Elastic net-based prediction of IFN-β treatment response of patients with multiple sclerosis using time series microarray gene expression profiles

Arika Fukushima¹, Masahiro Sugimoto²,³,⁴, Satoru Hiwa¹ & Tomoyuki Hiroyasu¹

INF-β has been widely used to treat patients with multiple sclerosis (MS) in relapse. Accurate prediction of treatment response is important for effective personalization of treatment. Microarray data have been frequently used to discover new genes and to predict treatment responses. However, conventional analytical methods suffer from three difficulties: high-dimensionality of datasets; high degree of multicollinearity; and achieving gene identification in time-course data. The use of Elastic net, a sparse modelling method, would decrease the first two issues; however, Elastic net is currently unable to solve these three issues simultaneously. Here, we improved Elastic net to accommodate time-course data analyses. Numerical experiments were conducted using two time-course microarray datasets derived from peripheral blood mononuclear cells collected from patients with MS. The proposed methods successfully identified genes showing a high predictive ability for INF-β treatment response. Bootstrap sampling resulted in an 81% and 78% accuracy for each dataset, which was significantly higher than the 71% and 73% accuracy obtained using conventional methods. Our methods selected genes showing consistent differentiation throughout all time-courses. These genes are expected to provide new predictive biomarkers that can influence INF-β treatment for MS patients.

Multiple sclerosis (MS) is one of the most common neurological disabilities of the central nervous system¹. The highest incidences of MS have been reported in North America and Europe (100/100,000), and the lowest occur in East Asia and sub-Saharan Africa (2/100,000)². This disease is the second most common neurological disability in young adulthood³. Approximately 80–90% of MS patients initially suffer from relapsing-remitting MS (RRMS) where MS repeatedly occurs with a variety of symptoms, including the stages of neurological disability (relapse) and recovery (remission)⁴. The disease gradually shifts to secondary progressive MS (SPMS) which is associated with frequent relapses. Therefore, a systematic treatment strategy to prevent and/or delay relapse is important for the improvement of the quality of life (QOL) of MS patients.

Interferon-β (INF-β) has been commonly used to prevent relapse of MS⁵,⁶ however, INF-β treatment has two issues. First, the treatment only works for a limited number of patients, where approximately half of the patients relapse within 2 years despite treatment⁶,⁷. Second, this treatment can cause side effects, such as spasticity and dermal reaction⁸. Thus, effective surveillance and appropriate intervention over a long period of time post-treatment is required. Although the pathogenesis of MS has yet to be fully elucidated, various genetic factors involved in this disease have been reported⁹. Gene expression data have been intensively analyzed to predict INF-β treatment responses⁹,¹⁰,¹¹. Hundreds of genes, such as Caspase2, Caspase10, and FLIP, showed promise in predicting treatment response¹¹; however, these genes were identified by conventional statistical methods which showed low prediction accuracies in some cases¹¹,¹². The MxA and ISG genes were reported to be predictive for

¹Doshisha University, Graduate School of Life and Medical Sciences, Kyoto, Japan. ²Research and Development Center for Minimally Invasive Therapies Health Promotion and Preemptive Medicine, Tokyo Medical University, Shinjuku, Tokyo, 160-8402, Japan. ³Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata, 997-0052, Japan. ⁴University of Tsukuba, Research and Development Center for Precision Medicine, Tukuba, Ibaraki, 305-8550, Japan. Correspondence and requests for materials should be addressed to T.H. (email: tomo@mis.doshisha.ac.jp)
IFN-β treatment response. The expression patterns of these genes, however, were not consistently differentiated throughout all of the time-courses. Given this, any predictions would only be accurate immediately after the observation of the gene expression levels, while the accuracy of prediction would be low for subsequent responses. Therefore, the identification of genes showing highly accurate prediction abilities throughout all time-courses is needed.

Generally, data analyses to identify biomarkers are categorized into single time-point and time-course-based approaches. Prediction using only the currently observable data to predict an outcome of treatment is the most useful but most challenging approach for optimizing patient treatment. Single time-point-based analyses are challenging because the gene expression levels observed during the progression of MS are dynamic. Prediction using time-course data consisting of multiple time-points would result in more accurate predictions by eliminating the selection of genes showing inconsistent differentiation throughout the observation period. In particular, the identification of genes showing highly accurate prediction abilities throughout all time-courses is important for both the static-longitudinal scenario and the static-longitudinal scenario.

Microarray data analyses present several difficulties, including the problem of high-dimensionality (a higher number of genes compared to sample size) and the high degree of multi-collinearity. Elastic net, a type of sparse modelling method, has been commonly utilized to identify differentiated genes to address these issues. To our knowledge, however, the identification of genes showing highly accurate prediction abilities throughout all time-courses for MS patients by sparse modelling has not been reported.

The purpose of this study was to identify new genes showing highly accurate prediction abilities throughout all time-courses for MS patients. Therefore, sparse modelling methods were modified, and two microarray time-course datasets collected from patients with MS were used for predictions of INF-β treatment responses by our proposed method.

