Changes of anti-citrullinated peptide antibodies titres after biologic treatment in patients with rheumatoid arthritis: a systematic literature review and retrospective study

S. Hilliquin[1], J. Herrou[1], L. Gutermann[2], C. Goulvestre[3], J. Avouac[1], J. Henry[4], P. Hilliquin[5], M. Dougados[1,6], A. Moltó[1,6]

[1]Service de Rhumatologie, Université de Paris-Cité, Hôpital Cochin, Assistance Publique - Hôpitaux de Paris, CUP, Paris; [2]Department of Pharmacy, Hôpital Cochin, APHP Centre, Paris; [3]Department of Immunology, Hôpital Cochin, APHP Centre, Paris; [4]Department of Rheumatology, Bicetre Hospital, APHP Centre, Le Kremlin Bicêtre; [5]Department of Rheumatology, Centre Hospitalier Sud-Francilien, Corbeil-Essones; [6]INSERM (U1153): Clinical Epidemiology and Biostatistics, PRES Sorbonne Paris-Cité, Paris, France.

Abstract

Objective

There is an increasing body of evidence suggesting a direct pathophysiological role of anti-citrullinated peptide antibodies (ACPA) in rheumatoid arthritis (RA), and immunological remission could be a target for treatment. However, data related to the ability of biologics to reduce ACPA titres are contradictory. We aimed to evaluate the changes in ACPA titres after treatment with different biologics in patients with RA.

Methods

As a first step, a systematic review of the literature available on 3 biologics (TNFi, abatacept and rituximab) and ACPA in patients with RA was performed in Pubmed and Cochrane. As a second step, a retrospective study was performed: all RA patients treated with the 3 above-mentioned biologics were identified. To be included in the analysis, patients had to have at least two titres of ACPA (one before and one after biologic treatment) available. ACPA titres were compared before and after treatment in each of the treatment groups.

Results

As a result of the literature review, 24 articles were retained confirming that the data on change in ACPA under biologics is contradictory, particularly for abatacept and TNFi. 144 RA patients (79.3% female, mean age: 56 years) were included in the retrospective analysis: 59 patients had received rituximab, 31 abatacept, 55 TNFi. ACPA titres decreased significantly with rituximab but not with abatacept nor TNFi. Modelling of ACPA titres over follow-up confirmed the significant decrease of ACPA over time rituximab.

Conclusion

In this real-life study, ACPA titres only significantly decreased after treatment with rituximab.

Key words

anti-citrullinated peptide antibodies, rheumatoid arthritis, rituximab, abatacept, TNF inhibitors

Stéphane Hilliquin, MD
Julia Herrou, MD
Loriane Gutermann, PharmD
Claire Goulvestre, MD
Jérôme Avouac, MD, PhD
Julien Henry, MD
Pascal Hilliquin, MD
Maxime Dougados, MD, PhD
Anna Moltó, MD, PhD

Please address correspondence to:
Stéphane Hilliquin
Service de Rhumatologie,
Hôpital Cochin,
27 rue du Faubourg Saint Jacques,
75014 Paris, France.
E-mail: stephane.hilliquin@gmail.com

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease, which can lead to joint destruction. One disease characteristics is the presence of anti-cyclic citrullinated peptide antibodies (ACPA) (1). ACPA play an important role on the diagnosis of RA at an early stage (2) and sensitivity and specificity of ACPA for the diagnosis are 66% and >90%, respectively (3). In this sense, in 2010, American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) decided to include ACPA in addition to rheumatoid factor (RF) as immunologic biomarkers (4) in the classification criteria to widen the spectrum of RA.

Citrullination is a post-translational conversion of the amino acid arginine to citrulline in a range of intra cellular and matrix proteins (fibronectin, collagen, fibrinogen, enolase and vimentin). ACPA are produced by B cells and plasma cells, with a specificity against citrullinated peptide.

RA development is determined by both a predisposing genotype upon which environmental (*e.g.* smoke and gingivitis, which induce citrullination) and genetic factors (*e.g.* presence of MHC class II) operate resulting in the inflammatory and destructive synovial response. Furthermore, a major gene-environment interaction was demonstrated between distinct HLA-DR variants and exposure to smoke and other agents such as silica dust, and this interaction was only specific for ACPA-positive subset of RA. These findings gave rise to the initial suggestion that anti-citrulline immunity may be involved in the onset of RA joint inflammation (5). ACPA could have a direct pathological role in development of RA, and it has been reported that transfer of ACPA in mice could induce pain-like behaviour, bone loss or synovitis/tenosynovitis on MRI (6, 7).

ACPA are also considered as a prognostic factors: it is well established that ACPA seropositivity is associated with disease severity, radiological progression and are considered poor prognosis factor with a predisposition to develop an erosive RA (5, 8-11). The presence of ACPA have also been associated with an increased likelihood of extra-articular manifestations, in particular RA-associated interstitial lung disease (8, 12).

