–5: −0.25; $P < 0.05$). The functional improvement was even more striking (ΔHAQ 0–5: −0.81; $P < 0.05$) among the 459 patients in clinical remission (44.43%; DAS28 < 2.6), and there was no disability after the 30th month of followup. Interestingly, there was a temporal dissociation between the time of clinical remission (21st month) and the time of maximum functional improvement (60th month).

The population was then stratified on the basis of the various clinical and demographic variables described in the Methods section, and the results are shown in Table 3. The 847 females (82%) had greater disability at baseline than the 186 males (18%) (ΔHAQ > MID; $P < 0.05$), and this difference was even greater at the end of the followup period.

The 245 patients aged ≥65 years (25.76%) showed greater disability at baseline (ΔHAQ > MID; $P < 0.05$) than the 788 patients aged 18–64 years (74.24%), and their improvement during followup was less striking.

The seronegative patients (75/668, 11.22%) and those with a low RF titre (110/668, 16.47%) showed similar disability at baseline and similarly improved during the five years of biological treatment (HAQ < MID; P: ns), whereas the baseline disability of the patients with a high titre (483/668, 72.31%) was worse (ΔHAQ > MID; $P < 0.05$) and remained so until the end of the followup.

The between-group differences in disease duration before the start of anti-TNF therapy (<3 years: 412/1017, 40.52%; 3–5 years: 105/1017, 10.32%; 5–10 years: 181/1017, 17.8%; 10–15 years: 140/1017, 13.76%; and >15 years: 179/1017, 17.6%) were significant at baseline and remained so until the end of the followup (ΔHAQ > MID; $P < 0.05$): in particular the longer the disease duration, the worse the disability.

The patients with higher baseline HAQ and DAS28 scores showed worse disability and achieved a less significant improvement (ΔHAQ > MID; $P < 0.05$). The disability subgroups were HAQ < 0.5: 53/1018, 5.2%; 0.5–1: 161/1018, 15.82%; 1-2: 599/1018, 58.85%; and ≥2: 205/1018, 20.13%. The disease activity subgroups were DAS28 < 3.2: 30/1021, 2.93%; 3.2–5.1: 218/1021, 21.36%; ≥5.1: 773/1021, 75.71%.

The patients were stratified into four subgroups on the basis of the number of comorbidities: 380/704 (53.98%) had one, 188/704 (26.7%) two, 86/704 (12.22%) three, and 50/704 (7.1%) four or more. The presence of comorbidities significantly reduced the recovery of joint function during anti-TNF treatment: the greater the number of comorbidities, the worse the improvement in disability (ΔHAQ > MID; $P < 0.05$).

The baseline difference between the patients receiving steroids (867/1033, 83.93%) or not (166/1033, 16.07%) was statistically significant (ΔHAQ > MID; $P < 0.05$), but there was no difference after 42 months (ΔHAQ > MID; P = ns).

The patients concurrently receiving DMARDs (950/1033, 91.94%) showed a better functional recovery than those receiving monotherapy (83/1033, 8.03%), and the difference became significant after 30 months of followup (ΔHAQ > MID; $P < 0.05$).

Table 1: Baseline clinical data of the 1033 study patients.

HAQ (SD)		1.42 ± 0.61
DAS28 (SD)		5.73 ± 1.15
	High	773 (74.83%)
	Moderate	218 (21.1%)
	Low	15 (1.45%)
	Remission	15 (1.45%)
	Missing	12 (1.16%)
Steinbrocker functional class	I	37 (3.58%)
	II	706 (68.34%)
	III	247 (23.91%)
	IV	37 (3.58%)
	Missing	6 (0.58%)
Pain visual analogue scale (SD)		62.09 ± 22.68
Global health assessment (SD)		59.81 ± 22.47
Swollen joint count (SD)		9.73 ± 5.54
Tender joint count (SD)		10.8 ± 6.49
	Adalimumab	305 (29.53%)
Anti-TNF drugs	Etanercept	231 (22.36%)
	Infliximab	497 (48.11%)
	1	67 (6.49%)
	2	364 (35.24%)
Number of previous DMARDs	3	309 (29.91%)
	4	172 (16.65%)
	≥5	113 (10.94%)

HAQ: Health Assessment Questionnaire; DAS28: 28-joint disease activity score; TNF: tumour necrosis factor; DMARDS: disease modifying antirheumatic drugs; SD: standard deviation.

Table 2: Baseline demographic data.

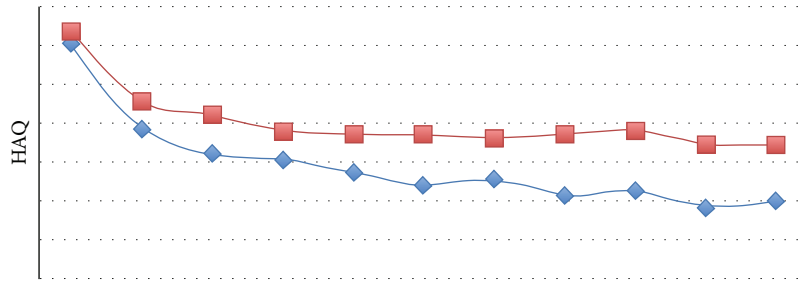
F/M	847/186 (81.99%)
Age at diagnosis (SD)	46.79 ± 14.62
Age at anti-TNF start, years (SD)	55.12 ± 13.4
Disease duration, years (SD)	7.68 ± 8.15
Comorbidities	704/1033 (68.15%)

F: female; M: male; SD: standard deviation.

Table 3: Patients stratified by their clinical and demographic characteristics. The table shows the number of patients in each subgroup and their mean HAQ scores (except in the case of missing data). The bold indicate that the difference between the subgroups is statistically significant ($P < 0.05$).

		Baseline	6th month	12th month	18th month	24th month	30th month	36th month	42th month	48th month	54th month	60th month
Gender	F; M	847-186	847-186	741-163	660-145	603-136	413-82	529-112	374-81	401-78	303-62	281-63
	HAQ	**1.47-1.21**	**1.06-0.75**	**0-0.96-0.59**	**0.91-0.56**	**0.87-0.54**	**0.8-0.55**	**0.82-0.5**	**0.8-0.44**	**0.81-0.42**	**0.73-0.43**	**0.76-0.41**
Age	<65; ≥65; missing	706-245-82	706-245-82	617-218-69	554-193-58	518-172-49	375-106-14	463-142-36	344-97-14	362-107-10	269-84-12	260-76-8
	HAQ	**1.39-1.62**	**0.94-1.21**	**0.81-1.16**	**0.75-1.15**	**0.72-1.09**	**0.68-1.05**	**0.68-1.08**	**0.64-1.04**	**0.67-1.01**	**0.59-0.96**	**0.61-0.98**
RF	neg; <3x; ≥3x; missing	75-110-483-365	75-110-483-365	63-104-416-321	55-95-371-284	52-91-327-269	32-57-181-225	47-84-276-234	34-51-165-205	29-56-198-196	24-43-138-160	22-39-139-144
	HAQ	**1.19-1.2-1.31**	0.82-0.89-0.96	**0.76-0.75-0.88**	**0.71-0.73-0.84**	**0.7-0.7-0.82**	**0.55-0.62-0.75**	0.62-0.68-0.79	0.62-0.56-0.76	**0.58-0.56-0.78**	0.56-0.49-0.72	0.64-0.54-0.71
Disease duration (years)	<3; 3-5;5-10; 10-15; ≥15	412-105-181-140-179-16	412-105-181-140-179-16	367-94-150-122-158-13	331-77-131-114-139-13	294-69-120-105-138-13	190-50-88-69-89-9	249-62-108-94-115-13	170-45-76-69-85-10	175-44-81-74-96-9	140-30-59-54-73-9	128-28-52-55-74-7
	HAQ	**1.23-1.36-1.44-1.64-1.74**	**0.96-0.8-0.86-1.12-1.3**	**0.8-0.75-0.76-1-1.24**	**0.75-0.72-0.68-1-1.21**	**0.69-0.69-0.72-0.89-1.13**	**0.63-0.68-0.67-0.85-1.11**	**0.64-0.67-0.67-0.9-1.08**	**0.65-0.65-0.59-0.78-1.03**	**0.62-0.71-0.66-0.86-0.99**	0.6-0.68-0.62-0.7-0.86	**0.56-0.61-0.58-0.83-0.93**
Comorbidity	1; 2; 3; ≥4; missing	380-188-86-50-329	380-188-86-50-329	335-163-78-45-283	305-146-67-41-246	284-128-62-38-227	190-92-42-30-141	249-114-50-34-194	166-94-39-29-127	177-98-44-27-133	131-80-34-20-100	129-73-32-20-90
	HAQ	**1.42-1.53-1.64-1.68**	**1-1.14-1.28-1.08**	**0.84-1.04-1.2-1.18**	**0.81-0.95-1.16-1.19**	**0.8-0.85-1.16-1.01**	**0.7-0.86-1.24-1.23**	**0.76-0.83-1-0.99**	**0.69-0.76-1.21-1.08**	**0.77-0.75-1.07-1.11**	**0.66-0.7-0.92-1.2**	**0.65-0.84-0.86-1.14**
Baseline HAQ score	<0.5; 0.5-1; 1-2; ≥2; missing	53-161-599-205-15	53-161-599-205-15	46-139-532-174-13	40-118-490-143-14	38-108-444-135-14	23-65-310-88-9	34-86-389-118-14	25-50-293-77-10	25-58-286-98-12	21-40-231-64-9	21-37-208-69-9
	HAQ	**0.48-0.79-1.37-2.35**	**0.3-0.56-0.99-1.61**	**0.31-0.5-0.85-1.52**	**0.25-0.5-0.81-1.44**	**0.29-0.47-0.77-1.35**	**0.14-0.46-0.71-1.33**	**0.15-0.5-0.71-1.32**	**0.14-0.47-0.71-1.17**	**0.15-0.43-0.69-1.23**	**0.14-0.51-0.64-1.1**	**0.14-0.43-0.63-1.15**
Baseline DAS28	<3.2; 3.2-5.1; ≥5.1; missing	30-218-773-12	30-218-773-12	29-194-671-10	26-161-607-11	21-160-547-11	11-105-370-9	18-135-477-11	12-98-335-10	17-99-354-9	13-73-272-7	15-65-258-6
	HAQ	**1.01-1.12-1.54**	**0.55-0.78-1.1**	**0.59-0.69-0.97**	**0.72-0.63-0.92**	**0.33-0.63-0.88**	**0.31-0.55-0.84**	**0.27-0.57-0.84**	**0.38-0.56-0.8**	**0.39-0.64-0.8**	**0.37-0.52-0.74**	**0.35-0.61-0.74**
Anti-TNF drug	ADA; ETA; INF; missing	301-227-494-11	301-227-494-11	260-193-442-9	220-171-404-10	208-153-368-10	159-97-231-8	183-128-320-10	143-84-219-9	133-84-254-8	102-68-188-7	80-57-201-6
	HAQ	**1.24-1.37-1.57**	**0.86-0.99-1.1**	**0.77-0.89-0.97**	0.76-0.88-0.89	**0.7-0.87-0.84**	0.73-0.84-0.75	0.7-0.86-0.77	0.67-0.86-0.72	0.65-0.92-0.74	0.67-0.74-0.66	0.72-0.71-0.67
Steroids	No; yes	166-867	166-867	148-756	132-673	124-615	87-408	107-534	83-372	85-394	68-297	65-279
	HAQ	**1.29-1.45**	**0.88-1.03**	**0.74-0.92**	**0.72-0.87**	**0.68-0.83**	**0.65-0.78**	**0.65-0.79**	0.67-0.75	**0.65-0.77**	0.64-0.69	0.61-0.71
DMARDs	No; yes	83-950	83-950	68-836	53-752	51-688	38-457	48-593	36-419	38-441	29-336	25-319
	HAQ	1.53-1.42	1.05-1	0.97-0.89	0.91-0.84	0.9-0.8	**1.11-0.73**	0.85-0.76	**0.89-0.72**	**0.97-0.73**	**0.9-0.66**	**1-0.67**

F: female; M: male; RF: rheumatoid factor; <3x RF: low titre [<3 times the upper normal limit of 42 IU/mL]; HAQ: Health Assessment Questionnaire; DAS28: disease activity score in 28 joints; TNF: tumour necrosis factor; ADA: adalimumab; ETA: etanercept; INFL: infliximab; DMARDs: disease modifying antirheumatic drugs.



	Baseline	6th month	12th month	18th month	24th month	30th month	36th month	42nd month	48th month	54th month	60th month
No switch	1.41	0.97	0.84	0.81	0.75	0.68	0.71	0.63	0.65	0.57	0.6
Switch	1.47	1.11	1.04	0.96	0.94	0.94	0.92	0.94	0.96	0.89	0.89

Figure 2

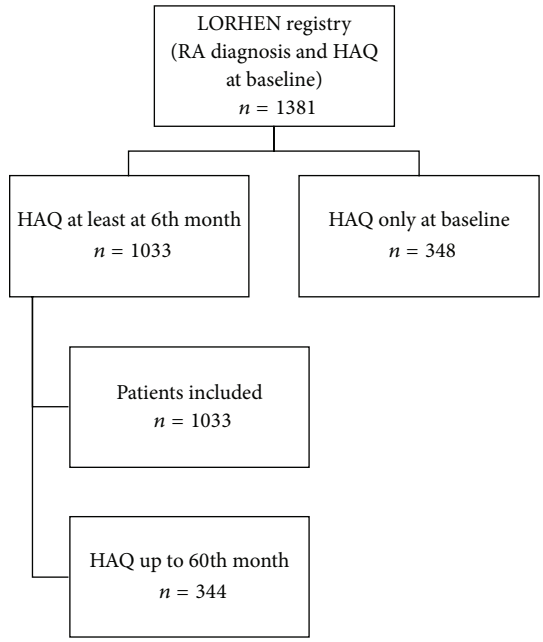


Figure 3

In relation to the anti-TNFα agents used (infliximab, 494/1022, 48.34%; etanercept 227/1022, 22.21%; and adalimumab 301/1022, 29.45%), the patients treated with infliximab had worse baseline disability (ΔHAQ > MID; $P < 0.05$), but the difference became nonsignificant after two years of anti-TNF treatment (ΔHAQ > MID; P = ns).

Two hundred and seventy-five patients (26.62%) switched from the first to a second TNF blocker because of a secondary lack of effectiveness (58.55%), adverse events (30.55%), or other reasons (10.9%): the most frequently discontinued drug was infliximab, which was most frequently replaced by etanercept. The patients who did not need to modify their biological treatment had less residual disability than those who had to switch (HAQ 0.6 versus 0.89; $P < 0.05$) (Figure 2), but the improvement in HAQ scores was significant after five years of followup in both subgroups (ΔHAQ > MID; $P < 0.05$).

Multivariate analyses identified the baseline variables correlated with a more than 0.5 decrease in HAQ scores after one and five years. The patients who were less likely to achieve an optimal functional recovery were mainly females, aged more than 65 years, had a baseline DAS28 score of >5.1, and were not taking steroids (Table 4). Functional recovery after five years was reduced in the patients treated with etanercept or adalimumab.

It was impossible to include the other variables in the multivariate analyses because of the missing data.

5. Discussion

Our results confirm the effectiveness of etanercept, adalimumab, and infliximab in reducing RA-related disability even in patients with a history of highly active and

Table 4: Univariate and multivariate analyses of baseline characteristics predicting complete disability recovery (HAQ < 0.5).

HAQ < 0.5 (1st year)	Univariate analysis		HAQ < 0.5 (1st year)	Multivariate analysis	
	OR (95% CI)	*P*		OR (95% CI)	*P*
Age (≥65 versus <65 years)	[0.974 (0.962–0.985)]	<0.0001	Age (≥65 versus <65 years)	[0.978 (0.965–0.99)]	0.0004
Gender (F versus M)	[0.354 (0.248–0.505)]	<0.0001	Gender (F versus M)	[0.417 (0.282–0.617)]	<0.0001
Disease duration (years)	[1.004 (0.985–1.023)]	ns	Disease duration	[0.998 (0.976–1.02)]	ns
DAS28 (high versus moderate versus low)	[0.622 (0.542–0.713)]	<0.0001	DAS28 (high versus moderate versus low)	[0.724 (0.626–0.837)]	<0.0001
DMARDs (no versus yes)	[1.248 (0.724–2.151)]	ns	DMARDs (no versus yes)	[0.85 (0.453–1.596)]	ns
Steroids (no versus yes)	[2.109 (1.445–3.078)]	0.0001	Steroids (no versus yes)	[1.775 (1.153–2.734)]	ns
Adalimumab versus infliximab	[1.495 (1.045–2.138)]	ns	Adalimumab versus infliximab	[1.547 (1.041–2.297)]	0.0307
Etanercept versus infliximab	[1.048 (0.69–1.59)]	ns	Etanercept versus infliximab	[0.871 (0.538–1.41)]	ns
HAQ < 0.5 (5th year)	Univariate analysis		HAQ < 0.5 (5th year)	Univariate analysis	
	OR (95% CI)	*P*		OR (95% CI)	*P*
Age (≥65 versus <65 years)	[0.978 (0.964–0.991)]	0.0014	Age (≥65 versus <65 years)	[0.982 (0.967–0.996)]	0.0142
Gender (F versus M)	[0.474 (0.31–0.726)]	0.0006	Gender (F versus M)	[0.571 (0.362–0.901)]	0.016
Disease duration	[1.002 (0.979–1.025)]	ns	Disease duration	[0.995 (0.97–1.022)]	ns
DAS28 (high versus moderate versus low)	[0.729 (0.624–0.853)]	0.0001	DAS28 (high versus moderate versus low)	[0.79 (0.668–0.936)]	0.0063
DMARDs (no versus yes)	[0.702 (0.322–1.53)]	ns	DMARDs (no versus yes)	[0.627 (0.255–1.54)]	ns
Steroids (no versus yes)	[1.851 (1.182–2.898)]	0.0071	Steroids (no versus yes)	[1.832 (1.102–3.045)]	0.0195
Adalimumab versus Infliximab	[0.607 (0.383–0.962)]	ns	Adalimumab versus infliximab	[0.554 (0.337–0.911)]	0.0199
Etanercept versus infliximab	[0.607 (0.364–1.011)]	ns	Etanercept versus infliximab	[0.502 (0.284–0.888)]	0.0179

OR: odds ratio; CI: confidence interval; F: female; M: male; DMARDs: disease modifying antirheumatic drugs; DAS28: 28-joint disease activity score; HAQ: Health Assessment Questionnaire.

long-standing RA. Similar findings have been reported in other, shorter observational studies [29, 30]. As suggested by Aletaha and Ward, we can also confirm that patients can achieve a good functional recovery even after years of illness because the reversible factors underlying HAQ scores (pain and inflammation) tend to prevail over joint damage [31]. Starting anti-TNF therapy not only generally reduced disability from moderate to mild, but also led to patients who achieved clinical remission during the followup (DAS28 score <2.6) completely recovering from disability (HAQ score <0.5) regardless of disease duration. Randomised clinical trials have shown that the well-timed use of biological treatment in the case of early failure with traditional DMARDs should induce the complete remission of functional dysfunction [32, 33], but these findings require confirmation in further studies aimed at this primary endpoint.

We found that the improvement in disability was biphasic, with a marked improvement during the first year of anti-TNF therapy, followed by slower but significant recovery over the subsequent four years. This was typically observed when a "step-up" therapeutic strategy was adopted and, in this case, it is important to wait five years for the stabilisation of HAQ scores in patients with long-standing RA. More aggressive and earlier treatment leads to a rapid phase of improvement (a J-shaped curve) and subsequent stabilisation after one year [24]. However, it is not enough to consider a state of clinical remission defined on the basis of DAS criteria because, in our study, DAS28 remission was observed an average of 21 months after the start of anti-TNF treatment, whereas it took five years before the improvement in HAQ scores was at its peak.

It is interesting to note that there was a clinical and statistical improvement in HAQ scores in all of the analysed groups during followup, although the results were less striking in patients aged ≥65 years, females, and those with a disease duration of more than 10 years, a higher comorbidity index, greater disease activity and disability, a high RF titre, or contraindications to combination therapy with traditional DMARDs. The chronic use of low-dose steroids seemed to contribute to reducing disability in the patients with greater impairment at baseline.

As expected, the patients treated with infliximab (the first biological treatment available in Italy) showed greater disability at baseline, but their improvement was better than that of the patients treated with etanercept or adalimumab.

The univariate and multivariate analyses confirmed that age (≥65 years), female gender, a DAS28 score of >5.1, and no use of steroids correlated with a lower level of recovery from disability during anti-TNF therapy. Although the probability of drug withdrawal was greater with infliximab, the use

of etanercept and adalimumab was also associated with a reduced probability of functional recovery, but this was only evident after five years of treatment. Disease duration does not seem to be a negative predictor.

One final aspect emerging from our study concerns switching from one anti-TNF agent to another: the results in the patients who had to switch because of inefficacy or adverse events were less striking than those in the patients who continued on the same drug. These findings are similar to those of other observational studies [16], but the effect on disability of using a drug with a different mechanism of action from the first still remains to be explored.

Recent studies have stressed the importance of measuring functional ability and considering it when making decisions concerning patient management. A Canadian observational study of 1086 RA patients (799 treated with anti-TNF agents) found that the annual mean (direct and indirect) costs of the disease were directly proportional to disability as measured by means of the HAQ: the costs were three times higher for patients with severe disability (HAQ > 2) than for patients without disability (HAQ < 0.5), with working disability being the major indirect cost [34]. Data from the British registry indicates that half of all biological agent-naïve RA patients are unable to work: HAQ scores closely correlated with working capacity, with patients who were severely disabled at baseline being more likely to become work disabled at followup [35].

The main strengths of our study are the large number and the heterogeneity of the enrolled patients, and the long-term followup. However, it also has some important limitations. First of all, as it was an observational study, there may be some patient selection bias. Secondly, RA disability was evaluated without using radiological parameters, and some other parameters (cigarette smoking, ACPAs) were not statistically analysed because there were too many missing data.

6. Conclusions

The effect of anti-TNF therapy on the disability of patients with RA is certainly substantial also in the case of long-standing disease and, in addition to other clinimetric indices such as the DAS28, HAQ scores are a good means of evaluating the efficacy of biological treatment.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' contribution

MF, CB FA participated in the design and helped to draft the paper. All of the authors participated in patients' recruitment. All of the authors read and approved the final paper PSP, RC, RG, LC, and FA commented on and participated in critical editing of this paper.

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Review Article

The Emerging Role of Biotechnological Drugs in the Treatment of Gout

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One of the most important therapeutic advances obtained in the field of rheumatology is the availability of the so-called bio(techno)logical drugs, which have deeply changed treatment perspectives in diseases such as rheumatoid arthritis and ankylosing spondylitis. According to the steadily increasing attention on gout, due to well-established prognostic and epidemiology implications, in the last 5 years, the same change of perspective has been observed also for this disease. In fact, several bio(techno)logical agents have been investigated both for the management of the articular gout symptoms, targeting mainly interleukin-1β, as well as urate-lowering therapies such as recombinant uricases. Among the IL-1β inhibitors, the majority of studies involve drugs such as anakinra, canakinumab, and rilonacept, but other compounds are under development. Moreover, other potential targets have been suggested, as, for example, the TNF alpha and IL-6, even if data obtained are less robust than those of IL-1β inhibitors. Regarding urate-lowering therapies, the recombinant uricases pegloticase and rasburicase clearly showed their effectiveness in gout patients. Also in this case, new compounds are under development. The aim of this review is to focus on the various aspects of different bio(techno)logical drugs in gouty patients.

