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Incidence and risk factors for adalimumab and infliximab anti-drug antibodies in rheumatoid arthritis: a European retrospective multicohort analysis

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ABSTRACT

Objectives

To evaluate the incidence of anti-drug antibody (ADA) occurrences and ADA-related risk factors under adalimumab and infliximab treatment in rheumatoid arthritis (RA) patients.

Methods

The study combined retrospective cohorts from the ABIRISK project totaling 366 RA patients treated with adalimumab (n= 240) or infliximab (n=126), 92.4% of them anti-TNF naive (n=328/355) and 96.6% of them co-treated with methotrexate (n=341/353) with up to 18 months follow-up. ADA positivity was measured by enzyme-linked immunosorbent assay. The cumulative incidence of ADA was estimated, and potential bio-clinical factors were investigated using a Cox regression model on interval-censored data.

Results

ADAs were detected within 18 months in 19.2% (n=46) of the adalimumab-treated patients and 29.4% (n=37) of the infliximab-treated patients. The cumulative incidence of ADA increased over time. In the adalimumab and infliximab groups respectively the incidence was 15.4% (5.2–20.2) and 0% (0–5.9) at 3 months, 17.6% (11.4–26.4) and 0% (0–25.9) at 6 months, 17.7% (12.6–37.5) and 34.1% (11.4–46.3) at 12 months, 50.0% (25.9–87.5) and 37.5% (25.9–77.4) at 15 months and 50.0% (25.9–87.5) and 66.7% (37.7–100) at 18 months. Factors associated with a higher risk of ADA development were: longer disease duration (1–3 vs. <1 year ; adalimumab: HR 3.0, 95% CI 1.0–8.7 ; infliximab: HR 2.7, 95% CI 1.1–6.8), moderate disease activity (DAS28 3.2–5.1 vs. <3.2 ; adalimumab: HR 6.6, 95% CI 1.3–33.7) and lifetime smoking (infliximab: HR 2.7, 95% CI 1.2–6.3).

Conclusions

The current study focusing on patients co-treated with methotrexate for more than 95% of them found a late occurrence of ADAs not previously observed, whereby the risk continued to increase over 18 months. Disease duration, DAS28 and lifetime smoking are clinical predictors of ADA development.

KEYWORDS

rheumatoid arthritis; anti-drug antibodies; anti-TNF treatment; incidence; risk factors

1. INTRODUCTION

Biopharmaceuticals are an important class of drug therapies commonly used in clinical practice. Nine biopharmaceuticals are now licensed for the treatment of rheumatoid arthritis (RA) in the EU, including infliximab since 1999 and adalimumab since 2003. Biopharmaceuticals are usually used as a second line treatment after failure of conventional synthetic disease-modifying anti-rheumatic drug therapy,[1]. In spite of this progress, primary or secondary failure in the response to biopharmaceuticals is frequent,[2-3]. One of the main potential causes of failure is the development of anti-drug antibodies (ADA),[4-5]. This unwanted immune response could induce biopharmaceutical neutralization and hypersensitivity reactions that are IgE or non-IgE mediated,[6]. ADA production is the final stage of a complex immune process from antigen presentation to activation of both adaptive and regulatory cellular immune responses,[7]. Importantly, primary nonresponse to anti-tumor necrosis factor (TNF) therapy could be related to disease mechanisms that are relatively TNF-independent, whilst secondary nonresponse could be explained by ADA formation,[8]. The measurement of ADAs could assist in predicting which patient could benefit from switching to a second TNF blocker rather than switching to a different mechanism of action,[8], and the prevention of ADA formation could increase the period during which the patient benefits from treatment. The identification, prediction and prevention of anti-drug immunization are thus major goals in biopharmaceutical development,[9].

ADA development has a multifactorial aetiology that has not yet been fully elucidated. Many factors (patient-, disease- or drug-related) contribute to the immunogenicity of biopharmaceuticals,[10]. Some of these bio-clinical factors such as the length and the dose of

the treatment or the route of exposure are easily actionable, while others, such as genetic factors, are risk factors for ADA production that could help to stratify patients. Only a few risk factors for the formation of ADAs, such as lack of concomitant use of methotrexate or not being naïve to TNF treatment, have already been identified,[11-12]. Therefore identifying additional risk factors for ADA development (and subsequent lack of treatment efficacy or hypersensitivity reactions) could be of great interest to the clinician,[13].

The frequency of ADA development varies across studies depending on the treatment and to the type of assay used. Methods to detect ADAs are as numerous as are the interpretations of the results, for instance the definition of a positive threshold or cut-off,[14]. A previous prospective observational cohort found ADAs against adalimumab in 28% of the patients after 3 years of treatment,[4]. The reported rate of ADA occurrence against infliximab in clinical studies ranges from 10 to 50%,[15].

The objective of the current study was to evaluate the incidence of ADA occurrences under adalimumab and infliximab treatment, to identify patient-related, disease-related and drug-related factors associated with the occurrence of ADAs, and finally to analyse the factors potentially influencing drug serum levels and the response to treatment.

2. PATIENTS AND METHODS

2.1 Patients

Demographic and clinical data from RA patients from 6 historical cohorts from 3 European countries were anonymized, standardized and uploaded into the ABIRISK (Anti-Biopharmaceutical Immunization: prediction and analysis of clinical relevance to minimize the RISK) database (tranSMART software). The ABIRISK tranSMART database has been described in more detail elsewhere,[16]. The populations eligible for inclusion in adalimumab and infliximab retrospective longitudinal analyses were selected on the basis of the following criteria: (i) at least 1 dose of adalimumab or infliximab, (ii) at least 1 serum sample in the time-slot 0-18 months following the first biopharmaceutical treatment date, (iii) age over 18 at date of first biopharmaceutical dose.

