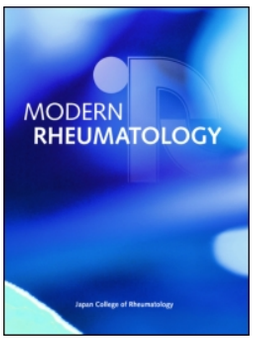

Modern Rheumatology

ISSN: 1439-7595 (Print) 1439-7609 (Online) Journal homepage: http://www.tandfonline.com/loi/imor20

A novel method predicting clinical response using only background clinical data in RA patients before treatment with infliximab

Fumihiko Miyoshi, Kyoko Honne, Seiji Minota, Masato Okada, Noriyoshi Ogawa & Toshihide Mimura

To cite this article: Fumihiko Miyoshi, Kyoko Honne, Seiji Minota, Masato Okada, Noriyoshi Ogawa & Toshihide Mimura (2016): A novel method predicting clinical response using only background clinical data in RA patients before treatment with infliximab, Modern Rheumatology, DOI: 10.3109/14397595.2016.1168536

To link to this article: http://dx.doi.org/10.3109/14397595.2016.1168536

View supplementary material

Published online: 05 May 2016.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=imor20

Download by: [Nanyang Technological University] **Date:** 06 May 2016, At: 21:04

Japan College of Rheumatology
MODERN RHEUMATOLOGY

http://www.tandfonline.com/imor
ISSN 1439-7595 (print), 1439-7609 (online)

Mod Rheumatol, 2016; Early Online: 1–4
© 2016 Japan College of Rheumatology
DOI: 10.3109/14397595.2016.1168536

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

A novel method predicting clinical response using only background clinical data in RA patients before treatment with infliximab

Fumihiko Miyoshi[1]*, Kyoko Honne[2], Seiji Minota[2], Masato Okada[3], Noriyoshi Ogawa[4], and Toshihide Mimura[1]

[1]Department of Rheumatology and Applied Immunology, Faculty of Medicine, Saitama Medical University, Saitama, Japan, [2]Division of Rheumatology and Clinical Immunology, Jichi Medical University, Tochigi, Japan, [3]Immuno-Rheumatology Center, St. Luke's International Hospital, Tokyo, Japan, and [4]Division of Immunology and Rheumatology, Internal Medicine 3, Hamamatsu University School of Medicine, Hamamatsu, Japan

Downloaded by [Nanyang Technological University] at 21:04 06 May 2016

Abstract

Objectives: The aim of the present study was to generate a novel method for predicting the clinical response to infliximab (IFX), using a machine-learning algorithm with only clinical data obtained before the treatment in rheumatoid arthritis (RA) patients.

Methods: We obtained 32 variables out of the clinical data on the patients from two independent hospitals. Next, we selected both clinical parameters and machine-learning algorithms and decided the candidates of prediction method. These candidates were verified by clinical variables on different patients from two other hospitals. Finally, we decided the prediction method to achieve the highest score.

Results: The combination of multilayer perceptron algorithm (neural network) and nine clinical parameters shows the best accuracy performance. This method could predict the good or moderate response to IFX with 92% accuracy. The sensitivity of this method was 96.7%, while the specificity was 75%.

Conclusions: We have developed a novel method for predicting the clinical response using only background clinical data in RA patients before treatment with IFX. Our method for predicting the response to IFX in RA patients may have advantages over the other previous methods in several points including easy usability, cost-effectiveness and accuracy.

Keywords

Clinical data, Infliximab, Machine-learning, Rheumatoid arthritis, The prediction of clinical response

History

Received 6 November 2015
Accepted 8 March 2016
Published online 28 April 2016

Introduction

Rheumatoid arthritis (RA) is the most common rheumatic disease that results in not only joint destruction, but also short life-expectancy [1]. Tumor necrosis factor alpha (TNF-α) plays a key role in the associated pathological phenomena in RA and has been identified as a therapeutic target. In fact, the neutralization of TNF-α is a widely used effective treatment for RA. Infliximab (IFX) is the first generation of anti-TNF-α agents to be used for the treatment of RA and is a genetically constructed immunoglobulin G1 murine-human chimeric monoclonal antibody that binds both to the soluble subunit and the membrane-bound precursor of TNF-α [2]. As with the other four approved anti-TNF blocking agents, i.e. etanercept, adalimumab, golimumab and certorizumab pegol, IFX has dramatically improved physiological function and proven to be an effective treatment for disease activity control in patients with RA [3]. Nevertheless, ∼30% of patients treated with anti-TNF blocking agents fail to achieve or maintain clinical improvement [3]. Moreover, TNF-α blockers are relatively expensive and may have potential serious side effects. Therefore, prediction of the clinical response to anti-TNF blocking agents prior to the administration is clearly an unmet need in RA treatment.

