Scandinavian Journal of Rheumatology

ISSN: 0300-9742 (Print) 1502-7732 (Online) Journal homepage: https://www.tandfonline.com/loi/irhe20

Association of female sex and positive rheumatoid factor with low serum infliximab and anti-drug antibodies, related to treatment failure in early rheumatoid arthritis: results from the SWEFOT trial population

K Hambardzumyan, C Hermanrud, P Marits, N Vivar, S Ernestam, JK Wallman, RF van Vollenhoven, A Fogdell-Hahn, S Saevarsdottir & for the SWEFOT study group

To cite this article: K Hambardzumyan, C Hermanrud, P Marits, N Vivar, S Ernestam, JK Wallman, RF van Vollenhoven, A Fogdell-Hahn, S Saevarsdottir & for the SWEFOT study group (2019) Association of female sex and positive rheumatoid factor with low serum infliximab and anti-drug antibodies, related to treatment failure in early rheumatoid arthritis: results from the SWEFOT trial population, Scandinavian Journal of Rheumatology, 48:5, 362-366, DOI: 10.1080/03009742.2019.1602670

To link to this article: https://doi.org/10.1080/03009742.2019.1602670

Association of female sex and positive rheumatoid factor with low serum infliximab and anti-drug antibodies, related to treatment failure in early rheumatoid arthritis: results from the SWEFOT trial population

K Hambardzumyan[1], C Hermanrud[2], P Marits[3], N Vivar[1], S Ernestam[4], JK Wallman[5], RF van Vollenhoven[1,6], A Fogdell-Hahn[2], S Saevarsdottir[1,7], for the SWEFOT study group

[1]Rheumatology Unit, Department of Medicine, Solna, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden
[2]Clinical Neuroimmunology, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
[3]Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden
[4]Academic Specialist Centre, Stockholm Health Services, Stockholm, Sweden
[5]Department of Clinical Sciences Lund, Rheumatology, Lund University, Lund, Sweden
[6]Amsterdam Rheumatology and Immunology Center, Amsterdam, The Netherlands
[7]Unit of Translational Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Objective: Infliximab-treated patients with rheumatoid arthritis (RA) may respond insufficiently due to low serum infliximab (sIFX) levels, caused by anti-drug antibodies (ADAs). However, monitoring of sIFX and ADAs is not routinely implemented, and levels for optimal outcome have not been validated. We searched for predictors for sIFX < 0.2 μg/mL and ADA development in a randomized setting.

Methods: In the SWEFOT trial, of 128 patients randomized to methotrexate + IFX therapy, 101 had serum samples at 3, 9, and 21 months that were analysed for sIFX [enzyme-linked immunosorbent assay (ELISA)] and ADAs [ELISA, and precipitation and acid dissociation (PandA) when sIFX > 0.2 μg/mL]. The primary and secondary outcome measures were low disease activity [LDA = 28-joint Disease Activity Score (DAS28) ≤ 3.2] and remission (DAS28 < 2.6). Baseline characteristics were assessed as potential predictors of sIFX < 0.2 μg/mL or ADA positivity, using logistic regression.

Results: Categorization of sIFX levels into < 0.2, 0.2–2.9, 3.0–7.0, and > 7.0 μg/mL showed a dose–response association with LDA (30%, 64%, 67%, and 79%, respectively, p = 0.008) and remission (10%, 45%, 39%, and 66%, p = 0.004) at trial cessation (21 months). Female patients had sIFX < 0.2 μg/mL more often than males (35% vs 7%, p = 0.006), with a similar trend for rheumatoid factor (RF)-positive vs RF-negative patients (34% vs 16%, p = 0.059). ADA positivity showed similar patterns, also after adjustment for potential confounders (female sex: p = 0.050; RF positivity: p = 0.067). PandA captured four highly ADA-reactive patients with sIFX > 0.2 μg/mL, of whom three were ADA positive at other time-points, all with high DAS28 at follow-up.