Methods

Elastic net, a sparse modelling method, was modified to analyse time-course data. Our method was designed to find genes showing consistent differentiation between the two given groups throughout multiple time-points. Here, we addressed the following problems:

1. High dimensionality. Microarray data includes a larger number of genes compared to a small sample size.
2. Multi-collinearity. Microarray data includes many genes showing highly positive correlations. The use of these genes for a prediction model would deteriorate generalization ability.
3. Time-courses. Genes showing consistent differentiation throughout multiple time-points should be identified.

Elastic net was designed to analyse single time-point data to identify differentiated genes by preventing multi-collinearity. We modified this method for the time-course data analyses.

Elastic net and stability selection. Sparse modelling is one of a variety of selection methods suitable for high dimensional data analyses. Among the different sparse modelling methods, Least Absolute Shrinkage and Selection Operator (LASSO) have been commonly used in various studies. LASSO, however, is limited in that it selects only one variable from two variables showing a high correlation (multi-collinearity), and the other variables are not selected despite being differentiated. The Ridge regression model is a method capable of solving this problem. This method can construct models from two variables showing a multi-collinearity; however, this method does not select genes. Elastic net is another sparse modelling method able to reduce those two limitations. Elastic net is comprised of LASSO and Ridge, which selects variable sets, and this method selects all variables, even those showing high multi-collinearities. Here, we employed Elastic net rather than LASSO to select gene candidates showing predictive abilities for subsequent analyses.
The proposed method used a logistic regression (eq. 1) to predict the INF-β treatment response based on differentiated genes:

\[
\Pr(y = 1 | X) = \frac{1}{1 + e^{-\beta X}}
\]

where, \(y = [y_1, y_2, \ldots, y_n]; y_i\) denotes the response variable that included good responders (labelled as 1) or poor responders (labelled as 0) to INF-β treatment, respectively. \(n\) denotes the sample size of MS patients. \(X = [x_{11}, x_{12}, \ldots, x_{1p}; x_{21}, x_{22}, \ldots, x_{2p}; \ldots; x_{n1}, x_{n2}, \ldots, x_{np}]; x_{ij}\) denotes the explanatory variables of gene expression levels at time-point \(t\), and \(p\) denotes the number of genes. \(\beta = [\beta_1, \beta_2, \ldots, \beta_p]; \beta\) denotes the regression coefficients.

The regression coefficient \(\beta\) in eq. 1 indicates the degree of association between the response to INF-β treatment and each gene. Therefore, a gene with a high absolute value of a regression coefficient was selected as a gene bearing the predictive ability of the treatment response. Regression coefficients, however, were difficult to calculate by Ordinary Least Squares (OLS), a general method for calculation of the regression coefficients, due to the high dimensionality of the microarray data (the number of genes \(p \gg n\) the sample size \(n\)). Therefore, a small number of differentiated genes should be selected prior to the use of OLS. Sparse modelling assumes that only several regression coefficients are needed for the prediction model and that the others are not needed. This assumption means that the regression coefficient values of several genes which were needed for the prediction model were non-zero while the other values were zero. Specifically, genes exhibiting non-zero regression coefficients were selected as genes able to predict responses to INF-β treatment. With the use of Elastic net, regression coefficients were calculated by adding a penalty term to a least-square loss function (eq. 2).

\[
\arg\min_{\beta} J(\gamma, X) + \lambda \sum_{j=1}^{p} w_j \left[ 1 - \alpha \right] \beta_j^2 + \alpha |\beta_j|
\]

where, \(J(\gamma, X)\) denotes loss of function of OLS, and \(\lambda\) denotes the hyper-parameter for the penalty term of Elastic net or poor responders (labelled as 0) to INF-β treatment, respectively. \(\lambda\) values were regarded as the selection bias of Elastic net as the selection bias of each gene at a given time-point \(t\). Thus, genes showing a larger or a lower weight were selected at a lower or a higher probability, respectively.

Cross validation is commonly used for optimizing the \(\lambda\) value in eq. 2. Inconsistent genes, however, are generally selected depending on the \(\lambda\) value. To prevent this problem, Stability Selection (SS) was used. SS selects for genes according to the following procedures:

1. A subset of samples was obtained from the gene expression data by random sampling.
2. An arbitrary \(\lambda\) value was provided to Elastic net to select genes using the data of (1).
3. (1–2) were repeated with multiple subsets.
4. The frequency of selection using an arbitrary \(\lambda\) value was calculated for multiple subsets.
5. (1–3) were repeated using multiple \(\lambda\) values.
6. For each gene, the maximum of the probability calculated in (4) among multiple \(\lambda\) values was regarded as the selection probability of the gene.
7. Genes showing a selection probability above the threshold \(\theta_n\) were selected.

