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Is it possible to predict which patients treated with biologic agents for rheumatic diseases will develop anti-drug antibodies?

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ABSTRACT

Objectives

Biologic agents are one of the main treatments for auto-immune diseases such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), and psoriasis arthritis (aPso). These drugs are often associated with good control of the disease, but in some cases, patients develop anti-drugs antibodies (ADABs) which can lead to a failure in the control of the disease. Why only certain patients develop these antibodies is not yet really understood.

The aim of this study is to look for clinical and biological predictors for the development of ADABs when tested in a real life cohort.

Method

In this retrospective study, 297 patients, followed at the unit of rheumatology of the Centre Hospitalier Universitaire Vaudois (CHUV) for a RA, SA, aPso and some with connective tissue disease, were included. The patients had to have at least one measurement of ADABs and drug trough level since 2013 to January 2016. They also had to be exposed to a treatment of anti-TNF agent or non-anti-TNF agent such as rituximab or tocilizumab. The method used for detecting the ADABs was a sandwich ELISA. The reproducibility of the ELISA method and the cut-off for ADABs have been tested among patients exposed and non-exposed to the medication.

Results

63 patients out of 297 developed ADABs, which represents 21% of the total cohort. In univariable analyses, many clinical and biological predictors were significantly associated with ADABs. In multivariable analyses, only four predictors remained significantly associated with ADABs. Clinical predictors independently associated with the development of ADABs were treatment with a monoclonal anti-TNF agent (OR:26, 95% CI:2.6-264), and previous exposure to other biologic agents (OR:5.9, 95% CI: 1.1-30). Two laboratory predictors were also independently associated with the development of ADABs an undetectable through level of the medication (OR:34, 95% CI: 7.2-160) and an high TNF through levels (OR:4.2, 95%

CI:1.1 -15). In patients exposed and tested for ADABs against more than one bDMARD, the percentage of ADABS was: 33 % (not significantly than against one agent: (p-value = 0.08)).

Conclusion

Our study confirms that ADABs can be found in a significant number of patients treated with biological DMARDs. The clinical predictors for developing such ADABs are limited and in line with those found in previous publications.

Key words: anti-drugs antibodies, ADABs, anti-TNF, rituximab, tocilizumab

Introduction:

Many clinical trials have shown that biologic agents such as anti-TNF drugs are highly effective in patients with chronic inflammatory disease (1). Biological disease-modifying anti-rheumatic drugs (bDMARDs) are now widely used in common practice for the treatment of auto-immune disease as rheumatoid arthritis (RA) or ankylosing spondylitis (AS).

Despite this efficacy, some patients are not responding to the biologic agents (primary failures) or lose initial response (secondary failures) which can lead to a progression of the inflammatory disease. For example, about 30% of patients with RA do not respond at all to the biological treatment (2). Recent studies have demonstrated that the development of anti-drugs antibodies (ADABs) could be part of this failure to treatment especially in secondary failures (1-11).

All biologic agents currently used can induce the development of ADABs, which will influence the drug level and the drug effectiveness. It is recognized that these ADABs are one possible mechanism to explain the failure of anti-TNF drugs in inflammatory disease (2). Neutralizing ADABs inhibit the effect of the medication by blocking the binding site to the target. .Non neutralizing ADABs can create immune complexes which results in an increased drug clearance (1;3;4). These ADABs are not only responsible of failure to treatment; they can also lead to acute and delayed infusion and injection site reaction (1;5).

The probability of ADABs development also depends on the type of treatment. Indeed, it is well known that the immunogenicity varies among the different types of bDMARDs. Parts of

differences depend on the structures of the bDMARDs. One of the most immunogenic bDMARD is infliximab. The rate of ADABs development with the infliximab varies between 6 % to 61 % (3).The high immunogenicity of this compound is partially due to the murine variable region present in this monoclonal antibody (1;2;3;6;7). On the opposite, the one of less immunogenic bDMARD is the etanercept (1;2;3;6;7;8). This bDMARD is a fusion protein of two TNFR2 receptor extracellular domains which is less immunogenic (6). When ADABs against etanercept are present, they are usually non-neutralizing. As mentioned above they can promote immune complexes that favor drug elimination, reducing therefore the effectiveness of the medication (1,2;3;6;8).