1. Introduction

Gout is an autoinflammatory disease associated with increased blood levels of urate and due to deposition of monosodium urate crystals in and around joints [1]. Over recent decades, the prevalence of this condition is steadily increasing and gout is becoming one of the most common causes of inflammatory arthritis in industrialised countries [2–7]. In fact, joints are the typical target of the disease and articular gout attacks are between the most painful conditions described [8]. But gout and hyperuricemia may also affect the kidneys [9] and cardiovascular system [10] and are frequently complicated by the metabolic syndrome [11]. Gout burden is substantial: joint flares, tophi, polyarticular involvement, and chronicization deeply impact patients' quality of life and workability [12–16], whereas gout by itself is an independent risk factor for cardiac and all-cause mortality [17, 18]. Current treatment is first based on lifestyle measures and then on a pharmacological approach [19, 20]. Recently, several biotechnological drugs have been employed and approved for gout treatment. This review is focused on the analysis of these treatments that potentially could reduce gout burden and the unmet needs of its pharmacological approach.

2. Gout Pharmacological Treatment: Targets of Bio(techno)logical Drugs

Gout pharmacological treatment is aimed at relieving articular symptoms and reducing hyperuricemia [19, 20]. Both targets are of primary importance and should be achieved in gouty patients. In the last years, several bio(techno)logical drugs have been found effective for these purposes.

Symptomatic relievers and urate-lowering therapies (ULTs) act on different pathways. Symptomatic relievers mainly target IL-1, a proinflammatory cytokine that has been linked to gout since late 1980s [21] and is now widely accepted as central to the initiation of the inflammatory

cascade that culminates in gouty arthritis. In particular, the activation of NALP3 inflammasome by uric acid crystals increases the production of IL-1 and the inflammatory state [22]. The understanding of these mechanisms thus opened a new perspective in acute and chronic gout management [23].

Even if IL-1 is pivotal in gout, we should consider that also other inflammatory cytokines could be potentially involved; in particular, previous studies showed that also TNFα [24–29] and interleukin-6 (IL-6) [28–30] are overexpressed in patients with gouty arthritis. The role of TNFα in gout is also suggested by the increased expression of soluble TNF receptors I and II (sTNFR-I/II) in synovial fluids from gouty patients during arthritis resolution phases [31]. The blockade of TNFα and IL-6 through biotechnolological drugs is well established and routinely performed in rheumatoid arthritis (RA) [32–34] and, limiting to TNFα, in ankylosing spondylitis (AS) [35], with a large literature in terms of treatment survival, side effects, and warnings [36, 37]. Recently, several authors underlined the central role of T lymphocyte in the appearance of gout articular damage [38–40] in particular through the upregulation of RANKL [39] and thus of osteoclastogenesis [41]. On this basis, also T cells targeting should be considered a potential treatment of gouty arthritis. Finally, we should also consider that increased levels of transforming growth factor (TGFβ1) [42, 43] and interleukin-10 [42] are typically found in the synovial fluid of patients during gouty arthritis resolution.

Although ULTs target several steps of the human enzymatic breakdown of uric acid as well as the renal system to increase urate urinary excretion, from the bio(techno)logical point of view, one of the most intriguing targets is an enzyme that humans lost due to a gene missense mutation: uricase [44]. This enzyme converts urate to allantoin, a substance more water-soluble and thus more readily eliminated than urate. The possibility of reducing serum urate levels by means of the action of an enzyme lost by humans during primate evolution is very fascinating, almost like an inversion of the evolutive process.

3. Symptoms Relievers Bio(techno)logical Drugs: IL-1 Inhibitors

3.1. Canakinumab. Canakinumab is a fully human, anti-IL-1β monoclonal antibody first approved for the treatment of cryopyrin associated periodic syndrome [45].

The effectiveness of canakinumab in acute gout was first reported in 2010 in a phase-2 dose ranging trial of 8 weeks [46]. Enrolled patients were randomized to receive a single dose of subcutaneous canakinumab (10, 25, 50, 90, or 150 mg; $n = 143$) or intramuscular triamcinolone acetonide (40 mg; $n = 57$). After 72 hours, a dose-related pain reduction was observed in canakinumab group for every dosage used. Moreover, canakinumab 150 mg was more effective than triamcinolone acetonide in every timepoint considered (e.g., 24, 48, and 72 hours and 4, 5, and 7 days after treatment—$P < 0.05$ in all cases), also reducing the risk of subsequent articular flares (relative risk reduction 94% for canakinumab 150 mg versus triamcinolone acetonide). The overall incidence of adverse events, generally mild or moderate in severity, was similar in both groups (41% and 42%, resp.).

Another study showed that the improvement of health-related quality of life (SF-36) was faster with canakinumab 150 mg compared to intramuscular triamcinolone acetonide 40 mg [47]. In another double-blind, double-dummy, dose-ranging study, involving 432 gout patients initiating allopurinol, a single canakinumab dose of 50–300 mg or 4-weekly dosing over 4 months was superior to colchicine (0.5 mg/day) in articular flares prophylaxis [48]. In particular, there was a 64% to 72% reduction in the risk of experiencing ≥1 flare for canakinumab doses ≥50 mg versus colchicine at 16 weeks (hazard ratio (HR): 0.28–0.36, $P \leq 0.05$). No differences were observed among groups in terms of adverse events, the treatments being generally well tolerated.

The β-RELIEVED and the β-RELIEVED-II were two 12-week randomised, multicentre, active-controlled, double-blind, parallel-group studies with double-blind 12-week extensions which aimed to assess canakinumab effectiveness in acute flares and reflares of gouty arthritis [49]. Only patients with a recent articular gout acute flare (VAS pain ≥50 mm), with at least three other flares in the previous 12 months, and with contraindications to NSAIDs and/or colchicine were considered for study inclusion. The comparators were canakinumab 150 mg by subcutaneous injection and intramuscular triamcinolone acetonide 40 mg. A total of 456 patients were enrolled in the core set study (227 canakinumab, 229 triamcinolone acetonide) and 365 entered the extension study (174 canakinumab, 161 triamcinolone acetonide). With respect to triamcinolone acetonide, canakinumab significantly reduced mean 72-h VAS pain score (difference of 10.7 mm, $P < 0.0001$) as well as physician-assessed tenderness and swelling (OR 2.16 and 2.74, both $P < 0.01$). The efficacy of canakinumab with respect to triamcinolone acetonide was evident also in terms of delayed time to first new flare on both core (62% reduced risk of a new flare over 12 weeks) and extension studies (56% reduced risk of a new flare over 24 weeks) and median C-reactive protein levels reduction at 72 h and 7 days (OR 4.4 and 2.1, both $P < 0.0001$). Adverse events were observed in 66.2% of canakinumab-treated patients and in 52.8% of patients receiving triamcinolone acetonide. Infections were reported in 20.4% (canakinumab) and 12.2% (triamcinolone acetonide) of patients, being serious infections observed in 1.8% and 0%, respectively.

Based on these data, subcutaneous canakinumab has been approved by the European Medicines Agency for the on-demand symptomatic treatment of frequent gouty arthritis attacks in adult patients in whom NSAIDs, colchicine, or corticosteroids are contraindicated, not tolerated, or not effective [50] and is potentially useful also in acute gouty prophylaxis during ULT initiation [48].

Recently, canakinumab has been found effective as adjunctive treatment in patients with type I diabetes [51], and possibly also in patients with type 2 diabetes [52]. A large secondary prevention trial with canakinumab in patients with prior acute myocardial infarction is ongoing [53]. By considering the burden of metabolic syndrome [11] and

cardiovascular complications [10] in gout patients, the results of these studies could be a further reason for the use of IL-1 inhibitors in this setting.

3.2. Rilonacept. Rilonacept (IL-1 TRAP) is a soluble decoy receptor Fc fusion protein that engages and inhibits both IL1α and IL1β. It is known also as a IL-1 Trap, because it is generated using Target-Related Affinity Profiling (Trap) technology [54]. Rilonacept has been first described as effective in gout in a 14-week, multicentre, nonrandomised, monosequence crossover study involving 10 patients. The active treatment period lasted 6 weeks, followed by other 6 weeks of withdrawal period that completed the study. Rilonacept was administered with a loading dose of 320 mg (two 2 mL injections) administered subcutaneously, followed by rilonacept 160 mg once a week. Only one patient withdrew from the study because of severe injection site erythema and induration. The remaining 9 patients significantly improved in terms of *patients' self-reported median pain visual analogue scale scores* and high-sensitivity C-reactive protein reduction; also two nonvalidated instruments, the *symptom adjusted* and the *severity-adjusted joint scores*, significantly improved, whereas no effects were observed on the number of affected joints.

Subsequently, further phase II and III studies have been performed. Phase II study involved 83 patients starting allopurinol (42 in the placebo group and 41 in the active treatment group). Rilonacept was administered subcutaneously once per week (loading dose 320 mg followed by 160 mg weekly) and the follow-up was 12 weeks [55]. Rilonacept significantly reduced the mean number of gout flares per patient during the entire follow-up (6 flares versus 33; $P < 0.0011$) and in particular during the first 4 weeks of follow-up ($P < 0.007$). The proportion of patients with articular flares during the study period was lower in the rilonacept than in the placebo group (14.6% versus 45.2%; $P < 0.0037$), whereas the most common adverse events were the occurrence of injection site reactions in patients treated with rilonacept.

The PRESURGE 1 was a 16-week follow-up phase III study involving 241 gout patients from USA and Canada with at least 2 previous articular flares and persistent hyperuricemia (>7.5 mg/dL). Concomitant to allopurinol treatment (300 mg/day), the patients were randomized to 16 once-weekly subcutaneous injections of placebo, rilonacept 80 mg, or rilonacept 160 mg, with a double (loading) dose on day 1. During the follow-up, the mean number of gout flares per patient was significantly reduced by rilonacept treatment, with respect to placebo (placebo: 1.06, rilonacept 80 mg: 0.29, rilonacept 160 mg: 0.21, $P < 0.001$ versus placebo). In particular, only 18.8% and 16.3% of patients had >1 gout flares, respectively, with rilonacept 80 and 160 mg: the differences with respect to placebo (46.8% of patients had >1 gout flares) were statistically significant ($P < 0.001$ for both). The number needed to treat (NNT) for the reduction of at least 1 gout flare was 2 for both rilonacept 160 and 80 mg groups [56].

The PRESURGE-2 was another phase III study 248 gout patients with the same selection criteria and treatment groups of the PRESURGE-1 study but involving patients from Germany, India, Indonesia, Republic of South Africa, and Taiwan [57]. In this study, both rilonacept dosages significantly reduced the occurrence of gout flares after the initiation of standard ULT, with >70% of patients having no flares. Similar to PRESURGE-1 study, safety and tolerability profile was acceptable with injection site reactions being the most common adverse events described in both rilonacept groups [56, 57].

By considering studies with active comparators, the effectiveness of rilonacept (320 mg at baseline) + indomethacin (50 mg *three times per day* for three days) after 72 hours was not superior to that of indometacin alone in terms of pain reduction in a group of 225 gout patients presenting within 48 hours since flare onset [58].

3.3. Anakinra. Anakinra is a recombinant human IL-1 receptor antagonist that differs from native human IL-1Ra because of the addition of a single methionine residue at its amino terminus [59]. Anakinra binds to both IL-1α and β [60] and is the first IL-1 inhibitor marketed and approved for RA treatment [61]. The first report on the effectiveness of this drug in gout has been published in 2007 [62]. In this open-labeled study, 10 patients with a long previous history of either recurrent gouty attacks or tophaceous gout that failed or not tolerated standard therapies were treated with anakinra and administered daily at a dose of 100 mg subcutaneously for 3 consecutive days. In all cases, treatment was rapidly effective and well tolerated during a short term follow-up (maximum: 2 months). Another retrospective study involving 10 patients with refractory gout showed less interesting results: in particular, 3 patients had a partial response and one was refractory to this treatment, whereas relapses were really common during follow-up [63]. This fact is not surprising if we consider the short half-life of anakinra and the short duration of treatment. One possible solution is the temporal prolongation of the treatment. In fact, further case reports confirmed this possibility, also in case of intermittent administration [64–67].

Anakinra has been tested in parallel with canakinumab as a potential treatment for type 1 diabetes patients [51]. Even if clinical trials on canakinumab in cardiovascular diseases are ongoing, more data are available for anakinra. In particular, anakinra improved left ventricular remodelling [68] and numerically lowered the incidence of heart failure [69] in patients with acute myocardial infarction and ST-segment elevation. IL-1 blockade with anakinra for 14 days significantly improved the aerobic exercise capacity of patients with heart failure with preserved ejection fraction and elevated plasma CRP levels [70]. On this basis, similar to canakinumab and rilonacept, also for anakinra, it is possible to suppose a wide range of positive effects in patients with gout. On this basis, similar to canakinumab and rilonacept, also for anakinra, it is possible to suppose a wide range of positive effects in patients with gout, by considering the impact of comorbidities (metabolic syndrome and cardiovascular disease) in the setting [10, 11].

When considering anakinra as a potential treatment for gout, some warnings must be regarded in particular

in patients with severe renal failure [71]. In fact, anakinra clearance is diminished by 75% in patients with severe renal failure [72]. In these cases, the injections could be given at wider intervals [71]. Other common side effects are injection site reactions, upper respiratory tract infections, headache, nausea, and diarrhoea [73]. Also when considering these warnings, a recent study clearly showed not only the effectiveness but also the safety of anakinra in a cohort of 26 complex hospitalized patients with gout arthritis, 15 of them characterized by the occurrence of chronic renal disease [74].

Despite these findings and suggestions, to date, no clinical trials on anakinra in gout have been performed or proposed.

4. Symptoms Relievers Bio(techno)logical Drugs: Other Bio(techno)logical Drugs (Anti-TNF Alpha, Tocilizumab, Abatacept)

Literature data on these drugs are scanty. The first anti-TNF alpha used in gout arthritis was etanercept in 2004 [75]. The patient treated was a 53-year-old man with recurrent tophaceous polyarticular gout complicated by kidney involvement. After the failure of every treatment tried (colchicine, diclofenac, methylprednisolone, and opioids), etanercept (25 mg subcutaneously twice weekly) was started with subsequent reduction of gout attacks, painful joints, ESR, and CRP, also during ULT. Subsequently, Fiehn and Zeier [76] described another patient with refractory chronic polyarticular tophaceous gout successfully treated with infliximab (5 mg/kg I.V. at weeks 0, 2, 6 and then every 8th week). But treatment failure has also been reported, as recently described with infliximab [77].

IL-6 inhibitor tocilizumab (8 mg/kg/month) completely stopped gouty attacks in a 44-year-old man with a 12-year history of severe uncontrolled tophaceous gout refractory to colchicine and diclofenac [78]. Another drug currently used in RA [79], abatacept, completely suppressed gout activity in a patient with a long-term history of gout and subsequent superimposed RA [80]. Finally, although in 1996 Lioté et al. observed that the recombinant human and the ultrapure TGFβ1 reduced the number of attacks in an experimental model of gout [81], no further studies on this potential therapeutic approach have been performed.

5. Urate-Lowering Bio(techno)logical Drugs

Even if nonrecombinant uricase from *Aspergillus flavus* was developed and used for reducing hyperuricemia in human tumour lysis syndrome in the late 1960s [82, 83], its use was complicated by production difficulties, the short half-life of the product, and the high frequency of severe allergic reactions (5%) [83]. Moreover, anaphylactic reactions may appear also in the long-term use of uricase, as evidenced in both animals [84] and humans [85] and repeated uricase injections can cause the production of antibodies that neutralise uricase enzyme activity [84, 85].

By considering its effectiveness in reducing serum urate levels, subsequent studies have been addressed to the identification of more stable and tolerated compounds.

5.1. Pegloticase. Pegloticase is a recombinant polyethylene glycol-conjugated form of uricase. With respect to the pure form of uricase, PEGylation should improve the half-life and reduce the immunogenicity of the enzyme [86]. Pegloticase was first tested via single subcutaneous injection in 13 gouty patients with high uricemia levels (>11 mg/dL) [87]. In the short term (7 days) dosages ranging from 4 to 24 mg led to normalization of urate levels in 11 cases. Patients treated with 8–24 mg of pegloticase had urate levels <6 mg/dL also after 21 days postinjection. In five subjects, the half-life and efficacy of pegloticase were reduced by the induction of antibodies, which, unexpectedly, were specific against the PEG residue rather than against the uricase itself.

Pegloticase was then tested intravenously on 24 patients with symptomatic gout (tophi, chronic synovitis, or flare within the past 6 months) and hyperuricemia (>7 mg/dL, not on conventional ULTs) [88]. The results of intravenous pegloticase were superior to those of subcutaneous injection for both hyperuricemia reduction and safety. The doses of 4 mg, 8 mg, and 12 mg reduced plasma urate concentrations to <2 mg/dL within 24 hours postinfusion, being the maximum decline (in average 10.2 mg/dL) obtained at 24–72 hours. With these doses, the results were satisfactory also 21 days after the infusion. Similar to other non-bio(techno)logical ULTs [89], gout flares and arthralgias were the main adverse events potentially linked to intravenous pegloticase [86], whereas subcutaneous delivery was frequently associated not only with injection site but also with widespread urticaria [85]. Interestingly, intravenous delivery seemed to be less immunogenic than the intramuscular one [86].

In a 16–18-week phase II study on 41 patients with gout, the highest proportion of patients who achieved and maintained the primary end-point (plasma urate level <6 mg/dL for at least 80% of the study period), the least pronounced increases in mean plasma urate levels between doses, and the highest proportion of time without hyperuricemia were obtained with the dose of 8 mg every 2 weeks, although all pegloticase doses were effective [90].

Two replicated, randomized, double-blind, placebo-controlled phase III trials (C0405 and C0406) were then conducted in patients with severe gout, refractory or intolerant to allopurinol, and serum uric acid concentration ≥8.0 mg/dL [91]. Two active treatment groups (Pegloticase 8 mg every 2 or 4 weeks) and one placebo group were planned. Prophylaxis against infusion-related reactions was given to all patients before each infusion (oral fexofenadine, 60 mg the evening before and again before infusion; acetaminophen, 1000 mg; and I.V. hydrocortisone, 200 mg, immediately before infusion). The primary end-point was the achievement of urate levels <6 mg/dL at months 3 and 6. A total of 225 patients participated: 109 in trial C0405 and 116 in C0406. When the 2 trials were pooled, the primary end-point was achieved in 36/85 patients in the biweekly group (42%; 95% CI, 32%–54%), 29/84 patients in the monthly group (35%; 95% CI, 24%–46%), and 0/43 patients in the placebo group (0%; 95% CI, 0%–8%; $P < 0.001$ for each comparison). Gout flares were reported in approximately 80% of patients across the 3 pooled study groups. Infusion-related reactions

were the second most common adverse events, occurring, respectively, in 26%, 42%, and 5% of patients receiving pegloticase biweekly, monthly, and placebo, some cases fulfilling criteria for anaphylaxis. Serious infusion-related reactions occurred in 5% (pegloticase biweekly) and 8% (pegloticase monthly) of patients. Infusion-related reactions were the most common reason for pegloticase discontinuation during the study (10% for biweekly; 13% for monthly). The majority of patients (89%) developed high titers of pegloticase antibody, in particular, in patients experiencing infusion-related reactions (79%). Interestingly, the preliminary loss of urate-lowering efficacy preceded the first infusion reaction in 91% of biweekly pegloticase and in 71% of monthly pegloticase: low titres (≤1:2430) of anti-pegloticase antibodies were less likely to be associated with loss of ULTs response.

A more detailed analysis of tophi response in these two replicated trials with the subsequent open-label extension study was subsequently performed [92]. Tophi were observed in the majority of patients (73%), accounting for 547 visible localizations recorded at baseline. After 6 months of treatment, pegloticase 8 mg biweekly group had the complete resolution of at least one tophus without the appearance or the enlargement of any other tophus (e.g., complete response) in the 45% of cases ($P = 0.002$ versus placebo), in comparison to 26% of pegloticase 8 mg monthly and 8% of placebo group. In the three groups treatment, respectively, the 28%, the 19%, and the 2% of tophi had a complete response. The results were more relevant in patients with sustained urate-lowering response to therapy and steadily increased during the open-label extension. Furthermore, pegloticase markedly improved patients reported outcomes (Health-related Quality of Life and Physical Function), with the results being more strong in the biweekly treatment group with respect to monthly group [93]. The long-term safety (up to three years) was not substantially different from that described in the randomized phase [91].

Pegloticase is approved for the treatment of chronic gout in patients not responsive to conventional therapy in USA and for disabling tophaceous gout in patients who may also have erosive joint involvement in Europe. The treatment is scheduled intravenously at the dosage of 8 mg every 2 weeks over at least 2 hours. No dosage adjustments are required in older patients or in those with renal impairment. Premedication with antihistamines and corticosteroids is recommended, as well as the administration in a medical context. In G6PD deficient patients, all uricases are contraindicated due to the risk of haemolysis and methemoglobinaemia [94].

5.2. Rasburicase. Rasburicase is a recombinant uricase obtained from *A. flavus* approved in the early 2000s in USA and in Europe for the treatment of tumor lysis syndrome. Generally, this formulation is tolerated better than non-recombinant urate oxidase. However, although potentially effective, up to now only few studies of rasburicase on gout have been performed.

Following the first case reports [95–97], Richette et al. [98] treated with intravenous rasburicase 10 patients with tophaceous gout, intolerant or refractory to allopurinol and suffering from moderate to severe chronic kidney disease. Five patients were treated daily for 5 days (group 1), as in tumour lysis syndrome [99], whereas the remaining five patients received 6 monthly injections of rasburicase (group 2). In all cases, premedication with 60 mg of methylprednisolone was administered. Even if group 1 had a rapid and marked decrease of serum urate levels, no differences were observed at 1 and 2 months with respect to baseline value; moreover, also tophi size did not change. Better results were observed in group 2, with the significant reduction of hyperuricemia after six infusions, and tophi size reduction in 2 cases. Adverse events were common, being observed in 8 out of 10 patients enrolled: 4 patients in group 1 and 2 in group 2 had a gouty articular flare, despite colchicine prophylaxis. Two patients in group 2 had an allergic reaction during the sixth infusion (bronchospasm and rash) requiring discontinuation of treatment.

Recently, some authors described the case of a patient with massive tophaceous gout that was concomitantly treated with 3 different ULTs, in particular allopurinol 600 mg/day, benzbromarone 100 mg/day, and 4 monthly rasburicase infusions that lead to the almost complete resolution of tophi before rasburicase withdrawal due to flushing and urticarial occurrence during the fifth infusion [100].