Several studies have been reported for the prediction of clinical response to IFX in RA patients. Biomarkers used for these predictions include gene profiling [4–8], specific gene expression [9], proteins [10] and inflammatory cytokines [11,12].

Despite the promising results, these methods have not been widely used. There are several possible explanations including the following: (1) available criteria for predicting the effect of IFX have not been described enough so that we are unable to verify many previous reports, (2) the predictive method evaluated in one cohort may not be effective in the other cohorts and (3) the prediction rates of these methods are within the range of 65–90%, which may not be high enough to be used.

Machine-learning approaches have been shown to be a well-established method for analyzing large dataset. For example, machine-learning methods have been used to screen a biomarker for predicting Alzheimer's Disease [13], and to make a method for predicting the therapeutic response to breast cancer patients after chemotherapy [14].

Therefore, in the present study, we have generated a novel method for predicting the clinical response to IFX, using machine-learning approaches with only clinical variables obtained before the treatment in RA patients.

*Present address: Fumihiko Miyoshi, Biology Research Laboratories, Sohyaku Innovative Research Division, Mitsubishi Tanabe Pharma Corporation, Saitama, Japan

Correspondence to: Toshihide Mimura, M.D., Department of Rheumatology and Applied Immunology, Faculty of Medicine, Saitama Medical University, Morohongo 38, Moroyama, Iruma-Gun, Saitama 350-0495, Japan. Tel: +81 49 276 1462. Fax: +81 49 295 4849. E-mail: toshim@saitama-med.ac.jp

Downloaded by [Nanyang Technological University] at 21:04 06 May 2016

Materials and methods

Patients and clinical procedures

Patients involved in this study were diagnosed with RA according to the 1987 revised ACR classification criteria of RA [15]. We obtained 32 components of the clinical variables from 90 patients with RA before IFX treatment at the Saitama Medical University Hospital, 52 patients at Jichi Medical University Hospital, 28 patients at St. Luke's International Hospital and 10 patients at Hamamatsu Medical University Hospital. Patients were assessed for overall disease activity using Disease Activity Score (DAS) 28-C-reactive protein (CRP) before IFX treatment and after 14 weeks. Almost all patients were scheduled to receive IFX (3 mg/kg) at weeks 0, 2, 6 and 14. This study was approved by the institutional review board or its equivalent at each hospital.

In the present study, those patients treated with IFX were divided at 14 weeks into two groups according to European League Against Rheumatism (EULAR) response criteria [16] which is based on DAS28-CRP; patients showing moderate or good response were classified as ''responders'', and those showing no response were classified as ''non-responders''.

Response predictor building and validation

In order to reduce the sample-bias among patients collected from four different hospitals and to ensure the robustness of prediction method, two data sets of patients from two hospitals were merged so that two groups of patients from four hospitals were made; Group 1 was composed of 141 patients from Saitama Medical University Hospital and Jichi Medical University Hospital and Group 2 was composed of 38 patients from St. Luke's International Hospital and Hamamatsu University Hospital. We used Group 1 as a training sample and Group 2 as a validation one.

At first, we try to predict the clinical response using only one clinical variable, such as DAS28-CRP score before IFX (at 0 week). Unfortunately, the prediction accuracy had been low, as compared with others previous reported methods. Therefore, we changed the strategy for choosing clinical variables sets described below. Clinical variables sets and prediction equation were obtained by using a stepwise discriminant function analysis and linear discriminant analysis of SPSS for Windows, ver. 18.0 (IBM Japan, Tokyo, Japan). As a criterion for selecting clinical variables sets, the accuracy of prediction was required over 80%. However, the predicted accuracies had been low, mainly in the 60–70% even when we used machine-learning algorithm. Therefore, all clinical variables were weighted as those of the DAS28. For example, CRP were weighted as follow:

$$\mathrm{CRP},\ \mathrm{CRP}^2,\ \sqrt{\mathrm{CRP}},\ \sqrt[3]{\mathrm{CRP}},\ \sqrt[4]{\mathrm{CRP}},\ 1+\ln(1+\mathrm{CRP}),\ \exp(\mathrm{CRP}),\ \exp\left(\frac{\mathrm{CRP}}{100}\right),\ \frac{1}{1+\mathrm{CRP}},\ \frac{1}{1+\mathrm{CRP}^2},\ \frac{1}{1+\sqrt{\mathrm{CRP}}},\ \frac{1}{1+\sqrt[3]{\mathrm{CRP}}},\ \frac{1}{1+\sqrt[4]{\mathrm{CRP}}},\ \frac{1}{1+\ln(1+\mathrm{CRP})},\ \frac{1}{1+\exp(\mathrm{CRP})},\ \frac{1}{1+\exp\left(\frac{\mathrm{CRP}}{100}\right)}$$

We re-identified clinical variables weighted according to the above example. Thereby, the prediction score resulted in ''>80%''.

To determine the best machine-learning algorithm in each clinical variables set, all algorithms in the WEKA software package [17], which consisted of a collection of machine-learning algorithms for data mining tasks, were compared using the training data (Group 1). In this study, all classifier algorithms were used with the default parameters. We determined the best machine-learning algorithm for each clinical variable set. As a criterion for selecting combinations of algorithm and parameter set, the accuracy of prediction was required over 95%. Next, the selected combination methods, composed of clinical variables sets and machine-learning algorithms, were applied to validation data (Group 2) and the predicted results were verified with the answer, ''responders'' or ''non-responders'' after 14 weeks. We decided the prediction method to achieve the highest score. Finally, the parameters of the selected classifier algorithm of WEKA were tuned and the prediction method was selected from the combinations of machine-learning algorithms and parameters. The protocol of the clinical variables and the machine-learning algorithm selection in our prediction method was available as Supplementary file 1 (Methods_in_detail.pdf).

Results

Clinical characteristics of RA patients before treatment with IFX

Characteristics of RA patients at baseline are outlined in Table 1. Before IFX treatment, two variables (ESR and PSL) were significantly different between responders and non-responders.

Selection of an optimal subset of clinical parameters and machine learning algorithm

The combination of multilayer perceptron algorithm (neural network) of machine-learning and nine clinical variables (ESR, TEN, ALB, MONO, RBC, PSL, MTX, HbA1c and Pre bio) shows the best accuracy performance. Training time was 50,000, learning rate was 0.1 and momentum was 0.1. Other parameters were default values. Used weighted clinical variables are shown in Table 2. This prediction method could completely reproduce (100% accuracy) the result of training data (Group 1).

Validation of the prediction method

Applying the prediction method to the clinical data of other hospitals (Group 2), it could predict the response to IFX with 92.1% accuracy. The incorrectly classified individuals were one

Table 1. Characteristics of RA patients before treatment with IFX.

Characteristic	Responder ($n=138$)	Non-responder ($n=41$)	p*
Gender (male/female)	25/113	11/30	0.27
Age (years)	54.6 ± 12.0	55.7 ± 12.3	0.43
Disease duration (years)	7.97 ± 8.20	7.79 ± 9.51	0.74
ESR (mm/h)	56.2 ± 33.0	73.1 ± 36.0	0.009
CRP (mg/dl)	2.16 ± 2.24	3.31 ± 3.31	0.1
MTX (mg/week)	7.99 ± 1.73	7.46 ± 2.30	0.39
PSL (mg/day)	2.75 ± 3.00	3.90 ± 2.91	0.018
DAS28-CRP	4.67 ± 1.11	4.62 ± 1.21	0.79
TEN (numbers)	7.15 ± 5.48	6.17 ± 5.49	0.23
ALB (g/dl)	3.79 ± 0.39	3.55 ± 0.48	0.0005
MONO (numbers/μl)	390 ± 191	433 ± 188	0.17
RBC (numbers $\times 10^6$/μl)	3.99 ± 0.44	3.98 ± 0.40	0.94
Pre bio (numbers)	0/113	7/30	0.0001
HbA1c (JDS, %)	5.35 ± 0.57	5.40 ± 0.58	0.68

Values are mean ± SD. *p value calculated using Fisher's exact test or Mann–Whitney U test when appropriate.