Several clinical trials have shown that it is possible to reduce the ADABs formation by adding some co-medication such as immunosuppressive drugs to the biological treatment. For example, azathioprine or methotrexate decrease the ADABs formation by a mechanism not yet known (1;3).

Why only certain patients develop these antibodies is not yet clear. The aim of this study was to look for predictive factors of such antibodies in a cohort of patients treated in real life setting.

Method:

In this retrospective cross-sectional study, we have evaluated 297 patients followed at our rheumatologic unit of the Chuv in Lausanne for rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriasis arthritis (aPso) and some other auto-immune disease such as familial Mediterranean fever and Behçet disease.

Since March 2013, we can measure ADABs and drug trough levels for all anti-TNF agents and also for rituximab and tocilizumab . To be included, the patients had to be at time or before the ADABs testing on a treatment of infliximab, adalimumab, golimumab, certolizumab, etanercept, rituximab or tocilizumab. .

All the patients had at least one measurement of ADABs and drug trough level up to January 2016. ADABs measurements were made either systemically for some drugs like infliximab, or when a loss in treatment effectiveness or a side effect (like hypersensitivity reaction) potentially due to ADABs was suspected.

We used a LISA-TRACKER® ELISA sandwich kit to detect neutralizing serum ADABs .Tthe

drug trough level (see figure 1) was measured by a double ELISA. Both kits were validated by the company Theradiag in collaboration with the immunology lab of the CHUV by cross-checking with:

- Patients with specific ADABs for a specific drug receiving another treatment
- Measure of ADABs on patient with another ELISA kit (Unilabs)
- Radio-immunologic dosages in some doubtful cases (Sanquin lab in the Netherlands).

The clinical predictors for the development of ADABs analysed were: sex and the age of the patients, the disease duration and the type of the disease, the treatment length and the type of treatment, the use of co-medication such as prednisone, methotrexate, leflunomide, sulfazalazine, hydroxychloroquine, azathioprine, cyclosporine and exposure to a previous biologic agent. We also analyzed some biologic predictors: the drug through level of the drug , the TNF level, the C reactive protein (CRP), the erythrocyte sedimentation rate (ESR). The activity rate of the disease was monitored, in particular via CRP and ESR.

Clinical and biological predictors of ADABs development were analyzed using univariable and multivariable regression analysis when the p-value under 0.1 on the univariate analysis. To be significant, the p value had to be under 0.05.

Results:

Among the 297 patients of our cohort, 124 patients were treated with a biologic agent for rheumatoid arthritis, 116 for ankylosing spondylitis, 30 for psoriatic arthritis, 27 for other diagnoses (like Behçet disease, familial Mediterranean fever).

63 patients developed ADABs against at least one bDMARD, which corresponds to a proportion of 21%. All of those were or had been exposed to the medication responsible for the ADABs except for 3 patients (98% specificity) .
The proportion of ADABs positivity varied according to the different biological agent, they are summarized in Table 1. ABAS were found much more frequently when the patients had been exposed to mab anti TNF agents than against other BDMARDs

On univariate analysis we found several significant clinical predictors .

The sex distribution showed a feminine predominance (54% in the ADABs positive group and 69% in the ADABs negative) with a p value of 0.035. The mean duration of the disease was higher in the ADABs positive group: 11 years versus 8 years in the other group, the p-value for this variant was 0.018.

The mean duration of biological treatment usage was also longer in the ADABs positive group 41 months against 28 months in the ADABs negative group, the p-value : 0.015.

Concerning the type of biologic treatment, we found that 58 out of the 63 patients (94%) had ADABS directed against an anti-TNF agent and only 5 patients (6 %) against an another type of biological treatment. The p-value between these variant is < 0.0001.