5.3. Bio(techno)logical Drugs under Development. Other symptoms relievers and ULTs bio(techno)logical drugs for gout treatment are under development. AC201 is an oral IL-1β inhibitor having also uric acid-lowering effects that is under evaluation in the prophylaxis against gout flares when initiating ULT [101]. Pegsiticase (Uricase-PEG 20, 3SBio, China) is another PEGylated derivative of a recombinant uricase from *C. utilis* [94]. Two phase 1 studies considering either the intramuscular [102] or the intravenous [103] administration of this compound for gout refractory to conventional therapy are ongoing.

6. Conclusions

In the last year, several bio(techno)logical drugs targeting particular points of gout and urate synthesis cascade have been approved for gout treatment by the US Food and Drug Administration and/or by the European Medicines Agency. As for RA and other rheumatology conditions, these drugs clearly opened a new era in the treatment of gouty patients, in particular in those with refractory disease or not tolerating conventional therapies. These drugs may act as symptom relievers or as ULTs. If IL-1 is the main target of symptomatic relievers agents (anakinra, canakinumab, and rilonacept), recombinant uricases (rasburicase and pegloticase) are the prototypical example of bio(techno)logical ULTs. Moreover, other bio(techno)logical compounds are at the pipeline.

By considering the burden of gout from the clinical and from the economic point of view [104], these new treatment possibilities may help the clinicians to improve patients' prognosis and impact. However, the high costs of these drugs clearly indicate that from the therapeutic point of view one of the most challenging points is patients' low

adherence to gout therapies, with negative consequences on success rate and on disease progression [105]. In fact, there is also increasing interest by clinicians in the improvement of patient education, self-management training, and urate-lowering medication titration, in order to use the right drugs at the right moment and to provide the optimal gout care.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Treatment Comparison in Rheumatoid Arthritis: Head-to-Head Trials and Innovative Study Designs

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Over the last decades, the increasing knowledge in the area of rheumatoid arthritis has progressively expanded the arsenal of available drugs, especially with the introduction of novel targeted therapies such as biological disease modifying antirheumatic drugs (DMARDs). In this situation, rheumatologists are offered a wide range of treatment options, but on the other side the need for comparisons between available drugs becomes more and more crucial in order to better define the strategies for the choice and the optimal sequencing. Indirect comparisons or meta-analyses of data coming from different randomised controlled trials (RCTs) are not immune to conceptual and technical challenges and often provide inconsistent results. In this review we examine some of the possible evolutions of traditional RCTs, such as the inclusion of active comparators, aimed at individualising treatments in real-life conditions. Although head-to-head RCTs may be considered the best tool to directly compare the efficacy and safety of two different DMARDs, surprisingly only 20 studies with such design have been published in the last 25 years. Given the recent advent of the first RCTs truly comparing biological DMARDs, we also review the state of the art of head-to-head trials in RA.

1. Introduction

Based on the wrong assumption of a possible interference with proliferation of connective tissues, methotrexate (MTX) was first trialled for the treatment of rheumatoid arthritis (RA) in 1962 [1]. Definite approval of MTX as a therapy for active RA came in 1988 after two placebo-controlled studies involving a total of 224 patients treated for a maximum of 24 weeks [2, 3]. Much has changed since then in drug discovery and trial design in RA. The identification of tumor necrosis factor (TNF) as a key player in the inflammatory and destructive pathways of the disease initiated a landmark shift of interest away from agents with poorly understood mechanisms of action towards therapies targeted to key molecules and cells involved in RA pathogenesis [4]. Advances in understanding of the role of T cells, B cells, and cytokines such as IL-6 have paved the way to the development of additional biological drugs beyond TNF-inhibitors, such as abatacept (ABT), rituximab, and tocilizumab (TCZ) [5–10]. These have come to formal approval after randomised controlled trials (RCTs) mostly adherent to the recommendations from the US Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMA). Specific requirements include long-term RCTs (12 to 24 months in duration) evaluating radiographic progression, and patient-reported physical function in addition to accepted outcomes assessing signs and symptoms [11, 12]. Table 1 summarises RCTs of biological disease-modifying antirheumatic drugs (DMARDs) that have supported regulatory labelling [13–45]. After more than ten years of experience, biological DMARDs have consistently shown good efficacy and safety in patients with RA [46–51].

The ever-increasing plethora of effective treatment options for patients with RA undoubtedly reflects the vitality of research in this area. The paradox, however, is that rheumatologists have little or no idea of how to approach an individual patient to best utilise this vast arsenal. As a proof, updated recommendations for the management of RA still refer to homogeneous disease populations (with the exception of few and ill-defined prognostic factors) and,

Table 1: Randomised controlled trials of biological disease-modifying anti-rheumatic drugs that have supported regulatory labeling.

	Trial	Study drug	Duration	Primary endpoints
MTX-naïve				
abatacept*	AGREE [13]	ABT + MTX versus MTX	12 months	DAS28-CRP < 2.6 at 12 months xRay progression at 12 months
adalimumab	PREMIER [14]	ADA + MTX versus MTX versus ADA	24 months	ACR50 at 12 months xRay progression at 12 months
	OPTIMA [15]	ADA + MTX versus PL + MTX	6 months	DAS28-CRP < 3.2 at 78 wks no xRay progression at 78 wks
certolizumab*	—			
etanercept	ERA [16]	ETN versus MTX	12 months	overall ACR response at 6 months xRay progression at 12 months
	COMET [17]	ETN + MTX versus MTX	24 months	DAS28 < 2.6 at 12 months xRay progression at 12 months
golimumab	GO-BEFORE [18]	GOL + MTX versus PL + GOL versus PL + MTX	6 months	ACR50 at 6 months
infliximab	ASPIRE [19]	IFL + MTX versus PL + MTX	12 months	overall ACR response at 12 months xRay progression at 12 months
rituximab**	IMAGE [20]	RTX + MTX versus MTX	12 months	xRay progression at 12 months
tocilizumab**	—			
MTX/DMARD-IR				
abatacept	AIM [21]	ABT + MTX versus PL + MTX	12 months	ACR20 at 6 months HAQ-DI at 12 months xRay progression at 12 months
adalimumab	ATTEST [22]	ABT + MTX versus PL + MTX versus PL + INF	12 months	DAS28 at 6 months
	ARMADA [23]	ADA + MTX versus PL + MTX	6 months	ACR20
	DE019 [24]	ADA + MTX versus PL + MTX	12 months	ACR20 at 6 months HAQ-DI at 12 months xRay progression at 12 months
certolizumab	RAPID-1 [25]	CER + MTX versus PL + MTX	12 months	ACR20 at 6 months xRay progression at 12 months
	RAPID-2 [26]	CER + MTX versus PL + MTX	6 months	ACR20 at 6 months
	FAST4WARD [27]	CER versus PL	6 months	ACR20 at 6 months
etanercept	Weinblatt [28]	ETN + MTX versus PL + MTX	6 months	ACR20 at 6 months
	TEMPO [29]	ETN versus MTX versus ETN + MTX	12 months	overall ACR response at 6 months xRay progression at 12 months
	ADORE [30]	ETN + MTX versus ETN	4 months	improvement of > 1.2 units in DAS28 at 4 months
golimumab	GO-FORWARD [31]	GOL + MTX versus PL + GOL versus PL + MTX	6 months	ACR20 at 14 weeks HAQ-DI at 6 months
infliximab	ATTRACT [32]	IFL + MTX versus PL + MTX	30 weeks	ACR20 at 30 weeks
rituximab***	DANCER [33]	RTX + MTX versus PL + MTX	6 months	ACR20 at 6 months
	SERENE [34]	RTX + MTX versus PL + MTX	12 months	ACR20 at 6 months
	MIRROR [35]	RTX + MTX versus PL + MTX	12 months	ACR20 at 12 months

Table 1: Continued.

	Trial	Study drug	Duration	Primary endpoints
tocilizumab	OPTION [36]	TCZ + MTX versus PL + MTX	6 months	ACR20 at 6 months
	LITHE [37]	TCZ + MTX versus PL + MTX	24 months	HAQ-DI at 12 months xRay progression at 12 months
	TOWARD [38]	TCZ + DMARD versus PL + DMARD	6 months	ACR20 at 6 months
	STREAM [39]	TCZ versus PL	3 months	ACR20 at 3 months
	ROSE [40]	TCZ + DMARD versus PL + DMARD	6 months	ACR20 at 6 months
Anti-TNF IR				
abatacept	ATTAIN [41]	ABT + DMARD versus PL + DMARD	6 months	ACR20 at 6 months HAQ-DI at 6 months
adalimumab	—			
certolizumab	—			
etanercept	—			
golimumab	GO-AFTER [42]	GOL + DMARDs versus PL + DMARDs	6 months	ACR20 at 14 weeks
infliximab	OPPOSITE [43]	IFL + MTX versus ETN + MTX	n.d.	none
rituximab	REFLEX [44]	RTX + MTX versus PL + MTX	24 months	ACR20 at 6 months
tocilizumab	RADIATE [45]	TCZ + MTX versus PL + MTX	6 months	ACR20 at 6 months

* approved by FDA, not approved by EMA in MTX-naïve patients.
** not approved by neither FDA nor by EMA in MTX-naïve patients.
*** not approved by neither FDA nor by EMA in MTX-IR patients.
ABT: abatacept; ACR: American College of Rheumatology; ADA: adalimumab; CER: certolizumab; CRP: C-reactive protein; DAS: disease activity score; DMARD: disease modifying anti-rheumatic drug; ETN: etanercept; GOL: golimumab; HAQ-DI: Health Assessment Questionnaire Disability Index; IFL: infliximab; IR: irresponsive; MTX: methotrexate; PL: placebo; RTX: rituximab; TCZ: tocilizumab; TNF: tumor necrosis factor.

most importantly, do not assist in the choice and optimal sequencing of available biological DMARDs [52–54]. This area of uncertainty is likely to increase with the upcoming introduction of biosimilar and targeted synthetic DMARDs on the market.

Classic RCTs sponsored by pharma industries to assess the efficacy and safety of new compounds clearly do not fit well with the urgent need of improving decisions that affect medical care at the levels of both policy and the individual. Most of the RCTs in RA indeed exclude commonly used comparator interventions and clinically relevant patient subgroups. These exclusions diminish the ability to understand the relative merits of different interventions and the generalisability of the trial results [55]. Although comparative efficacy and effectiveness can be informed by analysis of observational data, decision modelling, and other tools (reviewed in [56, 57]), the RCT still remains the most rigorous method for comparing interventions. However, trial designs should be substantially rethought in order to allow reliable assumptions on the effectiveness of different interventions among patients in typical day-to-day practice. In this review, we will first briefly summarise how and to what extent the dearth of evidence from comparative RCTs in RA can be partially counterweighted by indirect comparisons. We will then examine some of the possible implementations to classic RCTs, such as the inclusion of active comparators and new trial designs, to align research methods to current demands. In light of the recent advent of the first RCTs truly comparing biological DMARDs, we will also review the state of the art of head-to-head trials in RA.

2. Indirect Comparisons and Meta-Analysis

In contrast to direct within-trial comparison, in indirect comparisons, the effects of interventions are compared to each other by their performance against a common comparator. Quantitative results of several similar studies comparing the same intervention with the same comparator can be combined by means of meta-analysis to summarise the available evidence into a pooled estimate of the outcome of interest (pairwise meta-analysis). Furthermore, multiple different pairwise comparisons across a range of different interventions can be combined into network meta-analysis (also known as mixed treatment comparisons or multiple treatment meta-analyses) [58, 59].

Of key importance in indirect comparisons is not to break randomisation, thus preserving the advantages of RCTs. If one trial compares drug A versus placebo and a second trial compares drug B versus placebo, it is incorrect to simply compare the absolute efficacy observed with drug A with that observed with drug B. Indeed, part of the absolute efficacy can be attributed to the drug, whereas another part is due to a placebo effect. Furthermore, differences in absolute treatment effects may be a result of different baseline prognostic factors. In order not to break randomisation, one can only compare the relative effect of drug A versus placebo from one trial with the relative effect from other trials (adjusted indirect comparison) [60].

Basic assumptions underlying indirect comparisons include that results from different trials should be sufficiently homogeneous [61] by either fixed-effects models or random-effects models. In fixed-effects models, it is assumed that differences in true relative treatment effects are only caused by the difference in treatment and no other factors. In random-effects models, differences in study-specific treatment effects (beyond the differences attributable to the interventions compared) are exchangeable, and heterogeneity is constant between the different comparisons [62]. Another assumption for an adjusted indirect comparison to be valid is similarity [61]. This means that patients included should be sufficiently similar in the two sets of placebo-controlled trials, so that the relative effect estimated by trials of A versus C is generalisable to patients in trials of B versus C, and the relative effect estimated by trials of B versus C is generalisable to patients in trials of A versus C. Last, when both direct and indirect evidence is available, an assumption of consistency is required to quantitatively combine the direct and indirect estimates [61]. Possible causes of discrepancy (inconsistency) between the direct and indirect evidence include the play of chance, invalid indirect comparison, bias in head-to-head comparative trials, and clinically meaningful heterogeneity across trials.

Conclusions from meta-analysis are drawn by applying a statistical inference technique, which can be either frequentist or Bayesian [63]. With a frequentist approach, the result of the meta-analysis is a point estimate along with a 95% confidence interval. Bayesian methods involve a formal combination of a priori probability distribution (that reflects a priori belief of the possible values of the pooled effect) with a likelihood distribution of the pooled effect based on the observed data to obtain a posterior probability distribution of the pooled effect. The likelihood informs us about the extent to which different values for the parameter of interest is supported by the data. As such, the posterior distribution obtained with the Bayesian approach can be interpreted in terms of probabilities, which allows for a more intuitive interpretation of the results.

Although being increasingly adopted to compare the effects of different treatments in many medical areas, indirect comparisons are not immune to conceptual and technical challenges. As treatments being compared have not been randomised directly within the individual trials, standard meta-analysis provides evidence of an observational nature and thus suffers from the limitations of observational studies [61]. Furthermore, sometimes inconsistency cannot be explained after considering effect modifiers. A recent meta-epidemiological study indeed identified 14% of inconsistency between direct and indirect comparisons [64].

Surprisingly, evidence for conventional DMARDs (MTX, leflunomide [LEF], and sulfasalazine [SSZ]) in RA coming from network meta-analysis combining direct and indirect comparisons does not support the superiority of one DMARD over another [65]. Limitations may stem from the wide differences in MTX dosing across different trials. The preferred use of MTX in most patients versus other oral DMARDs is thus rather supported by extensive clinical experience over the years [66]. For biological DMARDs, as

head-to-head comparisons are only exceptions, thus, network meta-analysis is the sole informative tool for comparative effectiveness. Disappointingly, many of the published comparisons lead to different conclusions. As an example, a 2009 Cochrane overview failed to recognise significant differences in efficacy among the available biological DMARDs (with the exception of anakinra), whilst the safety profile favoured etanercept (ETN) [67]. In contrast, Schmitz and colleagues [68] reported superiority of ETN compared with infliximab (IFL) and golimumab and of certolizumab compared to infliximab and adalimumab (ADA). A number of factors may account for such inconsistency, including the RCTs being considered, the analysis of potential sources of heterogeneity, and the efficacy outcomes assessed [69]. Additionally, some confounders, such as period of enrolment of different RCTs, cannot be adequately corrected, limiting the possibility of indirect comparisons for specific outcomes (e.g., radiographic progression) [70]. These shortcomings currently hamper the use of available indirect comparisons as part of formal decision making strategies in RA.

3. Randomised Controlled Trials: New Designs to Improve Comparative Effectiveness

The classic RCT that most rheumatologists are familiar with is the two-armed, parallel-group efficacy trial comparing the experimental treatment with placebo. This type of RCT is clearly not aligned with the need of determining the optimal strategy for individual patients in a community-based setting. The use of active comparators instead of placebo will be discussed in a further section. Here, we will summarise possible trial implementations aimed at individualising treatments in real-life conditions.

3.1. Implementation by Study Design

3.1.1. N-of-1 Trials. The development of n-of-1 or single subject clinical trials is based on the recognition of the tremendous heterogeneity of diseased populations and the need of individualised treatments. In a n-of-1 trial, the individual patient is considered as the sole unit of observation, with the ultimate goal of determining the optimal or best intervention for that specific patient using objective data-driven criteria [71]. Although n-of-1 trials, by definition, eschew consideration of the population-level effects of an intervention, combining and evaluating multiple n-of-1 trials through meta-analysis can allow generalisability of the results [72]. The typical design of a n-of-1 trial is a within patient randomised, double-blind, and crossover trial. The unit of randomisation is the treatment sequence for an individual patient, and a treatment cycle includes an exposure to each therapy. In contrast to classic randomised crossover trials, where the individual is randomised to one group or another, in n-of-1 trials each participant receives each intervention at different time frames of the study.

N-of-1 trials have not been frequently adopted in rheumatology, with the exception of studies of pain medications in osteoarthritis. A study by Yelland et al. [73] provides a good example. A comparison of celecoxib and paracetamol was assessed. The design of the trial was based on a double-blind, crossover comparison where a subject took either celecoxib or sustained-release paracetamol for three pairs of 2-week periods. The order of the drugs during each pairing was random. Both patients and physicians did not know the order of the drug regimens until after the study was completed. Statistical analyses were conducted using Bayesian methods. The aggregate results showed that most (80%) patients completing the trial had a similar response to celecoxib as to paracetamol.

3.1.2. Cluster Randomised Trials. In cluster RCTs, randomisation is by group (such as communities, families, or medical practices) rather by individual patient. Advantages of cluster RCTs over individually randomised controlled trials include the ability to study interventions that cannot be directed toward selected individuals and the ability to control for "contamination" across individuals, that is, the unintentional spillover of intervention effects from one treatment group to another. However, because of the dependence (or clustering) between individual units sample, cluster RCTs require more participants to obtain the same statistical power and are more complex to design, execute, and analyse [74].

Cluster RCTs are becoming increasingly common in health services research, being particularly appropriate for evaluating interventions aimed at changing behaviour in patients or practitioners or changing organisation of services. In RA, the effectiveness of systematic monitoring of disease activity in daily practice was confirmed in a multicentre cluster RCTs published in 2005 [75]. Twenty-four rheumatology outpatient centres were randomly allocated to systematic monitoring (0–4–12–24 weeks) using 28 joints disease activity score (DAS28) versus usual care. At 24 weeks, low disease activity (DAS28 ≤ 3.2) was achieved by 31% of the patients in the DAS28 group compared to 16% of the patients receiving usual care ($P = 0.028$) due to prompt changes in DMARD treatment.

3.2. Implementation by Outcome of Interest

3.2.1. Pragmatic Trials. In efficacy (or explanatory) RCTs, extended inclusion and exclusion criteria are used to identify a clearly defined population of participants who would benefit from the intervention under investigation. Although efficacy trials, if correctly designed and executed, lead to statistically credible results, the applicability of these results to real-life practice may be questionable. Indeed, the same characteristics that account for the high internal validity (well-defined inclusion and exclusion criteria, blinding, and controlled environment) can hamper external validity, that is, the ability to generalise the results in an extended population and clinical setting. Pragmatic RCTs, on the other hand, are designed to test interventions in the full spectrum of everyday clinical practice in order to maximise applicability and generalisability [76]. Common elements of such trials include clinically effective comparators, study patients with common

comorbid conditions and diverse demographic characteristics, and providers from community settings. Primary and secondary outcomes are patient-centered. The distinction between an explanatory and a pragmatic trial in real life is not that easy. The Pragmatic-Explanatory Continuum Indicator Summary (PRECIS) provides a useful framework to help researchers design pragmatic trials [77]. This tool identifies important domains (such as eligibility criteria, flexibility of the intervention, patient adherence, practitioner expertise, follow-up intensity, and outcomes) that should be considered during protocol development of pragmatic RCTs. Pragmatic trials arguably combine the advantages of randomisation (high internal validity) and observational research (high external validity). However, they also have important shortcomings. The increased variance due to the inclusion of chronic/poorly responsive/comorbid patients, insensitive or problematic outcome parameters, and inadequate sample size increases the risk of a β-error (failure to detect a difference although there is one), and unblinded designs can induce different kinds of biases.

In RA, the most cited example of a pragmatic trial is the Dutch Behandel Strategieen (BeSt) study [78]. Patients with early, untreated, and active RA were randomly allocated to 1 of 4 treatment groups. Treatment strategies included sequential monotherapy (group 1), step-up combination therapy (group 2), initial combination therapy with tapered high-dose prednisone (group 3), or initial combination therapy with IFL (group 4). Treatment adjustments were made on the basis of the treat-to-target and tight control principles. Primary endpoints were functional ability and radiographic joint damage. Despite several limitations, such as unblinding and intention-to-treat analysis, the BeSt study has contributed to significant advances in the management of RA by demonstrating that, in the majority of patients, a goal-steered, dynamic treatment towards tight control of disease activity ensures good clinical and radiographic outcomes irrespective of the type, combination, and sequencing of therapies [79].

3.2.2. Adaptive Trials. A conventional study is planned using assumptions about critical elements of the study design, such as population means or event rates, variance, dose-response effect size, discontinuation rates, that are not precisely known but are only estimated. When the prestudy estimates are inaccurate, a conventional study may fail to achieve its goal. Data accumulating during the course of the study, however, could provide improved knowledge of relevant parameters if those data could be examined. Adaptive RCTs are designed to change or adapt in response to information generated during the trial [80, 81]. This could make the studies more efficient (e.g., shorter duration, fewer patients), more likely to demonstrate an effect of the drug if one exists, or more informative. Importantly, adaptations (or changes) should not be ad hoc, but by design, based on prospectively planned, prespecified analyses of interim data. Points of weakness of adaptive designs include feasibility, validity, integrity, efficiency, and flexibility [81, 82].

Of the various adaptive design trials [81], biomarker-driven adaptive studies perhaps offer the most attractive prospects. Predictive biomarkers can be selected from a wide array of prognostic biomarkers (which are useful for projecting the natural history of a disease independent of therapy) to define a specific subgroup of patients for which treatment will be beneficial. An example of a predictive marker is the presence or absence of K-Ras mutations in colorectal cancers; patients without K-Ras mutations benefit from antiepidermal growth factor receptor therapy, whilst patients with such mutations derive little, if any, benefit [83]. Where a single biomarker has been identified, several trial designs can be employed [84], including (i) biomarker-enrichment design, which involves only patients testing positive for the biomarker. This design is more appropriate when there is preliminary evidence that patients testing positive for the biomarker will likely benefit from the treatment; (ii) biomarker-stratified design involves first testing patients for the biomarker and then separately randomising patients who test positive and those who test negative. This design is more appropriate when there is no preliminary evidence to strongly favour a positive or negative biomarker. The medical literature in oncology provides good examples of biomarker-driven studies [85]. No similar trial strategies have been adopted in RA yet. Paradoxically, despite anticitrullinated protein antibodies (ACPA) are acknowledged as one of the strongest prognostic factors of worst disease outcomes [86], no clinical studies have tailored RA treatment based on a positive ACPA-test. However, evidence from the PROMPT study seems to suggest that early MTX treatment in patients with undifferentiated arthritis could significantly delay progression to RA specifically in ACPA-positive patients [87], confirming the possibility of identifying subgroups of patients with treatment benefit at least in the earliest phases of the disease [88]. The field of biomarker discovery is moving fast in RA, fuelled by the implementation of systems biology and omic technologies. The increased awareness of the systemic and multidistrict nature of the disease have expanded the possibility to search for novel biomarkers in different diseased compartments [89–93]. Promising prognostic biomarkers (to be further tested for their predictive ability) are emerging in the peripheral circulation [94–97], and the accessibility of the synovial tissue through minimally invasive techniques [98] allows more extensive studies aimed at investigating the clinical and prognostic significance of different pathological features [99, 100].