Conclusion: In early RA patients receiving IFX as a second-line agent, sIFX < 0.2 μg/mL and ADA development were associated with treatment failure and were more common in females, with a similar trend for RF positivity. Our findings support the use of therapeutic drug monitoring, and PandA in ADA-negative non-responders.

Trial registration: SWEFOT NCT00764725 (https://clinicaltrials.gov/ct2/show/NCT00764725).

Approximately one-third of patients with rheumatoid arthritis (RA) respond poorly and one-third lose the response to biological drugs, including the tumour necrosis factor (TNF) inhibitor infliximab (IFX) (1, 2). Immunogenicity is considered an important reason for treatment failure, and the proportion of RA patients reported to develop anti-drug antibodies (ADAs) against IFX ranges from 12% to 54% (3, 4). Furthermore, observational studies, mostly in established disease, have found an association between ADA and/or low serum infliximab (sIFX) levels and lack of response (5, 6). Despite this, sIFX or ADA measurement are still not incorporated into clinical guidelines, most probably owing to insufficient evidence concerning optimal methods, recommended drug levels, and how to act on the results

Saedis Saevarsdottir, Institute of Environmental Medicine, Nobels väg 13, Karolinska Institutet, SE-17176 Stockholm, Sweden.
E-mail: saedis.saevarsdottir@ki.se

Accepted 29 March 2019

DOI: 10.1080/03009742.2019.1602670

(3). IFX is still a widely used second-line treatment in early RA, being the first TNF inhibitor with biosimilar alternatives available at a lower cost. Thus, it is of high importance to optimize its therapeutic use.

Here, in an ad-hoc study on the IFX-treated arm of the randomized early RA SWEFOT trial (7), we studied associations of sIFX and ADA with recommended treatment goals [European League Against Rheumatism (EULAR) low disease activity (LDA) or remission], and looked for baseline predictors of low sIFX or ADA development.

Method

Study population

This study was based on the SWEFOT randomized, controlled trial of early RA patients (N = 493), which was conducted in accordance with the Declaration of Helsinki and approved by the regional ethics committee (7). Of 128 patients randomized to add-on IFX (3 mg/kg), following 3 months of methotrexate (MTX) monotherapy initiated at diagnosis, 101 had clinical data and serum available over 21 months of follow-up (the trial period). Eight patients lacked serum samples at 3 months and six patients at 9 months (6 and 12 months from inclusion/RA diagnosis, respectively). LDA [28-joint Disease Activity Score (DAS28) ≤ 3.2] was used as the primary, and remission (DAS28 < 2.6) as the secondary outcome measure. Rheumatoid factor (RF) and anti-CCP antibody positivity was tested at RA diagnosis/inclusion in routine clinic at recruiting centres.

Measurement of sIFX and ADA

Levels of sIFX and ADA were measured with a validated enzyme-linked immunosorbent assay (ELISA) (8) used in clinical routines at Swedish University Hospitals. ADAs were measured in those with very low sIFX levels (< 0.2 μg/mL). Patients were defined as ever ADA positive if they were positive at any follow-up time-point. Samples with sIFX levels ≥ 0.2 μg/mL were categorized as low (0.2–2.9 μg/mL), proposed optimal (3.0–7.0 μg/mL), and high (>7.0 μg/mL) levels, as suggested by a publication on inflammatory bowel disease (9). Since ELISA is unreliable for ADA detection if the drug level is ≥ 0.2 μg/mL, samples with a level < 7 μg/mL were also tested using the precipitation and acid dissociation (PandA) method (10) on the Meso Scale Discovery® platform. The cut-off point for ADA reactivity was statistically determined to be ≥ 1.15 relative electrochemiluminescence (RECL).