The exposure to a previous biologic agent before the ADABs measurement was also predictive for ADABs. In the ADABs positive group, 51 patients (79%) had a previous biologic agent and 13 (21 %) had no previous biological treatment. In the ADABs negative group, 149 patients (65%) received a previous biologic agent and 81 (35%) patients had no previous biological treatment (p= 0.027).

The age (mean of 50 in ADABs+ against 53 years in ADABS-) at measurement of ADABS was not different between the two groups(p= 0.11).

The type of the disease was distributed as follows: in the ADABs positive group, 26 patients with AS, 18 patients with RA, 9 patients with aPso and 3 patients with other diagnosis. In the ADABs negative group, we found 105 patients with RA, 87 patients with AS, 27 patients with another diagnosis and 21 patients with aPso. The p-value for the type of disease between the two groups was not significant with a value of 0.09.

The presence of a co-medication at time of measurement was also not different In the ADABs positive group, 22 patients (35%) versus 78 patients (38%) in the ADABs negative. The p-value for these variants is 0.6.

All the biological predictors analyzed the CRP, ESR, drug trough level and the TNF level were found to be predictive of ADABS in univariate analysis.

37% of the patients had an elevated CRP (> 5 mg/L) in the ADABs positive patients versus , 16% in ADABs negative patients (p-value = 0.0005). When considering a high ESR to be more than 20 mm/h, the ESR also was a significant predictor for ADABs development (p-value =0.0001). In the ADABs positive group, 33 (37%) patients had an elevated ESR. In the ADABs negative group, 25 patients (13%) had ESR over 20 mm/h and 162 had a normal one.

The drug trough level and an elevated TNF were both predictors for ADABs development with a p-value respectively at < 0.0001 and 0.0001

The drug trough level was undetectable in 41 patients (81%) in the ADABs positive group. In this group 15 patients had a drug trough level which could be detected. In the ADABs negative group, 36 patients (18%) had an undetectable drug trough level and 162 patients had a detectable drug level. We found an elevated tumor necrosis factor (TNF) in 30 patients (52%) in the ADABs positive group and 28 patients had a normal level. In the ADABs negative group, 51 patients (26%) had a high TNF level, 149 had a normal level.

On multivariable analysis, only four predictors remained significantly associated with the presence of ADABs, two clinical predictors and two laboratory predictors. The clinical predictors were a treatment by a monoclonal anti-TNF agent (OR: 26, 95%, CI: 2.6-264), previous exposure to another biologic agent (OR: 5.9, 95%, CI: 1.1-30). The laboratory predictor: an undetectable through level of the medication (OR: 34, CI: 7.2-160) and an elevated TNF levels (OR: 4.2, 95%, CI: 1.1 -15). All the details of these results are summarized on the table 1.

Discussion:

Our study confirm that in a real life cohort composed mainly of patients with a diagnosis of RA and AS treated with bDMARD that the prevalence of ADBs is nor negligible (around 20%). Our study also confirms that although several clinical and biological factors can be identified as predictors for appearance of ADABS, it remains quite difficult to predict at a individual level which patient will develop such ADABs.

The development of ADABs was significantly higher after the exposition to a monoclonal anti TNF antibody and after a previous exposition to another bDMARD. No other clinical predictors, prove to be significant, despite the data found in the literature (5;6;9). Indeed, in our study, neither the sex nor the age of patients, nor the duration of the disease, nor the type of disease or even the presence of co-medication came out to be significant factors.

In the literature, we found that the most immunogenic bDMARD was the infliximab. This could be easily understood because it is a chimeric biologic agent containing 25 % of murine sequences. (1;3;6). According to some studies, the prevalence of ADABs anti-infliximab is estimate at 14-40 % in RA patients (6). In other studies, the rate of ADABs of infliximab varies from 6 % to 61 %.(3). In our study, the rate of ADABs for the infliximab corresponded approximately to the data in the literature, more frequent than after exposure to a total

human anti TNF antibodies such as golimumab or adalimumab or fusion protein such etancerpt. However, the structure of the compound was not necessarily predictive for the appearance of ADABS. Indeed ADABs were rarely found in patients exposed to a non anti TNF agent even when the antibodies was chimeric like rituximab or not fully humanized such as tocilizumab (see table 1). The target of the compound seems to play a important role and TNF blockers were more immunogenic than non anti TNF agents.