The dataset analysed included biopharmaceutical-treated RA patients from France, Sweden and the Netherlands (Amsterdam and Leiden). The cohorts were heterogeneous in terms of numbers of patients and monitoring schemes (Supplementary Figure 1). In France, the data came from the ESPOIR cohort (Etude et Suivi des Polyarthrites Indifférenciées Récentes) which is a prospective study on patients with early arthritis from 15 centers followed for more than 10 years,[17]. These patients had 1 or 2 samples collected at random time points in the period up to 18 months after the start of therapy. In Sweden, the patients were participants in the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) cohort with clinical follow-up data by the Swedish Rheumatology Register and those who had available blood samples within the given follow-up period were included,[18]. These patients had 1 sample collected at random time in the period up to 18 months after the start of therapy. The cohort data from EIRA database, Swedish Rheumatology Register, and RA Biobank were retrieved,

integrated and queried using the methods described in [19-20]. In Amsterdam, the data was collected from a cohort of consecutive patients treated with adalimumab,[21] and infliximab,[22]. The adalimumab group had 1 to 3 samples collected and the infliximab group had 1 to 6 samples collected at time points fixed by the studies. In Leiden, the data originated from 2 clinical trials: IMPROVED (Induction therapy with MTX and Prednisone in Rheumatoid Or Very Early arthritic Disease) and BeSt (Behandel Strategieën),[23-24]. From IMPROVED, we selected patients treated with methotrexate plus adalimumab who had 1 sample collected at a fixed visit at 4 months,[23]. From BeSt, we selected patients treated with infliximab and who had samples collected once after 12 months of follow-up,[24].

Ethical approval and subject consent for using these samples for research on RA were obtained in each country by the cohort investigators.

2.2 Biological sample testing

Biologic drug (adalimumab or infliximab) and anti-drug antibody serum levels in treated patients were measured using the Lisa-Tracker® Duo enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instruction (Theradiag®, Marne-la-Vallée, France). This assay enables the simultaneous detection of both drug and ADA, in a micro-well plate format in which half of the plate is coated with TNF-α, to measure drug concentration, and the other half is coated with the drug (adalimumab or infliximab), for the detection of the corresponding ADAs. A calibration range enabled cut-off concentrations to be determined for biopharmaceuticals at 0.3 µg/mL and for ADA at 10 ng/mL. All these assays were performed in a single site, an ABIRISK reference laboratory (Clinical Immunology Laboratory, Kremlin Bicêtre Hospital, AP-HP, France). Patients were defined as positive for ADAs if titres were above 10 ng/mL on at least 1 occasion. The original study protocols were specific to each

cohort, therefore patients could have 1 or several visits and stored serum samples (Supplementary Figure 1). Time lapses between the serum collections at each visit and the previous drug injection were not clearly known. Therefore drug serum levels were not considered as trough concentrations.

2.3 ADA outcome

The primary outcome event of interest was the occurrence of a positive follow-up sample in the ADA assay. The time-to-event for positive ADA was calculated from the date of the first treatment to the time of the first positive sample or last follow-up (drop-out, drug switch or censoring at 18 months). The available information was obtained from the monitoring schemes. Thus for this study, the only available information on the time-to-occurrence for positive ADA was whether or not it exceeded some given time points. This particular kind of data is known as interval-censored data and requires specific methods that differ widely from those used for classic right-censored data,[25-26]. Interval-censored data are often encountered in longitudinal studies where the event of interest is not directly observed but is only known to lie within the interval of two pre-scheduled visits. Moreover, in this study there was an unexpected increase in the level of complexity due to the fact that we had to deal with very different monitoring schemes. In practice, for some cohorts there was a fixed monitoring time point (e.g. Leiden) whereas for others there were random monitoring time points. In this latter case, single (Sweden) or multiple time points (France, ESPOIR or Amsterdam) were possible. This heterogeneity in the sampling pattern is important to take into account since it can lead to some informative censoring problems that can induce false associations. The censoring is called informative when it provides information regarding the survival distribution and the factors under study,[27]. Thus, in order to cope with this complex data,

we used interval-censored methods and analysed cohorts having a similar sampling scheme. This led us to exclude the Leiden cohort, IMPROVED, for adalimumab and the Amsterdam cohort for infliximab when investigating prognostic factors for ADA.

2.4 Response to treatment

We also investigated the clinical response to treatment, which was assessed according to the European League Against Rheumatism (EULAR) response criteria,[28] based on the disease activity score in 28 joints (DAS28),[29], calculated between the date of each sample collection and just before the date of the first biopharmaceutical dose. EULAR response was therefore calculated at the same time as sample collections and considered as a binary outcome, a non-response versus a moderate/good response.

2.5 Statistical analysis

We present the results of separate analyses for adalimumab and infliximab. The baseline characteristics of the patients were compared between groups using Fisher's exact tests (for small samples). For quantitative variables, Kruskal-Wallis tests were used (no normality assumption). The Kruskal-Wallis test was used to compare drug serum levels between positive and negative ADA patients. When significant, exact p-values were calculated using Dunn's nonparametric test. To measure the correlation between ADA serum levels and drug serum levels, Kendall's rank correlation coefficient was used. The cumulative incidence of ADA over the study was calculated as the complement of the survival function S under interval censoring. The non-parametric maximum likelihood estimation (NPLME) of the survival function $\hat{S}$ was obtained using Turnbull's algorithm,[30]. The 95% confidence intervals (95% CIs) were calculated with a modified bootstrap method. In order to describe