Statistical analysis

We used the non-parametric Mann–Whitney U test and the chi-squared test to compare continuous and categorical variables, respectively. The relationship of sIFX categories and ADA positivity with clinical outcomes was assessed by the Mantel–Haenszel test for trend. Baseline parameters were assessed as potential predictors of developing ADAs by univariate and multivariate logistic regression analyses, adjusting for sex, RF, current smoking, and Health Assessment Questionnaire score, i.e. parameters showing a trend association ($p < 0.2$) with ADA development. Parameters with high collinearity, i.e. anti-CCP antibodies and RF, as well as DAS28 and its components, were not included simultaneously in the model. Analyses were performed using SPSS Statistics, version 25 (IBM Corp., Armonk, NY, USA).

Results

Baseline characteristics between IFX-treated patients included in this study (N = 101) and the remaining patients (N = 157) randomized to triple therapy or lacking serum samples did not differ, except for pain and patient global assessment on a 100 mm visual analogue scale (median 47 vs 39, $p = 0.050$, and 49 vs 44, $p = 0.003$, respectively).

Association of sIFX levels and ADAs with treatment outcome

The frequencies of very low sIFX levels increased over time, with 15%, 23%, and 28% at 3, 9, and 21 months from IFX start, respectively. The majority of patients with very low sIFX levels were ADA positive at these time-points [71% (10/14), 82% (18/22), and 68% (19/28), respectively]. Of patients with very low sIFX levels at any time-point (n = 39), 34 (87%) were ADA positive at least once, and we refer to this group as ever ADA positive.

The proportion of patients with LDA was numerically higher at all follow-up time-points among those with sIFX ≥ 0.2 μg/mL compared with patients who had sIFX < 0.2 μg/mL and positive ADAs (Figure 1A), although only significant at 21 months (67% and 26%, $p = 0.002$). Similar results were observed when remission was the outcome measure (47% vs 11%, $p = 0.004$) (Figure 1B).

Next, we stratified patients into four groups of sIFX levels (< 0.2, 0.2–2.9, 3.0–7.0, and > 7.0 μg/mL) (9), and observed a dose–response association between sIFX and likelihood of LDA (30%, 64%, 67%, and 79% respectively, $p = 0.008$) (Figure 1C) or remission (10%, 45%, 39%, and 66%, respectively, $p = 0.004$) (Figure 1D).

Serum samples with sIFX between 0.2 and 7 μg/mL (n = 82) were tested for free and bound ADAs with the PandA method (10) (Figure 2). Of these, 42 were ADA reactive (≥ 1.15 RECL), but only four had high