With regards to the rate of ADABs for etanercept, it is known that this fusion TNF blocker protein is less immunogenic than the other monoclonal anti-TNF antibodies. In the literature, we found that the rate of development ADABs for etanercept varied from 0 to 18 %. On the other hand, ADABs against etanercept are reported to be essentially non-neutralizing. In our study, we initially found that 5 % of patients developed neutralizing anti-bodies against this biologic agent. However, in those patients, ADABs were just above the cut-off considered to be positive. We modified the cut-off after the ADABs turned out to be absent when evaluated with a radio-immunoassay test done by Sanquin Company in Netherlands. These cases illustrate some of the problems induced by the dosages of ADABs in particular when using ELISA kits and some of the difficulties to set up cut-offs clinically relevant.

In our study, the most immunogenic agent was certolizumab with a rate of 50 % of ADABs. It doesn't correspond to the literature data. We found that the range of ADABs development for the certolizumab is approximately 3 to 25% (3). Our results may be linked to a selection bias. Indeed, in our study we only had 4 patients under a treatment of certolizumab which probably not a large enough population for a statistical analysis.

It should also be noted that we found ADABs for 3 patients who were not exposed to a treatment of bDMARDs confirming the high specificity of these dosages Moreover we cannot exclude an error either due to a cross-reaction with other antibodies presents in the patient when detecting ADABs or due to the fact that patients have been exposed to bDMARDs without our knowledge

More surprisingly, the association between co-medication and the development of antibodies was only borderline significant (p-value 0.06). This result is probably due to the retrospective design of the study. The ADABs assays were done in a transverse manner, which did not allow us to know precisely when the antibodies appeared in relation to the exposure to co-medication. In some patients, co-medication had been stopped before dosing ADABs (for example in case of good control of the disease, adverse effects etc). Furthermore, some

patients were no more followed at our rheumatologic unit but made the ADABs measurement in our lab. Maybe we did not have the entire list of treatment for these patients which could distort the statistical analysis.

The only two clinical predictors that remained significant after multivariate analysis. We have largely discussed the importance of the target since the treatment with an anti-TNF monoclonal anti-body (OR: 26) were highly associated with ADADBs than non TNF blocker agents

The exposition to a previous biological treatment (OR: 5.9) was also found to be a more often associated with the development of ADABs although the presence of ADBAS against a previous agent was not associated with a significant increase risk of developing ADABS against a new agent confirming literature data suggesting that ADABS are quite agent specific .

Concerning the biological predictors, our data are in line with literature: a high TNF trough level (OR: 4.2) and an undetectable drug trough level (OR:34) are significant predictors of the developing of ADABs. These observations can be easily explained by the fact that when ADABs are produced, they bind to the circulating drug which cannot be detected anymore since only the free drug is revealed by the ELISA kit. Moreover as discussed in the introduction, the immune complexes: drug + ADABs enhance the clearance of the drug leading to a shortened half life. In presence ADABs and low through level of the drug, the disease has more chance to be active inducing an elevated TNF level (1;2;7;8). Why only TNF and not CRP nor ESR two other markers of disease activity remained the single predictive factor of development of ADABs after multivariable analysis remains unclear and need to be further evaluated .

This study has several limitations**.** It is a retrospective one, with a single center experience. The ADABs measurements were made in a cross-sectional fashion and at different times in the management of the patients. This means that some patients had an ADAB dosage at the beginning of their treatment and others while they had a bDMARD for many years .

The number of patients followed at our unit for some of the compounds was limited for some biological treatment (for example, only 4 patients treated by certolizumab).

Moreover, according to the design of the study, no definite causal inference between the potential predictors and ADABs developments can be drawn but rather just an association.

Two predictors could just a reflection of some degree of therapeutic resistance and probably pre-existing ADAB formation: "previous exposure to other biologic agents" (patients who have failed several bDMARDs due to prior ADAB development against the prior agent…) and "high TNF through levels" (patients who did not respond well to common bDMARD dosages and required increasing drug dose…). From an epidemiology point of view, this could be described as a "channelling bias" (or a form of selection bias) in this study population, rather then a true independent predictor of ADAB development.

