

EXTENDED REPORT

The immunogenicity of anti-TNF therapy in immune-mediated inflammatory diseases: a systematic review of the literature with a meta-analysis

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ABSTRACT

Background Immunogenicity of aTNFs is one of the mechanisms behind treatment failure.

Objective To assess the effect of anti-drug antibodies (ADA) on drug response to infliximab, adalimumab and etanercept, and the effect of immunosuppression on ADA detection, in patients with Rheumatoid Arthritis, Spondyloarthritis, Psoriasis and Inflammatory Bowel Diseases.

Data sources PubMed, EMBASE, Cochrane databases, article reference lists (through August 19 2012).

Study selection Out of 2082 studies, 17 were used in the meta-analysis (1RCT; 16 observational studies).

Data extraction Two reviewers extracted data. Risk ratios (RR), 95% CI, using random-effect models, sensitivity analysis, meta-regressions and Egger's test were calculated.

Data synthesis Of 865 patients, ADA against infliximab or adalimumab reduced drug response rate by 68% (RR=0.68, 95% CI=0.12 to 0.36), an effect attenuated by concomitant methotrexate (MTX): <74% MTX+: RR=0.23, 95% CI=0.15 to 0.36; ≥74% MTX+: RR=0.32, 95% CI=0.22 to 0.48. Anti-etanercept antibodies were not detected. Of 936 patients, concomitant MTX or azathioprine/mercaptopurine reduced ADA frequency by 47% (RR=0.53, 95% CI=0.42 to 0.67), particularly when ADA were assessed by RIA (RR=0.36, 95% CI=0.23 to 0.55) compared with ELISA (RR=0.63, 95% CI=0.53 to 0.74).

Conclusions ADA reduces drug response, an effect that can be attenuated by concomitant immunosuppression, which reduces ADA frequency. Drug immunogenicity should be considered for the management of patients receiving biological therapies.

INTRODUCTION

Anti-Tumor Necrosis Factor (aTNF) biological therapies, such as infliximab, adalimumab and etanercept, are effective in treating Rheumatoid Arthritis (RA), Spondyloarthritis (SpA), Psoriasis (Ps) and Inflammatory Bowel Diseases (IBD), such as Crohn's Disease and Ulcerative Colitis. Some patients maintain active disease and others show loss of efficacy after continued treatment.¹ Recent studies have highlighted drug immunogenicity as a mechanism behind treatment failure.^{2–4}

Immunogenicity is the ability that biotechnology-derived therapeutic proteins have in generating antibodies against themselves, since they contain

unique sequences that can elicit an immune response.⁵

The European Medicines Agency and Food & Drug Administration define the assessment of immunogenicity as mandatory for the approval of biopharmaceuticals; however, clinical trials may not reflect true immunogenicity induced by long-term treatment.

Quantification of such antibodies is challenging and undertaken by different assays^{6–10}; assays have been optimised with improved sensitivity and specificity.^{11–15} New evidence reveals a significant impact of immunogenicity on treatment response to biologics.²

A systematic review (SR) and meta-analysis (MA) were undertaken to evaluate the impact of anti-drug antibodies (ADA) on therapeutic response and the effect of immunosuppression (IS) on ADA detection.

METHODS

We followed the PRISMA guidelines for reporting SRs and MAs and Meta-analysis Of Observational Studies in Epidemiology recommendations for observational studies.^{16 17}

Eligibility criteria

To be included in this review, studies had to meet the following predefined eligibility criteria, defined according to the 'PICOS' strategy.¹⁷ The rationale for these criteria is also provided—table 1.

Data sources

A comprehensive search strategy was designed to retrieve relevant clinical data from published literature. The following databases were examined up to 19 August 2012: PubMed, EMBASE and Cochrane Library. Article reference lists were also scanned. The following search terms were used: (('Arthritis, Rheumatoid') OR ('Spondylitis, Ankylosing'(Mesh)) OR ('Arthritis, Psoriatic'(Mesh)) OR ('Psoriasis'(Mesh)) OR ('Colitis, Ulcerative'(Mesh)) OR ('Crohn Disease'(Mesh))) AND 'Treatment Outcome'(Mesh) AND (('TNFR-Fc fusion protein'(Substance Name)) OR ('infliximab'(Substance Name)) OR ('Adalimumab'(Substance Name))).

Study selection

Two independent authors selected studies and extracted data. Disagreements were resolved by

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Table 1 Eligibility criteria for studies included in the systematic review (SR)

