

Spectroscopic diagnosis of chronic fatigue syndrome by visible and near-infrared spectroscopy in serum samples

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Abstract

To investigate visible and near-infrared (Vis–NIR) spectroscopy enabling chronic fatigue syndrome (CFS) diagnosis, we subjected sera from CFS patients as well as healthy donors to Vis–NIR spectroscopy. Vis–NIR spectra in the 600–1100 nm region for sera from 77 CFS patients and 71 healthy donors were subjected to principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA) to develop multivariate models to discriminate between CFS patients and healthy donors. The model was further assessed by the prediction of 99 masked other determinations (54 in the healthy group and 45 in the CFS patient group). The PCA model predicted successful discrimination of the masked samples. The SIMCA model predicted 54 of 54 (100%) healthy donors and 42 of 45 (93.3%) CFS patients of Vis–NIR spectra from masked serum samples correctly. These results suggest that Vis–NIR spectroscopy for sera combined with chemometrics analysis could provide a promising tool to objectively diagnose CFS.

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Chronic fatigue syndrome (CFS) is a persistent weakened condition associated with a variety of somatic and psychological symptoms [1]. The prominent features are self-reported impairments in concentration and short-term memory, sleep disturbances, and musculoskeletal pain [1]. Although cytokines, neuropeptides, or neurotransmitters are considered to be responsible for the abnormal immune response [2] and disrupted hypothalamo–pituitary–adrenal (HPA) axis [2,3], which is found in CFS patients, the precise pathophysiology is unknown to date. Recently, the association of the polymorphism of serotonin-transporter gene promoter with CFS has been reported [2]. However,

almost all failed to find candidate genes associated with CFS. Some studies reported that serotonergic activity was increased [4], but others showed activity to be normal [5]. Levels of serum acetyl-L-carnitine [2], immunological abnormalities [2], dehydroepiandrosterone (DHEA) and its sulphate (DHEA-S) [2], cortisol [6], prolactin [7], adrenocorticotrophic hormone (ACTH) [7], serum metals [8], oxidative stress markers [9], plasma-free tryptophan [10], and melatonin [11] have been also reported to be changed in CFS patients. Furthermore, association with viruses such as Epstein–Barr virus (EBV) [12], human herpesvirus 6 (HHV-6) [12], coxsackie B virus [13], Borna disease virus (BDV) [14], parvovirus B19 [15], and human cytomegalovirus (HCMV) [16] has been observed in some CFS patients. However, none of the changes show a clear consensus [17–19]. Therefore, the diagnosis of CFS is currently based on

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clinical symptoms due to the absence of reliable biochemical markers [20]. As this diagnostic procedure relies on the experience and skill of medical doctors, CFS can be diagnosed only by limited numbers of skilled medical doctors. To overcome these problems, an additional diagnostic method using instruments, which enables objective diagnosis, is needed.

Visible and near-infrared (Vis–NIR) spectroscopy is a spectroscopic method using visible light and NIR radiation. Moreover, Vis–NIR spectroscopy requires no sample preparation and no reagents and enables non-invasive and non-destructive analysis [21]. Therefore, Vis–NIR spectroscopy has become a widely used analytical method in agricultural, pharmaceutical, chemical, and petrochemical industries [21,22]. Vis–NIR spectroscopy has also been used for a broad range of clinical applications [21,23] including CFS [24]. However, most of the work done on Vis–NIR in the medical field has been based on oxygenated or deoxygenated hemoglobin. Those works have been mainly used for functional analyses of the brain and muscle but not diagnosis.

The purpose of this study was to investigate the ability of Vis–NIR spectroscopy to diagnose CFS by multivariate analysis of Vis–NIR spectra from the sera from CFS patients and healthy donors. We report here the first evidence that CFS can be objectively diagnosed by principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA) of Vis–NIR spectra of sera. Therefore, this suggests the possibility of Vis–NIR spectroscopy for sera coupled with multivariate analysis for a novel procedure of CFS diagnosis.

