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Original article

Prealbumin, platelet factor 4 and S100A12 combination at baseline predicts good response to TNF alpha inhibitors in rheumatoid arthritis

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ABSTRACT

Objectives: Tumour necrosis factor-alpha inhibitors (TNFi) are effective treatments for Rheumatoid Arthritis (RA). Responses to treatment are barely predictable. As these treatments are costly and may induce a number of side effects, we aimed at identifying a panel of protein biomarkers that could be used to predict clinical response to TNFi for RA patients.
Methods: Baseline blood levels of C-reactive protein, platelet factor 4, apolipoprotein A1, prealbumin, α1-antitrypsin, haptoglobin, S100A8/A9 and S100A12 proteins in bDMARD naive patients at the time of TNFi treatment initiation were assessed in a multicentric prospective French cohort. Patients fulfilling good EULAR response at 6 months were considered as responders. Logistic regression was used to determine best biomarker set that could predict good clinical response to TNFi.
Results: A combination of biomarkers (prealbumin, platelet factor 4 and S100A12) was identified and could predict response to TNFi in RA with sensitivity of 78%, specificity of 77%, positive predictive values (PPV) of 72%, negative predictive values (NPV) of 82%, positive likelihood ratio (LR+) of 3.35 and negative likelihood ratio (LR−) of 0.28. Lower levels of prealbumin and S100A12 and higher level of platelet factor 4 than the determined cutoff at baseline in RA patients are good predictors for response to TNFi treatment globally as well as to Infliximab, Etanercept and Adalimumab individually.
Conclusion: A multivariate model combining 3 biomarkers (prealbumin, platelet factor 4 and S100A12) accurately predicted response of RA patients to TNFi and has potential in a daily practice personalized treatment.

1. Introduction

Rheumatoid Arthritis (RA) is a frequent inflammatory chronic disease characterized by joint inflammation leading to joint destruction which is responsible for functional disability [1]. Biologic disease-modifying anti-rheumatic drugs (bDMARD) such as tumor necrosis factor-alpha inhibitors (TNFi) have completely changed the outcomes and the prognosis of patients suffering from RA. Nowadays we have access to a broader range of DMARDs targeting different molecular mechanisms such as TNFα, B cells, T cells, anti-IL6 or Janus Kinase pathways. Those new molecules appeared to be as effective as TNFi [2]. Nevertheless despite the overall improvement of quality of life, around 30–50% of patients

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do not respond to bDMARDs [3] and some patients respond to TNFi and other rather to non-TNFi biotherapy without known reason [4]. European League Against Rheumatism (EULAR) recommends the prescription of bDMARD in RA patient with insufficient response to conventional synthetic DMARD (csDMARD) [5]. Currently, rheumatologists do not have evidence-based knowledge to choose the most suitable bDMARD for his patients and these biologics are often prescribed in a 'trial-and-error' manner. From this situation non-responders are unnecessarily exposed to undesired side-effects along with the worsening of their physical condition. Furthermore, the ineffective use of biologics has a dramatic burden on the medico-economic resources regarding the important cost of these treatments [6]. Thus, the major current challenge in RA therapy is being able to predict drug responsiveness *prior* to treatment initiation [7], mainly by identifying relevant predictive biomarkers.

In a previous study, we observed two distinct protein profiles in RA patients associated with a good or bad response to infliximab [8]. Apolipoprotein A-1 was predictive of a good response to infliximab, whereas platelet factor 4 was associated with non-responders [8] suggesting the possibility of a personalized treatment strategy. We have previously identified S100A8/A9 proteins, involved in the inflammatory response in RA, as biomarkers allowing discriminating RA subjects from other miscellaneous inflammatory arthritides subjects [9]. Furthermore dimer S100A8/A9 has been reported as good predictive biomarker to etanercept in RA [10]. Haptoglobin, α-1 antitrypsin, C-reactive protein (CRP) and prealbumin which are involved in inflammatory processes and/or described in RA [11–14] may represent potential predictive biomarkers.

In this study, we aimed to identify predictive biomarkers allowing generating an algorithm capable of discriminating good responders to TNFi (adalimumab [ADA], etanercept (ETN) and infliximab [IFX]) in RA before treatment initiation.