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; MTX: methotrexate; PSL: prednisolone; DAS: disease activity score; TEN: 28 tender joint count; MONO: monocytes; RBC: red blood cells; pre bio: previous use of biologic agents before infliximab.

Downloaded by [Nanyang Technological University] at 21:04 06 May 2016

IFX responder and two non-responders. The sensitivity of this method was 96.7%, while the specificity was 75%. A comparison with previous studies of predicting the response of IFX is summarized in Table 3. The receiver operating characteristic curve is shown in Figure 1. Area under the curve was 0.75.

The set of prediction rule file and answer file were available as Supplementary file 2 (rule.csv) and Supplementary file 3 (answer.csv). The protocol of our prediction method in WEKA was available as Supplementary file 4 (protocol.docx).

Discussion

We have generated a novel prediction method of response to IFX therapy, using only nine clinical variables before the treatment with IFX. Though many previous methods have used gene expression profiles [4,7,8], this study used only clinical variables available in the clinical setting. It may be not enough to compare the prediction accuracy of our method to that of other previous methods. However, the prediction accuracy of our method was almost equal to those of other previous methods (Table 3). We believe it important to know that the use of both machine-learning approach and weighted clinical variables resulted in a high accuracy rate. Two studies previously have shown that the predictive accuracies of machine-learning approaches are superior to those of traditional statistical approaches [18,19]. In our studies, machine-learning approaches achieved better performance than traditional statistical methods and the use of weighted clinical variables achieved better result than the use of default ones (described in Methods section). These results suggest that the use of both machine-learning approaches and weighted clinical variables are critical to the development of a method for predicting the clinical response. To our knowledge, this is the first study that has only clinical variables before IFX to predict response of IFX in patients with RA. It may be easy and cost-effective to use our prediction method in the real-world clinical settings in world wide.

The strategy in some previous reports was searching for the best response gene from peripheral blood mononuclear cells (PBMCs) in RA patients in anti-TNF-α therapy [4,6–10,12]. Toonen et al. [20] validated previously reported prediction methods for anti-TNF-α therapy response utilizing gene expression profiles from their original genome-wide expression data. They reported that a set of 20 genes showed by Lequerré et al. [4] obtained the highest score in their validation study. However, they also reported in their article that the obtained sensitivity rate (71%) and specificity rate (61%) were not high enough for use in real-world clinical setting. Additionally, the alteration of certain genes

Table 2. A list of selected variables used in this prediction method.

Clinical variables	Weighted clinical variables
ESR (mm/h)	ESR^2
TEN (numbers)	$\frac{1}{1+\sqrt[4]{\mathrm{TEN}}}$
ALB (g/dl)	$\frac{1}{1+\mathrm{ALB}^2}$
MONO (numbers/μl)	$\frac{1}{1+\mathrm{MONO}^2}$
RBC (numbers $\times 10^6$/μl)	$\sqrt{\mathrm{RBC}}$
PSL (mg/day)	PSL^2
MTX (mg/week)	$\frac{1}{1+\mathrm{MTX}^2}$, $\frac{1}{1+\exp(\mathrm{MTX})}$
HbA1c (JDS, %)	HbAlc^2
Pre bio (numbers)	$\exp(\frac{\mathrm{Pre\,bio}}{100})$

TEN: 28 tender joint count; MONO: monocytes.

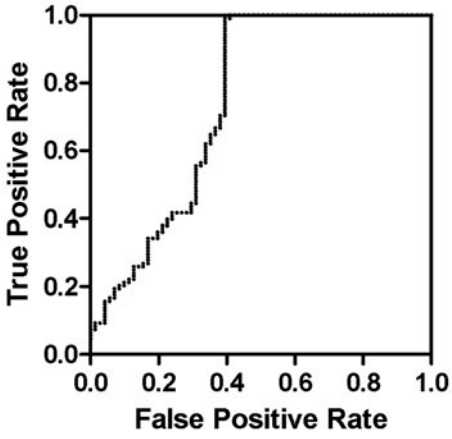

Figure 1. The receiver operating characteristic curve of the prediction result.

Table 3. A comparison with previous studies of predicting the response of IFX.