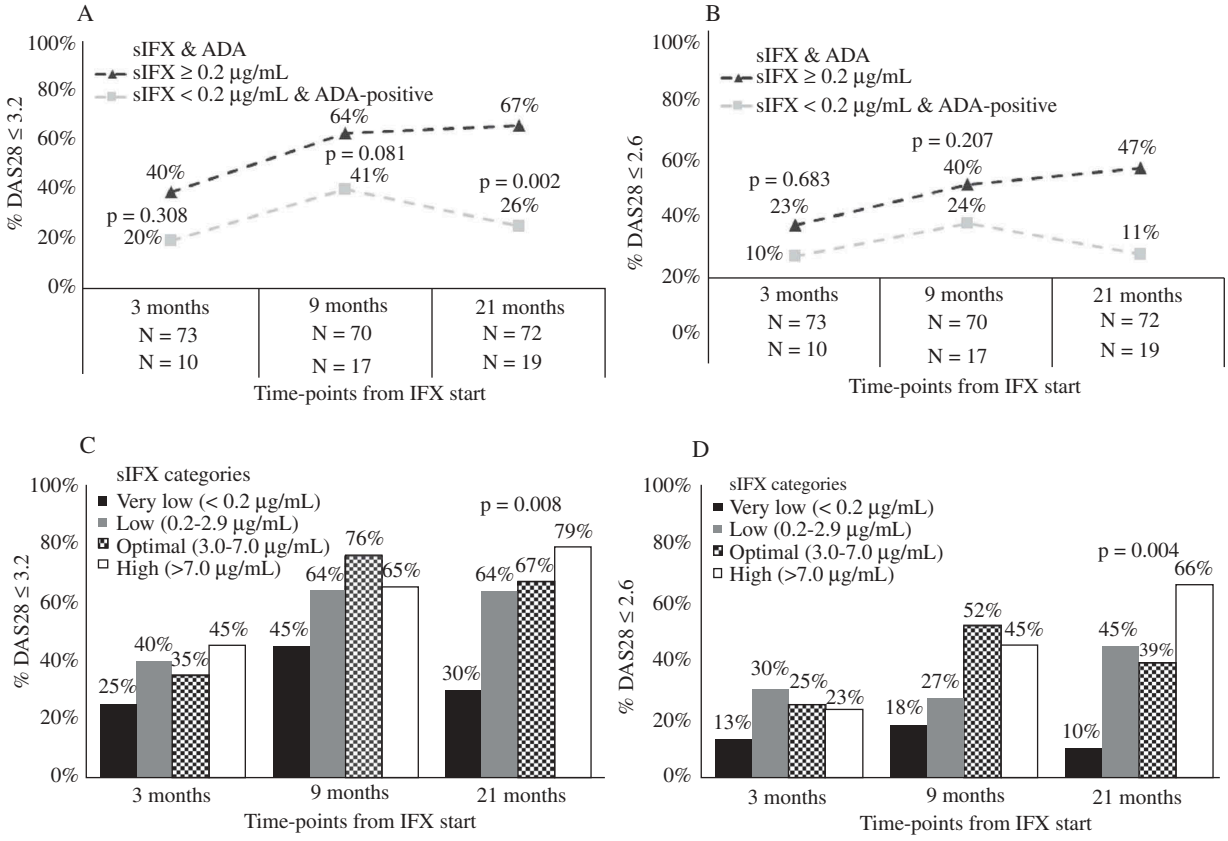

Figure 1. Clinical outcome of early rheumatoid arthritis (RA) patients at 3, 9, and 21 months after infliximab treatment start, stratified for serum infliximab (sIFX) and anti-drug antibody (ADA) status. Proportion of patients (A) in low disease activity (LDA) and (B) in remission among patients with sIFX level ≥ 0.2 µg/mL (black triangles) and ADA-positive patients with sIFX levels < 0.2 µg/mL (grey squares). Proportion of patients (C) in LDA and (D) in remission among four subgroups of patients according to sIFX level: very low sIFX level (black bars), 0.2–2.9 µg/mL (grey bars), 3.0–7.0 µg/mL (chequered bars), and > 7.0 µg/mL (white bars). DAS28, 28-joint Disease Activity Score.

reactivity (> 25 RECL). Three of those were ADA positive at other time-points and all four had high DAS28 at follow-up. The proportion of ADA-reactive patients increased during follow-up: 37% at 3 months and 55% at 21 months (data not shown).

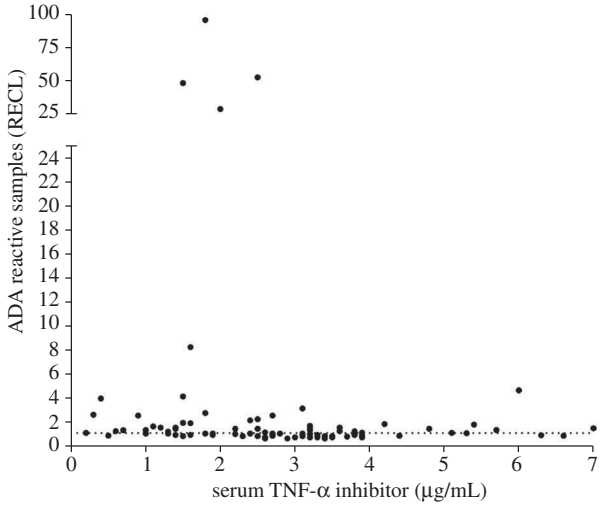

Figure 2. Levels of anti-drug antibodies (ADA) [expressed as relative electrochemiluminescence (RECL)] in serum of early rheumatoid arthritis patients with infliximab (IFX) levels ≥ 0.2 µg/mL (0.2–7.0 µg/mL), using the precipitation and acid dissociation (PandA) method. TNF-α, tumour necrosis factor-α.