2. Methods

2.1. Patient samples and study design

Fifty-three RA patients were included in a French multicentric (Grenoble, Saint-Etienne, Clermont-Ferrand, and Lyon) prospective study approved by the Medical Ethics Committee of the institute (04PHR06) and registered in *Agence nationale de sécurité du médicament et des produits de santé* (ANSM) (number 050218). This number of patients was considered as relevant since sample size calculation indicated that 39 patients was sufficient to detect a high area under the curve (AUC) value above 0.75. All patients: (i) were naïve from bDMARD, (ii) fulfilled the 1987 American college of Rheumatology (ACR) criteria for RA [15] and displayed (iii) either contraindication or insufficient response to conventional synthetic (cs) DMARD or nonsteroidal anti-inflammatory drugs. The type of TNFi therapy were left to the discretion of the rheumatologist. Plasma samples were collected before the initiation of the TNFi treatment and did not modify biomedical or health-related outcome of the patient. Patients were evaluated clinically at baseline and 6 months of treatment. Within the RA cohort, 12 patients were given IFX 3 mg/kg at week 0, 2, 6 and every 8 weeks thereafter; 14 received ETN 50 mg/week, and 27 patients were treated with ADA 40 mg/14 days.

2.2. Assessment of clinical response

Demographic parameters and disease activity were collected at baseline and 6 months following TNFi instauration using the disease activity score (DAS-ESR) in 28 joints [16]. Response to TNFi treatment was assessed by the EULAR criteria, based on the 28-joint disease activity score (DAS28) [17]. The patients were categorized into responders and non-responders based on the change in the EULAR response [18]. Patient is considered as responder (R) in case of good EULAR response and as non-responder (NR) in case of moderate or absence of EULAR response.

2.3. Assessment of blood biomarkers

Plasma collection protocol was standardized and homogeneously performed in all hospital centers participating to the study. Briefly, blood was collected in EDTA tube (Becton Dickinson) and centrifuged at 1800 g for 10 minutes at room temperature. Plasma was aliquoted in presence of proteases inhibitors (Complete EDTA free protease inhibitor cocktail–Roche) and frozen at −80 °C. Baseline plasma levels of apolipoprotein A1, haptoglobin, prealbumin, C-reactive protein and α-1 antitrypsin were evaluated by nephelometry on a Dimension Vista® lab system (Siemens Healthcare). Platelet factor 4 (PF4) (Abcam®), S100A12 (CircuLex™), and S100A8/S100A9 (Hycult) dimer were evaluated using commercial ELISA kits according to the manufacturers' instructions.

2.4. Statistics

Biomarkers and demographic characteristics at baseline were compared using Fisher's exact test and the Mann–Whitney non-parametric test. The associations between baseline biomarkers and clinical response at 6 months were tested in univariate and multivariate logistic regression models. Biomarker values were log transformed to reach normal distribution and were analyzed through quantitative and dichotomized values. Dichotomization into high or low values was based on median. Biomarkers with a $P < 0.20$ and Area Under the Curve (AUC) > 0.6 in univariate logistic regression test were selected and combined into a multivariate model. Multivariate logistic regression with stepwise forward selection was performed to build the final combined model. Using the fitted model, a probability of response could be obtained for any patient by applying the logistic function $(\exp(L)/(1+\exp(L))$ in which the estimated logit L was calculated from the coefficients of the regression model. Model performances were studied using Receiver Operating Characteristic (ROC) curves and the AUC calculation. Sensibility, specificity, positive and negative predictive values and likelihood ratios were calculated based on the optimal cut-off (Youden's Index). Exact binomial confidence limits are calculated for test sensitivity, specificity, and positive and negative predictive value. Confidence intervals for positive and negative likelihood ratios are based on formulae provided by [19].