Materials and methods

Samples. At the Medical Hospital of Osaka City University, sera were examined from 77 CFS patients (33.0 ± 8.8 years old; male/female: 29/48), which were diagnosed on the basis of clinical criteria proposed by the Centers for Disease Control and Prevention (CDC) [1]. Sera from 71 healthy donors (41.7 ± 10.4 years old; male/female: 33/38) were also used. A venous-blood sample (10 ml) was obtained from an antecubital vein from each subject. Sera from 77 CFS patients and 71 healthy donors were used as test samples to develop a calibration model of PCA [25] and SIMCA [26], whereas another 99 determinations (33 samples \times 3 spectra) including 54 determinations [18 healthy donor samples (35.9 ± 9.1 years old; male/female: 11/7) \times 3 spectra] and 45 determinations [15 CFS patient samples (34.9 ± 7.0 years old; male/female: 8/7) \times 3 spectra] were masked and used for prediction. Among all 92 CFS patients used as test and masked samples, 76 CFS patients were divided into the following five groups before collecting serum samples [28 non-treated patients (36.8%); 6 patients treated with vitamin B family and/or ascorbic acid 7.9%; 19 patients treated with herbal medicine such as hochu-ekki-to (25.0%); 9 patients treated with selective serotonin reuptake inhibitor such as paroxetine and fluvoxamine maleate (11.8%); and 14 patients treated with psychotropic agents such as sulpiride, etizolam, maprotiline hydrochloride, bromazepam, and methylphenidate hydrochloride (18.4%)]. This research project was approved by the Ethics Committee of Osaka City University and written informed consent was obtained from all CFS patients and healthy donors. All samples were diluted 10-fold with phosphate-buffered saline and adjusted to a constant volume (2 ml) in a polystyrene cuvette (SARSTEDT, Aktiengesellschaft, Germany) before Vis–NIR spectroscopy measurement.

Instrument and data collection. Three consecutive Vis–NIR spectra were measured at 2 nm resolution with an NIRGUN (Japan Fantec Research Institute, Shizuoka, Japan) at 37 °C. The spectral data were collected as absorbance values [$\log(1/T)$], where T = transmittance in the wavelength ranged from 600 to 1100 nm.

Data processing. Pirouette software (ver. 3.11; Infometrics, Woodinville, WA) was employed for data processing. To minimize differences between spectra caused by baseline shifts and noise, prior to calibration, spectral data were mean-centered and transformed by standard normal variates (SNV) [27] and smoothing based on the Savitsky–Golay algorithm [28]. To identify the predominant absorbance peaks in the spectra, PCA [25] and SIMCA methods [26] were further applied to develop PCA and SIMCA models for CFS diagnosis, respectively. We used a method of visualizing the SIMCA approach, the Coomans plot [29], which plots class distances against each other. Coomans plot was applied to assess the classification performance of the SIMCA model by predicting class membership in terms of distance from the model. The critical distance from the model used corresponded to the 0.05 level and defined 95% tolerance interval. The mathematical formulas used are available in the Pirouette manual.

Results and discussion

The main problems in CFS studies can be attributed to the objectivity of diagnosis, because the diagnosis of CFS is based on clinical symptoms [20]. To test the possibility of spectroscopic diagnosis for CFS by Vis–NIR spectroscopy, sera from CFS patients as well as healthy donors were subjected to Vis–NIR spectroscopy coupled with multivariate analysis such as PCA and SIMCA, which may provide a novel method to objectively diagnose CFS.

Clear discrimination of the sera of CFS patients from those of healthy donors was seen in PCA scores using first principal component (PC1) and second principal component (PC2) (Fig. 1A). The SIMCA model allowed correct separation of Vis–NIR spectra of 209 of 213 (98.1%) healthy donor sera and 220 of 231 (95.2%) CFS patient sera. SIMCA analysis using the Coomans plot demonstrated that sera classes from healthy donors and CFS patients did not share multivariate space, providing validation for the class separation (Fig. 2A). Furthermore, the masked samples were applied to Vis–NIR spectroscopy and further predicted by the above PCA and SIMCA models. Complete discrimination of the masked serum samples between CFS patients and healthy donors was attained using the above PCA and SIMCA models of Vis–NIR spectra (Figs. 1 and 2B). PCA showed clear discrimination of the masked samples between healthy donors and CFS patients. SIMCA predicted 54 of 54 (100%) healthy donors and 42 of 45 (93.3%) CFS patients of Vis–NIR spectra from masked serum samples correctly.