Author	Factors	Used data	Responders definition
Kayakabe	1	The concentration of cytokine	Good or moderate responders according to the DAS28 before and after 24 weeks
Tsuzaka	1	Gene expression data	Good responders according to the DAS28 before and after 38 weeks
Trocme	14	The concentration of proteins	Responders according to the ACR 70 positive before and after 30 weeks
Tanino	10	Gene expression data	Responders according to CRP <0.3 mg/dl at 14 weeks
Julià	8	Gene expression data	Good or moderate responders according to the DAS28 before and after 14 weeks
Lequerré	20	Gene expression data	Responders according to a change of DAS28 = 1.2 obtained at 3 months
Lequerré	8	Gene expression data	Responders according to a change of DAS28 = 1.2 obtained at 3 months
Miyoshi	9	Clinical variables	Good or moderate responders according to the DAS28 before and after 14 weeks

Author	Training data				Test data			
	The number of patients	Accuracy (%)	Sensitivity (%)	Specificity (%)	The number of patients	Accuracy (%)	Sensitivity (%)	Specificity (%)
Kayakabe	13		78.1	77.8				
Tsuzaka	73		27.3	97.5				
Trocme	60		97.1	97.5				
Tanino	42	97.6	100	96.4	26	65.4	73.3	54.5
Julià	30	96.6			14	85.7	91.7	50
Lequerré	13				20	80	90	70
Lequerré	13				20	90	80	100
Miyoshi	141	100	100	100	38	92.1	96.7	75

in PBMCs from RA patients on TNF inhibition therapy may not represent the changes of overall disease activity. We believe that RA may be a syndrome with a similar phenotype under possible different pathological mechanisms, therefore, that it may be reasonable to use clinical data, which could be more universal than biomarkers like gene expressions, for the prediction. Additionally, in the present study we used clinical data of a patient-group from two individual hospitals as test samples and another patient-group from two other hospitals as validation samples to make our result more generalized. By using machine-learning approach with weighted clinical variables, we have developed a novel prediction method from multiple cohorts and verified other multiple ones. Therefore, our method may be a robust predictor of response to IFX therapy.

In conclusion, we have developed a novel method for predicting the clinical response to IFX, using Multilayer Perceptron algorithm, which is one of machine-learning algorithms, with nine clinical parameters of RA patients before treatment. This method has predicted the clinical response of IFX on different groups of RA patients with 92% accuracy. We believe that our method for predicting the response to IFX in RA patients has advantages over the other methods in several points including easy usability, cost-effectiveness and accuracy.

Downloaded by [Nanyang Technological University] at 21:04 06 May 2016

Acknowledgments

The authors acknowledge Dr K. Sato for his critical reading of this article.

Conflict of interest

S.M. has received honoraria from Eisai Pharmaceutical, Takeda Pharmaceutical, Asahi Kasei, Santen Pharmaceutical, Mitsubishi-Tanabe Pharma, Pfizer, Astellas Pharmaceutical, Daiichi Sankyo, Abbvie, Actelion, Bristol Myers, Eli Lilly, Ono Pharmaceutical and GlaxoSmithKline, and unlimited research funds from Takeda Pharmaceutical, Mitsubishi-Tanabe Pharma, Asahi Kasei, Shionogi, Astellas Pharmaceutical, Teijin, Ono Pharmaceutical, Pfizer, Eisai, Abbvie, Chugai Pharmaceutical and MSD, and royalty from Chugai Pharmaceutical. T.M. has received research grants from Abbvie, Bristol Myers Squibb, Chugai Pharmaceutical, Eisai Pharmaceutical, Mitsubishi-Tanabe Pharma, Takeda Pharmaceutical, Astellas Pharmaceutical and Pfizer and received lecture fees from Chugai Pharmaceutical, Janssen Pharmaceutical, Mitsubishi-Tanabe Pharma, and Takeda Pharmaceutical. All other authors have declared no conflicts of interest. F.M. has become an employee of Mitsubishi-Tanabe Pharma after this study finished.