Criteria	Inclusion criteria	Rationale
Inclusion criteria	Population	
	Age: adults (≥ 18 years)	The patient population has been restricted to adults, both genders, with diagnoses of RA, SpA, Ps, CD or IBD
	Gender: any	
	Race: any	The review included pharmacological interventions, which are used in clinical practice for RA, SpA, Ps, CD or IBD. Different formulations or route of administration of the same drug were included as supplement evidences
	Disease: RA, SpA, Ps, CD or UC	
	Intervention	
	Infliximab	These comparators were selected to potentially enable the comparison of different drug responses
	Adalimumab	
	Etanercept	
	Comparator	Any of the included interventions
Placebo		
Outcomes	Drug response, assessed by:	Therapeutic responses were defined according to the internationally defined criteria for each disease. In IBD patients, therapeutic responses are often defined by an expert physician, in routine clinical practice. The review was not restrictive regarding the assay employed to assess ADA, if described.
– the European League Against Rheumatism (EULAR) criteria for RA. ⁵⁹ EULAR good and moderate responders were included within the responder group.		
– the Assessment in Ankylosing Spondylitis 20% response criteria or Ankylosing Spondylitis Disease Activity Score for SpA, ^{60 61}		
– the Psoriasis Area and Severity Index for Ps ⁵²		
– Empirically by an expert physician for IBD.		
ADA detection		Randomized Clinical Trials (RCTs) are the gold standard of clinical evidence, minimising the risk of confounding and allowing the comparison of the efficacy of interventions. To enhance the level of evidence, observational studies were included
Study design	Controlled Clinical Trials and Observational studies published in peer-reviewed journals. Unpublished data, 'recommendations', reviews and MAs were excluded from the analysis but used to hand-search additional bibliographic references.	
Phases of trial	All phases	
Language restrictions	No Language Restriction	
Publication timeframe	No date or publication status restrictions were imposed.	
Exclusion criteria	Population Study population limited to a known type of drug response (ex. non-responders)	
Exposure	ADA assessed outside the trough period or before week 6	
	Assay not specified	
	Studies without a specified IS or administration schedule (to address the influence of IS on ADA detection).	

ADA, anti-drug antibodies; CD, Crohn's Disease; IBD, Inflammatory Bowel Diseases; IS, immunosuppression; MA, meta-analysis; Ps, Psoriasis; RA, Rheumatoid Arthritis; RCTs, Randomized Clinical Trials; SpA, Spondyloarthritis; UC, Ulcerative Colitis.

discussion between the two reviewers. To ascertain the validity of eligible studies and to minimise the risk of bias, two blinded reviewers determined the adequacy of study characteristics. We decided to use more broad inclusion criteria and then to perform meta-regressions, subgroup analyses and sensitivity analysis, rather than using highly restrictive study quality assessment scores.¹⁶

Data extraction

The following information was extracted from each study: (1) population demographic characteristics: age, gender; (2) clinical

data: diagnostic criteria and disease activity; (3) treatment: aTNE, duration, dose, schedule, route of administration, immunosuppressors; (4) exposure and outcome: therapeutic response, serum trough ADA, time of assessment, assay; (5) publication: study design, follow-up period, author, year.

Statistical analysis

Data were analysed by calculating risk ratios (RR) for each study and performing forest plots; uncertainty was expressed by 95% CI. Assuming clinical and methodological heterogeneity in the available body of evidence, random-effects models, according to

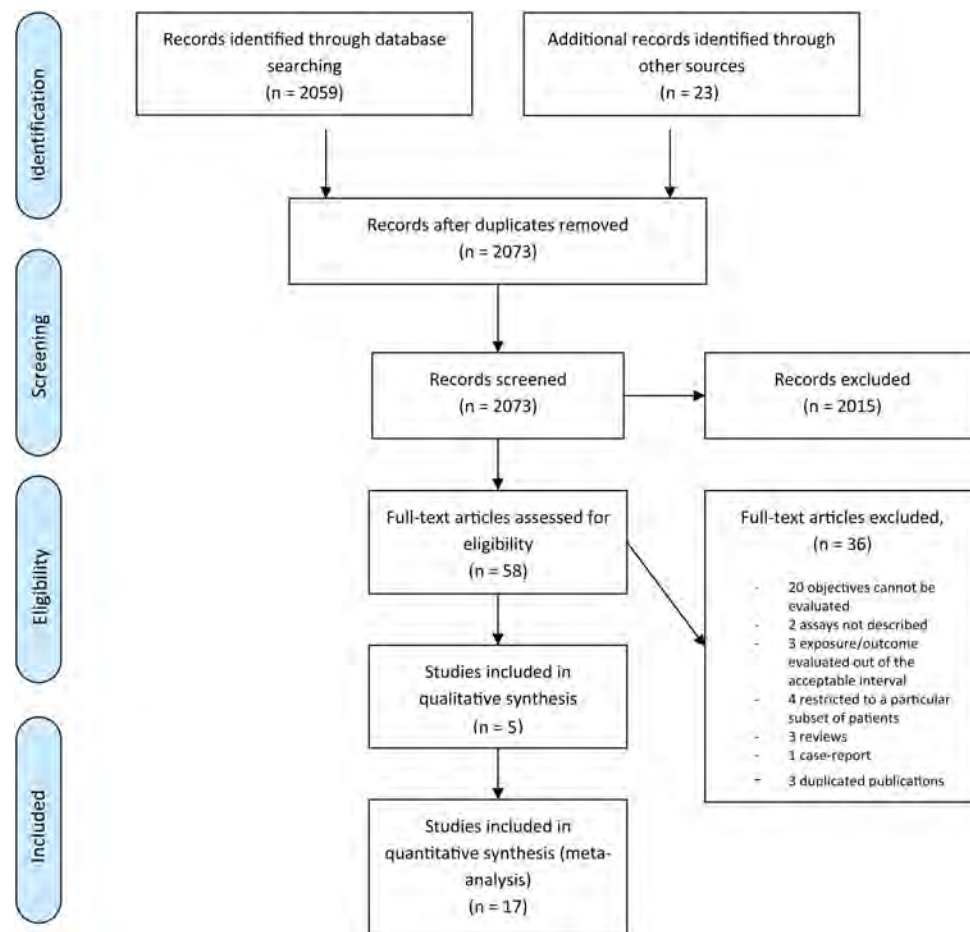


Figure 1 Flow of studies through the systematic review (SR) process. Access the article online to view this figure in colour.

