RHEUMATOLOGY

Rheumatology 2018;57:688–693
doi:10.1093/rheumatology/kex474
Advance Access publication 19 January 2018

Concise report

Drug immunogenicity in patients with inflammatory arthritis and secondary failure to tumour necrosis factor inhibitor therapies: the REASON study

Alejandro Balsa[1], Raimon Sanmarti[2], José Rosas[3], Victor Martin[4], Ana Cabez[5], Susana Gómez[6] and María Montoro[6]

Abstract

Objectives. The aims were to evaluate the prevalence of anti-drug antibodies (ADA) in patients with RA or SpA experiencing secondary failure to anti-TNF therapy and to correlate ADA presence with anti-TNF concentration and clinical response.

Methods. This was a cross-sectional, observational study of patients with active RA or SpA experiencing secondary failure to etanercept (ETN), infliximab (INF) or adalimumab (ADL). Concomitant non-biologic DMARDs were permitted. Serum anti-TNF and ADA levels were measured with two-site ELISA.

Results. Among 570 evaluable patients, those with RA ($n = 276$) were mostly female (80 *vs* 39%), older (56 *vs* 48 years), received concomitant DMARDs (83 *vs* 47%) and had maintained good clinical disease control for longer (202 *vs* 170 weeks) compared with patients with SpA ($n = 294$). ADA were found in 114/570 (20.0%) patients; 51/188 (27.1%) against INF and 63/217 (29.0%) against ADL; none against ETN. Of these 114 patients, 92 (81%) had no detectable serum drug concentrations. Proportionately more patients with SpA (31.3%) had anti-INF antibodies than those with RA (21.1%; $P = 0.014$). A significantly lower proportion of patients receiving concomitant DMARDs (16.5%) developed ADA than those on monotherapy (26.4%; $P < 0.05$).

Conclusion. In patients with RA or SpA and secondary failure, the development of ADA against ADL or INF, but not ETN, appears to be one of the main reasons for secondary treatment failure, but not the only one. Further investigations are needed to determine other causes of anti-TNF failure.

Key words: biologic, TNF inhibitor (anti-TNF), immunogenicity, secondary failure, antibody, rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis

CLINICAL SCIENCE

Rheumatology key messages

- In Spanish patients with RA or SpA, anti-drug antibodies appear to contribute to secondary treatment failure.
- Anti-drug antibodies appear to be more prevalent among adalimumab- or INF-, but not etanercept-treated patients.
- Additional studies are needed to determine other causes for secondary treatment failure in RA and SpA.

Introduction

The treatment of RA, AS and PsA has been greatly improved by the introduction of anti-TNF agents [1–3]. However, a proportion of patients receiving these biologic therapies persist with active disease, either because the treatment fails to initiate a response (primary failure) or because initial responsiveness gives rise to non-response (secondary failure). Secondary failure can occur in as many as 30% of patients [4]. A potential reason for

[1]Department of Rheumatology, IdiPAZ, Hospital Universitario La Paz, Madrid, [2]Arthritis Unit, Department of Rheumatology, Hospital Clinic de Barcelona and IDIBAPS, Barcelona, [3]Department of Rheumatology, Hospital Marina Baixa, Villajoyosa, Alicante, [4]Biostatistics, [5]Medical Affairs, Pfizer España and [6]Inflammation Rheumatology, Medical Department, Pfizer SLU, Madrid, Spain

Submitted 14 December 2016; revised version accepted 20 November 2017

Correspondence to: Alejandro Balsa, Department of Rheumatology, IdiPAZ, Hospital Universitario La Paz, Paseo de la Castellana 261, 28046 Madrid, Spain.
E-mail: alejandro.balsa@salud.madrid.org

secondary failure is the development of anti-drug antibodies (ADA), which can neutralize the drug or decrease the serum drug concentrations to sub-therapeutic levels, resulting in a loss of clinical response [5–7]. There are reports showing loss of efficacy owing to development of ADA against infliximab (INF) and adalimumab (ADL) [8]. Furthermore, ADA may also cause acute or delayed reactions, such as infusion- and injection-site reactions [9].