2.5. Bootstrapping

The performance of a predictive model is overestimated when calculated on the sample used to construct the model. More accurate estimations of performance can be obtained with internal validation methods, and more particularly with bootstrap methods which provided stable estimates with low bias. Bootstrapping relies on random sampling with replacement from the original data set, of the same size as the original data set. The approach used here to internally validate the model is described by Frank Harrell and colleagues [20] and implemented in rms package (R software). First, a bootstrap sample, with replacement, was taken. The logistic model was fitted to this bootstrap dataset. This model was evaluated in the bootstrap sample and in the original sample. The difference between performances in the bootstrap sample and in the original sample represents an estimate of the optimism. This difference was averaged to obtain a stable estimate of the optimism, on about a thousand of bootstrap samples. This estimate of optimism was then subtracted from the naïve estimate of predictive ability, to obtain an optimism adjusted measure.

Table 1
Baseline demographics and clinical assessments of the studied population.

Baseline variable	All ($n = 53$)	Responders($n = 23$)	Non-responders($n = 30$)	P-value
Age, median (IQR) years	54 (46; 60)	55 (44; 63)	54 (47.2; 57.8)	0.95
Female, %	75	74	77	0.86
Disease duration, median (IQR) years	6 (3; 12)	4.5 (2; 11.5)	6.5 (3.8; 11.5)	0.25
BMI, median (IQR) kg/m^2	23.9 (22.3; 25.9)	23.7 (22.4; 25.7)	23.9 (22; 25.9)	0.95
DAS28 ESR baseline, median (IQR) units	5.1 (4.3; 5.8)	5 (4.2; 5.5)	5.5 (4.7; 6.4)	0.074
Morning stiffness, median (IQR) min	60 (30; 120)	60 (45; 120)	52.5 (22.5; 120)	0.27
HAQ, median (IQR) units	1.1 (0.8; 1.6)	1.2 (0.8; 1.4)	1.1 (0.9; 2)	0.44
RF, median (IQR) IU/ml	118 (49; 383)	103 (46; 146)	128 (64; 500)	0.38
ESR, median (IQR) mm/hour	24 (13; 34)	24 (12; 34)	24 (14.2; 36)	0.55
CRP, median (IQR) mg/l	14.5 (6.8; 27.2)	11.5 (7; 28.5)	15.5 (6.2; 21)	0.88

IQR: Interquartile Range; BMI: Body Mass Index; DAS28: Disease Activity Score 28; HAQ: Health Assessment Questionnaire; RF: Rheumatoid Factor; ESR: Erythrocyte Sedimentation Rate; CRP: C-reactive protein.