the Laird method, were used to cluster the results. Heterogeneity of study results was examined by calculating the χ^2 test for heterogeneity (when the χ^2 test had a $p < 0.1$) and the I^2 measure of inconsistency.¹⁸ Potential sources of heterogeneity were explored through analysis of the following predefined subgroups and confirmed through meta-regression: (1) primary diagnosis; (2) proportion of patients co-treated with IS: methotrexate (MTX) or azathioprine/6-mercaptopurine (AZA/MCP); (3) proportion of patients who underwent aTNF dose escalation; (4) proportion of patients who started with higher initial doses of aTNF; (5) scheduled treatment regimens; (6) assay for ADA detection; (7) population characteristics and (8) study characteristics. Publication bias was analysed through the Egger's plot and test.

STATA VSE 12 was used.

RESULTS

Figure 1 shows the flow of studies through the SR process. Search of literature databases yielded 2082 references. Seventeen eligible studies were evaluated quantitatively, through MA. Five additional studies were exclusively qualitatively assessed because of significant missing data, but considered important evidence.

To address the impact of ADA on drug response, 865 patients (540 RA, 132 SpA, 58 Ps, 130 IBD) from 12 observational prospective cohort studies were analysed;^{2 3 12 14 19–26} and five additional studies were described qualitatively^{27–31}—table 2.

Two publications used the same cohort of patients.^{2 20} We selected 'Bartelds 2007' for the MA based on it being the original study and showing less heterogeneity in the pooled analysis—online supplementary figures S1 and S2.

Two studies had multiple time points of assessment.^{14 24} The 12-month time point was selected for the MA, because of consistency with the included studies and less heterogeneity in the pooled analysis—online supplementary figures S3–S5.

Overall, detectable ADA reduced the drug response rate by 68% (RR=0.32, 95% CI=0.22 to 0.48)—figure 2. Significant between-study heterogeneity was observed ($I^2=45.5\%$, $p=0.037$). Seeking for potential sources of heterogeneity, we observed a trend to an increased effect size in studies where the proportion of patients co-treated with IS (%IS) was $<67\%$ —online supplementary figure S6. However, univariate meta-regression did not detect %IS as a significant effect modifier of the effect of ADA on response rates—table 3. A closer look at these subgroups showed that among studies where %IS $<67\%$, detectable ADA was associated with a reduction in therapeutic response by 78% (RR=0.22, 95% CI=0.12 to 0.39) and in studies where %IS $\geq 67\%$, the effect size reduction of drug response was attenuated to 59% (RR=0.41, 95% CI=0.27 to 0.62)—online supplementary figure S6.

Univariate meta-regression detects the proportion of patients co-treated with MTX (%MTX) as a significant effect modifier of ADA on drug response—table 3. In the subgroup analysis, we observed that in studies where %MTX $<74\%$, the presence of ADA reduced therapeutic response by 77% (RR=0.23, 95%

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Table 2 Study and baseline patient characteristics