The purpose of this observational study was to evaluate the prevalence of ADA against anti-TNF agents in Spanish patients with RA or SpA with secondary failure, and to assess whether the presence of ADA and low serum drug concentrations were the reasons for the lack of clinical response.

Methods

Patients and study design

The REASON study was a cross-sectional, observational investigation carried out in 45 rheumatology centres across Spain between November 2012 and July 2014. Patients were eligible for inclusion if they were aged $\geqslant$18 years, had active RA or SpA, including PsA, and had received and complied with anti-TNF [etanercept (ETN), INF or ADL] treatment as prescribed by a rheumatologist. Patients with secondary failure to anti-TNF treatment at the time of the study visit were included consecutively. Secondary failure was defined as good response (DAS28 $<$3.2, AS DAS $\leqslant$ 2.1 or BASDAI $<$ 4) in response to the administered anti-TNF treatment for at least 3 months and moderate-to-high disease activity (DAS28 $\geqslant$3.2 or AS DAS $>$2.1 or BASDAI $\geqslant$4) at the time of evaluation in the present study. Patients with low disease activity (RA: DAS28 $<$ 3.2; SpA: AS DAS $<$ 2.1 or BASDAI $\leqslant$ 4) were excluded. Treatment with concomitant non-biologic DMARDs during the study was permitted.

All patients provided signed and dated informed consent, and the study was approved by the ethics committee of La Paz University Hospital in Madrid. This study was conducted in compliance with the ethical principles originating in or derived from the Declaration of Helsinki, and in compliance with all International Conference on Harmonization Good Clinical Practice Guidelines.

Assessments

Serum drug and ADA concentrations were measured with two-site ELISA using Promonitor (Proteomika) kits [10]. All assays were performed in a central reference laboratory. The limit of detection for serum INF, ADL and ETN was 2, 0.4 and 1.2 μg/ml, respectively, and for the corresponding ADA was 1, 1.7 and 6.9 a.u./ml (100% specificity), respectively.

Statistical analysis

It was assumed that a sample size of 552 patients diagnosed with RA or SpA on anti-TNF treatment and with secondary failure to treatment would allow the estimation of the prevalence of anti-TNF antibodies with 95% CI, a power of 80%, and a precision of 0.0425 and 0.044, respectively. A prevalence of patients with RA or SpA who develop anti-TNF antibodies of 32 and 28%, respectively, and a reposition rate of 10% were assumed. Demographic and clinical characteristics were summarized using descriptive statistics. Quantitative and qualitative variables were analysed using measurements of central tendency (mean, median) and of dispersion (95% CI). Qualitative variables were defined according to their absolute and relative frequencies. Pearson's χ^2 test was used for qualitative variables. Data were analysed using SPSS V17.0 statistical software.

Results

Patient population

A total of 583 patients were recruited, and 570 were considered evaluable (RA: n = 276; SpA: n = 294, of whom 103 were diagnosed with PsA). At baseline, the demographics and characteristics of patients with RA were significantly different from those with SpA (Table 1). The majority of patients with RA were female (80%), whereas the majority of patients with SpA were male (61%). Patients with RA were also older than those with SpA (56.3 *vs* 47.9 years) and maintained good clinical disease control for longer (202 *vs* 170 weeks). Slightly more patients with RA than SpA were receiving ADL (40 *vs* 36%) and ETN (33 *vs* 26%) therapies, whereas more patients with SpA than RA received INF (38 *vs* 28%). Significantly more patients with RA than SpA were taking concomitant DMARDs other than MTX (83 *vs* 47%), but ~75% of patients in both groups took concomitant MTX.

Prevalence of ADA

Overall, 114/570 (20.0%) patients developed ADA, of whom 63/215 (29.3%) patients tested positive for anti-ADL antibodies and 51/187 (27.3%) patients tested positive for anti-INF antibodies; none of the patients treated with ETN developed ADA (Fig. 1A). Significantly more patients with SpA [70/294 (23.8%)] had ADA than did patients with RA [44/276 (15.9%); P = 0.015). There was no significant difference between these two patient groups testing positive for anti-ADL antibodies [SpA: 35/107 (32.7%) *vs* RA: 28/110 (25.5%); P = 0.221] or anti-INF antibodies [SpA: 35/112 (31.3%) *vs* RA: 16/76 (21.1%); P = 0.114].