ADA development over time, ADA survival distribution curves were plotted using the cumulative hazard estimation obtained previously. To compare the survival curves we used logrank tests adapted for interval-censored data and we reported the p-values. The test is an extension of the usual logrank test from right-censored data, as developed by Sun,[31]. In the multivariable analyses, we considered the Cox proportional hazards model via a multiple imputation strategy for unobserved survival times as proposed by Pan,[32]. Briefly, the interval-censored data are considered as missing event times and are imputed by the asymptotic normal data augmentation scheme based on the current estimates of the observed data. Then a Cox proportional hazards model is applied to the augmented data to update the estimates. Hazard ratios (HRs) and their 95% CIs were reported. The multivariable model was adjusted for cohort as a covariate and included all the variables explored in univariable analyses: age (18-50 years old vs. >50), sex, anti-TNF naivety (yes/no, for adalimumab group only), lifetime smoking status (yes/no), disease duration before start of the biopharmaceutical treatment (<1 year, 1-3 years, >3 years), positivity for rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) (yes/no), baseline DAS28 (<3.2, 3.2-5.1, >5.1), concomitant use of methotrexate (yes/no, for adalimumab group only), concomitant non-steroid anti-inflammatory drugs (NSAIDs) and corticosteroids (yes/no). Patients with missing data for at least one explanatory variable were excluded. A linear mixed-effects model was fitted to evaluate the association between drug serum levels and ADA status. The multivariable model included variables that may be potential confounders. Some patients could have been tested several times, therefore, a random effect parameter was estimated in order to consider the dependency of repeated measures. A Cox proportional hazards model was used to assess the benefit of the treatment (i.e. moderate or good versus non-response according to the EULAR criteria) as a function of the ADA status and drug serum levels.

Some patients could have had several visits and it was assumed that the model identified dependent observations.

For all tests, statistical significance was considered to be a p-value under 0.05. All analyses were carried out using R software (version 3.0.2) and related packages “survival”, “interval” and “MIICD”,33-36].

3. RESULTS

A total of 366 RA patients fulfilled the inclusion criteria. Of these, 240 were treated with adalimumab and 126 with infliximab. The flowchart shown in Figure 1 gives the details of the selection process. Patients’ characteristics at baseline are shown in Tables 1 and 2. Among adalimumab-treated patients there were significant differences between cohorts regarding age, percentages of previous anti-TNF treatment, follow-up, disease duration, ACPA positivity, DAS28 score and concomitant use of methotrexate, NSAIDs and corticosteroids. Among infliximab-treated patients there were significant differences between cohorts regarding age, lifetime smoking status, follow-up, disease duration, ACPA positivity, DAS28 score and the concomitant use of corticosteroids. Over a maximum follow-up of 18 months, ADA were detected in 46 adalimumab-treated patients (19.2%) and 37 infliximab-treated patients (29.4%). The median time to ADA occurrence was 4.5 months (interquartile range IQR 3.7–11.3) in adalimumab-treated patients and 13 months (IQR 11.9–15.0) in infliximab-treated patients. There were 341 and 171 samples available respectively for adalimumab- and infliximab-treated patients, (Supplementary Tables 1 and 2). Positive ADA samples had lower adalimumab and infliximab serum levels than negative ADA samples, significant for adalimumab at 0-6 months ($p=0.002$) and 6-12 months ($p<0.001$) and for infliximab at 6-12

months ($p<0.001$) and 12-18 months ($p<0.001$) after the start of therapy (Supplementary Figure 2). There was a reverse correlation between ADA levels and drug serum levels, significant for both adalimumab (correlation coefficient $\tau=-0.20$, $p<0.001$) and infliximab (correlation coefficient $\tau=-0.51$, $p<0.001$) (Supplementary Figure 2).

Table 1. Demographic and clinical characteristics at baseline in adalimumab-treated patients stratified by cohort

Characteristics	Sweden, EIRA (n = 18)	France, ESPOIR (n = 68)	the Netherlands, Leiden, IMPROVED (n = 62) [b]	the Netherlands, Amsterdam (n = 92)	P-value[c]
Age, mean (SD), years	49.7±14	47.0±11	52.2±13	52.3±13	0.03
Female (%)	16 (88.9)	51 (75.0)	45 (72.6)	68 (74.0)	0.58
Lifetime smoking (%)[a]	10 (55.6)	34 (50.0)	32 (51.6)	49 (63.7)	0.35
Anti-TNF naivety (%)[a]	18 (100)	62(91.2)	62 (100)	61 (75.3)	<0.001
Follow-up, median (IQR), months	13.3 (8.2–11.1)	5.9 (3.3–7.0)	3.7 (3.6–4.1)	9.1 (8.7–9.3)	<0.001
Disease status					
Disease duration, median (IQR), years[a]	1.0 (1.0–2.0)	1.9 (1.1–4.0)	0.8 (0.5–1.1)	5.0 (2.5–11.2)	<0.001
RF positivity (%)[a]	12 (66.7)	47 (69.1)	33 (56.0)	60 (66.0)	0.46
ACPA positivity (%)[a]	14 (82.4)	44 (64.7)	32 (51.6)	68 (74.7)	0.01
DAS28, mean (SD)[a]	5.1±1.5	4.9±1.6	3.6±1.2	5.3±1.1	<0.001
Concomitant anti-rheumatic therapy					
Methotrexate use (%)[a]	15 (93.8)	59 (86.8)	62 (100)	81 (98.8)	<0.001
NSAIDs use (%)	8 (44.4)	35 (51.5)	12 (19.4)	56 (60.9)	<0.001
Corticosteroids use (%)	8 (44.4)	31 (45.6)	0 (0.0)	26 (28.3)	<0.001

[a]Data for categorical or quantitative variables is presented as percentage, mean or median of non-missing data

[b]Cohort excluded from the univariable and multivariable analyses of anti-drug antibody risk factors

[c]P-values of comparison tests between cohorts

SD, standard deviation; IQR, interquartile range; Anti-TNF, anti-tumor necrosis factor; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody; DAS28, disease activity score in 28 joints; NSAIDs, non-steroid anti-inflammatory drugs

Table 2. Demographic and clinical characteristics at baseline in infliximab-treated patients stratified by cohort