Proposed method: marker identification using time-course data. The proposed method consisted of the following three procedures (Fig. 2):

1. Screening of gene candidates (Fig. 2a). Due to the difficulties associated with high dimensional problems, Elastic net along with SS was used for the screening of gene candidates, known as the gene pool, from the data at each time-point. Only genes selected at least one time by Elastic net using SS were selected in the gene pool and the rest were eliminated.
2. Ranking of genes showing consistent differentiation throughout multiple time-points (Fig. 2b). Modified Elastic net was used to select genes showing consistent differentiation throughout multiple time-points from the gene pool. Initially, Elastic net incorporating SS selected predictive genes from the gene pool at the first time-point. Then, at the next time-point \(t\), Elastic net (eq. 2) using SS was conducted with a higher selection bias to select genes which were selected at the previous time-point \(t - 1\). Therefore, Elastic net sets the weights of genes selected at \(t - 1\) to values smaller than genes not selected (eq. 3). This procedure was repeatedly performed at subsequent time-points. Consequently, genes showing consistent differentiation throughout multiple time-points were identified using the following:

\[
w_{ij} = \begin{cases} 1, & \text{if } g_j \in GL_{t-1} \\ \gamma, & \text{if } g_j \notin GL_{t-1} \end{cases}
\]

where \(w_{ij}\) denotes the weight of the \(j^{th}\) gene in Elastic net at \(t\) in eq. 2, \(\gamma (\gamma \in \mathbb{R}_{>0}; \gamma > 1)\) denotes the selection bias; \(g_j\) denotes the \(j^{th}\) gene, and \(GL_{t-1}\) denotes a gene list at \(t - 1\). The gene list was constructed using selected genes at \(t - 1\).
Finally, the product $SP_{final}$ was calculated by selection probability at each time-point for each gene, and the genes were ranked in descending order according to selection probabilities. The product $SP_{final}$ denoted the probabilities based on the frequency of selection of each gene throughout all time-points (eq. 4). The product $SP_{final}$ was ranked in descending order. According to this ranking, the gene list for the prediction model was created for use in the third step of the model using the following:

$$SP_{final} = \{SP_1, SP_2, \ldots, SP_p\}$$

$$SP_j = \prod_{t=1}^{T} SP_{t,j}$$

(4)

where $SP_{t,j}$ denotes selection probability of the $j^{th}$ gene by stability selection at $t$.

(3) Construction of a prediction model using the ranked genes (Fig. 2c). Genes for the prediction model were identified based on the gene list ranked in the second step. The time-point for data to be used for constructing the prediction model was also selected simultaneously. Here, prediction models of treatment response were constructed using combinations of various groups of genes and time-points of gene expression data. To identify the genes and select a time-point of data for the prediction model, these models were evaluated (Fig. 2d). An evaluation value was calculated by the prediction model that was constructed by one group of genes using time-point data. The genes in the group with the best evaluation value were identified as the genes showing consistent differentiation. These time-point data were selected for the prediction model. This group of genes was created by individually adding genes from the gene list generated in the second step in descending order. The prediction models of all gene groups were constructed and evaluated at each time-point of the time-course data.

In this step, prediction accuracy, a ratio describing the prediction accuracy of model data against data not used for constructing the model, was used as an evaluation value. A prediction model was constructed from a group of genes using data at a given time-point, and the prediction accuracy was calculated. Prediction accuracies were calculated at each time-point for model construction, as shown in the following two cases.

Case 1: Time-point for model construction = time-point for prediction. A constructed model was used for the prediction of data at identical time-points. Leave-one-out (LOO) was used to evaluate the prediction accuracy ($ACC_c$). In LOO, one sample of data was used as test data, and other data were used for model construction. LOO was repeated until all the samples became test data.
Case 2: Time-point for model construction ≠ time-point for prediction. A constructed model was used for the prediction of the data at a time-point not used for model construction. The prediction accuracies (ACCₜ) were calculated using the data at time-points for prediction.

The mean of prediction accuracies (ACCₘₑᵃₙ) was calculated for each group of genes and for each time-point of model construction in eq. 5.

\[
\text{ACC}_{\text{mean}} = \frac{1}{T} \sum_{d \in D} \left( \text{ACC}_{\text{t}}^{(d)} + \text{ACC}_{o} \right)
\]

where ACCₘₑᵃₙ denotes the mean of the prediction accuracy for the prediction model constructed by a group of genes and a time-point of data. T denotes the length of all time-points. ACCₜ⁽ⁿ⁾ denotes the prediction accuracy using data at d. D = {t₁, t₂, ..., t₉} denotes the time-points of ACCₜ. t was not included for the time-point used for model construction. ACCₒ denotes the prediction accuracy using data at the time-point used for model construction.

The genes in the group with the best ACCₘₑᵃₙ were identified as the selected genes. This model was constructed using the data from a given time-point. This time-point was selected as the time-point for model construction.

Numerical experiments. The prediction accuracies of the developed models were evaluated for the prediction of INF-β treatment responses. The prediction accuracies of the proposed and conventional methods were compared.

Material and pre-processing. The evaluated data consisted of the time-course gene expression data from two MS patients who underwent INF-β treatment. The two datasets of GSE24427 (Dataset A)³⁴ and GSE19285 (Dataset B)³⁵ were used. These datasets included time from start of therapy to the first relapse; however, the definition of response is different for the two datasets³⁴,³⁵. Table 1 shows the number of time-course points in each data platform, and the method of normalization. Log₂-fold change and quantile normalization were performed for pre-processing of gene expression data. Subsequently, the expression levels of each gene were converted to Z-scores.