Overall, a significantly lower proportion of patients receiving concomitant DMARDs [61/369 (16.5%)] *vs* those receiving anti-TNF monotherapy [53/201 (26.4%)] tested positive for ADA (P = 0.004; Fig. 1B). This difference in ADA response between patients receiving concomitant DMARDs and those not was also observed for patients treated with ADL [32/133 (24.1%) *vs* 31/84 (36.9%); P = 0.037] and those treated with INF [29/130 (22.3%) *vs* 22/58 (37.9%); P = 0.021; Fig. 1B]. A statistical analysis could not be performed for patients treated with ETN because no patient developed ADA (Fig. 1B).

Correlation between ADA, serum drug concentrations and RA markers

Overall, 92/114 (80.7%) patients who tested positive for ADA had no detectable drug in the serum (Fig. 1C). In

Table 1 Patient demographics (whole cohort; $n = 570$)

Parameter		SpA	RA	*P*-value[a]	Total
Female	*N*	293	275	<0.001	568
	n (%)	114 (38.9)	220 (80.0)		334 (58.8)
Age, years	*N*	292	274	<0.001	566
	Mean (S.D.)	47.9 (11.5)	56.3 (12.1)		52.0 (12.5)
Disease duration, years	*N*	288	266	0.075	554
	Mean (S.D.)	12.5 (10.2)	13.9 (8.7)		13.2 (9.5)
Anti-TNF drug treatment	*N*	294	276	<0.05	570
Adalimumab	*n* (%)	107 (36.4)	110 (39.9)		217 (38.1)
Etanercept	*n* (%)	75 (25.5)	90 (32.6)		165 (28.9)
Infliximab	*n* (%)	112 (38.1)	76 (27.5)		188 (33.0)
Duration of current treatment, months	*N*	290	272	0.004	562
	Mean (S.D.)	50.7 (40.7)	60.9 (42.8)		55.6 (42.0)
Concomitant DMARDs	*N*	294	276	<0.0001	570
	n (%)	138 (46.9)	229 (83.0)		367 (64.4)
MTX	*n* (%)	103 (74.6)	176 (76.9)		279 (76.0)
LEF	*n* (%)	16 (11.6)	50 (21.8)		66 (18.0)
SSZ	*n* (%)	24 (17.4)	7 (3.1)		31 (8.4)

[a]Pearson's χ^2 test. N: total number of patients evaluated; n: number of patients in that parameter.

patients treated with ADL, 52/63 (82.5%) who tested positive for ADA had no detectable drug in the serum. Likewise, in patients treated with INF, 40/51 (78.4%) testing positive for ADA had no detectable drug in the serum. This analysis could not be performed on patients treated with ETN, because none of them developed ADA. One patient each in the ADL- and INF-treated groups had drug concentrations within the normal range even though they tested positive for ADA.

Discussion

In this observational study of patients with RA or SpA and secondary failure treated with anti-TNF agents, we found that 20% of the study population tested positive for ADA. All these patients were treated with either ADL or INF; no patient treated with ETN tested positive for ADA. Overall, the rates of ADA formation were similar among patients treated with ADL and patients treated with INF. Of the 114 patients who developed ADA, 81% had no detectable drug concentrations in their serum. The development of ADA, therefore, appears to be a major contributor to reduced serum drug concentrations and, consequently, secondary treatment failure in this population. Other factors contributing to secondary failure potentially include compliance, obesity and monotherapy with biologics; however, these were beyond the scope of the present study and, consequently, not evaluated.