4. Head-to-Head Trials

Undoubtedly, the use of a placebo control in RCTs offers several advantages. Inclusion of placebo increases the efficiency of a trial, as statistical significance can be achieved with the smallest number of participants. Secondly, the results of a placebo-controlled trial are usually unequivocal, with clear evidence of whether the experimental drug being tested is efficacious or not. There are few ethical concerns in using placebo-controlled trials when no therapy of proven effectiveness exists. In contrast, when standard therapy does exist, controlling for placebo raises not only ethical but also practical issues, in that the usefulness of the results is ambiguous.

Despite the fact the regulatory agencies recommend that placebo should not be continued for more that 3–6 months in RA trials [101], Estellat and Ravaud [102], through a revision of all RCTs of biological DMARDs ended after 2002, highlighted that 6,518 RA patients enrolled in control arms were continuing their previously ineffective treatment for more than 6 months. As such, a significant revision in the requirements for the investigation and approval of new drugs for the treatment of RA is needed. An International Committee has recently proposed that placebo can be acceptable for no more than 3 months and new biological DMARDs should be tested against an active comparator [103]. In light of their tightest confidence intervals for efficacy, TNF-inhibitors (+ MTX) should be the comparator of choice [103].

Active control or comparative or head-to-head trials refer to all studies in which the control arm is an active one. Based on the scientific hypothesis behind the trial, comparative trials may be classified as superiority, equivalence, or noninferiority trials [104, 105].

In a superiority trial, the aim is to show that a new treatment is better than standard therapy. The null hypothesis is that the difference between the means of the two groups is zero or negative (i.e., favouring the standard treatment) versus the one-tailed alternative hypothesis that the new drug is better. The desirable difference in treatment effects should be decided on clinical grounds, considering the specific features of the disease, the known efficacy of the control therapy, and what may reasonably be expected from the new therapy. The rationale for a one-sided test of significance is that investigators are not interested in results that show that the new drug is equal to or inferior than the standard. However, a shortcoming of one-tailed tests is that significant results in the opposite direction must be dismissed as chance findings, despite having the potential of being clinically meaningful. Superiority trials are almost never seen in active-control trials because of the high risks of failure intrinsic in their design and the required sample size, which can be unachievable in certain conditions. Indeed, the smallest the difference between the standard and the experimental drug is expected, the largest will be the population required.

Equivalence trials test whether the effects of two drugs are the same within prespecified limits. As it is fundamentally impossible to prove that two treatments have exactly equivalent effects, "clinical equivalence intervals" must be determined. The null hypothesis is that the difference between treatments falls outside the interval versus the alternative hypothesis that the differences lie within the equivalence interval. Equivalence trials are based on two-sided tests, which increase the sample size and the cost of the study. Furthermore, equivalence margins are often far too large to be clinically meaningful and a claim of equivalence may be misleading if a trial has not been conducted to an appropriately high standard. Equivalence trials are often run when biosimilars are entering the market.

Noninferiority trials test whether the effect of a new treatment is not worse than that of an active control by more than a prespecified margin. Again, an interval of noninferiority must be determined. The null hypothesis is that the new drug is worse than the standard one by at least some amount, against the alternative hypothesis that the superiority of the standard drug does not exceed this interval. However, since noninferiority trials do not include a true negative control group, results of these trials are viewed with caution and are not generally accepted as being as strong as those from a superiority trial. Noninferiority trials are carried out when (1) a placebo-controlled trial is not ethically feasible and (2) the treatment under test is not expected to be better that the standard or reference intervention in terms of efficacy but is supposedly better regarding other secondary endpoints, safety, costs, compliance, or convenience.

5. Head-to-Head Randomised Controlled Trials in Rheumatoid Arthritis: State of the Art

5.1. Synthetic DMARD versus Synthetic DMARD. The comparison between two different synthetic DMARD monotherapies for RA was reported in 14 RCTs, all having MTX as basis for comparison (Table 2).

5.1.1. Auranofin (AUR). In a 36-week RCT randomising 281 patients with active RA to MTX or AUR, the clinical response (swollen joint count [SJC] and tender joint count [TJC]) with MTX occurred earlier and was consistently greater ($P < 0.01$) than the one with AUR. Adverse reactions were reported more frequently in the AUR group [106]. However, in a subsequent RCT involving 335 patients and comparing MTX, AUR, and the combination of both, no statistically significant differences were found among the treatment groups in terms of clinical response and safety profiles, even if patients taking AUR alone had a slower onset of response than those taking MTX alone [107].

5.1.2. Azathioprine (AZA). AZA was directly compared with MTX in two RCTs with similar results. In the first one, 64 patients were randomly assigned to receive either AZA (100 mg daily) or oral MTX (7.5 mg weekly): both clinical responses (SJC, erythrocyte sedimentation rate [ESR], C-reactive protein level [CRP], and DAS) at 24 and 48 weeks checkpoints [108] and radiographic progression [109] were significantly better in MTX treated group, accompanied by a lower rate of serious adverse reactions. In the second study [110], 209 enrolled patients were randomised to receive escalating doses of MTX (5–15 mg/week), AZA (50–150 mg/day), or the combination of both. The proportion of responders was significantly higher in MTX treated group compared with AZA (45% versus 26%, resp.) and a trend toward decreased radiologic progression was seen only in MTX-treated patients.

5.1.3. Cyclosporine A (CSA). Two RCTs compared CSA with MTX with different findings. Drosos et al. [111] demonstrated a similar clinical response (SJC, TJC, ESR, and CRP) and radiographic progression in two groups of early RA patients (disease duration < 3 years) randomly assigned to receive oral CSA (3 mg/kg/day) or oral MTX (0.15 mg/kg/week). More recently, in an open RCT randomising 126 patients to MTX,

Table 2: Summary of head-to-head trials in rheumatoid arthritis.

Reference	Study design	Drugs	Follow-up	Number of patients	Primary endpoint	Results
sDMARDs versus sDMARDs						
Weinblatt et al., 1990 [106]	Randomized double-blind controlled	AUR versus MTX	36 wks	138 versus 142	TJC, SJC, PhGA, PtGA	MTX is more effective and better tolerated than AUR
Williams et al., 1992 [107]	Randomized double-blind controlled	AUR versus MTX versus AUR + MTX	48 wks	115 versus 114 versus 106	TJC, SJC, PhGA, PtGA	No differences
Jeurissen et al., 1991 [108, 109]	Randomized double-blind controlled	AZA versus MTX	48 wks	33 versus 31	Ritchie index, TJC, SJC, VAS pain, PtGA	MTX is more efficacious and more rapid than AZA
Willkens et al., 1995 [110]	Randomized double-blind controlled	AZA versus MTX versus AZA + MTX	48 wks	73 versus 67 versus 69	TJC, SJC, PhGA, PtGA, HAQ, mTSS	MTX is more efficacious than AZA. Trend toward decrease radiographic progression only in MTX
Drosos et al., 1998 [111]	Randomized open labeled trial	CSA versus MTX	104 wks	52 versus 51	TJC, SJC, VAS pain, Larsen score	No differences in efficacy and radiographic progression
Ferraccioli et al., 2002 [112]	Open randomized controlled	SSZ versus MTX versus CsA	24 wks	42 versus 42 versus 42	ACR50	MTX is more efficacious than CSA and SSZ
Hamilton et al., 2001 [113]	Randomized open labeled trial	GST versus MTX	48 wks	72 versus 69	Paulus response criteria	GST and low dose MTX showed equivalent efficacy, but toxicity was more common in GST
Rau et al., 2002 [114]	Randomized double-blind controlled	GST versus MTX	156 wks	87 versus 87	Ratingen score	No differences in clinical efficacy and radiographic progression
Strand et al., 1999 [116]	Randomized double-blind controlled	LFN versus Placebo versus MTX	52 wks	182 versus 118 versus 182	ACR20	No differences in the efficacy of MTX versu LFN
Emery et al., 2000 [119]	Randomized double-blind controlled	LFN versus MTX	52 wks	501 versus 498	TJC, SJC, PhGA, PtGA	MTX is more efficacious than LEF, with low 2-yrs radiographic progression
Bao et al., 2003 [120]	Open randomized controlled	LFN versus MTX	24 wks	291 versus 213	ACR20	LFN is as effective but safer than MTX
Haagsma et al., 1997 [121]	Randomized double-blind controlled	SSZ versus MTX versus SSZ + MTX	52 wks	34 versus 35 versus 36	DAS	No differences in efficacy and radiographic progression between MTX and SSZ
Dougados et al., 1999 [122]	Randomized double-blind controlled	SSZ versus MTX versus SSZ + MTX	52 wks	68 versus 69 versus 68	DAS	No differences in efficacy and radiographic progression between MTX and SSZ
Capell et al., 2007 [123]	Randomized double-blind controlled	SSZ versus MTX versus SSZ + MTX	52 wks	55 versus 54 versus 56	DAS	No differences in efficacy and radiographic progression between MTX and SSZ
sDMARDs versus bDMARDs						
Bathon et al., 2000 [16]	Randomized double-blind controlled	ETN 25 mg versus ETN 10 mg versus MTX	52 wks	207 versus 208 versus 217	ACR-N AUC (24 wks), mTSS (52 wks)	ETN had a more rapid rate of improvement than MTX
Jones et al., 2010 [125]	Randomized double-blind controlled	TCZ versus MTX	24 wks	288 versus 284	ACR20	TCZ monotherapy is more efficacious than MTX

Table 2: Continued.

Reference	Study design	Drugs	Follow-up	Number of patients	Primary endpoint	Results
Klareskog et al., 2004 [29]	Randomized double-blind controlled	ETN + MTX versus ETN Versus MTX	52 wks	231 versus 223 versus 228	ACR-N AUC (24 wks), mTSS (52 wks)	Combination therapy and ETN are more efficacious than MTX (combo > ETN).
Breedveld et al., 2006 [14]	Randomized double-blind controlled	ADA + MTX versus ADA versus MTX	104 wks	268 versus 274 versus 257	ACR50, mTSS	Combination therapy was superior to both mono-therapies. No differences between ADA and MTX.
bDMARDs versus bDMARDs						
Gabay et al., 2013 [127]	Randomized double-blind controlled	TCZ versus ADA	24 wks	163 versus 162	DAS28	TCZ is superior to ADA
Weinblatt et al., 2012 [128]	Randomized double-blind controlled	ABT versus ADA	52 wks	318 versus 328	ACR20, mTSS	ABT is noninferior to ADA

sDMARDs: synthetic disease modifying antirheumatic drugs; LFN: leflunomide; SSZ; sulfasalazine; TJC: tender joint count; SJC: swollen joint count; ACR: American College of Rheumatology; HAQ: Health Assessment Questionnaire; AZA: azathioprine; MTX: methotrexate; VAS: visual anagogic scale; GST: gold sodium thiomalate; AUR: auranofin; MRI: magnetic resonance imaging; LOCF: last observation carried forward; HRQOL: health related quality of life, SF-36: 36-item short form health survey, DAS: disease activity score; CsA: cyclosporine A; ETN: etanercept; ACR-N AUC: numeric index of the ACR response area under the curve; ADA: adalimumab; TCZ: tocilizumab; and ABT: abatacept.

CSA, or SSZ, American College of Rheumatology (ACR) 50 responses were significantly higher in MTX treated patients compared with CSA (57% versus 31%, resp.; $P = 0.002$) [112]. In both the studies, the proportion of adverse events (AEs) was similar in MTX and CSA treated groups.

5.1.4. Intramuscular Gold (Gold Sodium Thiomalate [GST]). GST showed a similar clinical response (ESR, CRP, Ritchie Articular Index, and pain score) and a significantly higher proportion of withdrawals for toxicity (43% GST versus 19% MTX, $P = 0.0026$) compared to MTX in a 48-week head-to-head RCT [113]. It should be noticed that in this study MTX was used at relatively lower doses (median dose: 10 mg/weekly) than the currently recommended optimal doses. In a second double-blind RCT evaluating damage progression, 174 patients were assigned to receive weekly intramuscular injections of either 15 mg MTX or 50 mg GST for 3 years. No statistically significant differences in clinical efficacy and radiographic progression between the 2 treatment groups at all follow-up points were found [114].

5.1.5. Leflunomide (LEF). As part of the clinical development program for LEF provided by the Leflunomide Rheumatoid Arthritis Investigators Groups, patients were enrolled in 2 RCTs comparing LEF with SSZ and MTX, respectively. Since the first one [115] was not powered to show equivalence between the active treatments but only to indirectly compare LEF and SSZ, the study has not been included in the current review. In the second one, 482 patients were randomly assigned to receive LEF (100 mg daily on days 1–3, then 20 mg daily), placebo, or MTX (7.5 mg weekly, titrated to 15 mg weekly over 7 weeks). No statistically significant differences were found in the comparison of LEF and MTX treated patients regarding ACR20 response at 1- (52% versus 46%, resp.) [116] and 2-years follow-up (79% versus 67%, resp.) [117]. Moreover, radiographic progression at 1- and 2-year evaluations [116, 117] and improvements in physical function and health related quality of life at 2 years [118] were similar in the 2 treatment groups.

A direct comparison between LEF and MTX was performed in another RCT including 999 RA subjects randomised to LEF (loading dose 100 mg/day for 3 days, maintenance dose 20 mg/day) or MTX (10–15 mg/week) for 52 weeks [119]. After 1 year, improvements seen with MTX were significantly greater than those with LEF in terms of ACR20 response (64.8 versus 50.5%; $P < 0.0001$) and mean change from baseline of TJC (-9.7 versus -8.3; $P = 0.006$), SJC (-9.0 versus -6.8; $P = 0.0001$), physician global assessment (-1.2 versus -0.9; $P < 0.001$). Radiographic progression was similar with both treatment protocols at 1 year, whereas a significant difference in mean change from baseline of Larsen score in favour of MTX was found at 2 years. The proportion of AEs leading to withdrawal at 2 years (MTX 21%, LEF 27%) and the overall frequency of serious AEs (MTX 8%, LEF 7%) were comparable.

Besides, in a third comparative trial including 504 RA patients evaluated during a 24-week follow-up period, LFN was found as effective but safer than MTX [120]. In particular, 62% patients in the LFN group met the ACR20 criteria versus 60% in the MTX one, but the incidences of AEs were significantly lower in LFN than in MTX treated patients (16.8% versus 28.1%; $P = 0.002$).

5.1.6. Sulfasalazine (SSZ). A direct comparison of SSZ with MTX was performed in 3 RCTs [121–123], each designed by the randomisation of study population (105, 205, and 165 patients, resp.) into 3 treatment arms (SSZ alone [2000 to maximum 3000 mg daily], MTX alone [7.5 to maximum 25 mg weekly], and the combination of two). In all these studies, no significant differences emerged in the 1-year head-to-head comparison between SSZ and MTX in terms of clinical efficacy (measured as DAS), radiographic progression (total sharp score), and frequency of AEs.

On the contrary, in the previously mentioned study by Ferraccioli et al. [112] directly comparing MTX, CSA, and SSZ, the proportion of patients achieving ACR50 response at 12 months was significantly higher in MTX than in SSZ treated group (57% versus 33%, $P < 0.01$), with a similar safety profile.

5.2. Synthetic DMARD versus Biological DMARD. The vast majority of biotherapy related RCTs are designed to compare MTX monotherapy with the combination of MTX and a biological drug. Thus, only 4 RCTs provided data on the direct comparison between a synthetic and a biologic DMARD monotherapy: the ERA study and the TEMPO trial evaluated ETN, the PREMIER study ADA, and the AMBITION trial TCZ, all head-to-head compared against MTX (Table 2).

The ERA study is a 24-month RCT with both clinical and radiographic primary endpoints [14, 124]. In the study, 632 MTX-naïve early RA patients were randomised to receive either twice weekly subcutaneous ETN (10 or 25 mg) or weekly escalating doses of oral MTX (7.5–20 mg/week). The patients in the group assigned to the higher ETN dose had significantly greater areas under the curve for the numeric index of the ACR response [ACR-N AUC] at 3, 6, 9, and 12 months than did the patients in the MTX group ($P < 0.05$). However, no differences in the proportion of patients achieving ACR20 (72% versus 65%; $P = 0.16$), 50, and 70 responses at 12 months were found in the comparison of ETN and MTX treated groups. The mean increase in the erosion score was significantly lower in the 25-mg ETN group than in the MTX group at both 6-month (0.30 versus 0.68; $P = 0.001$) and 12-month (0.47 versus 1.03; $P = 0.002$) evaluations, as well as the mean total modified sharp score (mTSS) increases at 6 months (0.57 versus 1.06; $P = 0.001$), but not at 12 months (1.00 versus 1.59; $P = 0.11$) [14]. At 24 months, significantly more patients in the 25-mg ETN group than in the MTX group achieved an ACR20 response (72% versus 59%; $P = 0.005$) and the mean changes in mTSS and erosion score in the 25-mg ETN treated patients (1.3 and 0.66 units, resp.) were significantly lower than those in the MTX group (3.2 and 1.86 units, resp.; $P < 0.001$) [124]. The safety outcomes through the entire 2-year follow-up period were comparable between the two treatment drugs in terms of both infectious and noninfectious events, with the only exception of injection

site reactions (ETN 25 mg 39% versus MTX 9%; $P < 0.05$), nausea (ETN 25 mg 14% versus MTX 31%; $P < 0.05$), alopecia (ETN 25 mg 6% versus MTX 12%; $P < 0.05$), and mouth ulcers (ETN 25 mg 5% versus MTX 17%; $P < 0.05$).

The AMBITION trial [125] is a 6-month RCT including 673 patients with active RA for whom previous treatment with MTX had not failed. Study population was randomly assigned either TCZ 8 mg/kg every 4 weeks, or MTX (starting at 7.5 mg/weekly and titrated to 20 mg/weekly within 8 weeks), with the proportion of patients achieving ACR20 response at week 24 as the primary endpoint. The intention-to-treat analysis demonstrated that TCZ monotherapy was better than MTX with higher ACR20 (69.9% versus 52.5%; $P < 0.001$), ACR50 (44.1% versus 33.5%; $P = 0.002$), and ACR70 (28% versus 15.1%; $P < 0.001$) responses. Moreover, TCZ patients were five times more likely to achieve DAS28 remission (odds ratio versus MTX: 5.83; 95% confidence interval [CI] 3.27 to 10.40), and approximately four times more likely to achieve at least a moderate EULAR response (odds ratio versus MTX: 4.24, 95% CI 2.92 to 6.14). No significant differences between TCZ and MTX were found in the incidence of AEs (79.9% versus 77.5%; $P = 0.484$), serious AEs (3.8% versus 2.8%, $P = 0.50$), and serious infections (1.4% versus 0.7%). Furthermore, TCZ treated patients showed a higher incidence of reversible grade-3 neutropenia (3.1% TCZ versus 0.4% MTX) and increased total cholesterol ≥240 mg/dL (13.2% TCZ versus 0.4% MTX), and a lower incidence of alanine aminotransferase elevations >3x–<5x upper limit of normal (1.0% TCZ versus 2.5% MTX).

In the TEMPO trial [27] 686 RA patients were randomised to receive oral MTX (up to 20 mg/week), ETN (25 mg twice a week), or the combination of both, with clinical (24 weeks ACR-N AUC) and radiological (52 weeks mTSS change from baseline) primary endpoints. The combination therapy was significantly better than ETN and MTX monotherapies in reduction of disease activity, improvement of functional disability, and retardation of radiographic progression.

Focusing on direct comparison between ETN and MTX groups, no statistically significant differences emerged in terms of 1-year clinical response (ACR20 76% versus 75%, ACR50 48% versus 43%, and ACR70 24% versus 19%, resp.), whereas 1-year damage worsening was significantly lower in the ETN group compared with MTX (proportion of patient without progression 68% versus 57%, resp.; $P = 0.02$. Mean mTSS change from baseline 0.52 versus 2.80, resp.; $P = 0.04$). This finding was confirmed by 2-years follow-up data, showing a significantly lower mean change from baseline in mTTS in patients receiving ETN compared with those receiving MTX (1.10 versus 3.34, resp.; $P = 0.05$) [123]. The proportion of patients reporting AEs (ETN 86% versus MTX 81%) or serious infections (ETN 4% versus MTX 4%) was comparable between ETN and MTX groups.

As well as TEMPO trial, PREMIER study [12] was designed by randomising 799 early MTX-naïve RA patients into 3 treatment arms (oral MTX 20 mg/weekly, ADA 40 mg/every other week, or the combination of both). Coprimary endpoints at year 1 were ACR50 improvement and mean change from baseline in the mTTS. Similarly to what previously reported in the TEMPO trial, combination therapy was superior to both ADA and MTX monotherapies in all clinical and radiographic outcomes measured, whereas the proportion of 12- and 24-month ACR20 (54% versus 63% and 49% versus 56%, resp.), ACR50 (41% versus 46% and 37% versus 43%, resp.), and ACR70 (26% versus 28% and 28% versus 28%, resp.) responses were comparable between ADA and MTX groups. Otherwise, damage progression at both 1- and 2-year evaluations was significantly lower in ADA treated patients directly compared with MTX treated ones (mean change from baseline in mTTS 3.0 versus 5.7 and 5.5 versus 10.4, resp.; $P < 0.001$). The incidence of serious AEs and serious infections (21.1 versus 15.9 and 0.7 versus 1.6 per 100 patient years, resp.) was similar in both monotherapy groups.

5.3. Biological DMARDs versus Biological DMARDs. The head-to-head comparison between 2 different biological agents is available only in 2 RCTs, the ADACTA trial (comparing TCZ and ADA monotherapies) and the AMPLE trial (comparing ABT and ADA, both on top of MTX) (Table 2). The ATTEST study [20] and the ORAL STANDARD study [126] were excluded since they were designed with the statistical power for comparing biologic drugs against a common placebo group only but not against each other.

ADACTA is the first head-to-head superiority RCT comparing TCZ (8 mg/kg every 4 weeks) and ADA (40 mg every other week) monotherapy in a study population of MTX-insufficient responder RA patients ($n = 335$) [127]. TCZ monotherapy was superior to ADA monotherapy at 6 months according to all main efficacy endpoints: EULAR remission (39.9% versus 10.5%; $P < 0.0001$), EULAR low disease activity (51.5% versus 19.8%; $P < 0.0001$), ACR20 (65% versus 49.4%; $P = 0.003$), ACR50 (47.2% versus 27.8%; $P = 0.0002$), ACR70 (32.5% versus 17.9%; $P = 0.002$), and CDAI remission (17.2% versus 9.3%; $P = 0.03$). The kinetics of clinical response in the two groups were comparable through the entire follow-up period. The adverse event profiles of TCZ and ADA were similar and consistent with previous findings. In particular, no significant differences were found between the 2 treatment groups in the 6-month rate of overall (82% versus 83%) and serious (12% versus 10%) AEs, and overall (70% versus 65%) and serious (4% versus 4%) infections, as well as in the frequency of transaminase elevation ≤2.5x upper limit of normal (32% versus 25%). Otherwise, some differences in the effect of the two drugs on the neutrophil count have been registered, with a proportion of patients experiencing grade-2 neutropenia (<1500–1000x mm^3) with TCZ higher than with ADA (13% versus 4%), even if cases of severe neutropenia were very rare in both the treatment groups.