Therefore, based on the hypothesis of a direct pathogenic role of ACPAs, one could hypothesise that a significant decrease or even disappearance of ACPA titres, particularly at beginning, might be important to prevent RA onset or at least to prevent/limit structural damage in established RA. Thus, one could propose to aim for an immunological remission (*e.g.* disappearance of ACPA titres), on the top of the clinical remission. However, the potential capacity of the different biologics to suppress ACPA does not seem to be clearly established yet.

Based on these remarks, we decided to: 1. conduct a systematic literature review to evaluate the available data on the effect of biologics on ACPA titres changes, and 2. to determine, with a retrospective real-life setting study, whether different biologics had a significant impact on changes in ACPA titres.

Methods

Systematic literature review

A systematic literature review was performed in Pubmed, and Cochrane Controlled Trials Register until September 2022 of articles published over the last 10 years. EULAR and ACR scientific meeting abstracts from 2015 to 2022 were also included. The followings key-words were used: "Anti-cyclic citrullinated peptide autoantibody" [all fields] AND "rheumatoid arthritis" AND "treatment" [all fields], or "Anti-cyclic citrullinated peptide autoantibody" [all fields] AND "rheumatoid arthritis" AND "therapy" [all fields].

The search was limited to English language studies and studies including adults only.

To be selected, studies had to meet the following:

1. Prospective or retrospective, observational or interventional ; reviews, case-reports and case-series were excluded.
2. Patients had to be diagnosed with RA according to ACR/EULAR criteria (2010) (4).
3. The study drug had to be a biologic

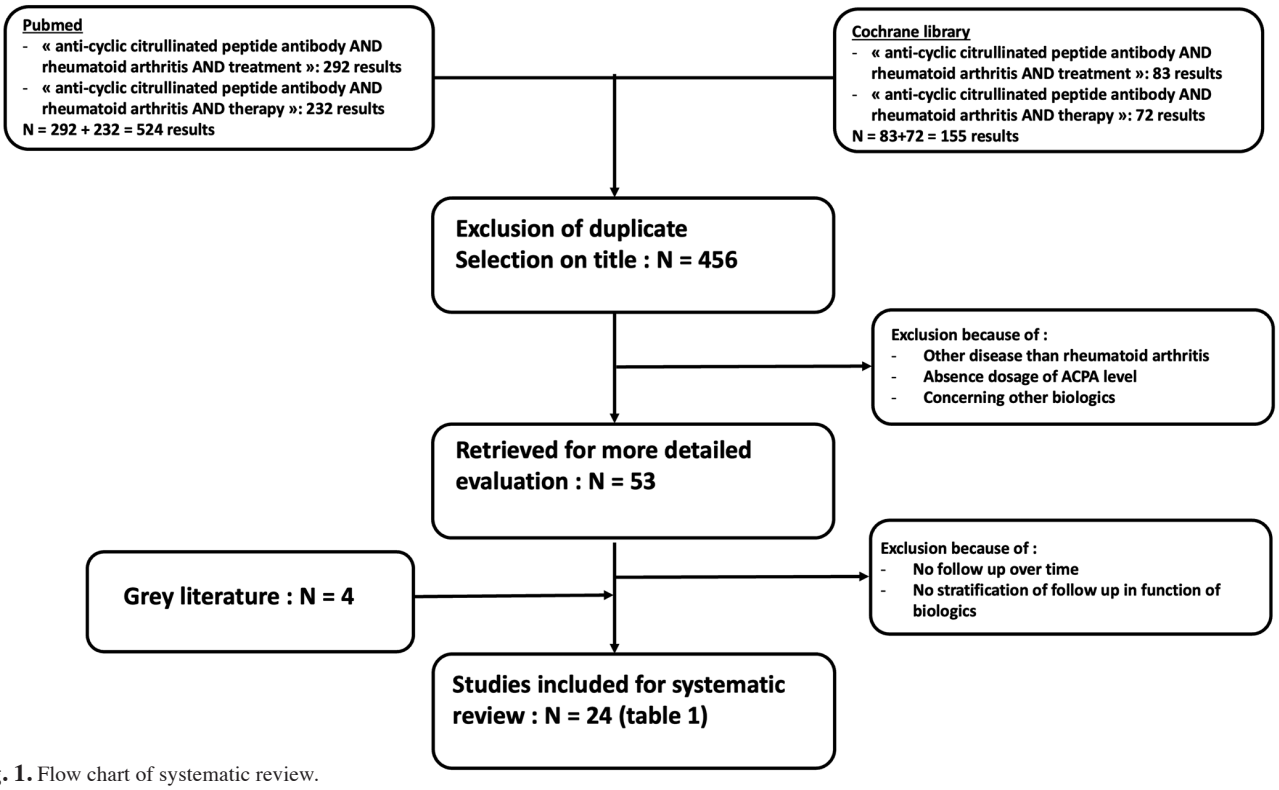

Fig. 1. Flow chart of systematic review.

therapy (TNF inhibitors, rituximab or abatacept).

4. Studies with an outcome measure of ACPA.

Studies were initially screened based on their titles and abstract, then selected based on the full text. Duplicate references were removed.