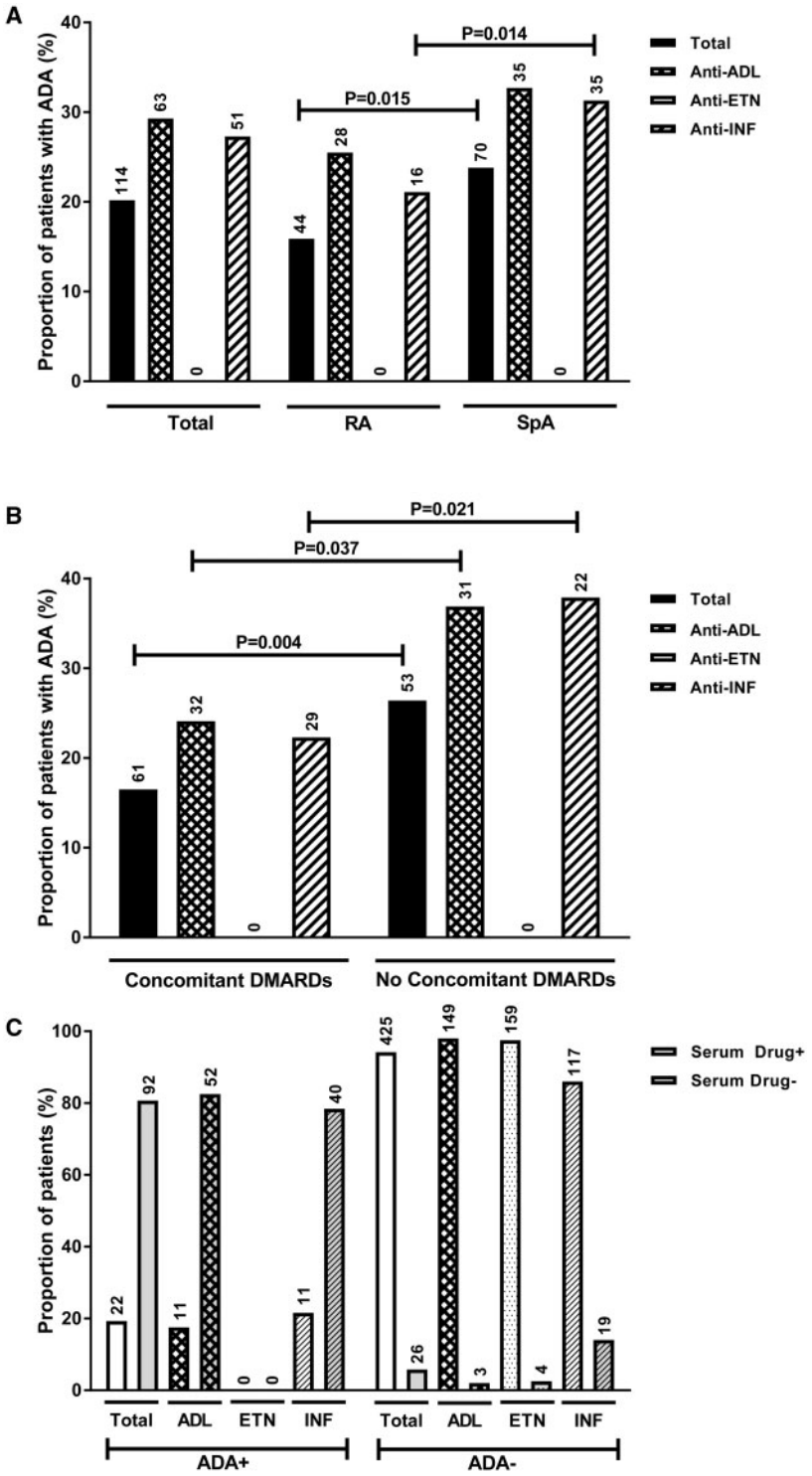
The absence of anti-ETN antibodies in our study is corroborated by other studies of the immunogenicity of ETN [4, 11, 12]. Similar studies in patients with RA have reported the presence of anti-ADL antibodies in 7–54% of patients [4, 13, 14] and anti-INF antibodies in 10–47% of patients [13, 15]. The wide variability of detection methods, thresholds, patient demographics, disease severity and the use of concomitant DMARDs in these studies could have contributed to the range in the rates of ADA reported. Nevertheless, the prevalence of ADA reported in our study, by far the largest, is consistent with previous reports.

In our study, patients with SpA appeared significantly more likely to develop anti-INF antibodies than those with RA. The reason for this is unclear. Our analysis showed that more than half the patients with SpA testing positive for ADA received INF monotherapy, whereas patients with RA all received concomitant DMARDs. This interpretation is supported by the fact that in the overall population, a significantly lower proportion of patients receiving concomitant DMARDs developed ADA compared with those receiving biologic monotherapy.