The AMPLE trial provided a noninferiority comparison of subcutaneous ABT and ADA, both administrated in combination with MTX, in a study population of 646 RA patients through a 2-year follow-up period [128, 129]. The clinical efficacy of ABT and ADA is comparable according to 1 and 2 years ACR20 (64.8% versus 63.8% and 60.1% versus 59.7%, resp.), ACR50 (46.2% versus 46% and 46.6% versus 44.7%, resp.), and ACR70 improvements (29.2% versus 26.2% and 31.1% versus 29.3, resp.), with similar kinetics of response

throughout the entire 2-year follow-up period. Similarly, no significant differences emerged in the comparison of radiographic progression in ABT and ADA treated patients, with more than 80% nonprogressor patients in both the groups. Finally, 2-year safety outcomes are balanced, but with some notable differences in the incidence of AEs (10.1% versus 9.1%), serious AEs (1.6% versus 4.9%), and serious infections (0 versus 2.7%) and injection site reactions (4.1% versus 10.4%), all in favour of ABT compared with ADA.

6. Conclusions

The arsenal of therapeutic options for RA is vast, but knowledge on the optimal use of different drugs in individual patients in typical day-to-day practice remains poor. Over a period of more than 25 years, only 20 head-to-head RCTs comparing two different DMARDs have been performed, providing some preliminary but encouraging suggestion on how to deal with the complexity of the available therapeutic armamentarium. Key messages emerging from direct comparisons are as follows.

(i) MTX overall risk/benefit ratio is the most favourable compared with other synthetic DMARDs, confirming its use as first line therapy and LEF or SSZ as an alternative treatment in newly diagnosed RA, as suggested by international guidelines [53].

(ii) TCZ is the only biologic DMARD with a demonstrated clinical superiority compared to MTX. ETN and ADA have been shown to be able only to slow damage progression better than MTX, without significant differences in clinical response.

(iii) TCZ monotherapy is superior to ADA monotherapy, with a similar safety profile.

(iv) Clinical efficacy, damage progression, and kinetics of response of sc ABT and ADA are comparable. Safety profiles are quite similar, slightly in favour of ABT.

It is hoped that future years will witness a radical shift in the way medical research is conceived and performed in RA. More direct comparisons and innovative trial designs will help achieving the final goal of treating the right patient at the right time with the right drug.

Conflict of Interests

Ennio Giulio Favalli has received lecture fees from BMS, Roche, MSD, UCB, Pfizer, and Abbvie. Roberto Caporali has received lecture fees from BMS, Roche, MSD, UCB, Pfizer, and Abbvie. Serena Bugatti and Martina Biggioggero have no conflict of interests to declare.

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Clinical Study

Ankylosing Spondylitis and Rheumatoid Arthritis: Serum Levels of TNF-α and Its Soluble Receptors during the Course of Therapy with Etanercept and Infliximab

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The effects of the TNF-α blockers infliximab or etanercept on the levels of TNF-α, TNF-receptor 1 (TNF-R1), and TNF-receptor 2 (TNF-R2), as well as the levels of the inflammation markers CRP and IL-6, were measured in ankylosing spondylitis (AS) and rheumatoid arthritis (RA) patients receiving treatment with either compound. We found that RA patients tend to have higher levels of TNF-α than both healthy individuals and AS patients prior to treatment ($P < 0.05$). We measured greatly increased levels of TNF-α in both the AS and RA etanercept patient groups during the course of treatment, while in the infliximab treated patients, the amount of TNF-α measured remained unchanged. Elevated TNF-α in the etanercept treated patients does not appear to be a significant risk factor for the spontaneous development of further autoimmune diseases in our study group. Increased levels of TNF-R1 were determined in both AS ($P < 0.05$) and RA ($P < 0.001$) patients when compared to healthy controls. In AS patients, the levels of TNF-R1 dropped significantly when treated with either infliximab ($P < 0.01$) or etanercept ($P < 0.001$). In contrast, the levels of this receptor remained unchanged in RA patients treated with either compound.

1. Background

Proinflammatory signaling that is activated by TNF-α is an important aspect in the pathology of rheumatic autoimmune diseases such as ankylosing spondylitis (AS) and rheumatoid arthritis (RA). TNF-α exerts its activity through binding to the membrane bound TNF-α receptors TNF-R1 and TNF-R2. In addition to their cell-bound forms, these receptors also exist as soluble molecules (sTNF-Rs) that are a result of enzymatic cleavage of the extracellular portions of the receptors [1]. These soluble receptors are free-floating in the serum and can bind to and act as natural TNF-α antagonists. It is thought that sTNF-R1 and sTNF-R2 modulate and balance the activity of TNF-α in the course of inflammatory events [2, 3].

Anti-TNF-α biologicals that complex TNF-α and neutralize its disease driving activities are beneficial drugs in the treatment of chronic inflammatory disorders. Infliximab, a humanized mouse monoclonal antibody against TNF-α, and etanercept, a soluble fusion protein comprising an epitope derived from the human TNF-receptor 2 fused to the Fc portion of human IgG, represent two different types of biotechnical engineered molecules developed for therapeutic targeting of TNF-α in inflammatory diseases. Both drugs are approved for the treatment of AS and RA as well as for other autoimmune diseases [4].

Although different autoimmune disorders share TNF-α as a major player in disease pathology, there are likely to be alterations in TNF-α regulation that can distinguish between different disease manifestations and respond to different types of TNF-α blocking strategies. Furthermore, studies have reported elevated levels of TNF-α in patients treated with TNF-α blockers and have speculated about the possible consequences thereof [5–8]. In order to see whether we could measure any differences in TNF-α regulation in patients with AS and RA being treated with infliximab and etanercept, we determined the serum concentration of TNF-α as well as

the levels of the soluble TNF-receptors 1 and 2. The measurement of interleukin 6 and C-reactive protein in the serum samples was used to further assess the inflammatory activity in these patients. To follow the course of the disease, we calculated the Bath ankylosing spondylitis disease activity index (BASDAI) and the disease activity score 28 (DAS28) for the AS and RA patients, respectively, at the start of the study and after 12 weeks into treatment.

2. Methods

2.1. Study Participants. The study cohort consists of 45 healthy blood donors, 50 patients with ankylosing spondylitis (AS), and 48 patients with rheumatoid arthritis (RA). AS patients met the criteria of the European Spondyloarthropathy Study Group [9] and RA was diagnosed according to the classification criteria of the American College of Rheumatology [10]. None of the patients exhibited any comorbidities such as malignancies or psoriasis vulgaris, nor showed symptoms of any other chronic diseases. Patients that had been recently immunized, or had a history of viral infection such as HBV or HCV, were excluded from the study. All patients underwent anti-TNF-α therapy for the first time with either etanercept or infliximab. Twenty-two AS and 20 RA patients received etanercept 50 mg subcutaneously weekly during the study, and patients being treated with infliximab received IV infusions of 3 mg/kg body weight (28 RA patients) or 5 mg/kg body weight (28 AS patients) at 0, 2, and 6 and then every 8 weeks (RA patients) or 6 weeks (AS patients) into therapy. Patients treated with infliximab had been treated previously only with NSAIDs, whereas RA patients had received 2 or more DMARDs, including methotrexate, before infliximab or etanercept. RA patients treated with infliximab or etanercept received concomitant methotrexate combined with low-dose glucocorticoids with mean dosages of 15 mg/week and 5 mg/day, respectively. After obtaining written informed consent, serum samples were taken at different time points and stored at −80°C until assayed. Clinical and laboratory assessments were conducted prior to administration of the respective TNF-α blocker at baseline and at 2 and 12 weeks into therapy. The serum concentration of C-reactive protein (CRP) and the disease activity were determined. Assessment of the disease activity was performed before and after 12 weeks of therapy following the Bath ankylosing spondylitis disease activity index (BASDAI) for the AS patients and the disease activity score 28 (DAS28) was calculated for the RA patients.

The study was approved by the local ethics committee of the University of Rostock.

2.2. ELISA. Quantification of sTNF-R1, sTNF-R2, sTNF-α, and IL-6 in serum samples was performed using ELISA-kits (R&D Systems, Wiesbaden, Germany, Catalogue Numbers DY225, DY726, DY210, DY206, resp.) following the manufacturer's protocol. The detection limit was 10 pg/mL for all determinations.

2.3. Statistical Analysis and Graphical Representation. Statistical analysis was performed using GraphPad Prism software (San Diego, USA). One way ANOVA was used to compare parameters measured in the different study groups and repeated measures ANOVA were used to compare values obtained from the same patients at the three time points. ANOVA analyses were followed by Tukey's multiple comparison tests. Paired t-test was used to analyze changes in the disease activity scores during the study period. Comparisons among groups and among time points are depicted as box and whiskers plots. The boxes represent the range from the 25th to the 75th percentile and the whiskers represent the maximum and minimum values. The horizontal line through the box indicates the median.

3. Results and Discussion

Comparing the three groups analyzed in the present study, the age of the healthy controls and the AS patients is very similar with an age (mean ± SD) of 45 ± 12 and 45 ± 13 years in both groups. With a mean age of 56 ± 12 years in the RA group, these patients are significantly older ($P < 0.001$ for both comparisons). The gender distribution within the study cohort shows a higher proportion of females in the control group (25 of 45) and the RA patients (35 of 48), whereas females are underrepresented among the AS patients (21 of 50).

In the AS patient groups treated either with etanercept or infliximab, the anti-TNF-α therapy led to a reduction of the inflammatory activity as seen by a significant drop of the CRP concentration after 2 and 12 weeks into therapy ($P < 0.01$ for both treatments). The RA patients show a trend towards decreasing CRP levels during both treatments, but this effect does not reach statistical significance. The proinflammatory cytokine IL-6 also tends to decrease during therapy in all treatment groups, but only reached statistical significance in the AS patients treated with etanercept and infliximab at the 2 week time point (both $P < 0.05$). All serum parameters determined before and during anti-TNF-α therapy are listed in Table 1.

All four treatment groups showed a significant decrease in the disease activity scores (BASDAI for AS and DAS28 for RA) determined before the first administration of a TNF-α blocker and after 12 weeks into therapy (Figure 1). This response shows that 12 weeks of treatment with infliximab and etanercept leads to clinical improvement in both diseases in our study cohort.

3.1. Serum TNF-α. Prior to treatment, 32% (infliximab group) and 20% (etanercept group) of RA patients were found to be TNF-α positive (>10 pg/mL). This is around twice as many as TNF-α positive AS patients prior to treatment (14% and 9%, infliximab and etanercept groups, respectively, Table 1). The mean TNF-α level prior to treatment of 8.7 ± 17.9 pg/mL in RA patients was significantly higher ($P < 0.05$) than in AS patients (2.4 ± 7.2 pg/mL) and in healthy participants (2.5 ± 7.9 pg/mL).

During the course of TNF-α blockade, no significant change in the level or in the percentage of TNF-α positive study subjects could be observed in the AS and the RA

Table 1: Serum parameters in AS and RA patients treated with infliximab and etanercept, respectively.

Week	Ankylosing spondylitis						Rheumatoid arthritis					
	Infliximab $N = 28$			Etanercept $N = 22$			Infliximab $N = 28$			Etanercept $N = 20$		
	0	2	12	0	2	12	0	2	12	0	2	12
CRP mg/mL	18.4 ±21.2	**5.9 ±6.9**	**7.2 ±9.5**	11.24 ±15.5	**2.4 ±5.0**	**3.1 ±4.8**	20.9 ±27.5	7.7 ±14.9	13.0 ±24.6	26.5 ±31.2	19.4 ±23.3	21.8 ±28.4
IL-6 pg/mL	3.1 ±7.4	**0**	0.4 ±1.9	15.2 ±18.3	**3.8 ±8.7**	6.3 ±10.8	11.7 ±28.8	7.5 ±21.4	5.2 ±7.1	19.8 ±25.7	13.3 ±27.5	10.9 ±20.4
TNF-α pg/mL	2.3 ±6.3	1.6 ±4.9	2.7 ±7.7	2.5 ±8.3	**25.6 ±14.8**	**23.4 ±14.8**	10.7 ±19.3	6.0 ±13.0	13.0 ±27.2	5.8 ±15.7	**19.2 ±15.8**	**23.1 ±28.7**
Patients >10 pg/mL	4 14%	3 11%	3 11%	2 9%	**21 96%**	**18 82%**	9 32%	5 18%	9 32%	4 20%	**14 70%**	**13 65%**
TNF-R1 ng/mL	1.01 ±0.47	**0.86 ±0.30**	**0.86 ±0.27**	0.74 ±0.16	**0.55 ±0.16**	**0.57 ±0.17**	1.23 ±0.58	1.06 ±0.45	1.20 ±0.79	1.21 ±0.35	1.10 ±0.36	1.17 ±0.39
TNF-R2 ng/mL	3.08 ±1.09	2.84 ±0.95	3.10 ±1.06	2.43 ±1.55	**474* ±319**	**570* ±348**	4.03 ±1.33	3.60 ±1.38	3.95 ±1.20	2.02 ±0.66	**278* ±144**	**280* ±198**

The serum concentrations of CRP, IL-6, TNF-α, TNF-R1, and TNF-R2 are reported as mean values ± SD. Numbers in bold indicate significant differences in the concentration at 2 or 12 weeks into therapy, when compared to the pretreatment level; *indicates TNF-R2 determinations which were interfered with by etanercept.

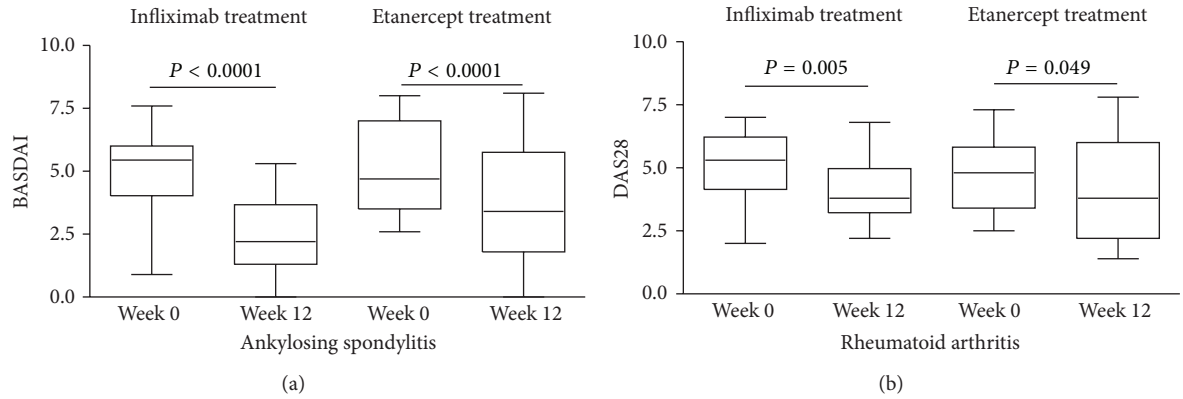

Figure 1: Changes in disease activity after 12 weeks of anti-TNF-α therapy. Disease activity scores were determined prior to the start of and at 12 weeks into treatment with infliximab and etanercept. Disease activity was assessed using the Bath ankylosing spondylitis disease activity index (BASDAI) and the disease activity score 28 (DAS28) in patients with ankylosing spondylitis (a) and rheumatoid arthritis (b), respectively. Significant differences in disease activity are indicated by the P values.

patients being treated with infliximab. Administration of etanercept led to a significant increase of the mean serum TNF-α concentrations in the AS patients (2.5 ± 8.3 to 23.4 ± 14.8 pg/mL, $P < 0.001$) as well as in the RA patients (5.8 ± 15.7 to 23.1 ± 28.7 pg/mL, $P < 0.05$) after 12 weeks into therapy (Figures 2(a) and 2(b)). This resulted in an increase in the number of TNF-α positive patients during etanercept treatment, with 96% and 82% of the AS patients, and 70% and 65% of the RA patients being TNF-α positive at the 2 week and 12 week time point, respectively. The amount of TNF-α detected in the presence of etanercept was not different when comparing the AS and RA patients during treatment (Table 1).

Our observation that the level of measured TNF-α does not increase in infliximab treated patients is in contrast to a previous study by Charles et al., in which a dose dependent increase in serum TNF-α in RA patients being treated with infliximab (cA2) was found [11]. We therefore tested whether the amount of TNF-α that is measured with the ELISA kits that we employed can be affected by either the presence of etanercept or infliximab in the sample. In order to do so, we mixed and incubated (30 minutes at room temperature) various concentrations of TNF-α with physiologically relevant amounts of either TNF-α blocker, and measured the amount of TNF-α using our anti-TNF-α ELISA assay. We found that both agents reduced the measured amount of TNF-α, with the effect by infliximab being much larger than that of etanercept (Figure 2(c)). As such, we are not able to interpret our measurements of TNF-α made in the presence of infliximab, since our assay system is not capable of measuring most of the TNF-α that is bound by this compound. Although we cannot rule out the possibility

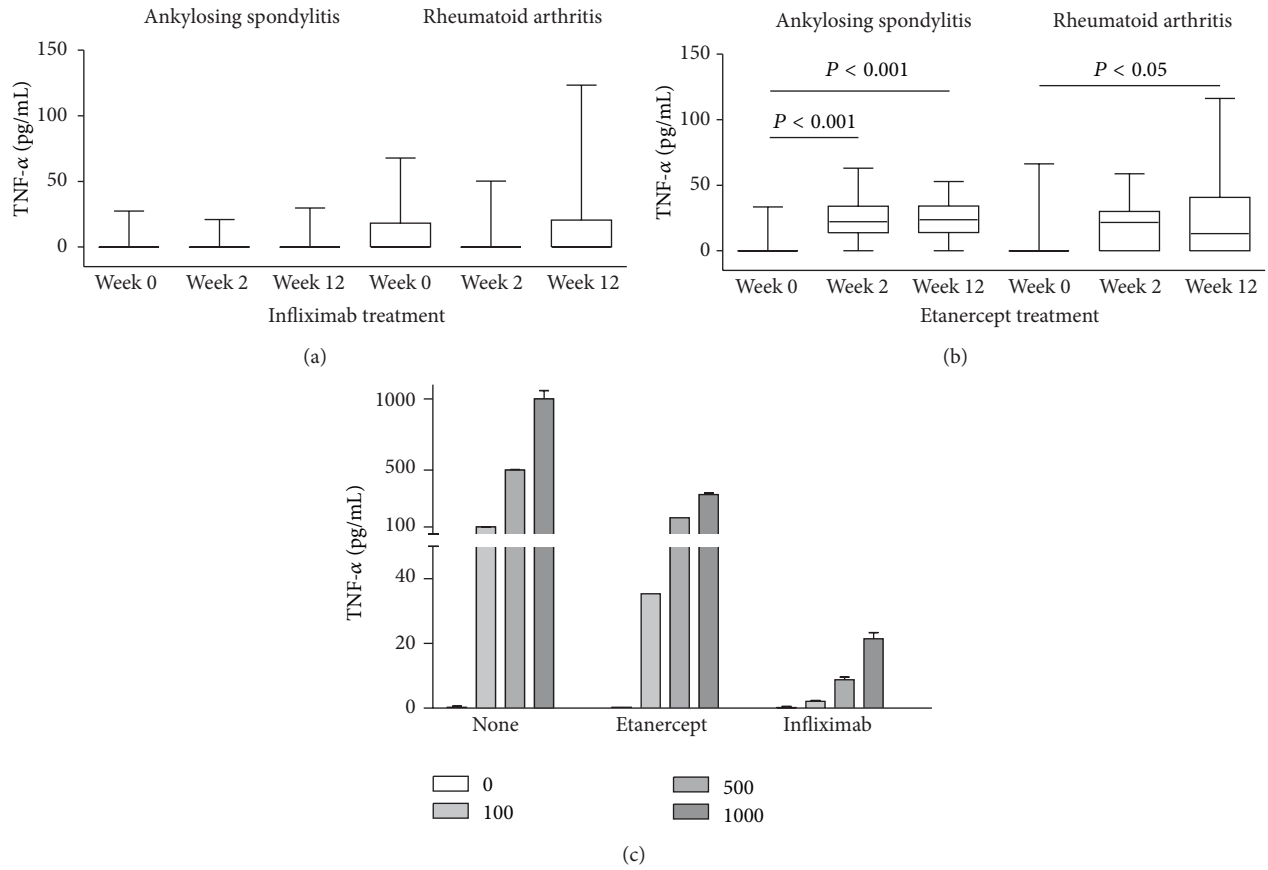

Figure 2: Influence of TNF-α blocking therapy on the serum TNF-α concentration in patients with ankylosing spondylitis and rheumatoid arthritis. Serum samples were taken at the indicated time points of therapy with infliximab (a) and etanercept (b). Significant changes in the TNF-α concentration during the course of therapy are indicated by the P values. To test for interference of TNF-α blockers, standard curves were performed using TNF-α ELISA in the presence of either 3 μg/mL etanercept or 5 μg/mL infliximab (c).

that autoantibodies to infliximab could also influence the quantitation of TNF-α, the fact that the presence of infliximab influences the amount of TNF-α measured in our assay might explain the different results that we obtained when compared with the study of Charles et al., that used a different ELISA assay system to measure TNF-α in the presence of infliximab.

In the past years, studies have emerged reporting increased serum concentrations of TNF-α as measured using ELISA assays in individuals being treated with etanercept for various conditions including rheumatic autoimmune disease [12]. The elevated serum levels could be explained by both an increased half-life of TNF-α when complexed with etanercept [13], and an upregulated expression and/or release of soluble TNF-α, that would be consistent with the observation that etanercept can enhance the capacity of immune cells to produce TNF-α [14], or possibly by the different biochemical and pharmacokinetic properties of the two anti-TNF-α agents.

While TNF-α is thought to be biologically inactive when complexed with the receptor fusion protein etanercept, the idea has been put forward that increased serum levels of TNF-α resulting from etanercept treatment can trigger autoimmune reactions such as psoriasis and Crohn's disease. The possible consequences thereof have been the subject of some debate, and it has been suggested that routine monitoring of serum TNF-α should be performed in all patients being treated with anti-TNF-α biologicals for this reason—even though the interpretation of such measurements remains unclear [5–8]. Although TNF-α is increased in the vast majority of the patients treated with etanercept in this study, we had not observed any new autoimmune symptoms such as psoriasis in these patients. Although we did measure differences in the levels of TNF-α in individual patients during the course of therapy with etanercept, the reasons for these differences are unclear, and we have no evidence to support the routine measurement of TNF-α in patients being treated with these compounds.