Study design

This was a retrospective, multi-centre study, conducted in three French Rheumatology Departments (Cochin hospital in Paris, Bicêtre hospital in Le Kremlin-Bicêtre and Sud Francilien hospital in Corbeil-Essones).

Patients

Patients were identified through the electronic medical records (EMR): to be included, patients had to be older than 18 years old, to have an RA diagnosis according ACR/EULAR criteria (2010) (4), and were followed for this disease in the department between 2005 and January 2018.

Afterwards, among the selected patients, those treated with biologics (rituximab, abatacept, and TNF inhibitors (TNFi) were identified by the pharmacy department (for intra-venous (iv) treatments) and the EMR (for subcutaneous (sc) treatments). To be included, patients should have received at least one dose of treatment: rituximab (iv, administered in a day-care hospital, at the dose of 500 mg or 1000 mg); abatacept (administered either in day-care hospital (iv dose of 500 mg, 750 mg or 1000 mg every 4 weeks) or at home, sc); two TNFi were selected, infliximab (in day-care hospital, 3 or 5 mg/kg every 8 weeks) and etanercept (sc at 50 mg per week at home).

Finally, among these patients, those with two available ACPA titre measurements (before -t1- and after -t2- treatment) performed in the hospital, were identified via the immunology department database. ACPA measurement was performed by ELISA test (2nd generation) in all 3 centres.

Data collection

The date of the biologic administration was retrieved from the pharmacy department files for the iv biologics and via the EMR for the sc biologics. The ACPA titres before and after treatment were retrieved from the Immunology department database.

Other clinical data were retrieved from the EMR: age, sex and disease activity (evaluated by DAS28) at the time of each available ACPA titre.

Statistical analysis

A descriptive analysis of the population according to the different biologics was performed (number and percentage and mean and standard deviation for categorical and continuous variables, respectively).

ACPA titres were compared before and after treatment in each of the treatment groups by Student t-test for paired data; as a sensitivity analysis, changes in ACPA over time were modelised by mixed models, including an interaction term between the drug and time. In case the interaction was significant, a subgroup (per drug) analysis was performed (mixed model with time*drug interaction term).

A secondary analysis was performed to determine whether ACPA titre changes over time could be explained by other parameters besides the drug: changes in disease activity (*i.e.* DAS28) over time, age or gender. For this, ACPA titre over time were modelled (mixed models) including in the model the parameter of interest as an interaction term with time.

Table I. Systematic review of the literature.