Our study was finally not designed to evaluate the correlation of ADABS and disease activity nor failure to the medication. Therefore the real clinical significance of ADABs and the usefulness of such dosages cannot be extrapolated from the present study.

Conclusion:

We found rate of developing of ADABs in a real life cohort was quite similar to the data in the literature. We only found only four predictors independently associated with the presence of ADABs. More studies with a larger population followed longitudinally need to be done to find reliable predictors for the development of ADABs, which ultimately could help to prevent the occurrence of these antibodies.

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12. en.wikipedia.org/wiki/ELISA

Annexes:

Table1:

Percentage of ADAB according to the biological agent

	ADAB+ numbers	ADAB+ %
infliximab	46/106	44% **
adalimumab	10/60	16%
certolizumab	2/4	50%
etanercept	1/20	5%*
golimumab	4/34	12%
tocilizumab	1/75	1%
rituximab	4/46	8%

* : 0% after RIA testing

** 22 % when only very high level (10 times above the cut-offs) taken into account

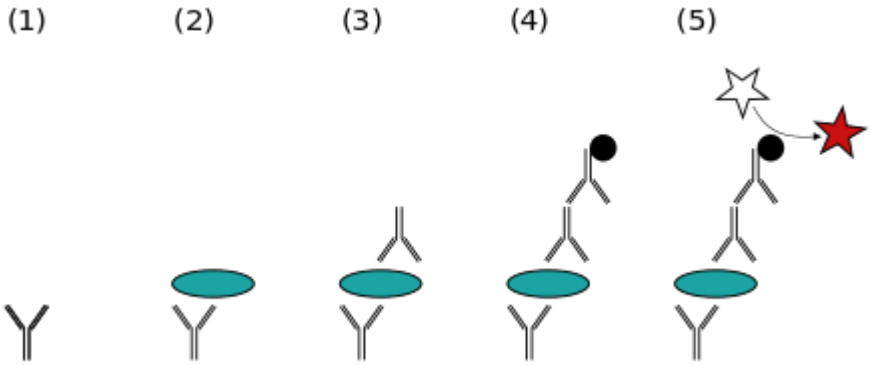
Table 2. :

Clinical and biological predictors for the development of ADABs after monovariate and multivariate analysis

	Monovariate analysis		p	multivariate	p
	ADBA+	**ADAB-**		**ADBA+ OR (CI)***	
Clinical predictors					
Age: mean (SD): years	50(13)	53(014)	0.11		
Sex (F/M/ n (%)	35/30(54%)	157/74 (69%))	0.035		
Type of disease (AS/apso/RA/ others)"	26/9/18/3	87/21/105/27	0.09+		
Duration of disease : mean (SD):years	8.(6)	11(10)	0.018		
Duration of treatments: mean (SD)/months)	41(33)	28(37)	0.015		
Type of treatments (mab ;antiTNF/others) n (%)	60/5 (94/6%))	137/60 (69/31%)	<0-0001	26(2.6-264)	0.005
Co-medication(Y/N/ n (%)	22/40 (35%)	78/122(38%)	0.6		
Previous biologic agent(Y/N/ (%))	51/13 (79%)	149/81 (65%)	0.027	5.9(1.14-30)	0.03
Biological predictors					
CRP: >5, (Y/N/ n (%)	26/44(37%)	31/152(16%)	0.0005		
ESR : >20 (Y/N/ n (%),	33/54(37%)	25/162(13%)	0.0001		
Trough level undetectable(Y/N (%))	41/15 (81%)	36/162/ (18%)	<0.00001	34(7.21-160.8)	0.0001
TNF level elevated (Y/N/ (%)	30/28(52%)	51/149 (26%)	0.00006	4.2(1.1-15)	0.025

Figure 1 :

Elisa double sandwich testing used in the study for the me measurement of ADABS



A sandwich ELISA. (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4)