3.2. Soluble TNF-α Receptor 1 and 2. Serum TNF-R1 showed elevated concentrations in both the AS and RA patient groups prior to anti-TNF-α treatment when compared to the controls (Figure 3). The mean concentration in the healthy blood donors was 0.66 ± 0.17 ng/mL compared to 0.89 ± 0.39 ng/mL in the AS patients and 1.22 ± 0.49 ng/mL in the RA patients. The differences among the groups reached statistical significance with $P < 0.05$ (AS versus control) and $P < 0.001$ (RA versus control and RA versus AS). No significant

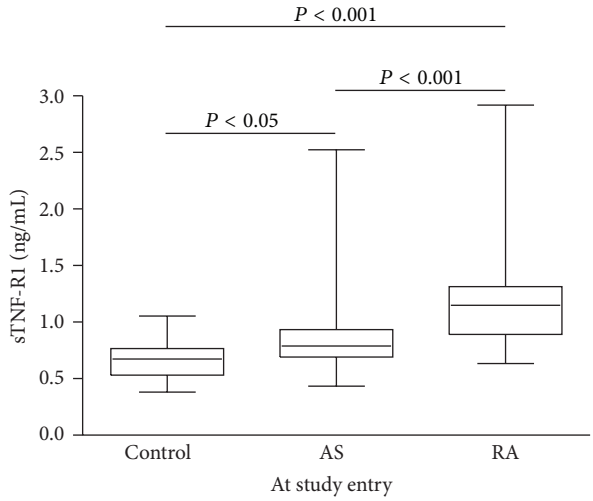

Figure 3: Comparison of serum TNF-R1 levels in healthy controls, AS, and RA patients. Patient serum samples were taken prior to the start of anti-TNF-α therapy. Significant differences in the receptor concentration found in the study groups are indicated by the P value.

differences in the serum TNF-R2 concentration could be detected when comparing the healthy control subjects with the two patient groups at the 0 week time point. The mean concentrations were 2.86 ± 1.80 ng/mL in the healthy subjects and 2.79 ± 1.33 and 3.22 ± 1.49 ng/mL in the AS and RA patient group, respectively. These results are in partial agreement with previously published data on RA patients, which showed elevated levels of both TNF-α receptors when compared with healthy controls [15, 16].

After 2 weeks into therapy, the AS patients showed a significant decrease in TNF-R1 levels when treated with either infliximab or etanercept. This effect was maintained at the 12 week time point, resulting in a 15 and 23% reduction of the receptor concentrations as compared to the pretreatment measurement ($P < 0.01$ and $P < 0.001$ in the infliximab and the etanercept groups, resp.). In the RA patient groups, that displayed the highest initial receptor levels, neither of the TNF-α blockers used affected the serum TNF-R1 concentrations during the study period (Table 1).

The decrease in serum concentration of TNF-R1 in AS patients treated with infliximab and etanercept is in line with the idea that the level of soluble TNF-R1 is connected to the activity of TNF-α and inflammatory events as discussed in context of rheumatoid arthritis and other diseases in which TNF-α is involved [17–19]. Although we cannot exclude the possibility that the higher dosage of infliximab used in treating AS patients might at least partly affect the levels of TNF-R1 in this group, the fact that the levels of TNF-R1 are not affected by either TNF-α blocker in RA patients might be explained by a distinct regulation of TNF-α signaling in AS compared to RA patients. Our study suggests that soluble TNF-R1 might be a good marker of inflammation in AS patients. It is an easily measured parameter that could be accurately quantified in every patient, whereas TNF-α, CRP, and IL-6 are often below the detection limits in routinely used assays.

No significant change in TNF-R2 was observed during infliximab treatment of the AS and RA patients. In the presence of etanercept, the measurement of serum TNF-R2 was interfered with by the TNF-R2 portion of the drug, and this resulted in values that were out of the range of the assay. We analyzed serial dilutions of etanercept using our TNF-R2 ELISA and found that the reactivity of etanercept in this immunoassay was about 10 to 15% when compared to the recombinant TNF-R2 standard calibrators (Figure 4(a)). We therefore diluted etanercept treated patient sera 1000-fold and reassayed them in the TNF-R2 ELISA. The ELISA determinations were about 100-fold higher as compared to endogenous TNF-R2 levels prior to the start of treatment, with mean concentrations corresponding to 570 ± 348 and 280 ± 198 ng/mL in AS and RA patient sera, respectively. Although etanercept is only partially recognized in the TNF-R2 ELISA, the assay can still be used to compare the relative concentration of etanercept in the patient sera. At the 12 week time point, when an established level of etanercept can be expected, the concentrations detected as well as the variation of the levels found in the group of AS patients were significantly higher ($P < 0.001$) when compared to the RA patients (Figure 4(b)). To test the possibility that high levels of TNF-α might interfere in the TNF-R2 ELISA, we mixed etanercept with varying concentrations of TNF-α and assayed these samples using ELISA. We found no effect of TNF-α up to a concentration of 1000 pg/mL (data not shown).

As both AS and RA are treated by self-administration of etanercept, both at the same dosage and time interval, our measurements indicate that distinct differences in drug metabolism between our patient groups exist. This is in contrast to a study where no significant change in etanercept pharmacokinetics could be detected when comparing AS patients with RA patients [20]. It is unlikely that the difference in the composition of our patient groups might account for the different etanercept levels we observe between AS and RA patients, since it has been shown that etanercept disposition does not differ between males and females and does not vary with age in adult patients [21, 22]. The fact that two patients with AS and two patients with RA displayed TNF-R2 concentrations at the 12 week time points similar to those found prior to treatment begins could be explained by noncompliance in these patients. This could also explain why elevated levels of TNF-α were not detected in these patients. Interestingly, a recent study found that the levels of etanercept at 3 months into treatment can predict response to therapy at 6 months, suggesting that the monitoring of etanercept during treatment might be of prognostic value [23].

4. Conclusions

The increase in measurable TNF-α frequently observed upon etanercept treatment likely reflects biochemical and physiological properties in the regulation of TNF-α and was not identified as being a risk factor for triggering further inflammatory reactions in our study. In AS patients, the measurement of TNF-α receptor 1 might be useful as a biological indicator of TNF-α blocker activity. The determination

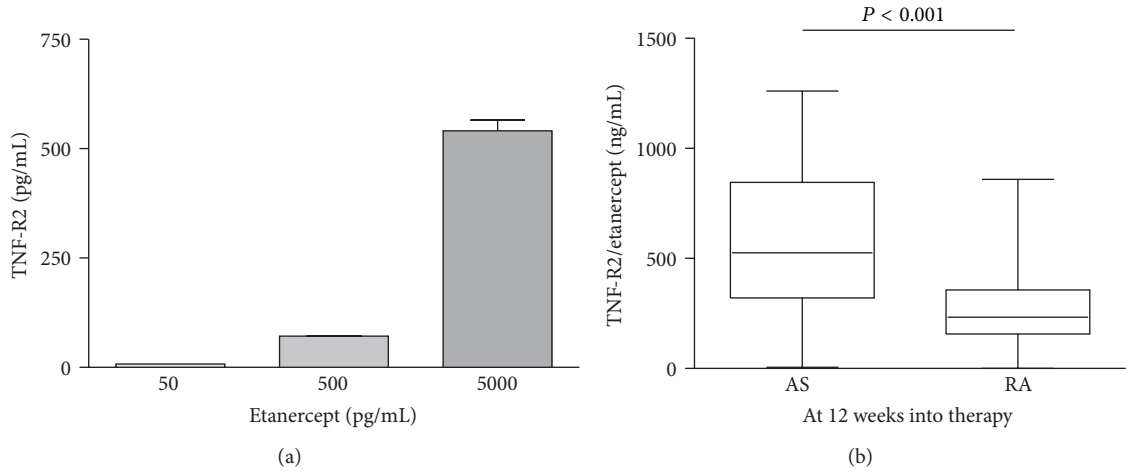

Figure 4: Detection of etanercept in the TNF-R2 ELISA. Serial dilutions of etanercept were assayed in the TNF-R2 ELISA and compared to recombinant standards (a). Patient sera at the 12 week time point of etanercept treatment were analyzed using TNF-R2 ELISA (b). The significant difference between the patient groups is indicated by the P value.

of serum etanercept might be useful in the monitoring of medication compliance and to allow for a quantification of the steady state concentration of these compounds in individual patients.

Abbreviations

ACR:	American College of Rheumatology
AS:	Ankylosing spondylitis
BASDAI:	Bath ankylosing spondylitis disease activity index
CRP:	C-reactive protein
DAS28:	Disease activity score 28
IL-6:	Interleukin-6
RA:	Rheumatoid arthritis
sTNF-α:	Soluble tumor necrosis factor α
sTNF-R1/2:	Soluble tumor necrosis factor receptor 1/2.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Martin Schulz, Helmut Dotzlaw, and Gunther Neeck participated in the design of the study. Gunther Neeck selected and performed clinical evaluation of study subjects. Helmut Dotzlaw performed ELISA analyses, and Martin Schulz performed statistical analyses. Martin Schulz, Helmut Dotzlaw, and Gunther Neeck discussed and interpreted all data. Martin Schulz drafted the paper, and Helmut Dotzlaw and Gunther Neeck critically read and revised the paper. All authors read and approved the final submitted paper.

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Review Article

Adipokines, Biomarkers of Endothelial Activation, and Metabolic Syndrome in Patients with Ankylosing Spondylitis

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Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease associated with accelerated atherosclerosis and increased risk of cardiovascular (CV) disease. AS patients also display a high prevalence of features clustered under the name of metabolic syndrome (MeS). Anti-TNF-α therapy was found to be effective to treat AS patients by suppressing inflammation and also improving endothelial function. Previously, it was demonstrated that a short infusion of anti-TNF-α monoclonal antibodyinfliximab induced a rapid and dramatic reduction in serum insulin levels and insulin resistance along with a rapid improvement of insulin sensitivity in nondiabetic AS patients. The role of adipokines, MeS-related biomarkers and biomarkers of endothelial cell activation and inflammation seem to be relevant in different chronic inflammatory diseases. However, its implication in AS has not been fully established. Therefore, in this review we summarize the recent advances in the study of the involvement of these molecules in CV disease or MeS in AS. The assessment of adipokines and biomarkers of endothelial cell activation and MeS may be of potential relevance in the stratification of the CV risk of patients with AS.

1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease, which mainly affects the axial joints, including the spine, sacroiliac joints, and entheses, but it may also involve peripheral joints [1]. Along with disease progression, inflamed joints tend to fuse (ankylosis) and there is also an ossification of the inflamed entheses, often leading to a loss of the well-known flexibility of the spine. AS is more prevalent in men than in women and usually appears around the third decade of life [1]. Moreover, extra-articular manifestations such as uveitis, psoriasis, or osteoporosis are frequently associated with this rheumatologic disease [2].

As observed in other rheumatologic diseases, such as rheumatoid arthritis (RA), AS patients disclose an increased risk of cardiovascular (CV) disease when compared to general population, being CV diseases one of the main causes of mortality in these patients [1]. Furthermore, an accelerated atherosclerotic process in these patients has also been reported [3].

AS patients also display a high prevalence of features such as obesity, dyslipidemia, hypertension, alterations in glucose metabolism, and insulin resistance (IR), which are clustered under the name of metabolic syndrome (MeS) [4]. Interestingly, individuals that suffer MeS also exhibit a dysregulation of adipokines, which are highly bioactive

substances secreted by adipocytes and immune cells and that are involved not only in metabolic functions but that also play an immunomodulatory role [5, 6]. This dysregulation leads to metabolic disorders such as IR [5], an essential feature of MeS that has been associated with inflammation [7]. In addition, multiple evidences show that IR promotes endothelial dysfunction [8, 9], an early key step in the atherogenic process which appears even before the structural changes associated with this process [10].

Regarding therapeutic approaches aimed to treat AS, anti-TNF-α therapy was found to be effective to treat patients with this disease and other types of spondyloarthritis [11–13]. Anti-TNF-α agents neutralize this cytokine leading to suppression of inflammation and, consequently, to a reduction of disease activity [14]. Moreover, it was demonstrated that this biologic therapy improves endothelial function in AS patients [15].

For the purpose of this review, we took advantage of data obtained from a series of 30 nondiabetic AS patients undergoing anti-TNF-α therapy with the chimeric anti-TNF-α monoclonal antibody infliximab [16]. At the time of assessment, these patients had been treated with this biologic agent for a median of 23 months. Since IR promotes endothelial dysfunction [8, 9], while anti-TNF-α treatment improves endothelial function in AS patients [15], our first objective was to evaluate short-term insulin response following anti-TNF-α infliximab therapy. We observed that our patients experienced a rapid and dramatic reduction in serum insulin levels and IR along with rapid improvement of insulin sensitivity after a single administration of infliximab [16]. This observation had previously been described in patients with RA undergoing anti-TNF-α infliximab therapy [17, 18].

Considering these results, we decided to further evaluate the short-term effect of anti-TNF-α therapy in our series of AS patients on periodical treatment with infliximab on MeS-related biomarkers, adipokines, and biomarkers of endothelial cell activation and inflammation. Figure 1 depicts the pathophysiologic context that encompasses all the molecules reviewed in this paper. Furthermore, the main results derived from these studies on the effect of an infliximab infusion are summarized in Table 1.

In this review, recent advances in the study of the involvement of these molecules in CV disease or MeS in AS patients are discussed, along with the possible link between these biomarkers and/or adipokines and clinical characteristics of this rheumatic disease.

Table 1: Effect of an infusion of the anti-TNF-α monoclonal antibody infliximab on MeS-related biomarkers, biomarkers of endothelial cell activation and inflammation, and adipokines in a series of AS patients undergoing periodic treatment with this drug.

Target	Biologic effect
MeS-related biomarkers	Reduction in serum insulin levels and IR Improvement of insulin sensitivity Reduction of RBP-4 serum levels No significant change on ghrelin serum levels
Biomarkers of endothelial cell activation and inflammation	Reduction of Angpt-2 serum levels Reduction of OPN serum levels No significant change on ADMA and GSN serum levels No significant reduction on OPG plasma levels
Adipokines	No significant change in the levels of the different adipokines (adiponectin, resistin, leptin, visfatin, and apelin)

2. Metabolic Syndrome-Related Biomarkers in AS

As previously mentioned, AS patients frequently display features of MeS [4]. Therefore, the study of potential biomarkers involved in the development of such features and its association with the pathogenesis of this spondyloarthritis could give us hints for the outcome and treatment of these patients.

2.1. Ghrelin. Ghrelin, a peptide predominantly expressed in the stomach, is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R), which regulates food intake and GH expression [19] and also acts as an anti-inflammatory molecule [20]. Previously, low levels of this peptide have been observed in obese individuals [21], a condition directly associated with hyperinsulinemia and IR. In our series we disclosed a significant correlation between ghrelin and IR and insulin sensitivity [22]. Similar results had previously been reported in individuals without rheumatic diseases [23, 24]. However, since only 10% of our patients were obese, possibly other mechanisms different from obesity may account for our findings. Furthermore, in this study we also observed a positive correlation between ghrelin and resistin, which is in accordance with their role in glucose homeostasis and the inflammatory process [22].

To our knowledge, the only previous study performed to evaluate ghrelin levels in AS patients was the one reported by Toussirot et al. [25]. However, in that study they described higher levels of ghrelin in AS patients when compared to controls. These results seem to be unexpected, since ghrelin is known to inhibit the production of inflammatory cytokines [20]. In line with this, RA patients show decreased ghrelin levels when compared to healthy controls [26]. Therefore, further studies are needed to elucidate whether the different inflammatory burden in RA and AS may account for these contradictory results.

We previously reported a significant elevation of ghrelin serum concentration upon a single infliximab infusion in RA patients with severe disease who despite receiving this anti-TNF-α agent had active disease with persistent elevation of laboratory markers of inflammation [27]. However, following a single infusion of infliximab, when we compared ghrelin levels found immediately before and after an infliximab infusion (the drug was administered in the fasting state, in saline solution over 120 minutes), we only disclosed a mild but not significant increase of ghrelin serum concentration in patients with AS [22]. These disparate results may be due to the absence of severe disease in our series of patients of AS

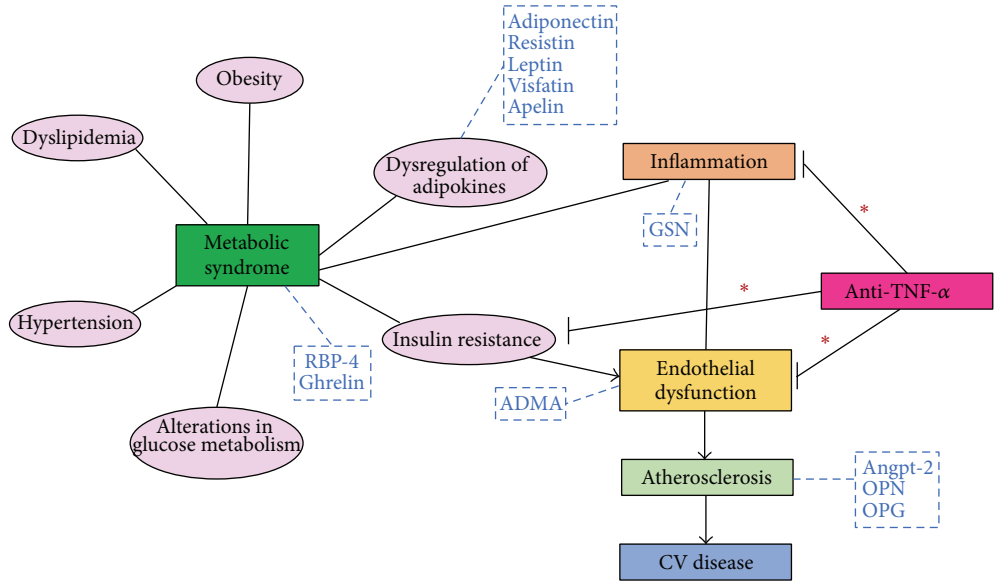

Figure 1: Pathophysiologic context that encompasses all the molecules reviewed in this paper. Ankylosing spondylitis patients display a high incidence of features clustered under the name of metabolic syndrome, which include obesity, dyslipidemia, hypertension, alterations in glucose metabolism, including insulin resistance, and also a dysregulation of adipokines. Moreover, all these pathologic features are associated with inflammation and lead to endothelial dysfunction and, consequently, to an enhanced risk of CV disease (mainly due to accelerated atherosclerosis) and CV death in these patients. Anti-TNF-α treatment not only suppresses inflammation, reducing thus ankylosing spondylitis activity, but it also improves endothelial function in these patients. The molecules that will be reviewed in this paper are included in this figure inside blue dashed boxes. *Anti-TNF-α improves insulin resistance and endothelial function and also reduces inflammation. ADMA: asymmetric dimethylarginine; Angpt-2: angiopoietin-2; OPG: osteoprotegerin; OPN: osteopontin; RBP-4: retinol binding protein-4.

at the time of assessment. It was not the case in RA patients undergoing infliximab therapy as the series of patients with RA still had active disease despite periodical treatment with this TNF-α inhibitor.

2.2. Retinol Binding Protein-4 (RBP-4). Retinol binding protein-4 (RBP-4) is another metabolic syndrome-related biomarker, also considered a new potential cardiometabolic risk factor. This proinflammatory protein is mainly released by adipocytes but also expressed in liver and macrophages [28] and has been associated with IR in individuals with obesity, impaired glucose tolerance or type 2 diabetes mellitus, and nonobese subjects with or without family history of type 2 diabetes [29–31]. In our study, we disclosed a marginally significant correlation between RBP-4 serum levels and IR and a correlation with systolic blood pressure. Besides, when our AS patients where stratified according to sex, men showed higher levels of this protein than women [32], which was in keeping with the results previously reported by Gavi et al. [30]. More importantly, when we evaluated the effect of a single infusion of anti-TNF-α infliximab on RBP-4 levels, we observed a statistically significant reduction in its levels. Furthermore, this change was more evident in those patients who had a higher IR index [32].

A former study disclosed that AS patients may have lower RBP-4 serum levels than controls [33]. However, when we compared our patients with healthy controls we did not find significant differences in RBP-4 serum levels [32]. The low disease activity at the time of study in our series of AS patients may explain the absence of differences in RBP-4 serum levels when compared with controls.

3. Adipokines in AS

Immune system and metabolism are linked through a network of soluble mediators widely known as adipokines, which take part in both metabolic and immunomodulatory functions [6]. Although the role of adipokines seems to be relevant in many chronic inflammatory diseases, the implication in AS has not been completely elucidated.

3.1. Adiponectin. Adiponectin is an adipokine mainly produced by adipocytes but that can also be found in endothelial cells, skeletal muscle cells, and cardiac myocytes [6]. Circulating adiponectin levels inversely correlate with adiposity [5], which suggests that this adipokine exerts a protective function against CV disease and obesity. Adiponectin increases fatty acid oxidation and reduces the synthesis of glucose in the liver and other tissues [34]. Depending on the context, this adipokine can have pro- or anti-inflammatory functions. Unlike observations in nonrheumatic patients, RA high levels of this adipokine have been reported in the inflamed joints, since it promotes matrix degradation [35]. However, results obtained in AS are contradictory. Some groups did not find differences in serum adiponectin levels between AS patients with active disease and controls [25], while others found

significantly higher levels of this adipokine in a series of AS patients under treatment with infliximab, when compared with controls [36].

In our series of AS patients we found a positive correlation between adiponectin serum levels and insulin sensitivity, suggesting that low circulating adiponectin concentrations may be associated with metabolic abnormalities that promote CV disease in AS [37]. The effect of adiponectin on insulin sensitivity is mediated in part by its ability to activate signaling pathways that lead to glucose uptake in muscle tissue and the inhibition of gluconeogenesis in the liver [38]. Furthermore, AS patients with hip involvement or synovitis and/or enthesitis in other peripheral joints had higher levels of adiponectin than those who did not have these complications [37]. These results are in keeping with the proinflammatory role described for adiponectin in the joints of RA patients [35]. Therefore, higher adiponectin levels might help to establish a subgroup of AS patients with predominant peripheral involvement.

As observed in RA patients undergoing anti-TNF-α infliximab therapy [39], a single infusion of this biologic agent did not lead to significant changes in the serum levels of adiponectin in patients with AS [37].

3.2. Resistin. Resistin is another proinflammatory adipokine mainly produced by monocytes and macrophages [40]. Proinflammatory factors such as TNF-α and IL-6 induce its expression [41]. Interestingly, a positive correlation between serum resistin and C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) has been observed in RA patients [42–44]. Moreover, resistin serum levels have been found increased in synovial fluid of RA patients [45]. In AS patients, likewise, higher levels of this adipokine have been observed as compared to controls [46].

In our cohort of AS patients treated with the TNF-α antagonist infliximab we did not observe any correlation between resistin concentration and disease activity or laboratory markers of inflammation, probably due to the low inflammatory burden as a result of prolonged treatment with anti-TNF-α [37]. These results are in agreement with those obtained by Kocabas et al., who did not find any correlation between resistin concentration and ESR, CRP, or BASDAI in their series of AS patients [46]. Once again, the lower inflammatory burden in our AS patients due to prolonged treatment with this biologic therapy could also explain the lack of effect observed after a single infusion of infliximab on resistin concentration [37].