Study	n of patients	Treatment	Study length	Effect on ACPA titres
Cambridge *et al*. *Arthritis Rheum* 2003; 48: 2146-54. (29)	22	Rituximab ± cyclophosphamide and/or GC	28 weeks	Responders: decrease from 950 to 236 (p=0.008). No decrease in no responder group.
Caramaschi *et al*. *Rheumatol Int* 2005; 26: 58-62 (20)	27	Infliximab 3mg/kg ± DMARD ± GC	22 weeks	No decrease of ACPA titres (in responder and no responder group).
De Rycke *et al*. *Ann Rheum Dis* 2005; 64: 299-302. (23)	62	Infliximab 3mg/kg W0, 2, 6 then at W8 ± DMARDS	30 weeks	No decrease of ACPA titres
Kolarz *et al*. *Rheumatol Int* 2011; 31: 1439-43. (21)	32	Infliximab 3 mg/kg W0, 2, 6 then at W8 ± DMARDS	6 months	No decrease of ACPA titres
Alessandri *et al*. *Ann Rheum Dis* 2004; 63: 1218-21. (22)	43	Infliximab 3 mg/kg W0, 2, 6 then at W8 ± DMARDS	24 weeks	Decrease of ACPA titres in all patients treated (greater in the responder group).
Vaz *et al*. *Isr Med Assoc J* 2014; 16: 17-9. (25)	139	Infliximab ± DMARDS	12 months	ACPA titres decrease in 65.3% of patients and increase in 34.7 % of patients.
Bruns *et al*. *Jt Bone Spine Rev Rhum* 2009; 76: 248-53. (19)	36	Infliximab 3mg/kg W0, 2, 6 then at W8 ± DMARDS	48 weeks	Non-significant decrease (p=0.053) of ACPA titres in all patients treated (greater in the responder group).
Bobbio-Pallavicini *et al*. *Arthritis Res Ther* 2004; 6: R264-72. (17)	39	Infliximab 3mg/kg W0, 2, 6 then at W8 ± GC	78 weeks	Non-significant decrease of ACPA titres
Atzeni *et al*. *Arthritis Res Ther* 2006; 8: R3. (18)	112	Adalimumab 40/14 days + Methotrexate	48 weeks	Significant decrease (p<0,001) of ACPA titres (greater in good responder group).
Bos *et al*. *J Rheumatol* 2008; 35: 1972-7. (15)	188	Adalimumab ± DMARD ± GC	28 weeks	No decrease of ACPA titres in every group (all, no response, moderate, and good response).
Chen *et al*. *Ann Rheum Dis* 2006; 65: 35-9. (16)	90	Etanercept 25 mg twice weekly for three months	3 months	Significant decrease of ACPA titres from 60 U/mL to 49 U/ML (p=0,007)
Onishi *et al*. *Mod Rheumatol* 2010; 20(5): 528-30. (35)	107	Etanercept twice a week + MTX	24 weeks	Significant decrease of ACPA titres
Connoly *et al*. *Ann Rheum Dis* 2014; 73: 395. (24)	251 in ADA group 257 in ABA group	Adalimumab; Abatacept	729 days	Reduction in ACPA were observed in two groups, with a greater reduction in ABA group.
Alemao *et al*. ACR abstract 2015. (14)	27 in ABA group 33 in TNFi group	Abatacept TNF inhibitor	2 years	Decrease of ACPA titre (median -40,5) in ABA No decrease of ACPA titres in TNFi
Sokolove J. *et al*. *Ann Rheum Dis* 2016; 75: 709-14. (32) Jabado O. *et al*. *Rheumatol Ther* 2022; 9(2): 391-409. (33)	328 in ADA group 318 in ABA group	Abatacept 125 mg Adalimumab 40 mg biweekly	2 years	Decrease of ACPA titres (anti-CCP2) only in abatacept group, with high levels of ACPA titres at inclusion (Q4)
Mariette X. *et al*. *Joint Bone Spine* 2019; 86: 753-9. (31)	390	Abatacept	2 years	No decrease of ACPA titres in abatacept group
Emery *et al*. *Ann Rheum Dis* 2010; 69: 510-6. (30)	50	Abatacept, 10 mg/kg ± GC	1 year	Non-significant decrease of ACPA titres (-6,5 U/mL at 1 year).
Endo *et al*. *Scand J Rheumatol* 2020; 49(1): 13-17. (36)	71 in ABA group 78 in TNFi group	Abatacept, TNFi	3 months	Significant decrease of ACPA titres only in abatacept group (not TNFi), associated with a persistence and sustained therapeutic response
Dinis V. *et al*. *Clin Rheumatol* 2020; 39(6): 1747-55. (28)	18 in ABA group 18 in TNFi group	Abatacept, TNFi	Every 6 months, up to 24 months	Significant decrease of ACPA titres only in abatacept group (not TNFi) at all time points (6, 12, 18 and 24 months
Yamada H. *et al*. *Immunol Med* 2020; 43(2): 87-91. (34)	60 in ABA group		12, 24 and 52 weeks	No decrease of ACPA titres in abatacept group and no correlation to disease activity
Iannone *et al*. *Clin Exp Rheumatol* 2016; 34: 424-9. (26)	71	TNFi, Rituximab, Tocilizumab	1 year	Significant decrease after treatment by TNFi, RTX and TCZ
Smolen *et al*. Poster presentation THU0135, EULAR congress 2016. (13)	728	Adalimumab + Methotrexate	Week 26-52-78	Significant decrease of ACPA titre in group of ADA + MTX, MTX + PBO and ADA + PBO
Martínez-Estupiñán L. *et al*. *Clin Exp Rheumatol* 2018; 36: 88-93 (27)	16	Infliximab	20 months	Significant decrease of ACPA titres only in patients with IFX-detectable patients.

ABA: Abatacept; TNFi: TNF inhibitors; ADA: Adalimumab; IFX: Infliximab; RTX: Rituximab; TCZ: Tocilizumab; MTX: Methotrexate; PBO: placebo.