When ADA are present, serum drug concentrations are usually undetectable. Secondary loss of efficacy attributable to low serum concentrations is not uncommon with TNF inhibitors. In the ATTRACT trial on patients with RA, 20–30% of patients receiving 3 mg/kg INF every 8 weeks had undetectable pre-infusion trough serum concentrations from week 22 to 54 of treatment; the majority of these patients also demonstrated poor clinical response [16]. In RA patients, low pre-infusion serum INF concentrations may herald the formation of anti-INF antibodies and are associated with a higher risk of treatment failure [15]. Development of ADA often results in the formation of drug–antibody complexes, which may be eliminated much faster than unbound drug, thereby changing the pharmacokinetic profile of the drug, resulting in potential loss of efficacy [17].

In our study, we identified a proportion of patients with ADA and detectable low concentrations of drug. Similar results were reported by Chen *et al*. [18] in a different population. Although this is not usual with bridging ELISA, drug interference has been reported in some assays [19]. There were also several patients in the ADL- and INF-treated groups who had low or no drug concentrations and no detectable ADA. Although poor compliance to therapy could be an explanation when it comes

Fig. 1 Proportion of patients testing positive for anti-drug antibodies by disease and concomitant DMARDs status



(**A**) Proportion of patients testing positive for ADA by disease. (**B**) Proportion of patients testing positive for ADA by concomitant DMARDs status. (**C**) Correlation between ADA status and serum drug concentration. Numbers above columns indicate the number of patients in that group. ADA: anti-drug antibodies; ADL: adalimumab; ETN: etanercept.

to patients receiving ADL, which is self-administered, it cannot be a cause of low drug concentrations for INF-treated patients, because this drug is administered in a hospital. Another possible reason is that those patients had ADA, but the real frequency of immunogenicity is underestimated when using bridging ELISA because free serum drug concentrations and drug–antibody complexes cannot always be detected by this method [20].

This cross-sectional observational study design provided some insights into medical practice in Spain but also uncovered some limitations. Sample collection from patients at a single time point without follow-up prevented any longitudinal analysis of clinical responses or the impact of historical treatments. It is also possible that there might be variations among health professionals in the routine use of the different clinical screening scales and treatment decisions. It is assumed that higher doses of biologics might have been administered by some physicians to counteract loss of efficacy. However, pharmacokinetic modelling suggests that decreasing the dose interval would raise low trough serum concentrations more effectively than increasing the dose [16].

ELISAs are subject to false-positive and false-negative results caused by non-specific binding or epitope masking [20]. Thus, owing to interference in the bridging ELISA, it is possible that patients identified as having low concentrations of drug in their serum might have no drug present. Furthermore, the kit used to measure ETN could not discriminate between drug concentrations, so analyses were limited to the presence or absence of ETN.

In conclusion, we identified ADA in almost 30% of the population of patients treated with ADL or INF for rheumatic disease. No ADA were detected against etanercept. Although the presence of ADA was clearly a contributing factor of secondary failure to therapy in most cases, immunogenicity could not explain all cases of failure. Further investigations could help to determine all possible causes of failure of anti-TNF therapy.

Acknowledgements

Medical writing support was provided by Samantha Forster, PhD and Mukund Nori, PhD, MBA, CMPP of Engage Scientific Solutions, and funded by Pfizer.

Author contributions: All authors were involved in drafting the article or revising it critically for important intellectual content and all authors approved the final version to be published. All authors had full access to all the study data. Drs Balsa and Montoro take responsibility for the integrity of the data and the accuracy of the data analysis.