Characteristics	Sweden, EIRA (n = 60)	France, ESPOIR (n = 11)	the Netherlands, Leiden, BeSt (n= 43)	the Netherlands, Amsterdam (n =12) [b]	P-value[c]
Age, mean (SD), years	47.7±13	42.6±14	55.5±13	56.7±10	0.004
Female (%)	43 (71.7)	8 (72.7)	26 (60.5)	7 (58.3)	0.56
Lifetime smoking (%)	41 (68.3)	5 (45.5)	16 (37.2)	2 (16.7)	<0.001
Anti-TNF naivety (%)	60(100)	10 (90.9)	43 (100)	12 (100)	0.09
Follow-up, median (IQR), months	13.6 (7.4–15.2)	8.1 (6.0–10.0)	12.0 (11.7–12.3)	5.5 (5.4–5.5)	<0.001
Disease status					
Disease duration, median (IQR), years[a]	1.0 (1.0–2.0)	2.1 (1.5–4.2)	0.4 (0.4–1.1)	7.1 (3.3–16.3)	<0.001
RF positivity (%)	40 (66.7)	7 (63.7)	26 (60.5)	9 (75.0)	0.78
ACPA positivity (%)	40 (66.7)	9 (81.8)	26 (60.5)	12 (100)	0.03
DAS28, mean (SD)	5.1±1.4	4.5±1.2	6.0±0.8	5.4±1.0	<0.001
Concomitant anti-rheumatic therapy					
Methotrexate use (%)[a]	59 (100)	10 (90.9)	43 (100)	12 (100)	0.09
NSAIDs use (%)	38 (63.3)	4 (36.4)	24 (55.8)	7 (58.3)	0.41
Corticosteroids use (%)	23 (38.3)	7 (63.6)	0 (0.0)	2 (16.7)	<0.001

[a]Data for categorical or quantitative variables is presented as percentage or median of non-missing data

[b]Cohort excluded from the univariable and multivariable analyses of anti-drug antibody risk factors

[c]P-values of comparison tests between cohorts

SD, standard deviation; IQR, interquartile range; Anti-TNF, anti-tumor necrosis factor; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody; DAS28, disease activity score in 28 joints; NSAIDs, non-steroid anti-inflammatory drugs

3.1 Incidence and risk factors for ADA development

The cumulative incidences of ADA in the adalimumab group and in the infliximab group were respectively 15.4% (95% CI 5.2–20.2) and 0% (95% CI 0–5.9) at 3 months, 17.6% (95% CI 11.4–26.4) and 0% (95% CI 0–25.9) at 6 months, 17.7% (95% CI 12.6–37.5) and 34.1% (95% CI 11.4–46.3) at 12 months, 50.0% (95% CI 25.9–87.5) and 37.5% (95% CI 25.9–77.4) at 15 months and 50.0% (25.9–87.5) and 66.7% (37.7–100) at 18 months (Figure 2 A-B).

In the univariable analyses of the adalimumab-treated patients from Sweden, France and the Netherlands (Amsterdam), patients with a disease duration over 1 year (p=0.04) and concomitant use of corticosteroids (p=0.003) were at significantly higher risk for the development of ADAs (Supplementary Figure 3). A multivariable Cox regression model was performed on 148 adalimumab-treated patients. The results are reported in Table 3. Patients with longer disease duration (>1 year) had a higher risk of ADA positivity as compared to those with a short disease duration (<1 year), with a significant difference in the years 1-3 (HR, 3.0 95% CI 1.0–8.7). Patients with an initial DAS28 over 3.2 had a higher risk than those having low initial DAS28 (<3.2), with a significant difference for moderate DAS28 between 3.2 and 5.1 (HR, 6.6 95% CI 1.3–33.7).

Table 3. Baseline risk factors for anti-adalimumab antibodies in 148 patients[a]

		HR[c]	95% CI[d]
Multivariable model[b]			
Age	18-50	1.0	reference
	50+	2.0	(0.9 – 4.4)
Sex	Female	1.0	reference
	Male	1.7	(0.6 – 5.2)
Lifetime smoking	No	1.0	reference
	Yes	1.1	(0.5 – 2.3)
Anti-TNF naivety	No	1.0	reference
	Yes	0.8	(0.2 – 3.5)
Disease duration	0-1	1.0	reference
	1-3	**3.0**	**(1.0 – 8.7)**
	3+	2.4	(0.7 – 8.2)
RF positivity	No	1.0	reference
	Yes	1.1	(0.4 – 2.7)
ACPA positivity	No	1.0	reference
	Yes	0.8	(0.3 – 2.3)
Baseline DAS28	Low (0-3.2)	1.0	reference
	Moderate (3.2-5.1)	**6.6**	**(1.3 – 33.7)**
	High (5.1+)	4.0	(0.9 – 18.5)
Methotrexate use	No	1.0	reference
	Yes	0.6	(0.2 – 1.9)
NSAIDs use	No	1.0	reference
	Yes	2.1	(0.9 – 4.6)
Corticosteroids use	No	1.0	reference
	Yes	2.1	(0.9 – 3.9)

[a]Patients from the Netherlands, Leiden, IMPROVED cohort and patients with missing data for at least one risk factor were excluded
[b]Adjusted on cohorts (Sweden EIRA, France ESPOIR, the Netherlands, Amsterdam)
[c]Hazard ratio
[d]95% confidence interval
RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody; DAS28, disease activity score in 28 joints; NSAIDs, non-steroid anti-inflammatory drugs
Bold text indicates a statistically significant hazard ratio

Among the infliximab-treated patients from Sweden, France and the Netherlands (Leiden), there was no statistically significant variable associated with the development of ADA (Supplementary Figure 3). In a multivariable Cox regression model for infliximab-treated patients (n=113) with the same variables as for the adalimumab analysis shown in Table 4 except anti-TNF naivety and concomitant use of methotrexate, we found similar association

of ADAs with disease duration between 1 and 3 years (HR, 2.7 95% CI 1.1–6.8) compared to disease duration shorter than 1 year. Lifetime smoking was associated with a higher risk of ADA (HR, 2.7 95% CI 1.2–6.3). An increase in DAS28 was of borderline significance (HR, 1.3 95% CI 0.9–1.9).