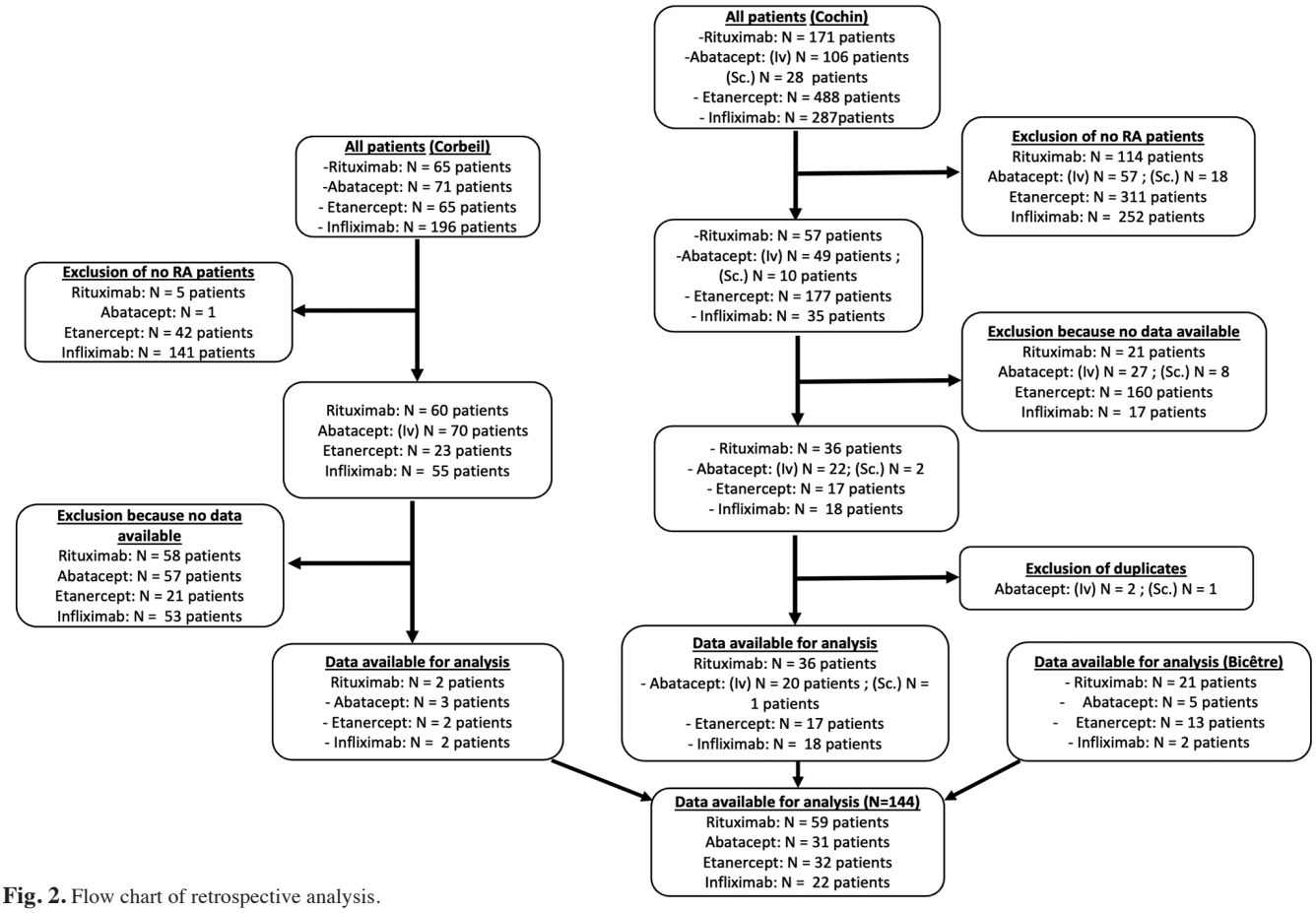

Fig. 2. Flow chart of retrospective analysis.

A subgroup analysis was performed, by categorising patients depending on the 'level' of ACPA titres pre-treatment, *i.e*. high-ACPA (*i.e*. ACPA above the mean of the group at baseline) *vs*. low-ACPA.; identically, changes in ACPA over time were modellised by mixed models, including an interaction term between time and the other potential explanatory variables (drug, disease activity, sex and age) in each of the two subgroups.

Results

Systematic literature review

After exclusion of duplicates, the search retrieved 456 abstracts, and 24 studies were finally selected (including 2 congress abstracts) (13, 14) (Fig. 1). The main reasons for exclusion were the absence of available ACPA over time and the absence of ACPA titre changes for a given treatment.

We identified 17 articles related to TNFi (13-28), 2 to rituximab (26, 29) and 9 to abatacept (14, 24, 28, 30-34) (Table I).

TNFi: Among the 17 articles related to TNFi, 8 reported results for infliximab (17, 19-23, 25, 27), 4 adalimumab (13, 15, 18, 24) and 2 etanercept (16, 35); one EULAR abstract considered the whole TNFi class (13, 14); 16 were prospective studies (15-16, 17-25, 28, 32, 35, 36); 1 was retrospective (14). The effect of TNFi on ACPA titres was different depending on the study and molecule: 8/17 (47%) studies did not report any significant change in ACPA titres (14, 15, 17, 19-21, 23, 24, 28). In 4 studies, rheumatoid factor (RF) titres significantly decreased whereas ACPA titres did not. (15, 17, 19, 23) One study evaluated ACPA titres according to EULAR response and ACPA titres significantly decreased after 28 weeks in the good-response group, but not in the non-response group (15). Seven studies reported a significant ACPA decrease after TNFi treatment (16-19, 22, 25, 35): 2 studies reported a statistically significant decrease in ACPA titres at 6 months (22) and 48 weeks (19), respectively; in these 2 studies, ACPA titre changes were more pronounced in good-responders.

Abatacept: Changes in ACPA titres in patients with RA treated with abatacept were available in 9 studies: 7 prospective studies (28, 30-34), 1 EULAR abstract (24), and 1 ACR abstract (14). Three were prospective studies (24, 30, 32); 3 were retrospective (14, 30, 31). At the ACR congress 2015 (14), Alemao *et al*. reported a reduction of ACPA titres with abatacept at one year from baseline and at 2 years from baseline (14). In the ADJUST trial, ACPA titres decreased at 6 months and at one year from baseline (30). Data from AMPLE, published in 2016, found a significant decrease of ACPA titres with abatacept only for patients with a high levels of ACPA titres (Q4 : 1060–4894 U/mL) at baseline (32, 33). In the ACTION cohort, there was no significant decrease of ACPA titres (31). The study by Endo *et al*. reported that a sustained therapeutic response was associated with an early significant reduction in ACPA titre only with abatacept (and not TNFi) (36).