1. Quantitative evidence													
Study	Disease	Total Population No.	Women %	Age, mean (SD)/median (IQR), y	Disease duration, mean (SD)/median (IQR), year	Disease activity,* mean (SD)	MTX %	AZA/MCP %	CST %	Other IS %	No IS %	Biological	Posology
Objective 1													
Wolbink 2006 ¹²	RA	51	82	56† (13)	12† (9)	6.0 (1.3)	86	na	na	8	6	INF	Ind‡+3 mg/Kg q8w
Radstake 2009 ¹⁹	RA	34	79	56† (10)	na	5.7 (1.0)	41	na	26	0	59	ADL	40 mg q2w
Radstake 2009 ¹⁹	RA	35	86	57† (10)	na	5.6 (1.2)	100	na	29	0	0	INF	Ind‡+3 mg/Kg q8w
Pascual-Salcedo 2011 ¹⁴	RA	85	81	54† (14)	na	5.5 (1.3)	34	na	74	18	1	INF	Ind‡+3 mg/Kg q8w
de Vries 2007 ³	AS	38	31	40† (10)	na	6.4 (1.2)	0	na	8	16	na	INF	Ind§+5 mg/Kg q6w
Hoffman 2011 ²⁶	Ps	29	45	na	na	14.7(10)	24	na	na	7	na	INF	Ind§+5 mg/Kg q8w
Objective 1 and 2													
Bartelds 2007 ²⁰	RA	121	79	53† (13)	12† (10)	5.3 (1.1)	79	na	34	12	20	ADL	40 mg q2w
Bender 2007 ²¹	RA	15	67	56† (8.1)	12† (8.2)	6.5 (1.2)	67	0	100	7	27	INF	40 mg q2w
Bartelds 2011 ²	RA	272	81	54† (12)	8¶ (3–17)	5.2 (1.2)	74	na	91 (33)	19 (7)	19	ADL	40 mg q2w
Lecluse 2010 ²⁵	Ps	29	31	44 (11)	22 (na)	15.5(na)	10	na	na	na	0	ADL	Ind**+40 mg q2w
West 2008 ²²	CD	30	77	36†† (21–73)	na	na	13	5 (17)	13	0	57	ADL	Ind‡‡+40 mg q2w
Study	Disease	Total population no.	Female %	Age, mean (sd)/median (IQR), year	Disease duration mean (sd)/median (IQR), y	Disease activity,* mean (SD)	MTX %	AZA/MCP %	CST %	Other IS %	No IS %	Biologic	Scheme
1. Quantitative evidence													
Objective 1 and 2													
Steenholdt 2011 ²³	CD/UC	106	54	30–41§§	3–10§§	na	7	62	1	20 (19)	na	INF	Ind§+5 mg/Kg q4–12w
Plasencia 2012 ²⁴	SpA	94	44	50 (11)	na	3.1 (1.3)	22	na	42	27	23	INF	Ind§+5 mg/Kg q6w
Objective 2													
Baert 2003 ⁴	CD	125	66	35†† (17–73)	na	260†† (0–575)	2	45	42	na	14	INF	5 mg/Kg SD or Ind§+5 mg/Kg OD
Vermiere 2007 ³²	CD	174	61	39†† (18–73)	na	na	29	37	na	0	34	INF	5 mg/Kg SD or Ind§+5 mg/Kg OD
Afif 2009 ³³	CD/UC	155	55	39†† (26–50)	14¶ (na)	na	8	37	10	0	42	INF	5 mg/Kg SD or Ind§+5 mg/Kg q8w
Maini 1998 ³⁵	RA	29	72	51† (na)	10† (na)	6.7	52	0	55	0	0	INF	Ind‡+3 mg/Kg q4w
Bartelds 2010 ³⁴	RA	235	79	53† (12)	9¶ (4–17)	5.2 (1.2)	82	na	34	na	na	ADL	40 mg q2w
2. Qualitative evidence													
Objective 1													
Van Kuijk 2010 ²⁸	PsA	22	36	43†† (21–61)	6¶ (1–18)	4.9¶¶ (0.3)	55	na	na	na	na	ADA	40 mg q2w
Adisen 2010 ³¹	Ps	15	47	38–74§§	30–40§§	11–36§§	na	na	27	na	na	INF	Ind§+5 mg/Kg q8w
Jamnitski 2012 ²⁹	RA	292	82	53† (13)	8¶ (3–16)	5.2 (1.3)	76	na	28	36	na	ETA	25 mg biw or 50 mg qw
Hoshino 2012 ³⁰	RA	40	88	60.5¶ (23–80)	6.5¶ (1–45)	5.4 (3.1–8.1)	58	0	85	18	0	ETA	25 mg biw
De Vries 2009 ²⁷	AS	53	25	41† (11)	na	6.4 (1.3)	na	na	na	na	na	ETA	25 mg biw or 50 mg qw

*DAS28 for RA and PsA patients, ASAS 20 or ASDAS for SpA and AS patients, PASI for Ps patients.
†Mean (SD).
‡Induction dose of 3 mg/kg at 0, 2 and 6 weeks.
§Induction dose of 5 mg/kg at 0, 2 and 6 weeks.
¶Median (range/IQR).
**Induction dose of 80 mg at wk 0 and 40 mg at wk 2.
††Mean (range/IQR).
‡‡Induction dose of 160 mg at wk 0 and 80 mg at wk 2.
§§Range.
¶¶Mean (SEM).
ADA, anti-drug antibodies; ADL, Adalimumab s.c.; AS, Ankylosing Spondylitis; ASAS, Ankylosing Spondylitis; ASDAS, Ankylosing Spondylitis Disease Activity Score; AZA, Azathioprine; biw, twice a week; CD, Crohn's Disease; CST, Corticosteroids; ETA, Etanercept s.c.; INF, Infliximab i.v.; IS, Immunosuppression; MCP, 6-Mercaptopurine; MTX, Methotrexate; na, not available; OD, on demand; PASI, Psoriasis Area and Severity Index; Ps, Psoriasis; PsA, Psoriatic Arthritis; qw, every week; qXw, every X weeks; RA, Rheumatoid Arthritis; SD, single dose; SpA, Spondyloarthritis; UC, Ulcerative Colitis; wk, weeks.