Table 4. Baseline risk factors for anti-infliximab antibodies in 113 patients[a]

		HR[d]	95% CI[e]
Multivariable model[b]			
Age	18-50	1.0	reference
	50+	0.5	(0.2 – 1.1)
Sex	Female	1.0	reference
	Male	2.1	(0.9 – 5.1)
Lifetime smoking	No	1.0	reference
	Yes	**2.7**	**(1.2 – 6.3)**
Disease duration	0-1	1.0	reference
	1-3	**2.7**	**(1.1 – 6.8)**
	3+	0.4	(0.0 – 3.6)
RF positivity	No	1.0	reference
	Yes	1.2	(0.4 – 3.3)
ACPA positivity	No	1.0	reference
	Yes	1.1	(0.4 – 2.9)
Baseline DAS28[c]	Per unit increase	1.3	(0.9 – 1.9)
NSAIDs use	No	1.0	reference
	Yes	0.9	(0.4 – 1.9)
Corticosteroids use	No	1.0	reference
	Yes	1.2	(0.3 – 4.3)

[a]Patients from the Netherlands, Amsterdam cohort and patients with missing data for at least one risk factor were excluded
[b]Adjusted on cohorts (Sweden EIRA, France ESPOIR, the Netherlands, Leiden BeSt)
[c]DAS28 was considered as a continuous variable because of lack of cases in some categorical groups
[d]Hazard ratio
[e]95% confidence interval
RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody; DAS28, disease activity score in 28 joints; NSAIDs, non-steroid anti-inflammatory drugs
Bold text indicates a statistically significant hazard ratio

3.2 Association of drug serum levels with ADA status and baseline factors

In a univariable analysis of ADA status on 341 samples from 240 patients treated with adalimumab, ADA positivity was significantly associated with a lower adalimumab drug serum level (β coefficient, -3.4, 95% CI -5.7 ; -1.2). The mean concentrations of adalimumab were 6.7 µg/mL in ADA positive patients and 10.6 µg/mL in ADA negative patients. After adjusting for potential confounders in a multivariable model performed on 287 samples from 206 patients due to missing data, this association remained the same (β coefficient, -4.7, 95% CI -7.5 ; -1.8) (Supplementary Table 3).

A univariable analysis of 171 samples from 126 patients treated with infliximab showed that ADA positivity was significantly associated with a lower infliximab drug level (β coefficient, -16.1, 95% CI -26.3 ; -6.0). The mean concentrations of infliximab were respectively 0.8 µg/mL in ADA positive patients and 15.9 µg/mL in ADA negative patients. In a multivariable model performed on 170 samples from 125 patients due to missing data and taking into account potential confounders, ADA positivity was still significantly associated with the drug serum level (β coefficient, -20.2, 95% CI -32.0 ; -8.3) (Supplementary Table 3).

3.3 ADA status, drug serum levels and clinical response

The effect of ADA status and drug serum levels on the clinical response to treatment was analysed using a multivariable Cox regression model. ADA positivity was significantly associated with a lower probability of good or moderate EULAR response for 215 adalimumab-treated patients (278 clinical observations available ; HR, 0.58, 95% CI 0.39–0.86) and 125 infliximab-treated patients (149 clinical observations available ; HR, 0.61, 95% CI 0.32–0.76) (Supplementary Table 4).

4. DISCUSSION

This study confirms ADA occurrence in 20% to 30% of RA patients treated with adalimumab or infliximab, for most of them anti-TNF naive and co-treated with methotrexate. Interestingly we observed that ADA production occurred later than expected and increased over the 18 months of follow-up without reaching a plateau. Furthermore, we found that longer disease duration (over 1 year) was a risk factor of ADA development against both adalimumab and infliximab while a higher baseline DAS28 was associated with a higher risk of ADA against adalimumab and lifetime smoking status with a higher risk of ADA against infliximab. The data confirmed that ADA positivity was associated with lower drug serum levels, for both adalimumab and infliximab, and with a poorer clinical response.

This study is the first collaborative cohort analysis exploring the occurrence of ADAs in different populations of RA patients. Its main strength was that it used a large dataset that combined cohorts from 3 European countries and analysed censored time-to-ADA outcome. It is worth noting that the analyses were performed using interval-censored methods that took into account the fact that, in this study, ADA positivity outcome was known only to fall within a monitoring interval. Indeed, use of classic survival methodology for data of this sort can lead to inaccurate conclusions by underestimating the variability of the parameters estimated,[37].

An interesting result highlighted by our study is that, for both treatments, the cumulative incidence of ADAs exhibits a sigmoidal shape, with the appearance of ADAs mainly after 6 to 12 months of treatment. These results were different from those of a previous study which showed that almost 10% of the patients developed ADAs after only 4 weeks and two-thirds of the positive patients had ADAs within 28 weeks,[4]. This is most likely due to the type of

assay and appearance of ADA is highly dependent on the sensitivity of the assays,[38]. The currently ongoing ABIRISK prospective studies, which include 250 patients with RA, will give opportunity to confirm these new findings which, interestingly, could explain why there is a continuous and regular rate of secondary failures without any plateau effect in patients treated with TNF inhibitors. ADAs were detected more frequently in infliximab-treated patients (29.4%) than in adalimumab-treated patients (19.2%). But the median time to ADA occurrence was only 4.5 months in adalimumab-treated patients and 13 months in infliximab-treated patients. This could be due to the heterogeneity of serum collection dates in the different cohorts. Indeed, the first sample collection occurred before 12 months for 223/240 adalimumab-treated patients (92.9%) and 64/126 infliximab-treated patients (50.8%) (Supplementary Tables 5 and 6) and thus the probability of detecting ADA before 12 months was less with infliximab.