References

1. Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. Cell. 1996;85(3):307–10.
2. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet. 1994;344(8930):1105–10.
3. Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. Lancet. 2007;370(9602):1861–74.
4. Lequerre T, Gauthier-Jauneau AC, Bansard C, Derambure C, Hiron M, Vittecoq O, et al. Gene profiling in white blood cells predicts infliximab responsiveness in rheumatoid arthritis. Arthritis Res Ther. 2006;8(4):R105. doi: 10.1186/ar1990.
5. Lindberg J, af Klint E, Catrina AI, Nilsson P, Klareskog L, Ulfgren AK, et al. Effect of infliximab on mRNA expression profiles in synovial tissue of rheumatoid arthritis patients. Arthritis Res Ther. 2006;8(6):R179. doi: 10.1186/ar2090.
6. Sekiguchi N, Kawauchi S, Furuya T, Inaba N, Matsuda K, Ando S, et al. Messenger ribonucleic acid expression profile in peripheral blood cells from RA patients following treatment with an anti-TNF-alpha monoclonal antibody, infliximab. Rheumatology. 2008;47(6):780–8.
7. Julia A, Erra A, Palacio C, Tomas C, Sans X, Barcelo P, et al. An eight-gene blood expression profile predicts the response to infliximab in rheumatoid arthritis. PLoS One. 2009;4(10):e7556. doi: http://dx.doi.org/10.1371/journal.pone.0007556.
8. Tanino M, Matoba R, Nakamura S, Kameda H, Amano K, Okayama T, et al. Prediction of efficacy of anti-TNF biologic agent, infliximab, for rheumatoid arthritis patients using a comprehensive transcriptome analysis of white blood cells. Biochem Biophys Res Commun. 2009;387(2):261–5.
9. Tsuzaka K, Itami Y, Takeuchi T, Shinozaki N, Morishita T. ADAMTS5 is a biomarker for prediction of response to infliximab in patients with rheumatoid arthritis. J Rheumatol. 2010;37(7):1454–60.
10. Trocme C, Marotte H, Baillet A, Pallot-Prades B, Garin J, Grange L, et al. Apolipoprotein A-I and platelet factor 4 are biomarkers for infliximab response in rheumatoid arthritis. Ann Rheum Dis. 2009;68(8):1328–33.
11. Takeuchi T, Miyasaka N, Tatsuki Y, Yano T, Yoshinari T, Abe T, et al. Baseline tumour necrosis factor alpha levels predict the necessity for dose escalation of infliximab therapy in patients with rheumatoid arthritis. Ann Rheum Dis. 2011;70(7):1208–15.
12. Kayakabe K, Kuroiwa T, Sakurai N, Ikeuchi H, Kadiombo AT, Sakairi T, et al. Interleukin-1beta measurement in stimulated whole blood cultures is useful to predict response to anti-TNF therapies in rheumatoid arthritis. Rheumatology. 2012;51(9):1639–43.
13. Gomez Ravetti M, Moscato P. Identification of a 5-protein biomarker molecular signature for predicting Alzheimer's disease. PLoS One. 2008;3(9):e3111. doi: http://dx.doi.org/10.1371/journal.pone.0003111.
14. Mani S, Chen Y, Li X, Arlinghaus L, Chakravarthy AB, Abramson V, et al. Machine learning for predicting the response of breast cancer to neoadjuvant chemotherapy. J Am Med Inform Assoc. 2013;20(4):688–95.
15. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31(3):315–24.
16. Prevoo ML, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum. 1995;38(1):44–8.
17. Frank E, Hall M, Trigg L, Holmes G, Witten IH. Data mining in bioinformatics using Weka. Bioinformatics. 2004;20(15):2479–81.
18. Mecocci P, Grossi E, Buscema M, Intraligi M, Savare R, Rinaldi P, et al. Use of artificial networks in clinical trials: a pilot study to predict responsiveness to donepezil in Alzheimer's disease. J Am Geriatr Soc. 2002;50(11):1857–60.
19. Catto JW, Linkens DA, Abbod MF, Chen M, Burton JL, Feeley KM, et al. Artificial intelligence in predicting bladder cancer outcome: a comparison of neuro-fuzzy modeling and artificial neural networks. Clin Cancer Res. 2003;9(11):4172–7.
20. Toonen EJ, Gilissen C, Franke B, Kievit W, Eijsbouts AM, den Broeder AA, et al. Validation study of existing gene expression signatures for anti-TNF treatment in patients with rheumatoid arthritis. PLoS One. 2012;7(3):e33199. doi: http://dx.doi.org/10.1371/journal.pone.0033199.

Supplementary material available online