Effect of ADA-Positivity on aTNF Response

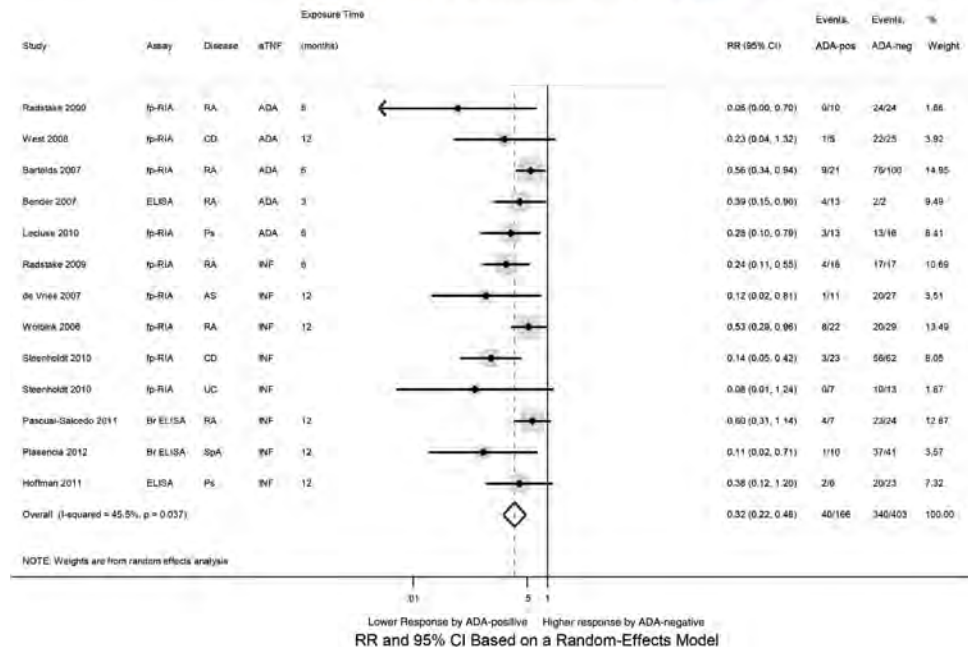


Figure 2 Effect of ADA positivity on aTNF response. ADA, anti-drug antibodies; AS, Ankylosing Spondylitis; Br ELISA, Bridging enzyme-linked immunosorbent assay; CD, Crohn's Disease; ELISA, enzyme-linked immunosorbent assay; fp-RIA, fluid-phase radioimmuno assay; INF, Infliximab; MTX, Methotrexate; Ps, Psoriasis; RA, Rheumatoid Arthritis; SpA, Spondyloarthritis; UC, Ulcerative Colitis. Access the article online to view this figure in colour.

CI=0.15 to 0.36), while in studies where that proportion was $\geq 74\%$, the effect size reduction was attenuated to 68% (RR=0.32, 95% CI=0.22 to 0.48)—figure 3.

Univariate meta-regression also showed primary diagnosis (RA vs others) and initial higher doses of biologicals to be significant effect modifiers of the effect of ADA on drug response—table 3. We verified a decreased effect size in studies evaluating RA patients and in studies where patients received initial

lower doses of biologicals—online supplementary figures S7–S9). These studies are also those with lower proportion of patients receiving IS and MTX. Significant heterogeneity in the pooled analysis was abrogated by dividing the studies according to the proportion of patients receiving IS, and particularly MTX, independently of the disease or the doses of biologicals that were used.

The impact of ADA on drug response was not significantly affected by the proportion of patients who underwent aTNF dose escalation—table 3.

We could not test the effect size modification by scheduled treatment regimens or by the assay used for ADA detection since most studies used schedule regimens and radioimmuno assays (RIAs) to assess ADA.

The Egger's test provided evidence for no significant publication bias—online supplementary figure S10.

Studies with Etanercept were not analysed quantitatively because they did not fit the eligibility criteria and/or because no anti-etanercept antibodies were detected. Three studies are described qualitatively. Both described absence of anti-etanercept antibodies, by using Bridging ELISA and fluid-phase RIA, in a total of 332 RA patients,^{29 30} and 53 Ankylosing Spondylitis (AS) patients²⁷ treated with 25 mg twice weekly or 50 mg weekly—table 1. The absence of anti-etanercept antibodies persisted 3 months after therapy withdrawal.²⁷

We found two additional studies among Ps patients, which could not be included in the MA due to missing data. In a study enrolling 15 Ps patients receiving infliximab, those with detectable ADA had higher Psoriasis Area and Severity Index values than patients without such antibodies (Psoriasis Area and Severity Index (mean, sd)=10(4.9) vs 5.3(2.4); $p=0.02$).³¹ Similarly, another study, among 22 PsA patients, revealed that ADA-positive patients had a significantly worse disease activity score in 28 joints (DAS28) at 12 months of adalimumab therapy, when compared with patients without such antibodies (DAS28 (SEM)=5.05 (0.84) vs 2.58 (0.32); $p=0.01$).²⁸

Table 3 Meta-regression stratified by clinical characteristics to address the effect of ADA on drug response and the effect of IS on ADA detection