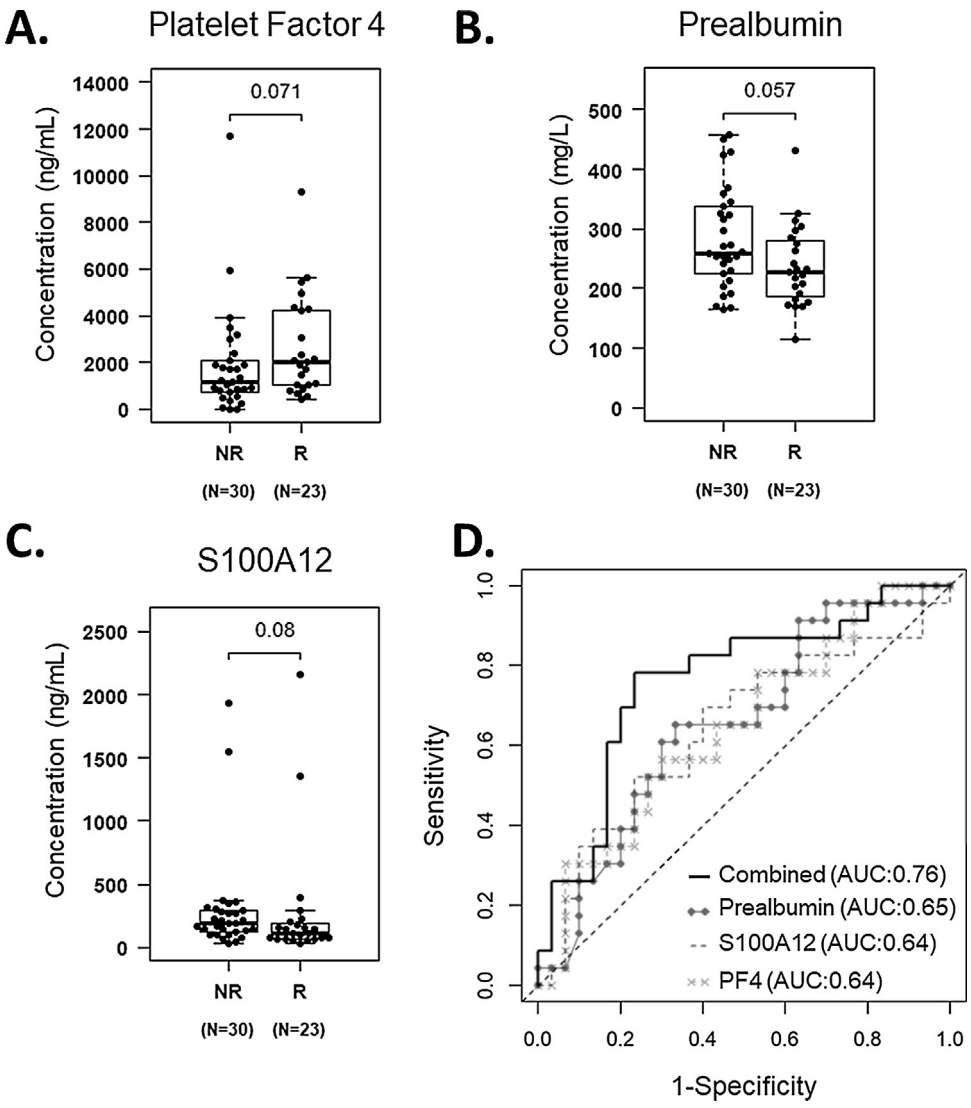

Fig. 1. A, B and C. Biomarkers' baseline concentrations in patients achieving good EULAR response (R) or not (NR) at 6 months upon TNFi treatment. The Mann-Whitney non-parametric test was used to evaluate the significance of differences of biomarkers. D. Overlay of the ROC curves of the predictive multivariate combined model and the univariate model (prealbumin, PF4 and S100A12).

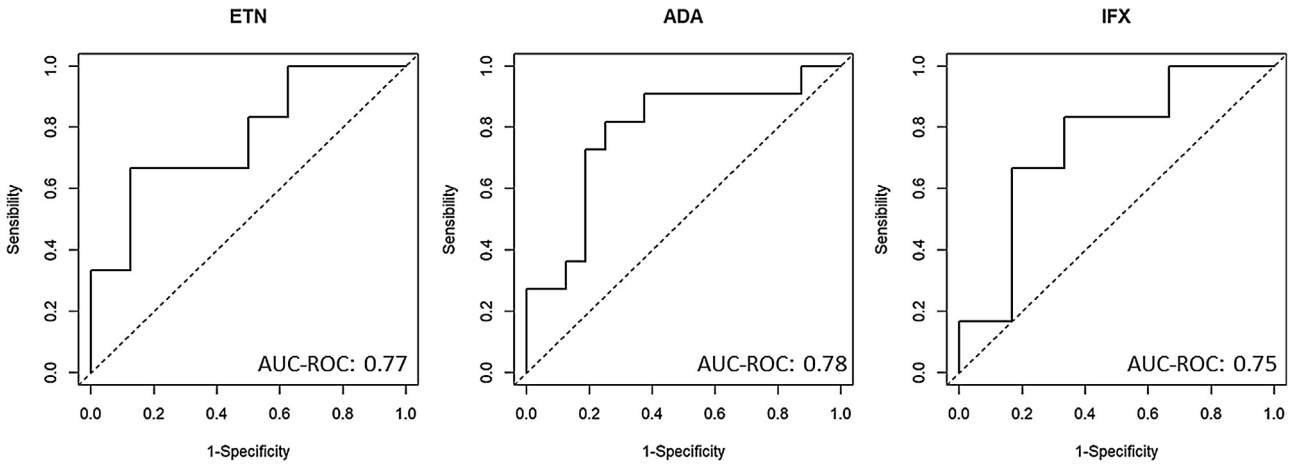

Figure 2. ROC curves corresponding to the application of the predictive combined model to specific biologic etanercept (ETN), adalimumab (ADA) and infliximab (IFX).