Factors predicting ADA development and very low sIFX levels during follow-up

Among baseline characteristics, RF positivity and female sex tended to be associated with both very low sIFX and ADA positivity (Figure 3 and Supplementary table S1). These findings remained after adjustment for potential confounders in a multivariate analysis (for ADA positivity: p = 0.067 and 0.050, respectively).

Discussion

In this randomized trial-based early RA study, where patients received add-on IFX if they did not respond to MTX as the first-line agent, we found that low sIFX and ADA positivity were associated strongly with treatment failure. Furthermore, a clinically meaningful dose–response association was observed between sIFX levels and likelihood of LDA or remission. Thus, our findings were consistent with, but extend, previous

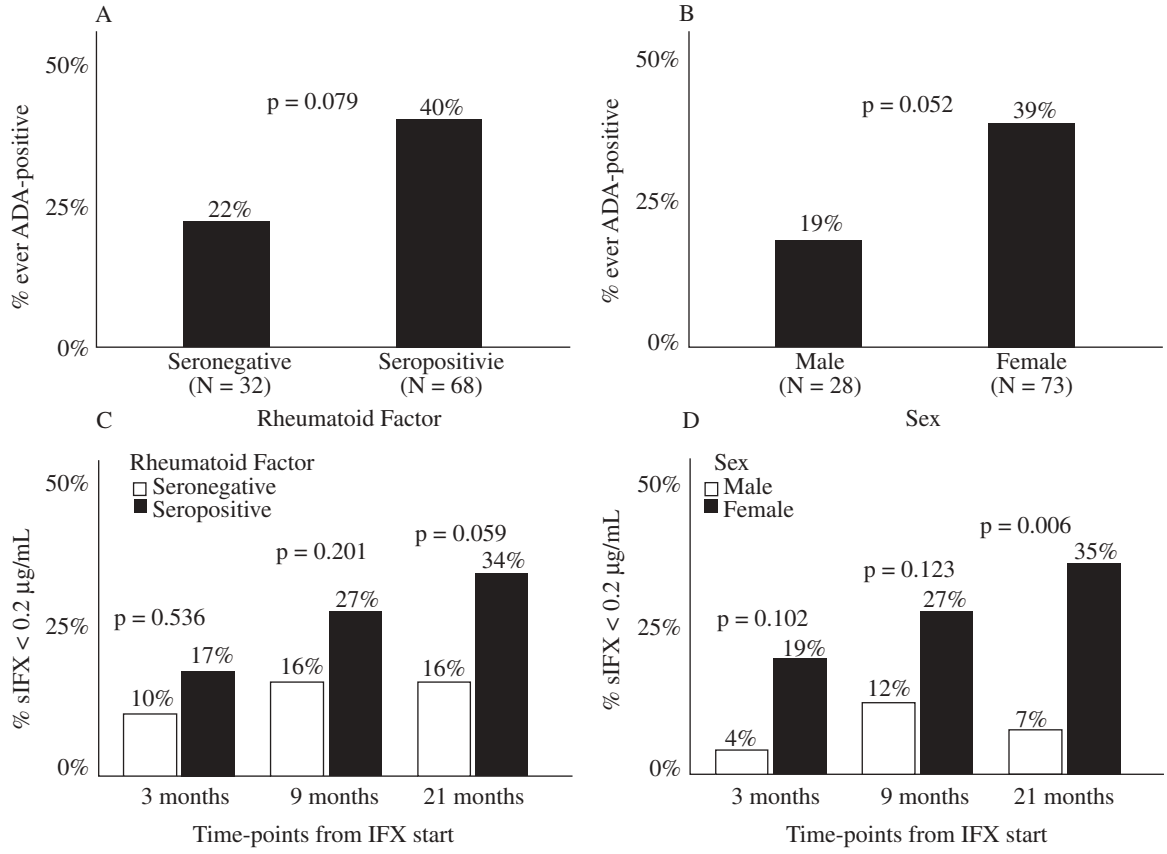

Figure 3. Associations of sex and rheumatoid factor (RF) status with ever anti-drug antibody (ADA) positivity and serum infliximab (sIFX) levels during follow-up of early rheumatoid arthritis (RA) patients. Proportion of ADA-positive patients (A) among RF-negative and RF-positive patients, and (B) among men and women. Proportion patients with sIFX levels < 0.2 µg/mL (C) among RF-negative (white bars) and RF-positive (black bars) patients, and (D) among men (white bars) and women (black bars).

reports, which are mostly non-trial based and consider established disease (1, 2, 5, 6, 11).

Previously reported cut-offs of sIFX that are used in routine clinical practice (8) worked reasonably well, except that sIFX > 7 µg/mL had better outcome than the recommended level of 3–7 µg/mL. The dose–response association we observed is in accordance with, but extends a recent non-trial-based report in RA patients with a mean disease duration of 14 years (12).

Female sex and RF positivity were related to lower sIFX and ADA positivity, which is, to our knowledge, a novel and clinically relevant finding. In two previous studies on established RA, there was no significant relationship between RF positivity and ADAs to different biological agents, but the study populations included varied widely (4, 13). Meanwhile, Takeuchi et al found in established RA an association between low autoantibody titres at baseline (RF and anti-CCP) and high levels of sIFX at follow-up (14). Although RF may interfere with standard cross-linking ELISA, our assay is an inhibition assay and control sera with high-titre RF do not give false-positive results. In female