Clinical characteristic	RR (95% CI)	p for interaction
Effect of ADA on response		
Immunosuppressors, %		
Lower proportion IS (<67%)	0.24 (0.11 to 0.53)	0.220
Higher proportion MTX ($\geq 67\%$)	0.40 (0.27 to 0.61)	
Methotrexate, %		
Lower proportion MTX (<74%)	0.24 (0.14 to 0.40)	0.028
Higher proportion MTX ($\geq 74\%$)	0.50 (0.35 to 0.71)	
Primary diagnosis		
RA	0.47 (0.33 to 0.65)	0.034
Other diseases	0.22 (0.12 to 0.40)	
Initiated higher biological doses		
No	0.47 (0.33 to 0.65)	0.034
Yes	0.22 (0.12 to 0.40)	
Dose escalation, %	0.31 (0.17 to 0.56)	0.57
Effect of IS on ADA detection		
Assay		
ELISAs	0.63 (0.50 to 0.79)	0.035
RIA	0.36 (0.23 to 0.57)	

ADA, anti-drug antibodies; ELISAs, Enzyme-linked immunosorbent assays; IS, immunosuppression; MTX, methotrexate; RA, Rheumatoid Arthritis; RIA, Radioimmuno assay; RR, Risk ratios.

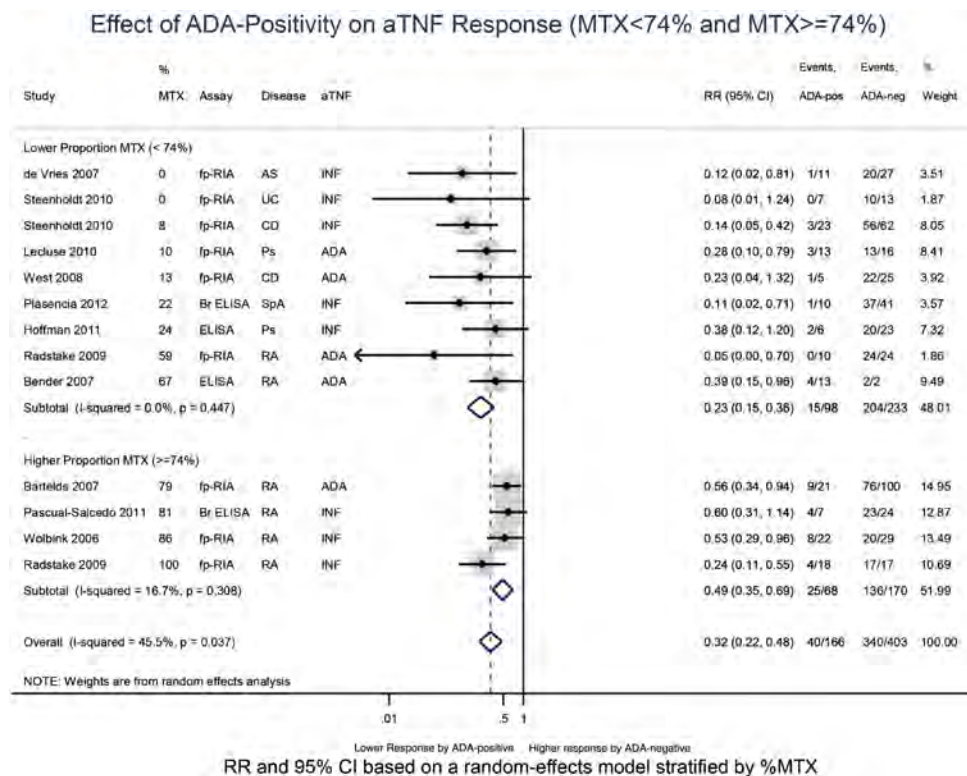


Figure 3 Effect of ADA positivity on aTNF response (MTX<74% and MTX ≥74%). ADA, anti-drug antibodies; AS, Ankylosing Spondylitis; BR ELISA, Bridging enzyme-linked immunosorbent assay; CD, Crohn's Disease; ELISA, enzyme-linked immunosorbent assay; fp-RIA, fluid-phase radioimmuno assay; INF, Infliximab; MTX, Methotrexate; Ps, Psoriasis; RA, Rheumatoid Arthritis; SpA, Spondyloarthritis; UC, Ulcerative Colitis. Access the article online to view this figure in colour.

To address the influence of IS on the detection of ADA 936 patients (376 RA, 94 SpA, 29 Ps, 437 IBD) from 12 studies were analysed quantitatively^{2, 4, 20–25, 32–35}—table 2.

MTX (dose range: 7.5–25 mg/week) was the main immunosuppressor used in RA patients; AZA (2–2.5 mg/kg/day) or MCP (1–1.25 mg/kg/day) were the main immunosuppressors used in IBD patients.

ADA were assessed through slightly different fluid-phase RIAs^{20, 22, 25, 34} and three different ELISA methods.^{4, 21, 22, 24, 32, 33, 35}

Two of the included studies used the same cohort of patients.^{2, 20, 34} We selected 'Bartelds 2007' for the MA based on the fact that it was the original study and also the one that offered lower heterogeneity in the pooled analysis—online supplementary figures S11–S13.