2.6. Role of the funding source

Data collection, data analysis, data interpretation, manuscript preparation, decision to publish was independent from the study funding.

3. Results

3.1. Study population

A total of 53 RA patients were included in this study. After 6 months of TNFi (adalimumab, etanercept or infliximab) treatment, 43% ($n=23$) of RA patients were judged as responders and 57% ($n=30$) as non-responders to anti-TNFα treatment. Baseline demographic, clinical and biological data of responders' and non-responders' populations were similar in the whole cohort (Table 1) and among patients treated with each bDMARD [Table S1; see the supplementary material associated with this article online]; showing that in this group, no classical marker of RA severity and aggressiveness could predict the patient response to a TNFi therapy.

3.2. Biomarker's distribution between responders and non-responders in the study population

To explore whether apolipoprotein A1, haptoglobin, prealbumin, C-reactive protein, α-1 antitrypsin, platelet factor 4, S100A12 and S100A8/S100A9 were differentially expressed between responders from non-responders we first compared baseline level of these 8 biomarkers between these two populations. Responders and non-responders baseline medians were presented in Table S2. No differences were observed for α-1 antitrypsin, apolipoprotein A1, CRP, S100A8/A9 and haptoglobin with a *P*-value > 0.3 (Fig. S1). Although the differences were not significant we observed a trend for prealbumin, S100A12 and PF4 with a *P*-value close to 0.05 (Fig. 1A, B and C).

3.3. Univariate analysis: prealbumin, PF4 or S100A12 exhibit potential predictive ability in the plasma of RA patients before anti-TNFα therapy

To evaluate the predictive value of each biomarker we performed a univariate logistic regression analysis. Predictor variables were examined through quantitative values and dichotomized values of each biomarker. The quantitative analysis revealed that only prealbumin and PF4 demonstrated a *P*-value lower than 0.1 while the qualitative analysis using median as cut-off value showed a *P*-value of 0.072 for S100A12 (Table 2). Furthermore, AUC-ROC of PF4, prealbumin and S100A12 were respectively 0.64, 0.65 and 0.64 (Fig. 1D).

Table 2
Univariate logistic regression analysis on biomarker's quantitative values and qualitative (dichotomized considering the median value as cut-off) values.

Univariate logistic regression	Quantitative analysis	Qualitative analysis	
	P-value	Median (cut-off)	*P*-value
S100A12	0.22	152.9	0.072
Prealbumin	0.045	254	0.18
C-Reactive protein	0.93	11	0.21
PF4	0.065	1687.97	0.34
Haptoglobin	0.63	1.6	0.43
S100A8/A9	0.49	370.3	0.48
Alpha 1 antitrypsin	0.57	1.57	0.48
Apolipoprotein A1	0.29	1.56	0.82

3.4. Multivariate analysis: prealbumin, PF4 and S100A12 combination exhibit discriminative power to differentiate future responders from non-responders to TNFi therapy

Since univariate analysis revealed that PF4, prealbumin and S100A12 could represent interesting biomarkers to discriminate future R from NR they were added to build the multivariate model.

A probability of response for each patient was calculated from the coefficients of the regression and allowed good discriminative ability to classify patients in responder's or non-responder's group with an AUC-ROC of 0.76 compared to that obtained when biomarkers were analyzed separately (Fig. 1 D). After determining the optimal cut-off using the Younden index at 0.45, sensitivity was 78% (Confidence Interval [CI], 95% CI: 56–93), specificity 77% (95% CI: 58–90), positive predictive values (PPV) 72% (95% CI: 51–88), negative predictive values (NPV) 82% (95% CI: 63–94), positive likelihood ratio (LR+) of 3.35 (95% CI: 1.69–6.64) and negative likelihood ratio (LR−) of 0.28 (95% CI: 0.13–0.63). To evaluate the overfitting in measures of our model we used a bootstrap method as indicated in the method session. We obtained an optimism-adjusted AUC value of 0.72 demonstrating that the discriminative ability of our model was still conserved.