Conventional method. The conventional method used only for the gene expression data at a single time-point. Elastic net with SS using data at a single time-point was used as the conventional method. Genes were ranked according to the selection probabilities by SS. Finally, using the procedures of the proposed method (Fig. 2d), ACCₘₑᵃₙ was calculated using these selection probabilities. Thereafter, the genes in the group with the best ACCₘₑᵃₙ were regarded as identified genes. These genes were regarded as genes with the best performance throughout multiple time-points in the conventional method using data from a single time-point.

Evaluation method. The prediction accuracies were calculated by eq. 6. These were calculated using only test data which were not used for model construction. To evaluate the prediction accuracies using the data at the time-point used for model construction, LOO was conducted. To evaluate the prediction accuracies using the data at the other time-points, all available data were used.

\[
\text{ACC} \% = \frac{TP + TN}{TP + FP + FN + TN} \times 100
\]

where TP denotes the number of true positives, FP denotes the number of false positives, FN denotes the number of false negatives, and TN denotes the number of true negatives in the test data.

First, the prediction accuracies of the construction models were evaluated. In order to compare the prediction model of the proposed and the conventional method, the mean prediction accuracy (ACCₘₑᵃₙ) throughout all time-points was calculated using ACCₜ and ACCₒ at each time-point using eq. 3. The lowest prediction accuracy, specifically the minimum prediction accuracy (ACCₘₑᵃₙ) throughout all time-points, was selected from ACCₜ.

| Name of dataset | Dataset A | Dataset B |
|-----------------|-----------|-----------|
| GEO ID          | GSE19285  | GSE24427  |
| Type of INF-β   | Intramuscular Interferon beta 1a | Subcutaneous Interferon beta 1a |
| Time-points     | first (t₁), Second (t₂), fifth (t₅)  | first (t₁), Second (t₂), 1 month (t₃) 12 month (t₄), 24 month (t₅) |
| Number of good responders | 15        | 16        |
| Number of poor responders | 9         | 9         |
| Number of genes | 11220     | 13513     |
| Gene expression | Peripheral blood mononuclear cells | Peripheral blood mononuclear cells |
| Platform        | Affymetrix Human Genome U133A Array | Affymetrix Human Genome U133A Array |
| Preprocessing for microarray | MAS5.0 | MAS5.0 |

Table 1. Summary of gene expression datasets of INF-β treatments for MS patients. In this paper, symbols for time-points were presented as “t1”, “t2”, “t3”, etc.
Table 2. Accuracy of prediction models by the proposed method and conventional methods with dataset. A. Values in () were calculated by leave-one-out at the time-point of data used by the prediction model. Bold accuracy indicates the top accuracy at each time-point, but top accuracy of t1 was not presented as gene expression data at t1 was used data by the proposed method. Accuracy was the minimum accuracy (ACCmin) of each method.

| Method          | Accuracy [%] | t1 | t2 | t3 | Mean (ACCmean) |
|-----------------|--------------|----|----|----|----------------|
| Proposed method | (88)         | 92 | 79 | 86 |                |
| Conventional method | (100)       | 74 | 79 | 83 |                |

Comparing the proposed method to the conventional method. The proposed and conventional methods were evaluated by the analyses of datasets A and B. The genes showing the most ideal ACCmean were identified for each dataset using both the proposed and the conventional method. The prediction accuracies at each time-point and their mean from the first evaluation are listed at Tables 2 and 3.

As an analytical result of dataset A, the proposed method identified 11 genes and constructed the prediction model using the t1 data. With the conventional method, prediction models were constructed using the t1, t2, and t3 data, from which 9, 8, and 21 genes were identified, respectively. Table 2 showed the prediction accuracies at each time-point and their mean. The ACCmean and ACCmin values using the proposed method were 86% and 79%, respectively. From the conventional method using t2 data, the ACCmean was 86% and was comparable to that from the proposed method. The ACCmin obtained by the conventional method using the t2 data was, however, only 67%, which was lower than that obtained by the proposed method. The ACCmean obtained by the conventional method using the t1 and t3 data was 83% and 79%, respectively. The ACCmean from the proposed method was higher than that of the conventional method. Here, we focus on the results at different time-points in the first evaluation. The prediction accuracies generated by the proposed method were 92% at t2 and 79% at t3. The conventional method using t2 data could predict treatment responses at t3 with 92% accuracy; however, all other results were lower than those from the proposed method.

As a result of the use of dataset B, the proposed method identified 8 genes and constructed the prediction model using the t1 data. The conventional method identified 5, 19, 7, 6, and 19 genes using t1, t2, t3, t4, and t5 data, respectively. Table 3 lists the prediction accuracies at each time-point and the ACCmean. The ACCmean and ACCmin of the proposed method were 84% and 72%, respectively. The ACCmean values were 77%, 71%, 81%, 69%, and 74% using t1, t2, t3, t4, and t5 data for model construction by the conventional method, respectively.
| Method       | Accuracy [%] |
|--------------|--------------|
|              | t1 | t2 | t3 | t4 | t5 | Mean (ACC_{mean}) |
| Proposed method | (96) | 72 | 92 | 84 | 76 | 84 |
| Conventional method | (92) | 68 | 84 | 76 | 64 | 77 |

### Table 3. Accuracy of prediction models by the proposed method and conventional methods with dataset B.

Values in () were calculated by leave-one-out at the time-point of data used by the prediction model. “Bold accuracy” indicates the top accuracy of each time-point, but top accuracy of t1 was not presented as gene expression data at t1 were used by the proposed method. “Accuracy” indicates the minimum accuracy (ACC_{min}) of each method.