To our knowledge, in RA, effect of disease duration as a predictor of ADA formation has not been analysed. Although there is evidence of association between ADA formation and disease activity change,[4,5,39-40], baseline DAS28 as a risk factor that could likely influence the development of ADA while taking into account of time variation, repeated measures or other covariates has not been fully investigated. In our study, longer disease duration and a high DAS 28 increased the risk of ADA occurrence. The impact of disease duration on ADA induction could in part explain why disease duration is an important factor predicting negative response to treatment,[41]. It is worth noting that the borderline significance for either very long disease duration (> 3 years) or very high DAS28 activity (>5.1) could be related to the loss of statistical power on account of the small sample size. Finally we found an association between lifetime tobacco smoking and ADA production in infliximab. In the literature, few studies have described the influence of tobacco consumption on ADA occurrence. In multiple sclerosis (MS), tobacco smoking is associated with the risk of neutralizing antibody

production against interferon-beta and natalizumab therapy,[42-44], although another study has contradicted this association,[45] and the mechanism behind this association is not yet understood. Cigarette smoking affects both cell-mediated and humoral immune response,[46]. However, smoking is a well-known risk factor for RA mainly associated with ACPA formation,[47], suggesting that smoking could be associated with an overall B-cell response in RA. In our study anti-TNF naivety and concomitant use of methotrexate were not associated with ADA development in the adalimumab group although the prior use of anti-TNF is known to increase immunogenicity of a second anti-TNF treatment,[48-49], and combination therapy with methotrexate is known to reduce immunogenicity,[50-51]. However, our study was not powered to analyse the effect of prior anti-TNF treatment or concomitant use of methotrexate since a large majority of the patients (328/355 and 341/353 respectively) were in this situation. In the univariable analysis, patients with concomitant use of corticosteroids in the adalimumab group presented an unexpected risk of ADA occurrence. However it was not significant in the multivariable analysis of both treatments. Although corticosteroids have an immunosuppressive effect, their role in combined therapy to reduce anti-TNF immunogenicity is not clear. In a previous study in Crohn's disease, the hydrocortisone premedication reduced anti-infliximab antibodies concentration but not their formation,[52]. Other studies in RA don't support an influence of corticosteroids on ADA development,[5,53]. A possible explanation to the discordant results between the univariable and multivariate analysis might be that corticosteroids are more often given to patients with higher DAS28 at baseline and the association observed in the univariable test is only due to a confusion bias with disease severity, which is the real factor behind the association and is confirmed significant in the multivariable analysis. This also highlights the importance of taking into account other covariates, which has not been performed in previous studies on ADAs against anti-TNF treatments.

We also confirmed the previously established association between ADA positivity and a decreasing drug serum concentration for both treatments,[11,54]. Diminished drug serum levels are probably consequences of ADA development and can result either from an increased drug clearance via the formation of immune complexes,[55] or via the functional neutralization of the drug via a blocking of its binding to the target, which interferes with the ADA detection assay,[54]. The marked decrease of drug serum concentrations in blood as a result of ADA formation could probably explain the reverse association between ADA positivity and a better clinical response also found in a number of previous studies,[4,40,56-58].

One of the drawbacks of this study was that it gathered cohorts with heterogeneous sampling schemes. As shown in Supplementary Figure 1, the Leiden cohort for adalimumab and the Amsterdam cohort for infliximab were the only two cohorts with pre-scheduled visits while having a shorter follow-up period. Due to an unexpected very low number of events, the follow-up time may have been too short and not sufficient to observe the formation of ADAs. This hypothesis was confirmed by a consistently high drug level in these two cohorts. Furthermore a verification was done on the quality of the stored serum and integrity of immunoglobulins by retesting RF and ACPA on historically positive samples from Amsterdam which were confirmed positive (data not shown). This led us to focus our univariable and multivariable analyses on the cohorts with a similar sampling pattern. Thus, to investigate prognostic factors we excluded the Leiden cohort for adalimumab and the Amsterdam cohort for infliximab, to avoid informative censoring. In addition, the same analyses were performed including the two cohorts that led to identify the same risk factors (Supplementary Tables 7 and 8). In our study, we used a commercial ELISA kit to measure ADA serum level. Although they are more sensitive to drug interference which could have led to an underestimation of the incidence of ADA appearance (in particular at early time points

when the ADA titre was still low), ELISA assays have the advantage of being the most suitable tests for routine use in any hospital or laboratory for reasons of cost and simplicity. Another limitation was that serum samples were collected during routine visits, although ideally trough concentration serum samples should be used in studies to test immunogenicity and treatment response. This is less of a concern for adalimumab where non-trough drug serum levels have previously been shown to be associated with trough serum levels and with ADAs,[49]. In addition, drug serum levels were inversely associated with ADA positivity and concentration, as expected (Supplementary Figure 2). These data represent the real-life clinical practice, where the assessment of trough samples is not always practical.

To conclude, this study provides new insights into adalimumab and infliximab immunogenicity. ADA occurrence appears to be a delayed phenomenon. Even if more than 95% of the patients were co-treated with methotrexate, 20 to 30% of them treated with adalimumab or infliximab developed ADAs and the risk of occurrence continued to increase over time up to 18 months of follow-up, without reaching a plateau. It is interesting to find that in real-life patients, almost all treated with methotrexate, the rate of ADA may be significant and may increase over time. A longer disease duration, a higher baseline DAS28 and lifetime smoking were found to be risk factors for ADA development against adalimumab and/or infliximab. Since ADAs have a negative impact on clinical response and vary inversely with drug serum levels, these factors could be taken into account to tailor therapeutic drug monitoring to each patient.

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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.

COMPETING INTERESTS

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: J Quistrebert, S Hässler, D Bachelet, C Mbogning, A Musters, PP Tak, CA Wijbrandts, M Herenius, SA Bergstra, G Akdemir, M Johannesson, B Combe, B Fautrel, S Chollet-Martin, A Gleizes, N Donnellan, F Deisenhammer, A Hincelin-Mery, A Fogdell-Hahn, N De Vries, T Huizinga, I Abugessaisa, S Saevarsdottir, S Hacein-Bey-Abina, M Pallardy, P Broët: have nothing to disclose. X Mariette received honorarium (less than $10,000) from BMS, GSK, Pfizer, UCB. J Davidson reports being salaried and holding shares from GlaxoSmithKline R&D, outside the submitted work. P Dönnes reports grants from Innovative Medicines Initiative Joint Undertaking during the conduct of the study.

