—Regular Article—

Detailed Analysis of Clinical Test Data on Chemotherapy for Colorectal Cancer

Noboru SOTOISHI,*[,a,d] Kazuhiko YOSHIMATSU,[b] Hajime YOKOMIZO,[b] Takao KATSUBE,[b] Junji NISHIMURA,[c] Shigeru YAKOU,[a] Kenji OGAWA,[b] and Kozo TAKAYAMA[d]

[a]*Department of Pharmacy,* [b]*Department of Surgery, Tokyo Woman's Medical University Medical Center East, 2–1–10 Nishiogu, Arakawa, Tokyo 116–8567, Japan,* [c]*Yamatake Corporation, 19F Tokyo Building, 2–7–3 Marunouchi, Chiyoda, Tokyo 100–6419, Japan, and* [d]*Department of Pharmaceutics, Hoshi University, 2–4–41 Ebara, Shinagawa, Tokyo 142–8501, Japan*

(Received December 24, 2010; Accepted February 4, 2011; Published online February 14, 2011)

We reported previously that spline interpolation is effective as a pretreatment before analyzing clinical data by time series. However, further improvement is required to understand the detailed tendency of clinical data. In this study, the tendency of interpolated hematological data was investigated in the period between the most tolerated dose (MTD) and low-dose chemotherapy (LDC) for colorectal cancer. All patients were received both MTD and LDC. Hematological data, white blood cell count (WBC), red blood cell count (RBC) and mean corpuscular volume (MCV), were interpolated. The accuracy of interpolation was verified using leave-one-out cross-validation. The difference, Δ_i, was calculated from interpolated data and exhibited as a function of time. The predictions of RBC and MCV were accurate with high correlation coefficients, although the interpolation of WBC data was inaccurate. A marked difference was observed in the trend of Δ_i between LDC and MTD periods. SD-RBC showed significant differences between LDC and MTD periods. The SD-MCV average in the LDC period was larger than in the MTD period. SD-MCV showed no significant difference. An attractor plot of Δ_i in RBC clarified the tendency of the interpolated RBC data. There is a possibility that Δ_i of RBC and/or SD-RBC may contribute to monitoring adverse reactions and decision of medication. Moreover, it is also useful to check on attractor plot of Δ_i in RBC together with SD-RBC in order to find out untoward reactions and decision of medication.

Key words——spline interpolation; time series analysis; chemotherapy; bone-marrow suppression; clinical test

INTRODUCTION

When patients receive chemotherapy, many clinical tests are performed. In order to prevent adverse effects, liver, renal and bone-marrow function should be checked. In particular, bone-marrow function is important because chemotherapy usually causes hematotoxicity. Furthermore, it is crucial to understand the time-course tendency of blood test results, which may be understood according to data plots over time; however, objective evaluation of this tendency is difficult because of the low frequency and irregular intervals of tests under the pragmatic medical treatment of outpatients. We reported previously that spline interpolation is effective as a pretreatment before analyzing clinical data of patients with gastric cancer by time series.[1] Spline is a piecewise polynomial function that is connected smoothly and has several continuous derivatives. It passes exactly through each vertebral point (*i.e.*, it has zero errors locally around the chosen points).[2] Furthermore, it was indicated that red blood cell count (RBC) data can be predicted precisely every seven days by spline interpolation, and interpolated RBC is an important factor that can identify hematotoxicity. To predict untoward reactions to chemotherapy, it could be required to analyze hematological data by using an advanced analytical method. Hematological data of the patients with metastatic colorectal cancer (mCRC) were employed in this study, because a large number of clinical data of mCRC with chemotherapy were available in our facilities compared with those of gastric cancer which had been reported in the previous article.[1] In a few cases of gastric cancer, it was difficult to evaluate relative merits of spline interpolation with different algorithms. In this study, however, an appropriateness of spline interpolation with different algorithms can be evaluated because of sufficient number of mCRC data.