Rituximab: In the 2 prospective studies concerning rituximab, ACPA titres de-

creased significantly (26, 29). The median time to observe a 50% decrease in ACPA titres was 5 weeks. In responders (8 patients), ACPA titres decreased significantly from 950 U/mL to 236 U/mL (p=0.008). In non-responders, a trend toward a significant decrease in ACPA titres was also observed (2 282 to 1350) (29).

Retrospective study

– Description of population

Among the 1477 screened patients (397 Corbeil-Essones Hospital, 1080 from Cochin Hospital, 41 from Kremlin-Bicêtre), 144 patients were selected for the analysis: 59 patients were treated with rituximab, 31 abatacept, 32 patients etanercept and 21 patients infliximab (Fig. 2).

Among the 144 patients, 117 (81.25%) were females, with a mean age of 55.6 (±13.6) years. Mean titres of ACPA the whole group (pre-treatment) were 712.8 U/mL. Mean disease activity at inclusion, evaluated by DAS-28 was 4.49 (±1.41) (Table III). Mean time between the first ACPA titre pre-treatment and the first ACPA titre during the course of treatment was 1005 (±805) days, and was not significantly different between the different biologics (Fig. 3).

– Effect of the biologics on ACPA changes over time

In the whole population, ACPA titres increased over time from 712.8 (±1039.8) U/mL to 833.2 (±1120.8) U/mL (Fig. 3B). A significant decrease of ACPA was observed in patients treated with rituximab only, from 943.2±1169.1 U/mL to 604.9±1033.7 U/mL (p<0.01). Conversely, a significant increase of ACPA was observed in the TNFi group: 495.8 (± 778,5) to 774,8 (±1154.7) U/mL (p=0.02). No significant change on ACPA titres was observed in the group of patients treated with abatacept 659.5 (±1116.3) to 146.8 (±1378.5 U/mL), p=NS) (Table II).

A significant interaction of drug and time was confirmed by the modelling of ACPA titres over time and the subgroup analysis per drug confirmed a significant interaction between ACPA titres over time and the drug only in the rituximab group (p<0.01) (Fig. 4).

Fig. 3A. Changes of ACPA titres over the time, depending on treatment.

	ACPA titre at t0 (before treatment)	ACPA titre at t1	ACPA titre at t2
All treatments		Mean time (t1-t0): 1005±805 days	Mean time (t2-t0): 1456±811 days
	n=145 Mean: 712,8 U/mL SD: 1039.8 U/mL	n=145 Mean: 714.1 U/mL SD: 1128.0 U/mL	n=45 Mean: 833.2 U/mL SD:1120.8U/mL
Rituximab		Mean time (t1-t0): 973±694 days (p=0.8)	Mean time (t2-t0): 1546 ± 910 days (p=0.7)
	n=59 Mean: 943.2 U/mL SD: 1169,1 U/ML	n=59 Mean: 604.9 U/mL SD: 1033.65 U/mL	n=14 Mean: 417.0 U/mL SD: 564.59 U/mL
Abatacept		Mean time (t1-t0): 832±614 days (p=0.3)	Mean time (t2-t0): 1366 990 days (p=0.7)
	n=31 Mean: 659.5 U/mL SD: 1116.3 U/mL	n=31 Mean: 773.5 U/mL SD: 1194.2 U/mL	n=11 Mean: 1463.8 U/mL SD: 1378.5 U/mL
TNFi		Mean time (t1-t0): 1139±986 days (p=0,3)	Mean time (t2-t0): 1438±714 days (p=0.7)
	n=54 Mean: 491.8 U/mL SD: 778.8 U/mL	n=54 Mean: 799.4 U/mL SD: 1197.4 U/mL	n=19 Mean: 774.8 U/mL SD: 1154.8 U/mL

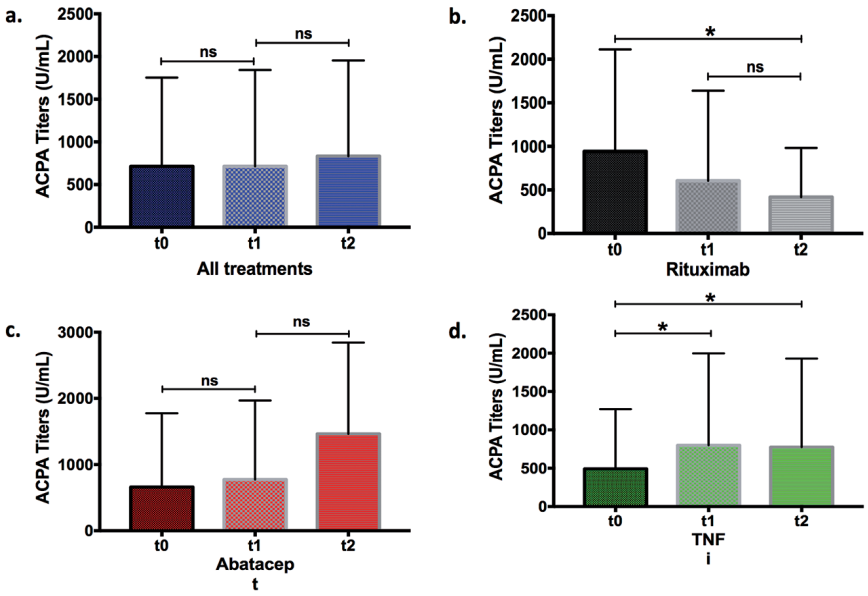

Fig. 3B. Changes of ACPA over the time for all treatment.
a. Change in the CSPA rate for all biologics. No significant change over time. **b.** Decrease of ACPA titre between t0 and t1. **c.** No change in ACPA titres with abatacept treatment. **d.** Significant increase in the ACPA titres between t0 and t1, and between t1 and t2. *p<0.05.