ACC_{min} values were 64%, 60%, 64%, 40%, and 60% using t1, t2, t3, t4, and t5 data for model construction by the conventional method, respectively. The ACC_{mean} and ACC_{min} values of the proposed method were higher than those of the conventional method. We focus on the results from different time-points in the first evaluation. The prediction accuracies of the proposed method using t1 data were 92%, 84%, and 76% at time-points t3, t4, and t5, respectively. The prediction accuracy at t2 by the conventional method using t3 data was 92%, which was higher than that obtained from the proposed method. The other accuracies generated by the proposed method were higher than those of the conventional method, with the exception of one case.

Bootstrap sampling was performed to evaluate the prediction accuracies at different time-points in the second evaluation. Figure 3 shows the mean and standard deviation of prediction accuracies given by the proposed and conventional methods at different time-points. As shown in Fig. 3a, in dataset A the mean accuracy of the different time-points (t2 and t3) was 81%. This prediction accuracy was significantly higher than 65% (p = 2.06 × 10^{-23}), 71% (p = 1.48 × 10^{-10}), and 68% (p = 1.16 × 10^{-16}) at t1, t2, and t3 in the conventional method (p < 0.001), respectively. As shown in Fig. 3b, in dataset B the mean accuracy of the different time-points (t2, t3, t4, and t5) provided by the proposed method was 78%. The prediction accuracies given by the conventional method at t1, t2, t3, t4, and t5 were 64% (p = 2.41 × 10^{-40}), 57% (p = 1.73 × 10^{-34}), 73% (p = 8.70 × 10^{-11}), 56% (p = 1.30 × 10^{-10}), and 55% (p = 1.46 × 10^{-78}), respectively. In dataset B, the mean accuracy of the different time-points given by the proposed method was significantly higher than those by the conventional method (p < 0.001). Therefore, the prediction accuracies at different time-points obtained using the proposed method were significantly higher than those given by the conventional method.

To assess the sensitivity and specificity of the prediction model of the proposed method, ROC curves and AUC in datasets A and B were measured (Fig. 3c,d). As shown in Fig. 3c, in dataset A the AUCs at t1, t2, and t3 were 0.95, 0.94, and 0.90 given by the proposed method, respectively, and all of these were higher than or equal to 0.9. The lower limits of the 95% confidence interval were 0.88, 0.82, and 0.77 at t1, t2, and t3, respectively. As shown in Fig. 3d, in dataset B the AUCs at t1, t2, t3, t4, and t5 were 0.99, 0.76, 0.95, 0.89, and 0.93, respectively. The lower limits of the 95% confidence interval were 0.97, 0.56, 0.87, 0.74, and 0.83 at t1, t2, t3, t4, and t5, respectively. In dataset B, the AUC and the lower limits of the 95% confidence interval of the proposed method at t2 were 0.76 and 0.56, which were lower than or equal to the other time-points as obtained by the conventional method (Figs S1 and S2). The AUC and lower limits of the 95% confidence interval of the proposed method were the highest in almost every case.

**Selected genes by the proposed method.** Eleven genes were identified in dataset A using the proposed method (Table 4) and eight genes were identified in dataset B using the proposed method (Table 5). These genes were expected to exhibit consistently higher expression levels of either good or poor responders at each time-point. The median levels of 9 genes in dataset A were consistently differentiated throughout all time-points (Table 4). In particular, the expression levels of the HPSS gene in poor responders at t1 and t2 were significantly higher than those in good responders (p < 0.05) (Fig. 4a). The median levels of 6 genes at each time-point were consistently higher in either group in dataset B (Table 5). In particular, the expression levels of the CDH2 gene in good responders at t1 and t3 were significantly higher than those in poor responders (p < 0.05) (Fig. 4b). Given this, the proposed method identified a number of genes where the expression levels were consistently different throughout all time-points.

**Discussion**

The genes identified by the proposed method showed consistent differentiation throughout all time-points and accurately predicted the responses of MS patients to INF-β treatment.