3.3. Leptin. Leptin also belongs to the group of proinflammatory adipokines, being produced by many cell types, including adipocytes [47]. This adipokine is involved in body weight regulation, since it inhibits food intake and stimulates energy expenditure [48]. Moreover, a proatherogenic role for leptin has been described [49]. Leptin induces the production of the proinflammatory cytokines IL-6, IL-12, and TNF-α by monocytes and macrophages [50], while it suppresses the production of IL-4, an anti-inflammatory cytokines [51]. Leptin levels are regulated by inflammatory mediators such as TNF-α and IL-1 [52]. While in RA leptin has a clear proinflammatory role, showing increased levels in patients when compared with healthy controls [53], the results obtained for this adipokine in AS are contradictory. Some groups found increased leptin levels in AS patients with active disease when compared to controls [54], while others found lower circulating levels of this adipokine than in controls [25, 55].

In contrast to previous reports which found a correlation between leptin levels and BASDAI in a series of AS patients [54], in our AS cohort, we did not observe any correlation between the levels of this adipokine and clinical and laboratory parameters of disease activity and inflammation [56]. Similar results were obtained in a series of RA patients undergoing periodical anti-TNF-α therapy [57]. Furthermore, as previously described [58], when our series of AS patients were stratified according to sex, we disclosed higher levels of leptin in women [56].

When we analyzed the effect of TNF-α blockade on circulating leptin levels, we found that they were not significantly altered [56]. Similar results were obtained by other groups, either in AS patients after 6 months of infliximab treatment [36] or in RA patients undergoing 2 weeks and 6 months TNF-α blockade [59].

3.4. Visfatin. Visfatin, also known as pre-B cell colony-enhancing factor or PBEF, is a proinflammatory adipokine with ubiquitous expression [60]. Visfatin was reported to act as an insulin-mimetic adipokine [61]. This adipokine positively correlates with visceral fat [62] and has also been described as an immunomodulatory molecule [63, 64]. In fact, visfatin can induce monocytes to produce proinflammatory cytokines such as IL-1, TNF, and IL-6 [64]. Previously, increased levels of this adipokine have been observed in patients with RA [53] when compared to controls. However, to our knowledge, in addition to our study [56], the only previous study performed in AS patients was performed by Hulejová et al. [65]. In that study, which included AS patients with mild to moderate disease, no correlation was found between visfatin levels and disease activity, functional status, or acute-phase reactants [65]. Similarly, in our series of AS patients on periodical treatment with the anti-TNF-α-blocker infliximab, we could not find correlation between serum visfatin levels and clinical and laboratory parameters of disease activity and inflammation [56]. This was also the case for RA patients undergoing anti-TNF-α infliximab therapy [66].

Although we could not find association between visfatin serum levels and metabolic syndrome in RA patients with severe disease undergoing anti-TNF-α therapy [66], we observed a positive correlation between visfatin serum levels and IR in AS [56]. A former study performed in lean women with polycystic ovary syndrome obtained similar results [67]. However, in our series of AS patients visfatin levels did not change upon infliximab administration [56].

3.5. Apelin. Apelin is a quite recently new adipokine produced by diverse cell types, including adipocytes and endothelial cells [68]. This adipokine is considered a potential

biomarker for CV disease risk since it stimulates nitric oxide (NO) release and, therefore, triggers arterial vasodilation [69]. Moreover, insulin directly upregulates the expression of apelin [70], making this adipokine an attractive candidate to be studied in metabolic disorders such as type-2 diabetes. Furthermore, low apelin levels have been associated with high LDL levels [71] and biomarkers of endothelial cell activation such as VCAM-1 and E-selectin correlated to apelin levels [72].

It has been postulated that apelin may have a role in the pathogenesis of the CV disease, since low levels of this adipokine have been observed in patients with ischemic heart disease [73]. However, contradictory results have been reported in patients with type 2 diabetes mellitus. In this regard, in a study fasting plasma apelin levels correlated positively with IR in patients with type 2 diabetes mellitus [74], while in another study plasma apelin levels were reduced in newly diagnosed and untreated patients with type 2 diabetes mellitus [75].

di Franco et al. measured apelin levels in early stage RA patients and found that they were lower than those observed in controls [76], suggesting a potential involvement of apelin in the pathogenesis of the rheumatic diseases. However, regarding AS, there are no previous reports performed to evaluate the levels of this adipokine in this disease. In a study of our group, apelin levels showed no association with markers of disease activity or MeS in patients with AS [77]. In line with our results, Ferraz-Amaro et al. did not find any correlation between the levels of apelin in RA patients treated with anti-TNF-α and BMI or IR [78].

In a further step, we analyzed the potential effect of anti-TNF-α infliximab treatment on apelin concentration in our series of AS patients. We found that even after the administration of a single dose of infliximab apelin serum levels were reduced, this decrease did not achieve statistical significance [77]. Similar results were previously described in RA patients after 12 months of anti-TNF-α treatment [78] or after 12 months of treatment with disease modifying antirheumatic drugs [76].

4. Biomarkers of Endothelial Cell Activation and Inflammation

In physiological conditions, NO acts as an anti-inflammatory molecule, maintaining the vascular wall in a quiescent state, also avoiding cellular proliferation. However, in the presence of inflammation or CV risk factors such as hypertension, hypercholesterolemia, or diabetes, the quiescent endothelium can switch to an activated phenotype. This leads to the secretion of proinflammatory factors such as cytokines and adipokines, to the expression of adhesion molecules for the recruitment of inflammatory cells to the vascular wall and to the generation of reactive oxygen species [79]. This pathological inflammatory condition also leads to a reduction in the release of NO into the arterial wall, either affecting its synthesis or due to oxidative inactivation of NO [80, 81], which further enhances the inflammatory status and maintains the endothelial activated phenotype [79].

Many biomarkers of endothelial cell activation and inflammation may be potentially used by clinicians to make an early diagnosis of CV disease and MeS. The implication of these biomarkers in the pathogenesis of the rheumatic diseases is also an issue of potential interest. However, as previously mentioned for adipokines, studies performed on AS patients are limited.

4.1. Asymmetric Dimethylarginine (ADMA). Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of the nitric oxide synthase (NOS), causing therefore a reduction in NO production and leading to endothelial dysfunction and CV events [82]. Consequently, ADMA has been proposed as a biomarker for endothelial dysfunction and a risk factor for CV disease [82, 83]. Furthermore, increased levels of ADMA have been associated with hypertension [84], hypertriglyceridemia [85], hypercholesterolemia [86], diabetes mellitus [87], and IR [88], as well as with inflammatory diseases such as RA [76] and AS [89–91].

We found higher concentrations of ADMA in AS patients with hypertension [92]. Unexpectedly, we observed a significant negative correlation between ADMA levels and total cholesterol (TC) and LDL-cholesterol in AS [92], which apparently seems to contradict previous results that associated high ADMA levels with patients with hypercholesterolemia [86]. We think that this negative association between ADMA and TC and LDL-cholesterol might probably be the result of the long-term treatment with anti-TNF-α in our series of AS patients (almost 2 years), which may have led to a reduction of the inflammatory burden in these patients [14] along with complex lipidic changes [93, 94]. With respect to this, our series of patients with AS were in a state of low disease activity, with low levels of CRP, showing a BASDAI less than 3, which is indicative of a favorable disease activity state [92]. This could be the reason why we could not see statistically significant associations between CRP/disease activity markers and ADMA levels in our series of patients.

Furthermore, we did not observe any change on ADMA levels after a single infusion of anti-TNF-α [92]. This was in accordance with previous studies that described no effect on ADMA concentration following long-term TNF-α blockade [95, 96].

4.2. Angiopoietin-2 (Angpt-2). Angiopoietin-2 (Angpt-2) is a proinflammatory marker of endothelial cell activation that is involved in angiogenesis and makes the endothelium responsive to inflammatory cytokines [97]. Angpt-2 levels have been found increased in recent onset RA patients with CV disease when compared with those without CV disease [97], which suggests that it could be a potential biomarker for the development of CV disease. Interestingly, a recent study of our group disclosed a correlation between age at the time of disease onset in patients with RA and Angpt-2 levels [98]. More importantly, after adjustment for sex, age at RA diagnosis,and CV risk factors, Angpt-2 levels were higher in RA patients with CV disease than in RA patients

without CV complications [98]. Interestingly, in our series of AS patients undergoing infliximab therapy, we also found an association between Angpt-2 serum levels and the age at the onset of symptoms of AS, as well as a marginally significant association with disease duration [99].

It has been reported that Angpt-2 promotes the proinflammatory activation of human macrophages in RA synovial tissue and that the neutralization of this molecule in an *in vivo* model of RA decreased disease severity, inflammation, neovascularisation, and joint destruction [100]. In line with this observation, we disclosed that a single infusion of anti-TNF-α infliximab was associated with a dramatic reduction of Angpt-2 serum levels [99], possibly as part complex mechanisms leading to a reduction of the risk of CV events associated with this intervention in patients with chronic inflammatory diseases [101].

4.3. Osteopontin (OPN). Osteopontin (OPN) is another biomarker of atherosclerosis, synthesized by osteoclasts, osteoblasts, chondrocytes, and by cells of the immune system [102, 103]. This protein has pleiotropic functions such as cellular adhesion, migration, angiogenesis, and inflammation [102, 104]. High OPN levels have been proposed to promote the development of atherosclerotic lesions [105] and atherosclerotic plaque rupture, acting as a chemotactic factor for inflammatory cells and leading thus to plaque rupture [106].

In a previous study performed by Choi et al., OPN levels were increased in AS when compared to healthy controls [102]. However, we did not observe significant differences in the levels of OPN between AS patients undergoing anti-TNF-α therapy and controls [107]. These different results may be due to the long-term treatment with anti-TNF-α therapy that our AS patients had received at the time of the study. It is possible that prolonged anti-TNF-α blockade may lead to reduction of the inflammatory burden and, therefore, to a reduction of OPN concentrations. In this regard, OPN levels observed in our cohort of AS patients and in the healthy matched controls were very similar to those observed in the control group reported by Choi et al. [102].

Interestingly, in our series of AS patients we found a positive correlation between serum levels of OPN and Angpt-2 [107]. Since, as described in the previous section, Angpt-2 is a marker of endothelial cell activation involved in angiogenesis, making the endothelium responsive to inflammatory cytokines [97], while OPN is also involved in the development of atherosclerotic disease, this result reinforces the idea of the potential use of OPN and Angpt-2 as biomarkers to predict CV risk in patients with AS.

As previously mentioned, a single infusion of the anti-TNF-α monoclonal antibody infliximab led to a significant reduction in Angpt-2 serum levels in our series of AS patients [99]. Likewise, a single infusion of infliximab also triggered a decrease in OPN serum levels in our cohort of AS patients [107]. This is in accordance with the proinflammatory role proposed for these biomarkers of endothelial cell activation and atherosclerosis and the beneficial effect against the development of CV disease mediated by the use of anti-TNF-α therapy.

4.4. Gelsolin (GSN). Gelsolin (GSN) is an anti-inflammatory protein mainly secreted by muscle cells, that is, involved in cytoskeleton reorganization [108]. GSN acts by binding to actin filaments (and probably to other extracellular matrix components) and thus prevents the activation of downstream inflammatory pathways by these filaments [109]. Reduced GSN levels have been reported in situations of acute injury or inflammation [109–111]. In this regard, it was proposed that in inflammatory diseases such as RA, circulating GSN may be potentially locally consumed by the interaction with macromolecules such as actin, fibrin, and fibronectin at the joints or other affected organs, leading thus to a reduction of the levels of GSN [109]. In keeping with these findings we observed lower levels of GSN in AS patients undergoing anti-TNF-α therapy when compared to healthy controls [112]. These data support the potential function of GSN as an anti-inflammatory molecule.

As observed for other biomarkers and adipokines, a single infusion of anti-TNF-α infliximab did not produce any significant change on GSN serum levels in our AS patients [112]. As pointed out before, a possible explanation for this steady level of GSN might be the low disease activity of our patients, since they had been receiving infliximab for a long period of time.

4.5. Osteoprotegerin (OPG). Osteoprotegerin (OPG) is a member of the TNF receptor superfamily, that is, implicated both in osteoporosis and in the atherosclerotic process. OPG acts as a decoy receptor for the receptor activator of nuclear factor-κB ligand (RANKL), inhibiting binding of RANKL to its receptor, RANK [113, 114]. OPG also acts as a soluble neutralizing receptor of TNF-related apoptosis-inducing ligand (TRAIL), an anti-inflammatory molecule with antiatherosclerotic properties [115–117]. Furthermore, it has been reported that OPG can upregulate the production of endothelial adhesion molecules [118].

OPG has previously been associated with increased risk of atherosclerotic disease in the general population [119]. Interestingly, a recent study of our group on patients with RA undergoing infliximab therapy disclosed that OPG concentrations were associated with biomarkers of endothelial activation (intercellular adhesion molecule-1), carotid intima-media wall thickness, and carotid plaques [120]. In keeping with these results, in our series of AS patients undergoing infliximab therapy we have disclosed an independent correlation with ADMA, another biomarker of endothelial cell activation [121]. This further supports the role of OPG as a valuable CV disease risk biomarker in chronic inflammatory rheumatic diseases such as AS.

Although a significant reduction of OPG levels upon infusion of this biologic agent was recently reported in long-standing RA patients with severe disease undergoing anti-TNF-α therapy [120], a single administration of anti-TNF-α infliximab did not lead to any significant reduction of

OPG levels in our series of AS patients [121]. The low disease activity and low inflammatory burden observed at the time of the study in our series of AS patients could probably explain the lack of significant reduction of OPG levels following administration of anti-TNF-α.

5. Therapeutic Potential Applications of These Biomarkers and Adipokines

Constantly, a progressively increasing list of new potential biomarkers and adipokines comes out, being subject to evaluation for their involvement in CV disease or MeS. However, as wisely suggested by other authors, these molecules exert such complex physiological effects that their use as potential therapeutic molecules must be carefully planned to obtain the adequate result and to avoid unknown side effects [5, 40].

Another point of potential interest is the possible use of these molecules as biomarkers of CV disease and predictors of increased risk for CV events or MeS in patients with diseases like AS that are associated with increased risk of CV death. However, up to now the routine use of these biomarkers in the daily clinical practice is still far from being well established. It is applicable not only to individuals with chronic inflammatory rheumatic diseases like AS or RA but also to the general population.

6. Conclusions

The assessment of adipokines and biomarkers of endothelial cell activation and MeS may be of potential interest for the improvement of the stratification of the CV risk of patients with AS. However, further studies are still needed to fully elucidate the clinical implication of these molecules in the mechanisms leading to accelerated atherosclerosis in AS and the benefits of the assessment of these molecules in the daily clinical practice.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Attenuation of Collagen-Induced Arthritis in Rat by Nicotinic Alpha7 Receptor Partial Agonist GTS-21

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This research was performed to observe the effect of GTS-21 on Collagen Induced Arthritis (CIA). CIA model was used and after the onset of arthritis, the rats were divided into three groups based on their clinical symptoms score. Two groups were intraperitoneally (IP) injected daily with GTS-21 (1 mg/kg, 2.5 mg/kg) for a week, whereas phosphate buffered saline (PBS) was used for the control group. Cytokine titers, radiological, and histological examinations were performed at different time points after treatment with GTS-21. Compared with those of the control, the levels of TNF-α, IL-1, and IL-6 in the serum were significantly reduced after GTS-21 management. In addition, radiological results show that bone degradation was inhibited as well. Moreover, the hematoxylin and eosin (H&E) staining indicated that the histological score was significantly alleviated in the therapeutic group. Tartrate-resistant acid phosphatase (TRAP) stain-positive cells were also detected in the destruction of the articular cartilage, which was significantly reduced compared with the control group. This study provides the first evidence on the effect of GTS-21 as a potential treatment for RA.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder of unknown etiology that mainly targets diarthrodial joints, synovial membrane first and then cartilage, ligaments, and subchondral bone [1]. The impact of RA is very important in terms of articular pain [2], patients' functional disability [3], and survival [4, 5], in particular when considering the occurrence of extraarticular manifestations [6, 7]. It has been reported that pro- and anti-inflammatory cytokines derived predominantly from cells of the macrophage lineage have a major role in the initiation and perpetuation of the chronic inflammatory process in the RA synovial membrane [8]. Therefore, several treatment methods for RA aim to block certain cytokines noted to be essential in the development of RA [9–11]. However, none of these methods can cure this disease at the current stage.

In the last decades, the treatment of RA is deeply changed; now it is well established that early treatment is mandatory in order to improve patients' prognosis and reach disease remission, which is the main goal that clinicians should now achieve [12–14]. The availability of new effective drugs that are able to modulate the inflammatory cascade of RA is another factor that contributes to this "change of perspective" in RA treatment; these drugs are generally known as bio(techno)logical agents and may target TNF [15], CD20+ cells [16], IL-6 [17], and T-cells costimulation [18]. The literature about these drugs is steadily increasing, in terms not only of effectiveness and safety profile description [19, 20], but also of responsiveness prediction [21, 22]. Despite the progresses, the number of unsatisfied needs in RA treatment still remains high, as the large number of biological agents under development suggests [23]. Therefore, new therapeutic methods for RA treatment are of great importance.

The nervous system has been demonstrated to be an important regulator of the immune system, and neuronal anti-inflammatory mechanisms have been selected by evolution to modulate inflammatory responses [24, 25]. These

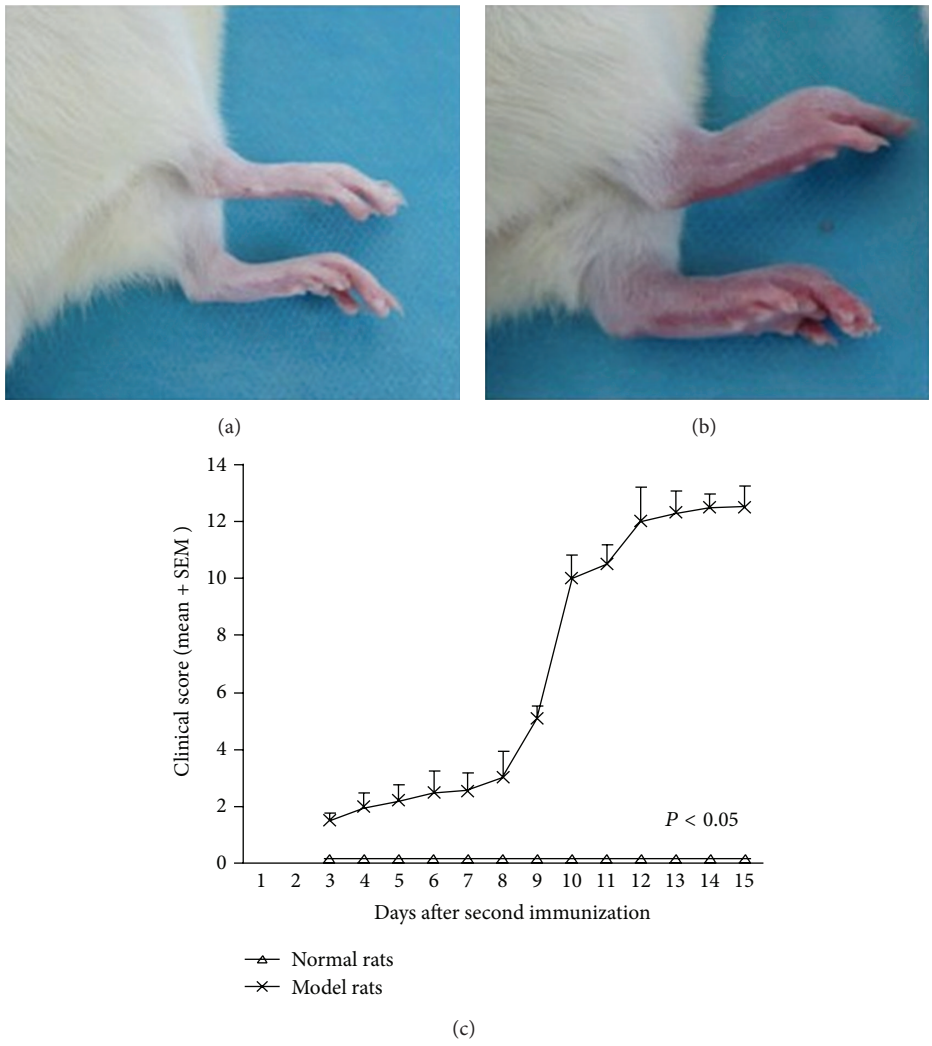

Figure 1: Macroscopic observation of joint swelling in rats with CIA. Arthritis was induced in 30 rats by treatment with type II collagen (5 normal rats as control), and the disease was scored clinically 3 times per week by the same person. (a) Normal rats, (b) arthritis onset of CIA model rats, and (c) the severity of arthritis clinical scoring. Differences between the control group and the model group were statistically significant ($P < 0.05$).

mechanisms can provide a major advantage for novel pharmacological anti-inflammatory strategies that control systemic inflammation [26]. Recent research indicated that acetylcholine, the principal neurotransmitter of the vagus nerve, is a key mediator of this cholinergic anti-inflammatory pathway. The neuronal nicotinic acetylcholine receptors (nAChRs) are named based on their subunit components, in which nicotinic α-7 acetylcholine receptor (α7nAChR) is a subunit of nAChRs [27]. The α7nAChR has been considered important for immune regulation in the absence of nerves; however, little is known about its therapeutic role in chronic joint inflammation. Interestingly, overexpressed α7nAChR in synovial biopsies from patients with RA may be a target in RA therapy [28].

Based on the above mentioned selective pharmacological stimulation of α7nAChR, it may have therapeutic potential for the treatment of inflammatory conditions. Consequently, more specific agonists of this receptor have been identified or developed and used in various studies. To date, one of the most effective α7 selective partial agonists for modulating inflammatory responses is GTS-21, which has been proven effective in attenuating the immune response and improving the outcome in animal models of pancreatitis [29], endotoxemia, sepsis [30], acute lung injury, and ischemia reperfusion injury [31–33]. GTS-21 has also been proven effective as an immunomodulatory drug that attenuates pro-inflammatory cytokine levels and improves survival in sepsis models [34], decreases severity in pancreatitis, and attenuates endotoxin-induced tumor necrosis factor (TNF) in lung tissue [31, 35].