The spectral information modeled by PCA and SIMCA can be inferred from the corresponding loadings or discriminating power, respectively. Concerning PC1, the loadings positively peaked around 950, negatively peaked around 1020 nm, were slightly low around 600–700 nm and slightly high around 700–900 nm (Fig. 1C). PC2 loadings negatively peaked around 950, positively peaked around 1020 nm, were slightly low around 600–700 nm and slightly high around 700–900 nm. PCA loadings were

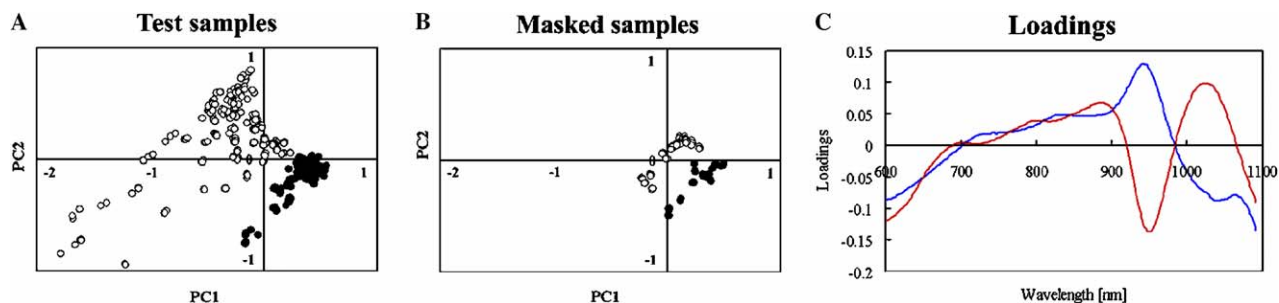


Fig. 1. Principal component analysis (PCA) (first two principal components) of visible and near-infrared (Vis–NIR) calibration and prediction of chronic fatigue syndrome (CFS) diagnosis. Serum samples from healthy donors and CFS patients were subjected to Vis–NIR spectroscopy. After the collection of Vis–NIR spectra of serum samples, the spectral data were pre-processed and subjected to PCA calibration modeling to develop a multivariate model to diagnose CFS, which was referenced on the basis of Centers for Disease Control and Prevention (CDC) criteria. The PCA score plot of the first principal component (PC1) versus the second principal component (PC2) for Vis–NIR spectra of test samples (A) and masked samples (B) from healthy donors and CFS patients by the PCA model showed clear discrimination between healthy donors (open circles) and CFS patients (closed circles). (C) PC1 (blue line) and PC2 (red line) loadings of the PCA.

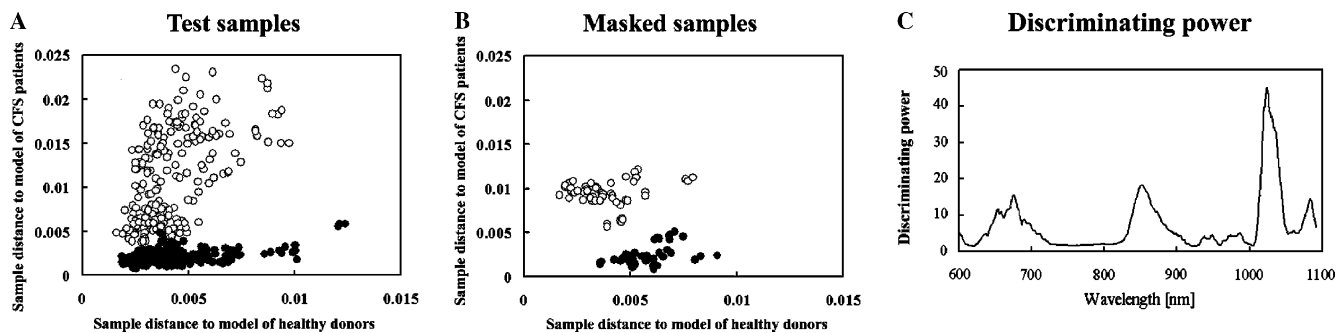


Fig. 2. Soft independent modeling of class analogy (SIMCA) of Vis–NIR calibration and prediction of CFS diagnosis. Vis–NIR spectral data of serum samples from healthy donors and CFS patients were pre-processed and subjected to SIMCA calibration modeling to develop a multivariate model to diagnose CFS. Coomans plot of SIMCA demonstrating that the healthy donor class (open circles) and CFS patient class (closed circles) of test samples (A) and masked samples (B) did not share multivariate space. (C) Discriminating power from the SIMCA calibration model.

generally consistent with the discriminating power of the SIMCA model, except for loadings of around 950 nm peaks (Figs. 1 and 2C). The most prominent discriminating power, which represents independent variables (wavelengths) important in discriminating two classes (CFS and healthy), were peaks at around 650, 850, 1020, and 1080 nm (Fig. 2C). The peak near 950 nm was previously reported to be related to water [22]. The peak around 750 nm was also close to a water band [22]. The peak near 800 nm was previously assigned to amine [22]. However, further studies will be necessary to accurately perform band assignment for the important peaks of PCA loadings and discriminating power. Further information obtained from detailed analysis of NIR spectra may make significant contributions not only to the diagnosis but also to the understanding of the pathogenesis of CFS.