patients, we observed ADAs more often and they were five times more likely to have undetectable sIFX, which is interesting since women are more prone to developing autoantibody-positive autoimmune diseases, and show stronger immunological responses to vaccination than men (15). Thus, although the mechanism is unclear, women may be more prone to develop ADAs towards biological drugs.

The strengths of our study are a well-characterized trial-based study group, where early RA patients received IFX add-on if not responding to MTX treatment, in accordance with current treatment guidelines. Few patients had missing data or samples, thus minimizing potential selection bias. The main limitation is that serum was taken at follow-up visits and not directly before the IFX infusion. This may skew the associations towards a false-negative finding, but at the same time, this is likely to make the observed associations more robust and applicable for the everyday clinical setting. Since ADAs cannot be measured with ELISA in patients with sIFX ≥ 0.2 µg/mL (thus assuming them to be ADA negative, as is current practice in routine clinic), we tested those samples with the PandA method and identified approximately half of them to be ADA reactive, but only four had high reactivity. Three of those four were ADA positive at other follow-up time-

points, and were thus included in the ever ADA-positive group. All had high DAS28 at follow-up. Therefore, adding the patients captured by PandA did not change our overall findings. However, this indicates that it may be worthwhile to go further with PandA measurement in non-responders without ADAs using ELISA.

Conclusion

In summary, early RA patients receiving IFX add-on to MTX, who had low serum IFX levels or ADAs at follow-up, had a lower likelihood of achieving LDA or remission. A clinically meaningful dose–response difference was observed. A higher proportion of female and RF-positive patients had very low sIFX and ADAs, which is of interest given the fact that these parameters are well known to be associated with worse outcome. Thus, our findings support the monitoring of serum drug levels, and call for validation in larger populations and for dose-adjustment studies, to optimize the outcome of IFX-treated RA patients.

Acknowledgements

We would like to thank all trial participants, nurses and rheumatologists who made the SWEFOT trial possible (listed in reference 7). In addition, we express our gratitude to people involved in the laboratory analyses.