However, no research was reported to identify the therapeutic effect of GTS-21 on RA. This study hypothesizes that α7nAChR provides a link between the neurologic system and the inflammatory process in the inflamed joint and that treatment with specific activators (GTS-21) of this receptor would reduce joint inflammation. For this purpose, we used a strain of rats susceptible to CIA, a widely used experimental

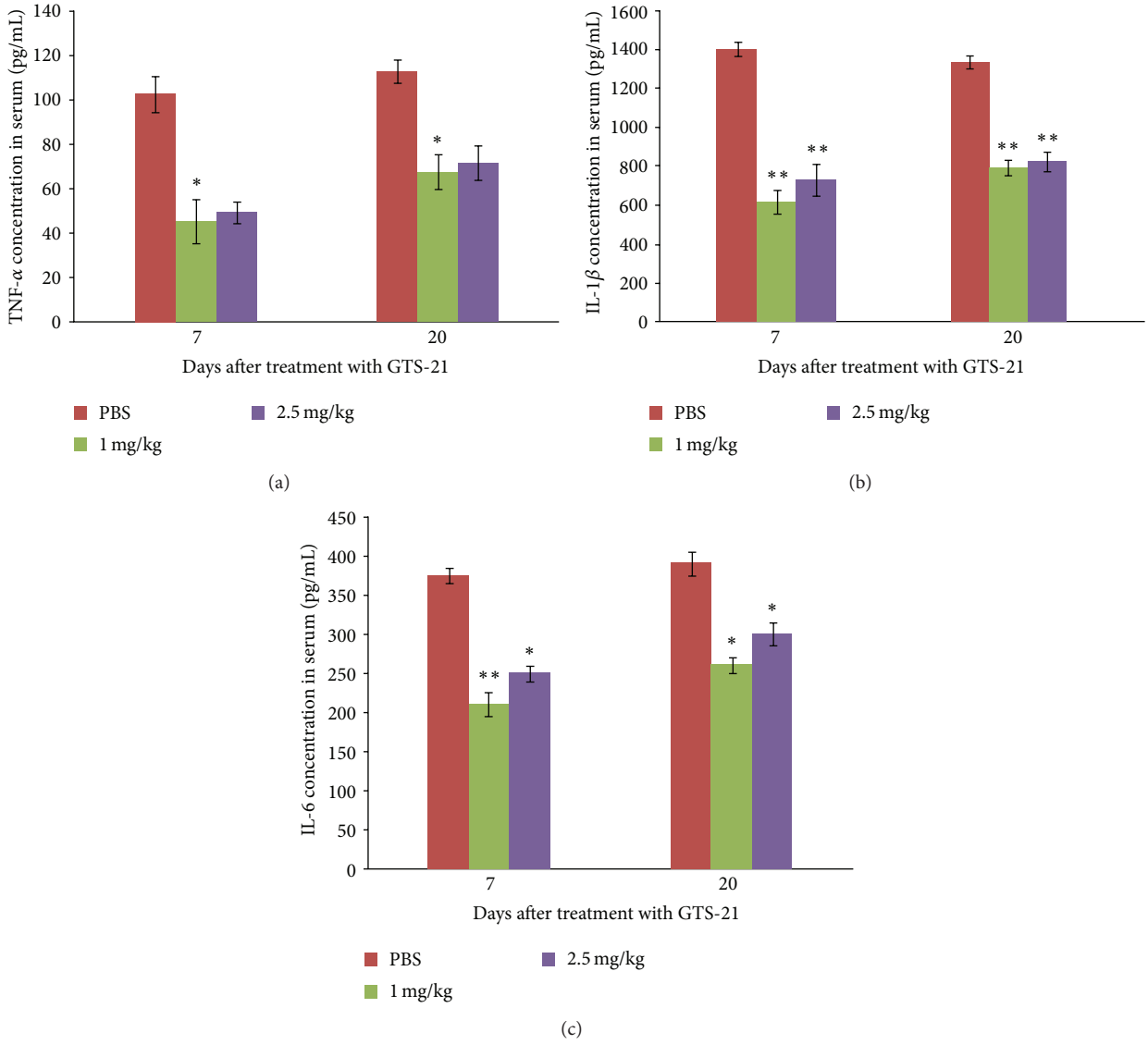

Figure 2: ELISA observation of inflammation-related cytokines. Serum concentrations of TNF-α, IL-1, and IL-6 are tested 7 and 20 days after treatment with GTS-21. (a) TNF-α, (b) IL-1, and (c) IL-6. $^{*}P < 0.05$ $^{**}P < 0.01$ versus treatment by PBS group.

model of RA, as it shares many histological and immunological features with this disease, such as pannus formation, bone and cartilage destruction, and synovitis as well [36]. In detail, CIA animal model in rat via intraperitoneally (IP) injecting the highly selective α7nAChR agonist GTS-21 was performed and the result was observed and analysed.

2. Materials and Methods

2.1. Animals. A total of 35 male Wistar rats (8 to 10 weeks of age) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The animals were housed under special pathogen-free conditions at the animal facility of the Shen Zhen Institute of Advanced Technology, Chinese Academy of Science. The Institutional Animal Care and Use Committee of the Shen Zhen Institute of Advanced Technology, Chinese Academy of Science approved all of the experiments.

2.2. Inducing CIA in Rats. Arthritis was induced in 30 rats by treatment with type II collagen (5 normal rats as control); CIA was induced using a modified method previously described by Trentham et al. [37]. In brief, bovine collagen-II ((CII) Chondrex, 2002, USA, dissolved in 0.05 M acetic acid) was emulsified with an equal volume of incomplete Freund's adjuvant ((IFA) Chondrex, 7002, USA). This CII-IFA emulsified liquid was administered as a 0.2 mL intradermal injection at the dorsum of each rat's tail, approximately 2 cm distal from the base. At 10 days after the first immunization, each rat received 0.1 mL of CII-IFA booster via intradermal injection

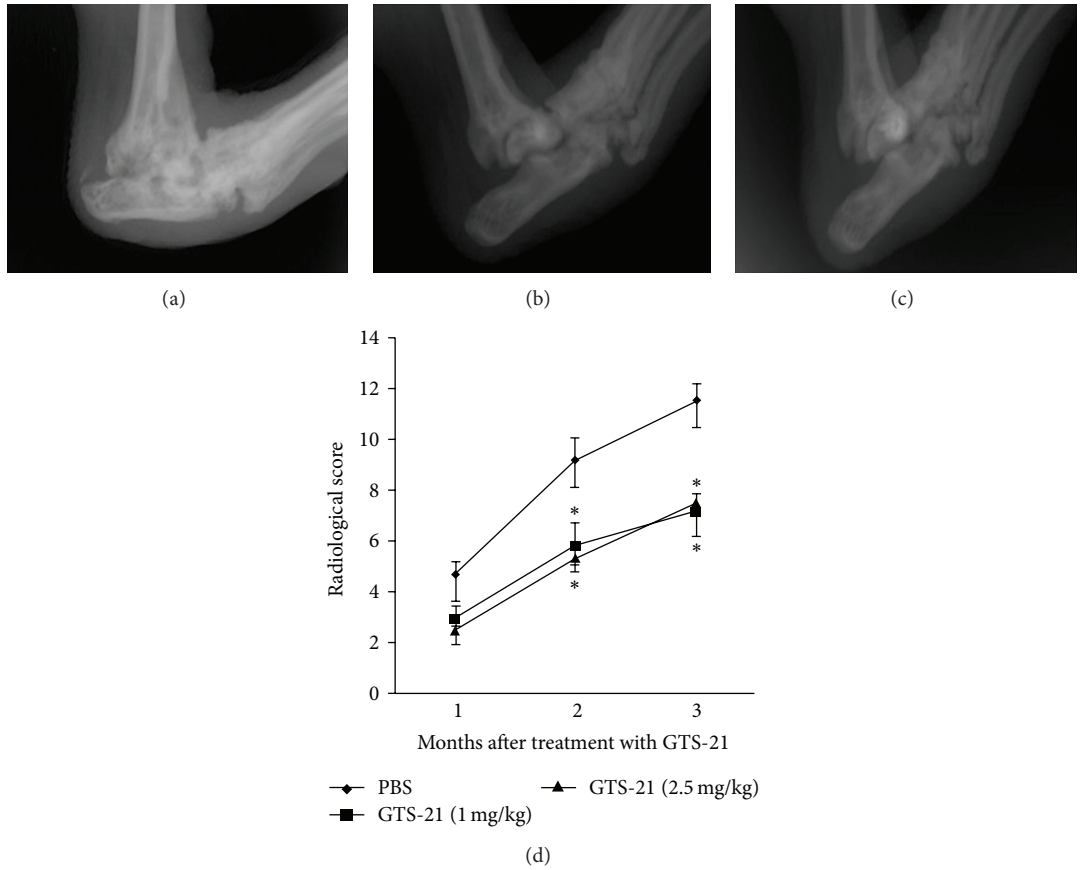

Figure 3: Inhibition of bone degradation in the joint by GTS-21 treatment. (a) Control group treated with PBS; (b) group treated with GTS-21 concentration of 1 mg/kg; (c) group treated with GTS-21 concentration of 2.5 mg/kg; (d) semiquantitative scoring of joint destruction. $^{*}P < 0.05$ versus PBS control group. Data represent mean ± standard errors of the mean and are representative of eight rats per group.

on the tail's ventral side. For arthritis assessment, all rats were monitored three times a week by the same person blinded to the treatment group, and the incidence of arthritis and clinical score were evaluated.

The severity of arthritis was assessed using an established semiquantitative scoring system of 0–4, where 0 = normal, 1 = swelling in 1 joint, 2 = swelling in >1 joint, 3 = swelling in the entire paw, and 4 = deformity and/or ankylosis [38]. The cumulative score for all four paws of each rat (maximum possible score of 16) was used to represent the overall disease severity and progression.

2.3. In Vivo Administration of GTS-21. To explore the effects of GTS-21 on the CIA rat model, GTS-21 was dissolved in PBS. 15 days after the second immunization, 24 of 30 induced rats showed symptoms of RA and they were divided into three groups, with each group comprising eight rats. The severity of arthritis score in these three groups was the same (each group score: 12) and treatment by drug that day after they were grouped, in which the two groups were receiving a once daily (IP) injection of GTS-21 (1.0 mg/Kg and 2.5 mg/Kg) for a week, whereas the other group was treated by PBS using the same method for a week.

2.4. Enzyme-Linked Immunosorbent Assay (ELISA). TNF-α, IL-1, and IL-6 levels in the serum were determined after treatment with GTS-21 (7 and 20 days after the treatment), using a commercially available ELISA kits, according to the recommendations of the manufacturer (Neobioscience Technology Co., Ltd.).

2.5. Radiological Analysis. 1, 2, and 3 months after being treated with GTS-21, radiographic scoring criteria (28 kv, 12 s, USA Fixitron X-ray) were assessed according to the method reported by Lin et al. [39]: 0 is normal intact bony outlines and normal joint space; 1 is slight abnormality with one or two exterior metatarsal bones showing slight bone erosion; 2 is definite early abnormality with bone erosion in three to five exterior metatarsal bones; 3 is medium destructive abnormality of all exterior metatarsal bones, as well as one to two interior metatarsal bones showing definite bone erosions; 4 is severe destructive abnormality of all the metatarsal bones showing definite bone erosion and at least one of the inner metatarsal joints completely eroded, leaving bony joint outlines partly preserved; and 5 is mutilated abnormality with the absence of decipherable bony outlines. All parameters were scored by at least two observers in a blind test manner.

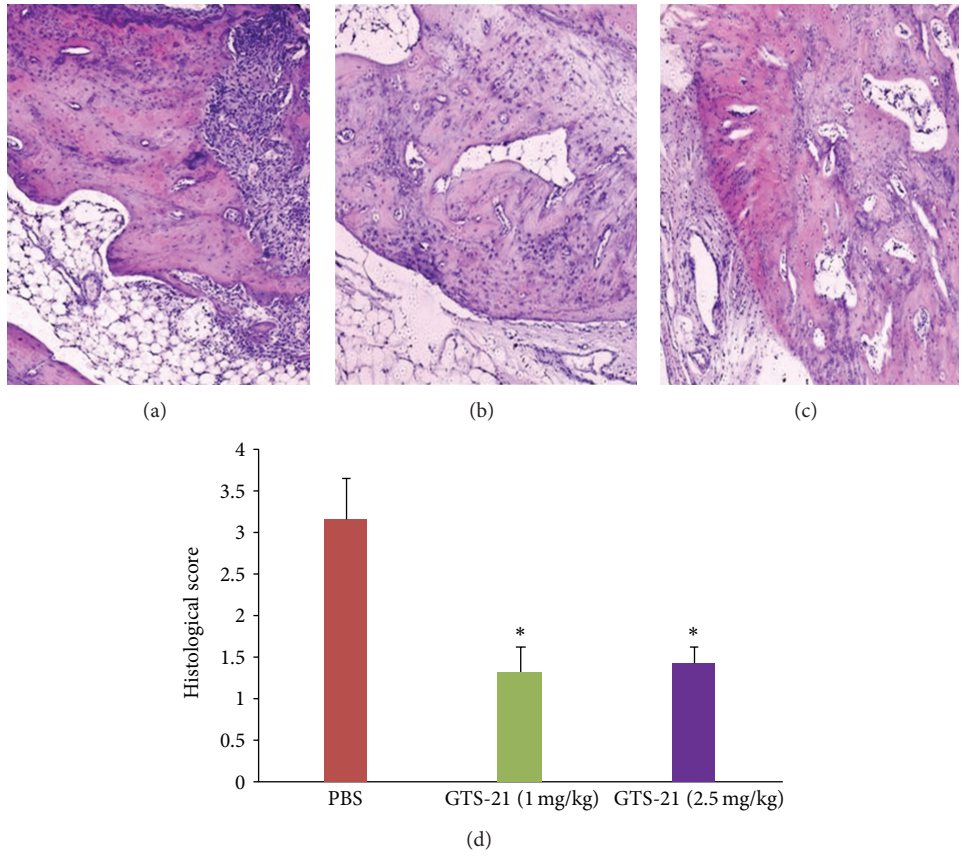

Figure 4: Histological observation of joint inflammation cell and bone damage. Tissue sections were stained with HE to study inflammatory cell influx and bone destruction (original magnification ×100). (a) Control group treated with PBS; (b) group treated with GTS-21 concentration of 1 mg/kg; (c) the group treated by GTS-21 concentration of 2.5 mg/kg; (d) histological score analysis of these three groups. Date was determined according to the scale described in Section 2. $^{*}P < 0.05$ versus PBS control group. Data represent mean + standard errors of the mean and are representative of eight rats per group.

2.6. Histological Analysis. 3 months after the treatment, the rats were sacrificed and hind paws were fixed in 4.0% formalin for 12 hours and then decalcified in 10% EDTA (Sigma) for 20 days at room temperature. The serial paraffin sections (5 μm) of the hind paws were stained with hematoxylin and eosin (HE) for assessment of synovial inflammation and bone erosions, with a leukocyte acid phosphatase staining kit (Sigma) for tartrate-resistant acid phosphatase (TRAP) to detect osteoclasts. All detailed processes were performed according to the recommendations of the manufacturer (Nanjing Jancheng Technology co., Ltd.).

To compare the histological differences among different foot joints, HE sections of different joints were evaluated using the following scale [40]: 0 is normal synovium; 1 is synovial membrane hypertrophy and cell infiltrates; 2 is pannus and cartilage erosions; 3 is major erosions of cartilage and subchondral bone; and 4 is loss of joint integrity and ankylosis. The assessment was performed by two independent investigators who were blinded to the identity of the specimens, and the average of the two scores was obtained. The sample size of each group was 16. TRAP-positive cells found in sections of the knee joints were collected. Specifically, collection of TRAP-positive cells found in six different microscopic fields per section were collected. Osteoclasts were quantified according to the following scores: 0 = normal (no osteoclasts), 1 = presence of a few osteoclasts (lining fewer than 5% of most affected bone surfaces), 2 = some osteoclasts (lining 5–25% of most affected bone surfaces), 3 = many osteoclasts (lining 30–50% of most affected bone surfaces), and 4 = abundant osteoclasts (lining >50% of most affected bone surfaces) [41].

2.7. Statistical Analysis. To evaluate the effects of different treatments, we determined the change in clinical arthritis scores in each mouse from the start of the treatment until the end of the experiment. A nonparametric test (Kruskal-Wallis test) was used to analyze the score data, including radiological and histological scores. The statistical significance level was set at a P value of 0.05 and 0.01. SPSS 17.0 was used for all experiments.

3. Results

3.1. CIA Model. The macroscopic observation of joint swelling is shown in Figure 1. Joint swelling in the CIA model rats (Figure 1(b)) was significantly higher than that

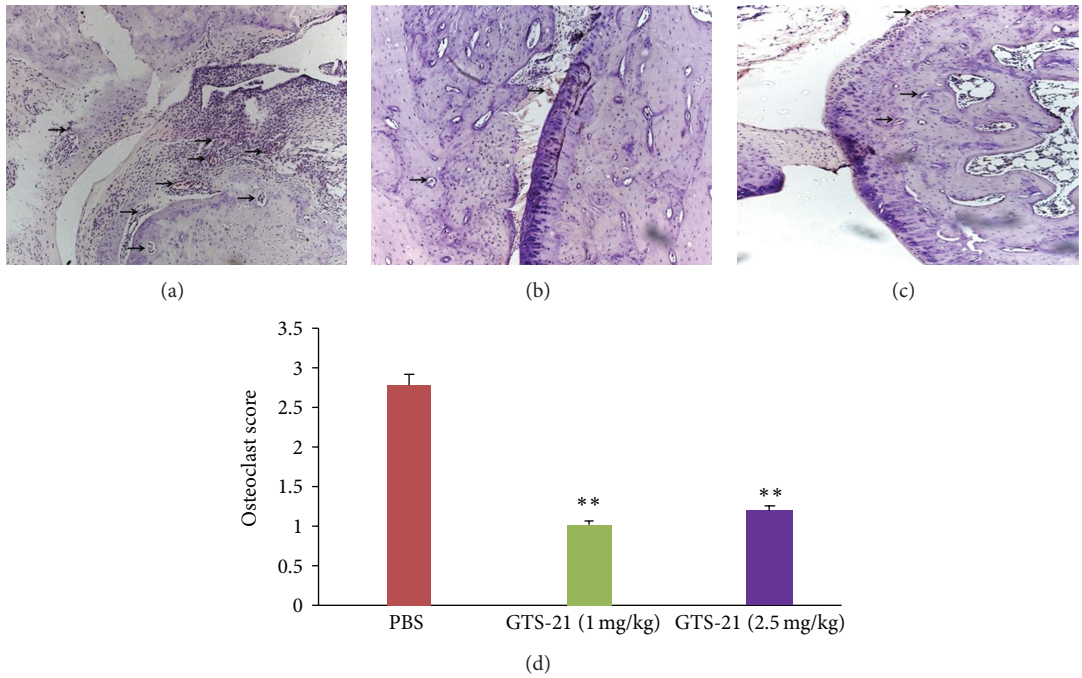

Figure 5: Osteoclast observation (the blank arrows). Tissue sections were stained with TRAP and restained with hematoxylin (original magnification ×100). (a) Control group treated with PBS; (b) group treated with GTS-21 concentration of 1 mg/kg; (c) group treated with GTS-21 concentration of 2.5 mg/kg; (d) statistical data of osteoclasts score in the knee joints of three different groups. $^{**}P < 0.01$ versus PBS control group. Data represent mean + standard errors of the mean and are representative of eight rats per group.

of the normal rats (Figure 1(a)). The severity of arthritis was assessed using an established semiquantitative scoring system, and the results are shown in Figure 1(c).

3.2. Cytokine Level in Peripheral Blood Serum. After treatment with GTS-21 and PBS (7 and 20 days), the serum concentrations of TNF-α, IL-1, and IL-6 were tested using ELISA. The results showed that the cytokine levels in the treatment groups were significantly lower than those in the control group, as shown in Figures 2(a), 2(b), and 2(c).

3.3. Radiological Observation. Radiographs of the knee and foot joints were evaluated to investigate the effects of GTS-21 on bone degradation. The X-ray results were shown in Figures 3(a), 3(b), and 3(c). In the third month after treatment, in the PBS treated group normal joints in the knees and toes could barely be seen, but GTS-21 treated group significantly improved on joint destruction, whereas the semiquantitative scoring of joint destruction was shown in Figure 3(d). All the dates indicated that joint destruction was significantly reduced in the group treated with GTS-21.

3.4. Histological Analysis. Synovial inflammation and joint erosions were assessed by HE staining of ankle joint specimens, as shown in Figures 4(a), 4(b), and 4(c). Histologic scoring revealed a significant reduction of inflammatory cell infiltration in rats treated with GTS-21 compared with the control group (Figure 4(d)). In the knee joint, many TRAP stain-positive cells adhered to the eroded surface of the cartilage, which directly contributed to the erosion of such cartilage (Figures 5(a), 5(b), and 5(c)). Meanwhile, subchondral side erosion severity and pannus abundant were observed in the PBS treatment group. Moreover, it destroyed the joint from outside the cartilage. The score of osteoclasts in the knee joint of the treatment groups was significantly lower than that of the control group (Figure 5(d)). By contrast, no difference was observed between two different drug concentrations in the treatment groups with regard to histological and radiological scoring.

4. Discussion

Studies have indicated that α7nAChR is important for immune regulation [42]. Specific stimulation of α7nAChR on monocytes leads to efficient suppression of pro-inflammatory cytokine production. This receptor is essential for the efficient cytokine regulation in neuroimmune mechanisms known as the cholinergic anti-inflammatory pathway [43, 44]. RA is a chronic, inflammatory autoimmune disease of unknown cause and may be related to several signaling pathways. M. Westman et al. [28] reported the strong expression of α7nAChR in synovium of RA patients. These results indicated the importance of α7nAChR and cholinergic mechanisms in arthritis pathogenesis and implicated specific cholinergic modulation as a potential anti-inflammatory therapeutic strategy in joint inflammation.

GTS-21 is a derivative of the natural product anabaseine which is an effective portion of α7nAChR agonists. Moreover,

GTS-21 is a characteristic α7nAChR-agonist that has been used in clinical trials and has been proven to be less toxic than nicotine [45, 46]. It has been reported that GTS-21 has been used in clinical trials to target neuronal α7nAChR in the brain of patients with Alzheimer's disease [47], since the cholinergic anti-inflammatory pathway is activated by stimulating the α7nAChR [33]. Meanwhile, the high expression of α7nAChR in the synovium of RA patients is a potential target of RA treatment and offers the possibility of GTS-21 as RA therapeutic drug.

In the present study, based on the newly discovered cholinergic anti-inflammatory pathway, we proposed a new method for the treatment of CIA using GTS-21. In our study, 7 and 20 days after treatment with GTS-21, those cytokines were significantly reduced relative to the control group. Based on observation of the inflammatory cell in the HE stained sections, the inflammatory environment was significantly improved in the treatment group. Furthermore, the treatment group had arthritis-related inflammatory cytokines that were much lower than those of the control group, which displayed significant inhibitory effects.

Bone and cartilage destruction are among the main symptoms of RA. We observed the destruction of knee joint at different times by radiology and measurement scores. As the disease developed, inhibitory effects were observed in the treatment group, opposite that of the control group, in which the joint was destroyed more seriously. This phenomenon was also observed in foot joints (results not shown). Osteoclast related erosion is one of the main factors of bone destruction in arthritis. In our observation of the osteoclast score, we found that the score of osteoclasts in the joints of the treatment group was lower than that of the control group. Many inflammatory cytokines, such as TNF-α, have already been proven to have important roles in osteoclast [48, 49]. Hence, the reduction of the secretion of cytokines in the treatment group could reduce the number of osteoclast, thereby reducing bone damage. Accordingly, we observed consistent results in our study, in which joint destruction was reduced in the treatments groups compared with the control group.

In summary, the results of the present study showed the anti-inflammatory effects of GTS-21 as a partial agonist of α7nAChR. GTS-21 may have a new function on the treatment of RA. However, our results showed that drug concentration has no obvious correlation with the effect of treatment. This observation may be attributed to the fact that the difference between the two concentrations of this study was not large enough. Using these questions, more in-depth study is needed in our next work. As the therapeutic value of GTS-21 is selective, this agonist may be a suitable candidate for development as a novel approach to RA treatment.

Conflict of Interests

The authors declare that there is no conflict of interests in this paper.

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