These findings indicate that Vis–NIR analysis for sera combined with chemometrics analysis of serum achieves complete separation of CFS patients from healthy controls. This approach deserves further evaluation as a potential novel strategy for instrumental diagnosis of CFS. More importantly, these results suggest that unknown factor(s) in serum are commonly present in all CFS patients.

Further studies will be necessary to accurately perform band assignment for the important peaks of PCA loadings and SIMCA discriminating power. Further information obtained from detailed analysis of Vis–NIR spectra of sera may make significant contributions not only to the diagnosis, such as finding reliable biochemical markers, but also to the understanding of CFS pathophysiology, which will lead to an effective treatment for the disease. Finally, we would like to emphasize that noninvasive approaches to CFS diagnosis are further attractive. Although noninvasive blood glucose monitoring using Vis–NIR spectroscopy is not approved by the US Food and Drug Administration, in large part because of the lack of reliability of the determinations [30], other systems using Vis–NIR spectroscopy have not been examined sufficiently. This endeavor is now ongoing.

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References

- [1] K. Fukuda, S.E. Straus, I. Hickie, M.C. Sharpe, J.G. Dobbins, A. Komaroff, The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group, *Ann. Intern. Med.* 121 (1994) 953–959.
- [2] Y. Watanabe, H. Kuratsune, Brain science on chronic fatigue, *JMAJ* 49 (2006) 19–26.
- [3] H. Kuratsune, K. Yamaguti, G. Lindh, B. Evengård, G. Hagberg, K. Matsumura, M. Iwase, H. Onoe, M. Takahashi, T. Machii, Y. Kanakura, T. Kitani, B. Långström, Y. Watanabe, Brain regions involved in fatigue sensation: reduced acetylcarnitine uptake into the brain, *Neuroimage* 17 (2002) 1256–1265.
- [4] A.J. Cleare, J. Bearn, T. Allain, A. McGregor, S. Wessely, R.M. Murray, V. O'Keane, Contrasting neuroendocrine responses in depression and chronic fatigue syndrome, *J. Affect. Disord.* 34 (1995) 283–289.
- [5] L.N. Yatham, R.L. Morehouse, B.T. Chisholm, D.A. Haase, D.D. MacDonald, T.J. Marrie, Neuroendocrine assessment of serotonin (5-HT) function in chronic fatigue syndrome, *Can. J. Psychiatry* 40 (1995) 93–96.
- [6] P. Strickland, R. Morriss, A. Wearden, B. Deakin, A comparison of salivary cortisol in chronic fatigue syndrome, community depression and healthy controls, *J. Affect. Disord.* 47 (1998) 191–194.
- [7] D. Racciatti, M.T. Guagnano, J. Vecchiet, P.L. De Remigis, E. Pizzigallo, R. Della Vecchia, T. Di Sciascio, D. Merlitti, S. Sensi, Chronic fatigue syndrome: circadian rhythm and hypothalamic–pituitary–adrenal (HPA) axis impairment, *Int. J. Immunopathol. Pharmacol.* 14 (2001) 11–15.
- [8] S.J. van Rensburg, F.C. Potocnik, T. Kiss, F. Hugo, P. van Zijl, E. Mansvelt, M.E. Carstens, P. Theodorou, P.R. Hurly, R.A. Emsley, J.J. Taljaard, Serum concentrations of some metals and steroids in patients with chronic fatigue syndrome with reference to neurological and cognitive abnormalities, *Brain Res. Bull.* 55 (2001) 319–325.
- [9] G. Kennedy, V.A. Spence, M. McLaren, A. Hill, C. Underwood, J.J. Belch, Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms, *Free Radic. Biol. Med.* 39 (2005) 584–589.
- [10] C.M. Vassallo, E. Feldman, T. Peto, L. Castell, A.L. Sharpley, P.J. Cowen, Decreased tryptophan availability but normal post-synaptic 5-HT_{2c} receptor sensitivity in chronic fatigue syndrome, *Psychol. Med.* 31 (2001) 585–591.