Immunosuppressors reduced the proportion of patients with detectable ADA by about 41% (RR=0.59, 95% CI=0.50 to 0.70)—online supplementary figure S14. Meta-regression confirmed the assay as a significant source of heterogeneity—table 3. Concomitant IS reduced detectable ADA by 64% (RR=0.36; 95% CI=0.23 to 0.55) when RIA was used to detect ADA, while when ELISA methods were used the effect size reduction of detectable ADA was attenuated to 37% (RR=0.63, 95% CI=0.42 to 0.67)—figure 4.

It was not possible to evaluate potential differences in detectable ADA among MTX and AZA/MCP, due to limited data.

The Egger's test provided evidence of significant publication bias for the studies evaluating the effect of IS on ADA production—online supplementary figure S15. Positive results may be more likely to be published. However, the presence of small and heterogeneous studies may also underlie that result.

DISCUSSION

Immunogenicity reduces therapeutic response to aTNFs, an effect that is attenuated by immunosuppressors. Detectable ADA decreases aTNF response by as much as 80%. This contrasts with descriptions in most randomized clinical trials (RCTs), considering immunogenicity as a minor problem.^{36–39} ADAs might reduce drug efficacy by competing with the endogenous ligand (neutralising antibodies) and/or by forming immune complexes, which accelerates the clearance of the drug from the circulation, decreasing its bioavailability. Anti-idiotypic antibodies, which include neutralising antibodies, represent the most significant part of the antibody response against infliximab and adalimumab.^{40–42} The detection of ADA is technically challenging and the assay may influence the results. The majority of studies in this paper used two independent but similar RIAs, with higher specificity than ELISAs.^{6, 10, 11, 15} They are both fluid-phase RIAs, which avoid the artifacts induced by solid-phase adsorption of proteins and they both detect ADA against drug-F(ab')₂, not favouring the detection of low-avidity antibodies. RIA is less susceptible than ELISA to drug interference and is able to detect monovalent IgG4 ADA, which may represent a significant proportion of ADA.^{10, 43} ELISAs have been the main method used in RCTs, perhaps explaining the contrasting descriptions in RCTs. We believe that the association remains underestimated: four of the included studies used ELISAs; the assays employed cannot detect ADA that is in complex with the drug, not even RIA and we included European League Against Rheumatism moderate response within the responder group.

Despite few published studies reporting anti-etanercept antibodies,^{44–47} all showed transient low titres, with no impact on drug response; this suggests the presence of 'binding antibodies'

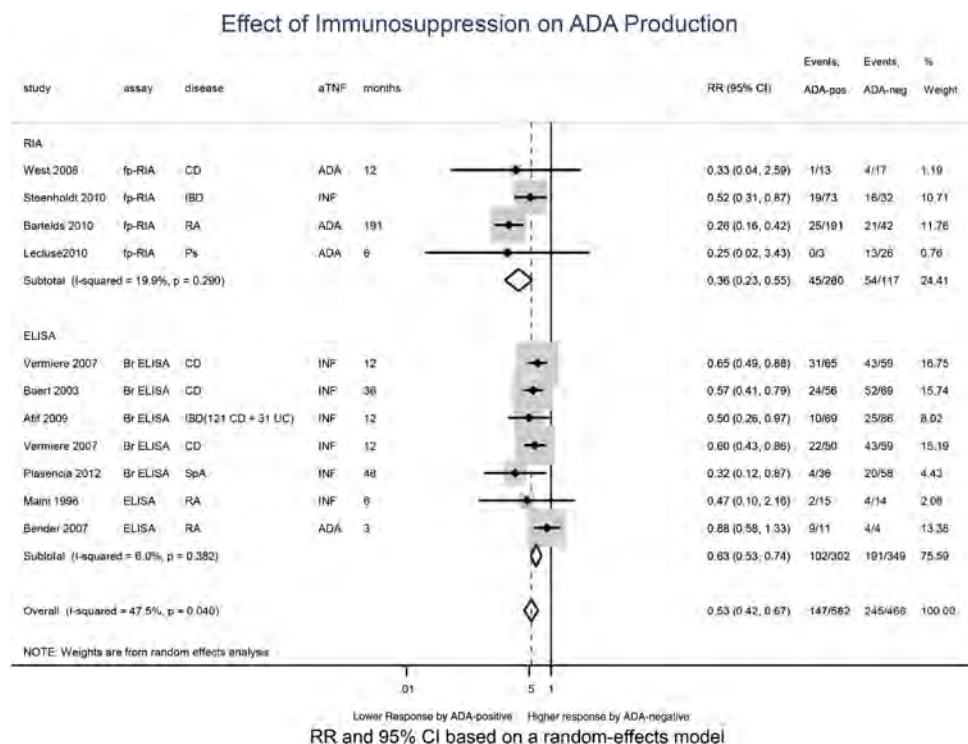


Figure 4 Effect of immunosuppression on ADA production (by assay). ADA, anti-drug antibodies; Br ELISA, Bridging enzyme-linked immunosorbent assay; CD, Crohn's Disease; ELISA, Enzyme-linked immunosorbent assay; fp-RIA, Fluid-phase radioimmuno assay; IBD, Inflammatory Bowel Diseases; INF, Infliximab; Ps, Psoriasis; RA, Rheumatoid Arthritis; SpA, Spondyloarthritis; UC, Ulcerative Colitis. Access the article online to view this figure in colour.