The ACC_{mean} and ACC_{min} values given by the proposed method in dataset A were 86% and 79%, respectively. The ACC_{mean} value was equal to or higher than that given by the conventional method (Table 2). The prediction model obtained from the conventional method using t2 data had a nearly identical ACC_{mean} value to that given by the proposed method; however, the ACC_{min} value of the proposed method was higher than that of the conventional method. The ACC_{mean} and ACC_{min} values of the proposed method in dataset B were 84% and 72%, respectively (Table 3). These values were higher than those of the conventional method. Thus, the proposed method yielded higher and more accurate predictions throughout most time-points in comparison to those given by
the conventional method (Tables 2 and 3). Additionally, the prediction accuracies at different time-points were evaluated by bootstrap sampling. Figure 3a,b provide the means and standard deviations of the prediction accuracies of different time-points calculated by bootstrap sampling. The mean accuracy of different time-points by the proposed method was 81%, which is higher than those obtained by the conventional method (Fig. 3a). This result indicates that the proposed method could achieve significantly higher prediction accuracies than the conventional method at different time-points. In dataset B, the mean accuracy of different time-points was 78%, and this was significantly higher than those given by the conventional method (Fig. 3b). Additionally, SES algorithm analysis of the static-longitudinal scenario is used as a conventional method, and this is compared with our proposed method. The static-longitudinal scenario in this method is expected to identify genes showing consistent differentiation throughout all time-points. Using the procedures of the proposed method (Fig. 2d), the ACC mean of the genes identified by SES algorithm was calculated, and a prediction model was created. The prediction accuracies at different time-points using SES algorithm were calculated by bootstrap sampling (Table S1). These mean accuracies obtained from our proposed method were higher than those given by this conventional method. Therefore, the proposed method using time-course data could achieve a high prediction accuracy compared with those provided by the conventional methods. Given this, the proposed method provided higher accuracy throughout all time-points.

Figure 3c,d show the sensitivity and specificity of the proposed method; and AUC was approximately 0.90 at most time-points in both datasets A and B. The AUC at t2 in dataset B given by the proposed method, however, was 0.76, which was lower than the AUC at other time-points and equivalent to the conventional method, as shown in Figs S1 and S2. The results at each time-point (Tables 2 and 3 and Fig. S3) revealed that the prediction accuracies did not depend upon the order of the time-course sampling, and the prediction accuracies by the proposed method were high at most time-points. There was, however, a case where the prediction accuracy was lower than that of the conventional method.

As shown in Tables 4 and 5, most genes from the proposed method showed different expression levels consistently throughout all time-points. Changes in those levels differentiated between good and poor responders consistently throughout the time-courses significantly (Fig. 4). Given this, the proposed method identified genes showing consistent differentiation throughout multiple time-points and could differentiate between good and poor responders.

The proposed method did not identify identical genes between datasets A and B. For dataset A, associations between MS and ZBTB16 and HOPX were reported. Th17 cells are a subset of T helper cells involved in several immune diseases, including MS. ZBTB16 was reported to activate differentiation of Th17 cells, and this contributed to the maintenance of the phenotype of Th17 cells in the human body. In regard to the relationship between
Table 4. Identified genes of dataset A by the proposed method. P values were adjusted using the BH method, and “Bold accuracy” exhibited significantly different gene expression (GE) levels between good and poor responders (p < 0.05). If GE levels of good responders at each gene were higher than those of poor responders at all time-points (TPs), “good” was represented in the final column.

| Gene symbol | Gene name | P value | Higher GE levels at all time-points |
|-------------|-----------|---------|-----------------------------------|
| ZBTB16      | Zinc Finger and BTB Domain Containing 16 | 0.064 | 0.013 0.137 good |
| ZFP37       | ZFP37 Zinc Finger Protein | 0.070 | 0.220 0.013 — |
| HPSS4       | HPSS4, Biogenesis of Lysosomal Organelles Complex 2 Subunit 2 | 0.013 | 0.013 0.084 poor |
| HOPX        | HOP Homeobox | 0.105 | 0.005 0.090 good |
| ARF6AP3     | ADP Ribosylation Factor GTase Activating Protein 3 | 0.013 | 0.162 0.105 good |
| CALML5      | Calmodulin Like 5 | 0.077 | 0.013 0.126 good |
| VPS26A      | VPS26, Retromer Complex Component A | 0.026 | 0.090 0.205 good |
| SLC5A4      | Solute Carrier Family 5 Member 4 | 0.190 | 0.022 0.190 good |
| MBL2        | Mannose Binding Lectin 2 | 0.149 | 0.013 0.640 — |
| DLGAP4      | DLG Associated Protein 4 | 0.007 | 0.115 0.390 good |
| CAGNC1C     | Calcium Voltage-Gated Channel Subunit Alpha1 C | 0.064 | 0.382 0.390 poor |

The functional defects of T cells and autoimmune encephalomyelitis, many experiments and reviews reported the deletion of the HOPX gene as responsible for decreasing suppressor ability of pTreg cells. For dataset B, there were reports detailing CDH2. Microglia, a type of glial cell of the central nervous system, are known as central immunocompetent cells, and CDH2 is involved in the context of these cells. Many genes identified in dataset A were related to cancer, but their association with MS remains unclear.

The proposed method possessed several limitations. First, there were time-points where the prediction accuracy given by the proposed method was lower. Second, the γ value as the weights of Elastic nets must be set adequately to ensure accurate prediction. Finally, we used genes as independent variables; however, the interactions of genes could also be considered as explanatory variables to obtain higher accuracy predictions.