Chemotherapy for colorectal cancer has greatly improved recently, and patients with mCRC have a life expectancy of 6 months.[3] Combination therapy of

*e-mail: sotoph@dnh.twmu.ac.jp

oxaliplatin, fluorouracil and leucovorin is called FOLFOX, and contributes to prolonging overall survival (OS) to 15 months or more in phase III studies.[4,5] Furthermore, FOLFOX combined with bevacizumab prolonged OS over 20 months;[6] however, it is known that adverse reactions, neutropenia, peripheral neuropathy, diarrhea, vomiting and so forth are frequently caused by FOLFOX.[7] It is thought that combination therapy at a MTD results in a low quality of life (QOL). LDC, such as metronomic chemotherapy, is applied when patients refuse or cannot tolerate MTD. Metronomic chemotherapy, which is a chronic low-dose administration schedule, has been recognized as a therapy with minimum toxicity.[8,9] Although LDC has a relatively low evidence, marked efficacy has been demonstrated in a few cases. The dosage as decided empirically since there was no marker to establish the optimal dose of LDC.[8,10] It is important to monitor therapeutic activity early during the course of treatment; because it is thought that the curative effect is related with its toxicity, it is meaningful to evaluate the tendency of hematological data over time.

In this study, in order to show clearly which algorithm is the most suitable among the available spline types, the leave-one-out cross-validation (LOOCV) of spline interpolations was studied. Hematological data of individual patients with mCRC were interpolated. The tendency of interpolated data was estimated by the rate of change in order to study differences in the period between MTD and LDC for mCRC. Furthermore, an attractor plot was applied as time series analysis.

METHODS

Patients and Chemotherapy Previous hematological data of 10 patients with mCRC were accumulated and are summarized in Table 1. All patients were treated at Tokyo Women's Medical University Medical Center East and had received both MTD and LDC. All MTD regimens included oxaliplatin, 5-fluorouracil (5-FU), and leucovorin. And these were m-FOLFOX6, shown in Fig. 1 except one case. The LDC regimen consisted of 80 mg/d of S-1; TS-1®

Table 1. Summary of Clinical Data for Patients in This Study

Case	Age	Sex	BSA (m^2)	Diagnosis	LDC regimen	MTD regimen
1	69	M	1.73	RK SE N1H3P0M0 mod. diff. adenocarcinoma	S-1/CPT-11 80 mg/body	m-FOLFOX6
2	68	M	1.69	RK (Rs) SE N1H2P3M0 mod. diff. adenocarcinoma		m-FOLFOX6
3	50	M	1.62	CK (A) SS N4P0H0M1 mod. diff. adenocarcinoma		m-FOLFOX6
4	57	M	1.77	RK (Rb) A P1 (recurrence) well diff. adenocarcinoma		m-FOLFOX6
5	77	M	1.64	RK SS N0H (multiple) P0 mod. diff. adenocarcinoma		Biweekly oxaliptatin: 100 mg and weekly 5-FU/LV: 500 mg/25 mg
6	75	F	1.32	CK (A) SS P0N0H0M1 mod. diff. adenocarcinoma		m-FOLFOX6
7	69	M	1.47	CK (A) SS H3P0M1 mod. diff. adenocarcinoma		m-FOLFOX6
8	77	M	1.51	CK SS N2H0P0M1 mod. diff. adenocarcinoma		m-FOLFOX6
9	72	F	1.42	CK (C) SS H2P0M1 well diff. adenocarcinoma		m-FOLFOX6
10	44	F	1.65	RK (Rs) SE N0P0H1 mod. diff. adenocarcinoma		m-FOLFOX6

BSA: body surface area.

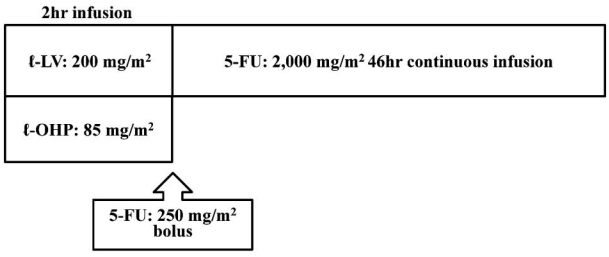

Fig. 1. Medical Treatment Schedule of m-FOLFOX6 (MTD) in This Study

l-LV; leucovorin, *l*-OHP; oxaliplatin. All of MTD were m-FOLFOX6 except one case. This regimen is repeated at intervals of over 2 weeks. The dose of 5-FU was smaller than in the general regimen.