– Effect of other variables on ACPA changes over time

Data on DAS28-CRP over time was available for 115 patients (80%).

No significant interaction was found for disease activity nor age with ACPA titre change (Supplementary Table S1).

A significant interaction with time was observed for sex: the subgroup analysis yielded a significantly increase in ACPA in males over time (p=0.02, Suppl. Fig. S1).

– Effect of biologics on ACPA changes over time depending on the ACPA titres

There were 72 patients in the High-

Table II. Baseline characteristics of patients.

Characteristics		Mean (SD)	*p*-value
Age (years)	All treatments (n=144)	55.7 (13.6)	0.66
	Rituximab (n=59)	57.7 (11.3)	
	TNFi (n=54)	53.4 (16)	
	Abatacept (n=31)	55.9 (14.2)	
Sex (male)	All treatments	27 (18.8%)	0.96
	Rituximab	11 (18.6%)	
	TNFi	10 (18.6%)	
	Abatacept	6 (19.4%)	
DAS28-CRP	All treatments	4.5 (1.4)	0.95
	Rituximab	4.6 (1.4)	
	TNFi	4.4 (1.4)	
	Abatacept	4.4 (1.5)	

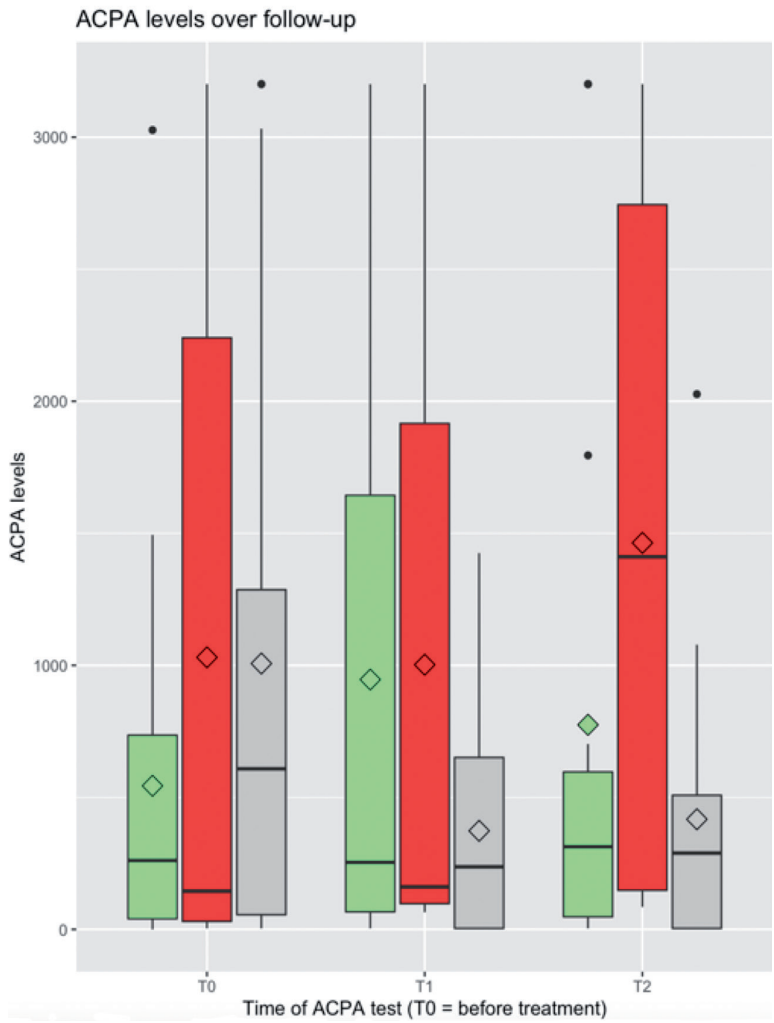

Fig. 4. Changes of ACPA titres (mixed models).
Time-specific interaction - treatment only for rituximab treatment. No significant association for TNFi and abatacept.

ACPA group and 72 in the Low-ACPA group. Changes in ACPA titres over time were comparable in both groups; 1344.81167 U/mL to 1254.7±1119.6 U/mL and 80.7±75.5 U/mL to 371.6±945.5 U/mL in the High-ACPA and Low-ACPA groups, respectively ($p>0.05$).

No significant interaction between ACPA titres and time was observed in the models.