DATA SHARING

Data availability is restricted due to patient data confidentiality. A dataset authorising repeats of the analyses presented in this article is available on request, and should be submitted to the ABIRISK steering committee via P Dönnes (contact: pierre@scicross.com)

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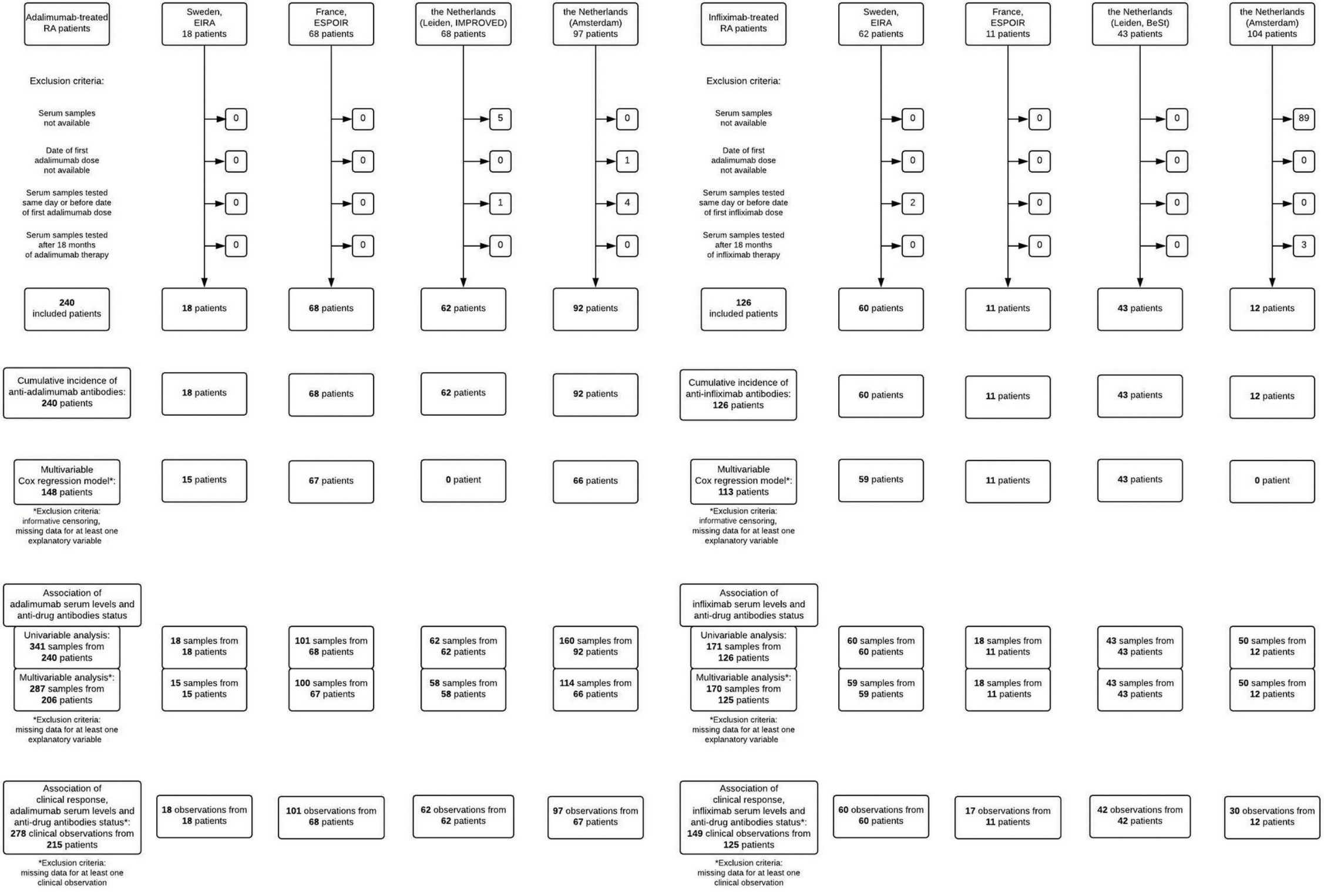
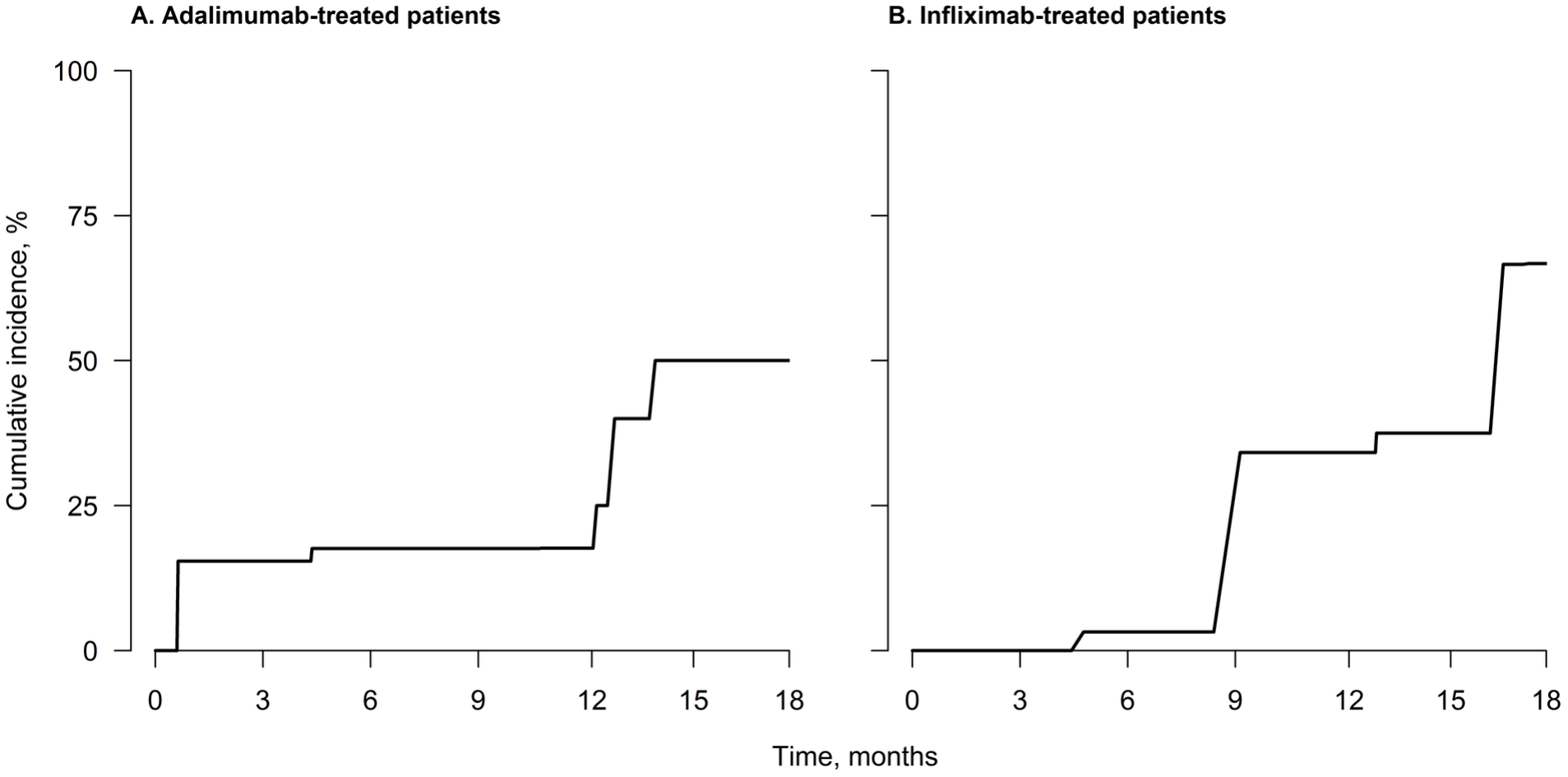
FIGURE LEGENDS