or false positive results, since ELISAs of low specificity were used. Etanercept is administered more often than other biologicals, possibly creating more drug interference in ADA detection. However, the absence of anti-etanercept antibodies has been confirmed months after therapy withdrawal.²⁷ Etanercept also blocks lymphotoxin- α , which among other functions is important for germinal centre formation.⁴⁸ The involvement of lymphotoxin- α on drug immunogenicity remains unknown. The absence of clinically significant immunogenicity attributable to etanercept is consistent with the higher drug survival that has been reported for etanercept in comparison with infliximab or adalimumab.^{49–51}

Factors able to modulate the clinical impact of immunogenicity need to be identified. We verified that concomitant IS attenuated the impact of ADA on drug response, particularly MTX. The exact mechanism remains unknown. Concomitant MTX has been shown to be efficient in reducing immunogenicity in a dose-dependent manner, either by reducing the frequency of detectable ADA or by delaying its detection.^{24 52 53} The time point of ADA assessment is also important. In our MA the majority of included studies assessed ADA at 12 months, showing a lower ADA frequency in patients receiving concomitant IS. That reduction was more apparent when RIA was used compared with ELISA methods (64% reduction vs 34%), which may be explained by the increased specificity and less drug interference of RIAs over ELISAs.

These results are of high clinical interest, since they reveal a putative beneficial role of concomitant IS in diseases such as SpA, by modulating immunogenicity, increasing drug survival and treatment effectiveness. A recent study in AS patients failed to demonstrate the influence of MTX on infliximab pharmacokinetics.⁵⁴ Infliximab is administered more frequently and at higher doses in AS, compared with RA, which may create drug

interference and false-negative results if highly drug-sensitive assays are used to detect ADA. That study was conducted up to 18 weeks of infliximab treatment, which for some patients may be too early for full development of immunogenicity. RCTs, with larger follow-up times should be conducted to show the beneficial effect of IS/MTX in these patients.

We could not assess differences between MTX and AZA/MCP regarding their impact on ADA detection, due to the low number of studies using AZA/MCP and the fact that some studies in IBD also enrolled patients receiving MTX, who cannot be separated from the group receiving IS. However, univariate meta-regression detected %MTX as a significant source of heterogeneity, but not %IS (MTX+AZA/MCP). Subgroups based on %MTX resulted in homogeneous groups, whereas, the same did not happen for %IS subgroups. Further studies are warranted to verify whether this effect modifier is extended to other immunosuppressive agents and whether dose and scheduled regimens influence response by detectable ADA.

In some trials, initial higher doses of infliximab or adalimumab have revealed to be less immunogenic.^{35 55} It is not clear if that is a true effect or just an assay limitation of detecting ADA in the presence of high drug concentration. We could not draw any conclusions in our MA because the studies that used higher biological doses enrolled only patients with SpA, Ps and IBD, who also had lower proportion of patients co-treated with IS, confounding the results. Moreover, the limited number of studies and the clinical heterogeneity among them also prevent robust conclusions. Despite the high costs of biological therapies, it would be of great clinical interest to know if higher induction doses of biologicals would reduce immunogenicity, which could result in an improvement of its cost-effectiveness over the long-term.

Some reports show that dose escalation decreased ADA detection, improving drug response^{12 20} but others³ show that the

procedure can indeed boost the immune response with serious consequences, such as infusion-related adverse events¹⁴ or severe thromboembolic phenomena.⁵⁶ The absence of ADA might be explained by drug interference, but it is striking why some ADA-positive patients do not boost the ADA production. In our analysis, the proportion of patients who underwent dose escalation did not interfere with the impact of ADA on drug response. Very limited data are available and more studies are warranted to specifically address this question, since dose escalation is often adopted in clinical practice in case of inadequate response. We believe that increasing the dose may be risky since, so far, there is no way to identify which patients are at risk of boosting an anti-biological response after dose escalation.

Scheduled regimens have been revealed to be less immunogenic, by poorly defined mechanisms.^{4 57 58} The limited number of studies prevented us from drawing such conclusions in our analysis.

A limitation to our study is that patient population, assessment of antibodies and therapeutic response definitions are not standardised across studies, although we addressed those sources of heterogeneity in the pooled analysis. The power of meta-regression analysis has limitations by the relatively small number of studies and the imprecision of the measurement techniques. The results obtained are driven largely by findings in patients with RA. Although we excluded significant effect size modification by the disease itself, generalisations should be done cautiously.

This is the first SR and MA on aTNF immunogenicity. Our study strongly supports the notion that drug immunogenicity should be considered in clinical practice, during long-term use of therapeutic proteins. It is also an exploratory study, trying to identify factors able to influence the clinical impact of drug immunogenicity. It adds some evidence that unwanted immunogenicity may be modulated. Increasing evidence shows that monitoring immunogenicity will help us to better understand the clinical heterogeneity among patients, representing a very promising tool for an optimised and more personalised usage of biological therapies.

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The immunogenicity of anti-TNF therapy in immune-mediated inflammatory diseases: a systematic review of the literature with a meta-analysis

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