The analyses of serum infliximab concentration and anti-drug antibodies were covered by a SWELIFE-VINNOVA grant. This work was supported in part by a grant from the Swedish Rheumatism Association. Some of the authors were supported by clinical research funds from Stockholm County (ALF funds). The original clinical trial received an unrestricted grant from Schering-Plough Sweden, which was used to support a study coordinator and a medical monitor.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. Rahman MU, Strusberg I, Geusens P, Berman A, Yocum D, Baker D, et al. Double-blinded infliximab dose escalation in patients with rheumatoid arthritis. Ann Rheum Dis 2007;66:1233–8.
2. Wolbink GJ, Voskuyl AE, Lems WF, de Groot E, Nurmohamed MT, Tak PP, et al. Relationship between serum trough infliximab levels, pretreatment C reactive protein levels, and clinical response to infliximab treatment in patients with rheumatoid arthritis. Ann Rheum Dis 2005;64:704–7.
3. Wadhwa M, Knezevic I, Kang HN, Thorpe R. Immunogenicity assessment of biotherapeutic products: an overview of assays and their utility. Biologicals 2015;43:298–306.
4. Quistrebert J, Hassler S, Bachelet D, Mbogning C, Musters A, Tak PP, et al. Incidence and risk factors for adalimumab and infliximab anti-drug antibodies in rheumatoid arthritis: a European retrospective multicohort analysis. Semin Arthritis Rheum. Published online 12 October 2018. doi:10.1016/j.semarthrit.2018.10.006.
5. Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT, et al. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. Arthritis Rheum 2006;54:711–15.
6. Bendtzen K, Geborek P, Svenson M, Larsson L, Kapetanovic MC, Saxne T. Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor α inhibitor infliximab. Arthritis Rheum 2006;54:3782–9.
7. van Vollenhoven RF, Ernestam S, Geborek P, Petersson IF, Cöstel L, Waltbrand E, et al. Addition of infliximab compared with addition of sulfasalazine and hydroxychloroquine to methotrexate in patients with early rheumatoid arthritis (Swefot trial): 1-year results of a randomised trial. Lancet 2009;374:459–66.
8. Marits P, Landucci L, Sundin U, Davidsdottir L, Nilsson J, Befrits R, et al. Trough s-infliximab and antibodies towards infliximab in a cohort of 79 IBD patients with maintenance infliximab treatment. J Crohns Colitis 2014;8:881–9.
9. Vande Casteele N, Ferrante M, Van Assche G, Ballet V, Compernolle G, Van Steen K, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology 2015;148:1320–9 e3.
10. Zoghbi J, Xu Y, Grabert R, Theobald V, Richards S. A breakthrough novel method to resolve the drug and target interference problem in immunogenicity assays. J Immunol Methods 2015;426:62–9.
11. Radstake TRDJ, Svenson M, Eijsbouts AM, van der Hoogen FH, Enevold C, van Riel PL, et al. Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. Ann Rheum Dis 2009;68:1739–45.
12. Siljehult F, Arlestig L, Eriksson C, Rantapaa-Dahlqvist S. Concentrations of infliximab and anti-drug antibodies in relation to clinical response in patients with rheumatoid arthritis. Scand J Rheumatol 2018;27:1–6.
13. Eng GP, Bouchelouche P, Bartels EM, Bliddal H, Bendtzen K, Stoltenberg M. Anti-drug antibodies, drug levels, interleukin-6 and soluble TNF receptors in rheumatoid arthritis patients during the first 6 months of treatment with adalimumab or infliximab: a descriptive cohort study. PLoS One 2016;11:e0162316.
14. Takeuchi T, Miyasaka N, Inui T, Yano T, Yoshinari T, Abe T, et al. High titers of both rheumatoid factor and anti-CCP antibodies at baseline in patients with rheumatoid arthritis are associated with increased circulating baseline TNF level, low drug levels, and reduced clinical responses: a post hoc analysis of the RISING study. Arthritis Res Ther 2017;19:194.
15. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol 2014;35:347–69.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Supplementary table S1. Characteristics of SWEFOT participants at the time of randomization to IFX stratified into ever and never anti-drug antibody-positive patients.

Please note that the editors are not responsible for the content or functionality of any supplementary material supplied by the authors. Any queries should be directed to the corresponding author.