- [11] A. Korszun, L. Sackett-Lundeen, E. Papadopoulos, C. Brucksch, L. Masterson, N.C. Engelberg, E. Haus, M.A. Demitrack, L. Crofford, Melatonin levels in women with fibromyalgia and chronic fatigue syndrome, *J. Rheumatol.* 26 (1999) 2675–2680.
- [12] K. Kondo, Post-infectious fatigue, *JMAJ* 49 (2006) 27–33.
- [13] J.W. Gow, W.M. Behan, K. Simpson, F. McGarry, S. Keir, P.O. Behan, Studies on enterovirus in patients with chronic fatigue syndrome, *Clin. Infect Dis.* 18 (Suppl 1) (1994) S126–S129.
- [14] T. Nakaya, H. Takahashi, Y. Nakamura, S. Asahi, M. Tobiume, H. Kuratsune, T. Kitani, K. Yamanishi, K. Ikuta, Demonstration of Borna disease virus RNA in peripheral blood mononuclear cells derived from Japanese patients with chronic fatigue syndrome, *FEBS Lett.* 378 (1996) 145–149.
- [15] J.R. Kerr, D.A. Tyrrell, Cytokines in parvovirus B19 infection as an aid to understanding chronic fatigue syndrome, *Curr. Pain Headache Rep.* 7 (2003) 333–341.
- [16] A.M. Lerner, H.J. Dworkin, T. Sayyed, C.H. Chang, J.T. Fitzgerald, S. Beqaj, R.G. Deeter, J. Goldstein, P. Gottipolu, W. O'Neill, Prevalence of abnormal cardiac wall motion in the cardiomyopathy associated with incomplete multiplication of Epstein–Barr Virus and/or cytomegalovirus in patients with chronic fatigue syndrome, *In Vivo* 18 (2004) 417–424.
- [17] P.M. Soetekouw, R.A. Wevers, P. Vreken, L.D. Elving, A.J. Janssen, Y. van der Veen, G. Bleijenberg, J.W. van der Meer, Normal carnitine levels in patients with chronic fatigue syndrome, *Neth. J. Med.* 57 (2000) 20–24.
- [18] W.J. Inder, T.C. Prickett, R.T. Mulder, Normal opioid tone and hypothalamic–pituitary–adrenal axis function in chronic fatigue syndrome despite marked functional impairment, *Hormon to Rinsho. Clin. Endocrinol.* 62 (2005) 343–348.
- [19] B. Evengård, T. Briese, G. Lindh, S. Lee, W.I. Lipkin, Absence of evidence of Borna disease virus infection in Swedish patients with Chronic Fatigue Syndrome, *J. Neurovirol.* 5 (1999) 495–499.
- [20] J.B. Prins, J.W. van der Meer, G. Bleijenberg, Chronic fatigue syndrome, *Lancet* 367 (2006) 346–355.
- [21] E.W. Ciurczak, J.K. Drennen, *Pharmaceutical and Medical Applications of Near-Infrared Applications (Practical Spectroscopy)*, Marcel Dekker, New York, 2002.
- [22] B.G. Osborne, T. Fearn, *Near-Infrared Spectroscopy in Food analysis*, Longman Scientific & Technical, UK, 1986.
- [23] A. Sakudo, Y. Saganuma, T. Kobayashi, T. Onodera, K. Ikuta, Near-infrared spectroscopy: promising diagnostic tool for viral infections, *Biochem. Biophys. Res. Commun.* 341 (2006) 279–284.
- [24] K.K. McCully, B.H. Natelson, Impaired oxygen delivery to muscle in chronic fatigue syndrome, *Clin. Sci.* 97 (1999) 603–608, discussion 611.
- [25] I.T. Jolliffe, *Principal Component Analysis*, Springer, New York, 2002.
- [26] S. Wold, Pattern recognition by means of disjoint principal components models, *Pattern Recognit.* 8 (1976) 127–139.
- [27] R.J. Barnes, M.S. Dhanoa, S.J. Lister, Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra, *Appl. Spectrosc.* 43 (1989) 772–777.
- [28] A. Savitzky, M.J.E. Golay, Smoothing and differentiation of data by simplified least-squares procedures, *Anal. Chem.* 36 (1964) 1627–1639.
- [29] D. Coomans, I. Broeckaert, M.P. Derde, A. Tassin, D.L. Massart, S. Wold, Use of a microcomputer for the definition of multivariate confidence regions in medical diagnosis based on clinical laboratory profiles, *Comput. Biomed. Res.* 17 (1984) 1–14.
- [30] H.M. Heise, R. Marbach, T. Koschinsky, F.A. Gries, Noninvasive blood glucose sensors based on near-infrared spectroscopy, *Artif. Organs* 18 (1994) 439–447.