Table 5. Identified gene list of dataset B by the proposed method. P values were adjusted using BH method, and “Bold accuracy” represents significantly different gene expression (GE) levels between good and poor responders (p < 0.05). If GE levels of good responders at each gene were higher than those of poor at all time-points (TPs), “good” was represented in the final column.

| Gene symbol | Gene name | P value | Higher GE levels of all TPs |
|-------------|-----------|---------|----------------------------|
| SMA4        | Survival of Motor Neuron 1, Telomeric | 0.072 | 0.250 0.009 0.082 0.082 good |
| MIR7114_NSFM | MicroRNA 7114/NMDA Receptor Synaptosomal Signaling and Neuronal Migration Factor | 0.072 | 0.082 0.005 0.130 0.314 good |
| LSM8        | LSM8 Homolog, U6 Small Nuclear RNA Associated | 0.452 | 0.009 0.082 0.082 0.441 — |
| FLAD1       | Flavin Adenine Dinucleotide Synthetase 1 | 0.071 | 0.009 0.344 0.056 0.072 poor |
| RRN3P1      | RRN3 Homolog, RNA Polymerase I Transcription Factor Pseudogene 1 | 0.419 | 0.179 0.082 0.082 0.023 poor |
| RASL10A     | RAS Like Family 10 Member A | 0.033 | 0.334 0.344 0.452 0.314 — |
| IER3IP1     | Immediate Early Response 3 Interacting Protein 1 | 0.115 | 0.072 0.005 0.216 0.082 poor |
| CDH2        | Cadherin 2 | 0.250 | 0.033 0.397 0.043 0.082 good |
the proposed method were identified as capable of high-accuracy prediction throughout multiple time-points. Additionally, these genes included genes previously reported to be related to MS. The proposed modified Elastic net method for the time-course data analyses was used to identify genes showing consistent differentiation between two outcome groups throughout time-courses. Here, we demonstrated the use of this modified Elastic net for the prediction of INF-β treatment responses in patients with MS. Additionally, this method could also be used for microarray time-course data analyses.

References
1. Hemmer, B., Archelos, J. I. & Hartung, H. P. New Concepts in The Immunopathogenesis of Multiple Sclerosis. *Nature Reviews* 3, 291–301 (2002).
2. Leray, E., Moreau, T., Fromont, A. & Edan, G. Epidemiology of Multiple Sclerosis. *Neuropediatrics* 172, 3–13 (2016).
3. Hausier, S. L. et al. B-Cell Depletion with Rituximab in Relapsing–Remitting Multiple Sclerosis. *The New England Journal of Medicine* 358, 676–688 (2008).
4. Singh, M. K. et al. Gene Expression Changes in Peripheral Blood Mononuclear Cells from Multiple Sclerosis Patients Undergoing β-Interferon Therapy. *Journal of the Neurological Sciences* 258, 52–59 (2007).
5. Baranzini, S. E. et al. Transcription-Based Prediction of Response to IFNb Using Supervised Computational Methods. *Plos Biology* 3, 166–176 (2005).
6. Rudick, R. A. et al. Excessive Biologic Response to IFNb Is Associated with Poor Treatment Response in Patients with Multiple Sclerosis. *Plos One* 6, e19262 (2011).
7. Río, J. et al. Defining the Response to Interferon-β in Relapsing-Remitting Multiple Sclerosis Patients. *Annals of Neurology* 59, 344–352 (2006).
8. Hundeshagen, A. et al. Elevated type I interferon-like activity in a subset of multiple sclerosis patients: molecular basis and clinical relevance. *Journal of Neuroinflammation* 9, 1–13 (2011).
9. Mallotra, S. et al. Search for Specific Biomarkers of IFNb Bioactivity in Patients with Multiple Sclerosis. *Plos One* 6, e23634 (2011).
10. Gilli, F. et al. Biological Markers of Interferon-Beta Therapy: Comparison Among Interferon-Stimulated Genes MxA, TRAIL and XAF-1. *Multiple Sclerosis Journal* 12, 47–57 (2006).
11. Hecker, M. et al. Reassessment of Blood Gene Expression Markers for the Prognosis of Relapsing-Remitting Multiple Sclerosis. *Plos One* 6, e29648 (2011).
12. Martire, S., Navone, N. D. & Montarolo, F. A. Gene Expression Study Denies the Ability of 25 Candidate Biomarkers to Predict the Interferon-β Treatment Response in Multiple Sclerosis Patients. *Journal of Neuroimmunology* 292, 34–39 (2016).
13. Elo, L. L. & Schwikowski, B. Analysis of Time-Resolved Gene Expression Measurements across Individuals. *Plos One* 8, e82340 (2013).
14. Joseph, Z. B., Gitter, A. & Simon, I. Studying and Modelling Dynamic Biological Processes Using Time-Series Gene Expression Data. *Nature Review Genetics* 13, 552–564 (2012).
15. Kayano, M. et al. Gene Set Differential Analysis of Time Course Expression Profiles Via Sparse Estimation in Functional Logistic Model with Application to Time-Dependent Biomarker Detection. *Biostatistics* 17, 1–14 (2016).
16. Phan, J. H. & Wang, M. D. Estimating Classification Error to Identify Biomarkers in Time Series Expression Data. *Proc of IEEE 7th International Symposium on Bioinformatics and BioEngineering* (2007).
17. Huang, T. et al. The Prediction of Interferon Treatment Effects Based on Time Series Microarray Gene Expression Profiles. *Journal of Translational Medicine* 6, 1–9 (2008).
18. Wang, H. W. et al. Discovering Monotonic Stemness Marker Genes from Time-Series Stem Cell Microarray Data. *BMC Genomics* 16, 52 (2015).
19. Camillo, D. B. et al. Significance Analysis of Microarray Transcript Levels in Time Series Experiments. *BMC Bioinformatics* 8, S10 (2007).
20. Leng, X. & Muller, H. G. Classification Using Functional Data Analysis for Temporal Gene Expression Data. *Bioinformatics* 22, 68–76 (2006).
21. Morino, K., Hirata, Y. & Tomioka, R. Predicting Disease Progression from Short Biomarker Series Using Expert Advice Algorithm. *Scientific Reports* 5, 8953 (2015).
22. Tsagris, M., Lagani, V. & Tsamardinos, I. Feature selection for high-dimensional temporal data. *BMC Bioinformatics* 19, 1–14 (2018).
23. Friedman, J., Hastie, T. & Tibshirani, R. Regularization Paths for Generalized Linear Models via Coordinate Descent. *Journal of Statistical Software* 33, 1–22 (2010).
24. Meier, L., Geer, S. V. D. & Buhlmann, P. The Group Lasso for Logistic Regression. *Journal of the Royal Statistical Society Series B* 70, 53–73 (2008).
25. Zou, H. & Hastie, T. Regularization and Variable Selection via the Elastic Net. *Journal of the Royal Statistical Society Series B* 67, 301–320 (2005).
26. Tibshirani, R. Regression Shrinkage and Selection via the Lasso. *Journal of the Royal Statistical Society Series B* 58, 267–288 (1996).