Figure 1. Inclusion flow chart. Flow chart of rheumatoid arthritis (RA) patients per cohort of adalimumab- and infliximab-treated patients

Figure 2. Cumulative incidence of anti-drug antibody (ADA). Cumulative incidence of ADA over a maximum of 18 months follow-up in 240 adalimumab-treated patients (**A**) and 126 infliximab-treated patients (**B**)


Adalimumab-treated RA patients
Sweden, EIRA 18 patients
France, ESPOIR 68 patients
the Netherlands (Leiden, IMPROVED) 68 patients
the Netherlands (Amsterdam) 97 patients
Infliximab-treated RA patients
Sweden, EIRA 62 patients
France, ESPOIR 11 patients
the Netherlands (Leiden, BeSt) 43 patients
the Netherlands (Amsterdam) 104 patients
Exclusion criteria:
Serum samples not available
Date of first adalimumab dose not available
Serum samples tested same day or before date of first adalimumab dose
Serum samples tested after 18 months of adalimumab therapy
0 0 0 0
0 0 0 0
5 0 1 0
0 1 4 0
Exclusion criteria:
Serum samples not available
Date of first adalimumab dose not available
Serum samples tested same day or before date of first infliximab dose
Serum samples tested after 18 months of infliximab therapy
0 0 2 0
0 0 0 0
0 0 0 0
89 0 0 3
240 included patients
18 patients
68 patients
62 patients
92 patients
126 included patients
60 patients
11 patients
43 patients
12 patients
Cumulative incidence of anti-adalimumab antibodies: 240 patients
18 patients
68 patients
62 patients
92 patients
Cumulative incidence of anti-infliximab antibodies: 126 patients
60 patients
11 patients
43 patients
12 patients
Multivariable Cox regression model*: 148 patients
15 patients
67 patients
0 patient
66 patients
*Exclusion criteria: informative censoring, missing data for at least one explanatory variable
Multivariable Cox regression model*: 113 patients
59 patients
11 patients
43 patients
0 patient
*Exclusion criteria: informative censoring, missing data for at least one explanatory variable
Association of adalimumab serum levels and anti-drug antibodies status
Univariable analysis: 341 samples from 240 patients
Multivariable analysis*: 287 samples from 206 patients
18 samples from 18 patients
15 samples from 15 patients
101 samples from 68 patients
100 samples from 67 patients
62 samples from 62 patients
58 samples from 58 patients
160 samples from 92 patients
114 samples from 66 patients
*Exclusion criteria: missing data for at least one explanatory variable
Association of infliximab serum levels and anti-drug antibodies status
Univariable analysis: 171 samples from 126 patients
Multivariable analysis*: 170 samples from 125 patients
60 samples from 60 patients
59 samples from 59 patients
18 samples from 11 patients
18 samples from 11 patients
43 samples from 43 patients
43 samples from 43 patients
50 samples from 12 patients
50 samples from 12 patients
*Exclusion criteria: missing data for at least one explanatory variable
Association of clinical response, adalimumab serum levels and anti-drug antibodies status*: 278 clinical observations from 215 patients
18 observations from 18 patients
101 observations from 68 patients
62 observations from 62 patients
97 observations from 67 patients
*Exclusion criteria: missing data for at least one clinical observation
Association of clinical response, infliximab serum levels and anti-drug antibodies status*: 149 clinical observations from 125 patients
60 observations from 60 patients
17 observations from 11 patients
42 observations from 42 patients
30 observations from 12 patients
*Exclusion criteria: missing data for at least one clinical observation


A. Adalimumab-treated patients
B. Infliximab-treated patients
Cumulative incidence, %
100
75
50
25
0
0
3
6
9
12
15
18
Time, months