Finally, we investigated whether the combination of prealbumin, PF4 and S100A12 was able to predict clinical response for each biologic separately. As observed for the whole cohort, combination of prealbumin, PF4 and S100A12 exhibited similar predictive value

Table 3
Evaluation of the predictive value of the TNFi multivariate model combining prealbumin, PF4 and S100A12 on the ETN, IFX or ADA population individually.

	IFX	ETN	ADA
ROC-AUC	0.75	0.77	0.78
Specificity, % [95% CI]	67 [22–96]	88 [47–100]	75 [48–93]
Sensitivity, % [95% CI]	83 [32–100]	67 [22–96]	82 [48–98]
PPV, % [95% CI]	71 [29–96]	80 [28–99]	69 [39–91]
NPV, % [95% CI]	80 [28–99]	78 [40–97]	86 [57–98)]

AUC: area under the curve from the ROC analysis; CI: confidence interval.

for IFX (AUC-ROC = 0.75), ETN (AUC-ROC = 0.77) and ADA (AUC-ROC = 0.78) (Fig. 2 and Table 3).

4. Discussion

In this study, we identified 3 biomarkers, *i.e.* prealbumin, PF4 and S100A12, whose combination predicted good EULAR response to IFX, ADA and ETN in RA patient with inadequate response to csDMARDs. Several studies attempted to determine biomarkers for prediction to bDMARD and proteomic approach was often used to characterize differentially expressed proteins among patient proteome profile [8], as the mechanism-based genomic [21], cellular settings [22], and cytokine profiling [23] approach often lead to the confirmation of the up- or down regulation of biomarkers but with a limited diagnostic value. Most of the studies focused on single biomarker to predict TNFi response but the results are often discordant [24]. For example, Marotte et al. found that the circulating TNF-alpha bioactivity was higher in good responders to infliximab. However, other studies found no association between TNF-alpha and the response status to TNFi [12,25].

Recent studies addressed the predictive response to biologic by biomarker analysis at baseline. Obry et al. [26] used such an approach to characterize the biomarkers associated with the response or the non-response to etanercept in RA. A total of 7 biomarkers were associated with good response to etanercept. The combination of only two of them (complement component 7, CO7 and vitamin K-dependent protein S, PROS) permitted to improve the identification of responders and non-responders with a sensitivity of 88.9% and a specificity of 100%. This strategy of combining biomarkers appears very interesting to improve the accuracy to predict TNFi response and our study confirm this. Our approach demonstrated that isolated assessment of biomarker concentration does not allowed discriminating at baseline TNFi responder from non-responder whereas specific combination identified through a systematic approach could lead to the generation of a predictive algorithm. More interestingly our predictive model based on the combination of prealbumin, PF4 and S100A12 could not only discriminate future responders to a class of bDMARD such as TNFi but also showed similar predictive performance to IFX, ADA and ETN taken individually. This suggest that those 3 proteins belong to a common pathway to those 3 TNFi even though the mechanism of inhibition are different, monoclonal antibodies for IFX and ADA and TNF decoy receptor for ETN, as well as their efficacy [27]. However, this observation should be tempered because of the low number of patients included for each biomarker separately explaining the large range of CI observed for the model characteristics.

A few limitations should be emphasized. First external validity of this promising combination of prealbumin, PF4 and S100A12 to predict good EULAR response to IFX, ADA and ETN when combined rather than used separately, in RA patient with inadequate response to conventional synthetics DMARDs should be examined. In order to estimate the external validity of this biomarker combination we undertook a statistical analysis by bootstrap method, which results suggest a promising potential of external validity. However, the performance of this biomarker combination to predict good EULAR response need to be validated in an independent cohort. Second, canonical biomarkers of RA such as CRP were not included in our biomarker combination. Third, EULAR response was chosen as the main outcome measure to be in line with recent therapeutic strategies in [4]. We acknowledge that EULAR recommendation mentioned that treatment should be aimed at reaching remission or low disease activity in every RA patient [28,29]. However, a significant proportion of patients in clinical practice do not reach remission [30], therefore we used EULAR response to bDMARD as the primary outcome rather than remission or low disease activity as primary outcome.