---

**Figure 4.** Gene expression levels of good and poor responders at each time-point. Expression levels of HPS5 in dataset A (a) and CDH2 in dataset B (b). Wilcoxon rank sum test. *FDR-corrected* $p < 0.05$. 
27. Hughey, J. J. & Butte, A. J. Robust Meta-Analysis of Gene Expression Using the Elastic Net. *Nucleic Acids Research* **43**, e79 1–11 (2015).
28. Fan, J. & Lv, J. Sure Independence Screening for Ultra-High Dimensional Feature Space. *Journal of the Royal Statistical Society Series B* **70**, 849–911 (2008).
29. Hoerl, A. E. & Kennard, R. W. Ridge Regression: Biased Estimation for Nonorthogonal Problems. *Technometrics* **12**, 55–67 (1970).
30. Wu, M. Y. et al. Regularized Logistic Regression with Network-Based Pairwise Interaction for Biomarker Identification in Breast Cancer. *BMC Bioinformatics* **17**, 1–18 (2016).
31. Shimamura, T. *et al.* Recursive Regularization for Inferring Gene Networks from Time-Course Gene Expression Profiles. *BMC Systems Biology* **3**, 1–13 (2009).
32. Ye, J. *et al.* Sparse Learning and Stability Selection for Predicting MCI to AD Conversion Using Baseline ADNI Data. *BMC Neurology* **12**, 1–12 (2012).
33. Meinsmausen, N. & Buhlmann, P. Stability Selection. *Journal of the Royal Statistical Society Series B* **72**, 417–473 (2010).
34. Hecker, M. *et al.* Network Analysis of Transcriptional Regulation in Response to Intramuscular Interferon-B-1a Multiple Sclerosis Treatment. *The Pharmacogenomics Journal* **12**, 134–146 (2012).
35. Goertsches, R. H. *et al.* Long-term genome-wide blood RNA expression profiles yield novel molecular response candidates for IFN-beta-1b treatment in relapsing remitting MS. *Pharmacogenomics* **11**, 147–161 (2010).
36. Kohavi, R. A. Study of Cross Validation and Bootstrap for Accuracy Estimation and Model Selection. *Proc of the 14th international joint conference on Artificial intelligence* **2**, 1137–1143 (1995).
37. Singh, S. P. *et al.* PLZF regulates CCR6 and is critical for the acquisition and maintenance of the Th17 phenotype in human cells. *The Journal of Immunology.* **194**, 4350–4361 (2015).
38. Jones, A. & Hawiger, D. Peripherally Induced Regulatory T Cells: Recruited Protectors of the Central Nervous System against Autoimmune Neuroinflammation. *Frontiers in Immunology* **8**, 532 (2017).
39. Jones, A. *et al.* Peripherally induced tolerance depends on pTreg cells that require Hopx to inhibit intrinsic IL-2 expression. *The Journal of Immunology.* **195**, 1489–1497 (2015).
40. Conant, K. *et al.* Matrix metalloproteinase activity stimulates N-cadherin shedding and the soluble N-cadherin ectodomain promotes classical microglial activation. *Journal of Neuroinflammation* **14**, 56 (2017).

**Acknowledgements**
This study was not supported by any grants.

**Author Contributions**
A.F., M.S. and H.T. designed the study and wrote the manuscript. A.F. conducted data analysis. S.H. advised on the proposed method. All authors reviewed the manuscript.

**Additional Information**
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-38441-2.

**Competing Interests:** The authors declare no competing interests.

**Publisher’s note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.