We found a significant association between changes in ACPA titres and DAS28 decrease only in patients treated by rituximab with low-levels of ACPA at t0 ($p<0.05$).

A trend was observed towards an association between the decrease in DAS28-CRP and ACPA titre in patients treated with abatacept with a "high level" of ACPA at baseline ($p=0.06$) (Fig. 4).

Discussion

Our systematic literature review summarised the contradictory evidence regarding the impact of biologics on ACPA titres. Consistent results were only observed with rituximab, with significant reduction of ACPA titres in the 2 available studies. On the other hand, our study confirms these findings, with an observed significant decrease only with rituximab, while an increase was observed with TNFi and no significant change with abatacept.

The decrease in ACPA titres with rituximab may be linked to the mechanism of action of the drug; directly targeting B cells, key cells for immunoglobulin and antibody production, including ACPA. As for abatacept, some studies have reported that it could module B-cells compartment with a normalisation of serum levels of total Ig and a decrease in circulating $CD38^+$ and/or $CD27^+$ memory B cells. (37-40). It has also been described that congenital CTLA4 deficiency (41) was responsible for a decrease in B-cell numbers, especially class-switched memory cells, with a hypogammaglobulinaemia, autoimmunity, granulomatous disease. This decrease of memory B cells could be linked to a decrease of follicular helper T cells, induce by abatacept (42). However, in our study, this we did not observe any significant changes in ACPA in patients with abatacept. One of the reasons might be that in our study measurements of ACPA titres after the initiation of abatacept were performed later compared to all of the studies cited. It is possible that the effect of abatacept on memory B-cells may have diminished over time (mean time for t1: 832 days; mean time for t2: 1366 days - Fig. 3A). As for TNFi, in

our study ACPA titres increased over time while in our systematic literature review (SLR), studies concerning TNFi showed heterogeneous effects (increase or decrease) concerning ACPA titres. The literature suggests that the main effect of TNFi on ACPA subclasses would be an isolated decrease in IgG4 (43). These results may explain the limited impact of TNFi on ACPA titres.

Furthermore, we found a specific association between ACPA titre changes and disease activity only in patients treated with rituximab. These results are consistent with our findings in the SLR, as some studies reported a greater decrease in ACPA titre in responders, although the studies focused on TNFi (18, 19, 22) and abatacept (32). On the other hand, it has been suggested by several studies that high ACPA titres might be a predictive factor for treatment response with either abatacept or rituximab (44, 45). In the REFLEX study, seropositivity (RF and/or ACPA) with elevated CRP was associated with a greater likelihood of response to rituximab. These results were confirmed in the SERENE and IMAGE trials (46-48). Regarding abatacept, many studies have shown that ACPA positive patients, especially those high ACPA titres had a significantly greater probability of clinical response (32, 44, 49, 50).

In our analysis, we observed a dissociation between clinical efficacy of abatacept and TNFi and the absence of a decrease in ACPA titres: disease activity decreased while ACPA titres did not. In some auto-immune diseases, particularly in systemic lupus erythematous, auto-antibodies titres seem to be linked to the disease activity (51), and thus, the decrease or disappearance of specific auto-antibodies could be a therapeutic target (51-53), and could define a immunological remission. In rheumatoid arthritis, this concept is currently discussed, especially because the possible direct pathogenic role of ACPA.

Our SLR and analysis had some limitations worth mentioning. First, in our SLR, the evaluation criteria for ACPA titres were not homogeneous among the selected studies, making it impossible to conduct a meta-analysis. In addition, some studies reported associations between the change in ACPA titres and inclusion titres or clinical response. Secondly, there were only 21 studies included in the end, and especially for abatacept (4 studies) and rituximab (2 studies), highlighting the scarce literature available on the subject. Finally, in studies reporting TNFi effect, results were reported for the whole drug-class and not for each molecule on ACPA titres.

Our analysis had also some limitation, namely the retrospective nature of the design; nevertheless, in this case, the analysed variables both treatment and ACPA titres were systematically and prospectively collected in the EMR, Pharmacy and Immunology departments, we limited the recall bias related to the retrospective design. In particular, we were unable to collect data on co-treatments (glucocorticoid, conventional DMARDs) due to missing data related to the retrospective nature. However, no reliable data suggest that glucocorticoids or csDMARDs have a clear effect on ACPA changes. (54-57) Secondly, this was an observational trial, and the drug was not randomly allocated to patients, leading to potentially different patients (with different characteristics) receiving the different drugs. Nevertheless, in our study, when we explored whether other clinical variables different from the drug had an impact on ACPA changes, we did not find any relevant interaction.

Several strengths should be underlined. First, thanks to the exhaustive way of selection of patients, through the Pharmacy and Immunology databases, we did not have any missing data on the main outcome nor on the drug of interest. Secondly, to our best knowledge, our study is the first one comparing three biologic and their influence on ACPA changes in patients.

Our study suggests that some biologics (*e.g.* rituximab) do decrease significantly ACPA titres. However, whether an immunological remission (*i.e.* ACPA disappearance) should be considered as a target and an important outcome in RA needs to be further explored.

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