Platelet factor 4 (PF4 or CXCL4) is a chemokine classically described as secreted only by activated platelets but recent study show a monocyte and macrophage production [31,32]. PF4 acts on monocyte and induce the release of TNFα [33]. PF4 with TNFα together play an important role to potentiate native immune response. In RA patient, several studies reported an increase of PF4 in synovial fluid [34], serum [35] and synovial tissue [32]. S100A12 is a protein secreted by activated neutrophils and macrophages. S100A12 is an interesting biomarker of phagocyte activation and inflammation [36]. This protein active mast cells and induced production of pro-inflammatory cytokines: IL6, IL8 and TNFα [37]. It has been showed that in return TNFα up-regulate transiently S100A12 gene expression [38] and trigger the secretion of S100A12 by neutrophils [39]. Some studies found an overexpressed of S100A12 in synovial fluid and serum in RA patients and this protein is correlated with clinical, inflammatory markers and US score in RA patients [9,40,41]. Our data demonstrated that responder's patients exhibited a lower S100A12 expression probably due to a better efficacy of TNF blockade in those patients. Interestingly two studies reported that S100A12 mRNA in patient PBMC [42] and protein expression level in serum decreased in patients who responded to TNFi treatment [40]. Prealbumin, also known as transthyretin, is a biomarker of malnutrition and has been reported in RA. Indeed, its levels in sera of patients with early RA were significantly increased compared to that of healthy control [14]. Prealbumin has been identified as one of the protein that showed significantly up regulated expression in the plasma of RA patients. Most importantly, the increase in expression of prealbumin with the progression of severity of RA condition has been observed [43]. The lower basal expression of prealbumin in patients that will respond to TNFi treatment observed in our study may indicate that those patients present a pathological status more suitable to respond to TNFi initiation.

Although the increasing burden of bDMARD on medico-economic resources are well recognized, only a few initiatives have been undertaken to design innovative tool for personalized medicine in the field of chronic inflammatory rheumatisms. Combination of multiple markers is a more promising approach to improve the performance of a predictive strategy in RA patients than hypothetico-deductive model that failed to provide any help for the prescription of bDMARD for more than a decade.

We demonstrated in our study with a limited number of patients that the strategy of multiple biomarkers combination (*i.e.* prealbumin, PF4 and S100A12) to generate a predictive algorithm has the potential to predict good EULAR response to a class of bDMARD such as TNFi. This will help physicians choosing the right bDMARD for the right RA patient, allowing to succeed the implementation of personalized medicine in daily practice.

Ethics approval and consent to participate

The study was approved by the local ethics committee (*Comité de protection des personnes*). All patients provided written informed consent.

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Disclosure of interest

AB, CT, and PG generated intellectual property that is owned by Grenoble Alpes University and Centre Hospitalier Universitaire Grenoble Alpes. AB and PG are co-founders of Sinnovial company. MVCN and AC are employees of Sinnovial company. TL reports grants from BMS and punctual interventions with personal fees from AbbVie, BMS, Janssen, Lilly, Merck, Novartis and Pfizer. OV has obtained grants from BMS, MSD and Pfizer and reports grants punctual interventions with personal fees from BMS, Pfizer, MSD, Abbvie, Roche-Chugai, Lilly, Novartis, Janssen and UCB. Data are protected by patent register number #FR1358503 and publication number #FR3010188.

Authors' contributions

PG conceived of the study, participated in its design and coordination, and critically revised the manuscript. AB participated in study design and coordination, and critically revised the manuscript. CT participated in study design and coordination of the samples dosages, and critically revised the manuscript. MVCN analyzed the data and drafted the manuscript. AC performed the statistical analysis and help to draft the manuscript. XR helped to draft the manuscript. BT revised the manuscript. HM, TT, MS, PM, JT, LG, TL and OV supplied patient samples, collected clinical data, and revised the manuscript.

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Appendix A. Supplementary data

Supplementary data (Fig. S1, Tables S1–S3) associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jbspin.2018.05.006.

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