REASON Study Group: Dr A. J. Alegre López, Hospital General Yagüe, Burgos; Dr C. Alegre de Miguel, Hospital Valle Hebrón, Barcelona; Dr A. Alonso Ruiz, Hospital de Cruces, Barakaldo, Vizcaya; Dra. M. Alperi López, Hospital Central de Asturias, Oviedo; Dr M. Álvarez de Mon Soto, Hospital Príncipe de Asturias, Alcalá de Henares, Madrid; Dr J. Belzunegui Otano, Hospital de Donosita, San Sebastián; Dr A. Bermúdez Torrente, Hospital de Arrixaca, Murcia; Dra. S. Bustabad Reyes, Hospital Universitario de Canarias, Tenerife; Dra. C. Campos Fernández, Hospital Gral. de Valencia, Valencia; Dra. V. Jovani Casano, Hospital Gral. Universitario de Alicante, Alicante; Dra. Mª C. Castro Villegas, Hospital Reina Sofía de Córdoba, Córdoba; Dr H. Corominas, Hospital Moises Broggi, Barcelona; Dr J. Cruz Martínez, complejo Hospitalario de Pontevedra, Pontevedra; Dra. C. Delgado Beltrán, Hospital Clínico Lozano Blesa, Zaragoza; Dra. C. Fernandez Carballido, Hospital de Elda, Alicante; Dr A. Fernández Nebro, Hospital Carlos Haya, Málaga; Dr F. Gamero Ruiz, Hospital San Pedro de Alcántara, Cáceres; Dra. R. Mª García Portales, Hospital Virgen de La Victoria, Málaga; Dra. R. García De Vicuña, Hospital Universitario de La Princesa, Madrid; Dr C. M. Gonzalez Fernandez, HGU Gregorio Marañón, Madrid; Dr A. Gómez, Hospital Parc Taulí, Sabadell, Barcelona; Dr X. Juanola Roura, Hospital de Bellvitge, Barcelona; Dr A. García Sánchez, Hospital Virgen de las Nieves, Granada. Dr J. Mª de Llobet Zubiaga, Hospital Santa Creu i Sant pau, Barcelona; Dr J. L. Marenco de la Fuente, Hospital de Valme, Sevilla; Dr J. M. Martin Santos, Hospital Rio Hortega, Valladolid; Dr V. Martínez Taboada, Hospital Marqués de Valdecilla, Santander; Dra. I. Mateo Bernardo, Hospital Doce de Octubre, Madrid; Dr J. Maymó Guarch, Hospital del Mar, Barcelona; Dr F. Medina Varo, Hospital Puerta del Mar, Cádiz; Dra. C. Moll Tuduri, Hospital Mateu Orfilla, Menorca, Baleares; Dr F. Navarro Sarabia, Hospital Virgen Macarena, Sevilla; Dr J. Orte Martinez, Hospital Ramón y Cajal, Madrid; Dra. T. Pérez Sandoval, Hospital de León, León; Dr J. del Pino Montes, Hospital de Salamanca, Salamanca; Dr C. Rodríguez Lozano, Hospital Dr Negrín, Las Palmas de Gran Canaria; Dr J. Mª Salazar Vallinas, Hospital Infanta Cristina, Badajoz; Dr J. Salvatierra Ossorio, Hospital San Cecilio, Granada; Dra. A. Sanchez-Andrade Fernández, Hospital Lucus Augusti, Lugo; Dr J. Santos Rey Rey, Hospital Virgen de la Salud, Toledo; Dr J. Victor Tovar Beltrán, Hospital de Elche, Alicante; Dra. P. Trenor Larraz, Hospital Clínico de Valencia, Valencia; Dr E. Úcar Angulo, Hospital de Basurto, Bilbao.

Funding: This study was sponsored and funded by Pfizer.

Disclosure statement: R.S. is a member of the Advisory Board of the la MHDA Evaluation Committee, Cat-Salut, Generalitat de Catalunya, has participated in conferences/advisory boards sponsored by Abbott/AbbVie, Bristol-Myers Squibb, Merck Sharp & Dohme, Roche, UCB and Pfizer and has received funding for research projects from AbbVie, Bristol-Myers Squibb, Merck Sharp & Dohme, Pfizer, Roche and UCB. M.M., A.C. and S.G. are employees of and own stock in Pfizer. V.M. was an employee of TFS working for Pfizer. A.B. reports receiving grant/research support from Pfizer, Roche, UCB, Bristol-Myers Squibb, Novartis and consultancy fees from AbbVie, Bristol-Myers Squibb, Lilly, Merck Sharp & Dohme, Novartis, Pfizer, Roche and UCB. J.R. is a member of Advisory Boards for AbbVie, Bristol-Myers Squibb, Gebro, Janssen, Lilly, Merck Sharp & Dohme, Pfizer, Roche and UCB.

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