(Taiho Pharmaceutical Co. Ltd., Tokyo, Japan) containing tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate (Oxo), and 80 mg/biweek (day 1, 15) of irinotecan (CPT-11). S-1 and CPT-11 regimens were prescribed for patients regardless of the body surface area. S-1 was administrated twice daily for 21 days, followed by a 7-day washout period.

This study was approved by the ethics committee of Tokyo Woman's Medical University.

Spline Interpolation Hematological data, WBC, RBC and MCV, were interpolated. The prediction interval was every seven days. The accuracy of prediction had a markedly shorter interval than the actual measurement; therefore, the prediction interval was determined in consideration of the life period and nadir intervals of blood cells.

The algorithms (biharmonic, thin-plate, and cubic spline) were discussed to decide which is suitable for spline interpolation of laboratory data. Green functions are used for interpolation of multi-dimensional data points in biharmonic and thin-plate algorithms.[11,12] The thin-plate algorithm is superior for interpolating observational data, including experimental errors, since interpolated data are rectified by thin-plate approximation.[13] On the other hand, cubic splines interpolate the data with piecewise cubic polynomials. The use of low-order polynomials is especially attractive for curve fitting because they reduce the computational requirements and numerical instabilities that arise with higher degree curves.[14]

dataNESIA® (Yamatake Corp., Tokyo, Japan), computer software for experimental data analysis, was used for spline interpolation. The leave-one-out cross-validation (LOOCV) method was carried out using software preinstalled in dataNESIA®. In order to determine the optimal method, various spline algorithms were investigated. The correlation coefficients of thin-plate, biharmonic, and cubic spline interpolation were compared.

Calculation of Δ_i and SD of Δ_i In order to emphasize the tendency of interpolated data, a difference $[\Delta_i;\ (Y_{i+1}-Y_i)]$ was calculated and exhibited as a function of time. Since spline data were obtained at regular 7-day intervals, the Δ_i value serves as a rate of change. Moreover, the standard deviation (SD) of Δ_i was calculated as an index of variance of Δ_i. The populations of SD of Δ_i in LDC and MTD were analyzed by the unpaired Welch's t test, accounting for unequal variance.

Attractor Plot The attractor plot of Δ_i in RBC is constructed by joining points defined by the time between a set of data (Δ_i, Δ_{i+1}) and subsequent data $(\Delta_{i+1}, \Delta_{i+2})$.

RESULTS

Predictive Accuracy of Spline Interpolation Three algorithms of spline interpolation were examined. The correlation coefficient in LOOCV when interpolating hematological data by the spline of several algorithms is shown in Table 2. The values of cubic spline were obviously higher than those of other algorithms. The correlation coefficients of thin-plate and biharmonic algorithms were almost identical for each inspection item.

Typical examples of data measured and predicted by spline interpolation are shown in Fig. 2. Although the interpolations of WBC data were inaccurate, the predictions of RBC and MCV were accurate with high correlation coefficients. These results were similar to our previous report. Typical examples (case 1) of predicted outcomes and LOOCV are shown in Fig. 3.

Conversion to "Δ_i" from Interpolated Data Time course of Δ_i of RBC data in case 1 is shown in Fig. 4. A marked difference was observed in the trend of Δ_i between LDC and MTD periods. Although moderate reductions and increases were repeated periodically, the Δ_i was stable during LDC. In the period of MTD, the Δ_i fluctuated wildly, compared with the LDC period. Time courses of interpolated data and Δ_i of MCV are shown in Fig. 5. Although a difference in the trend of Δ_i of MCV between periods of LDC and MTD was found, it was different from Δ_i of RBC.

The details of administration and Δ_i in case 1 are

Table 2. Correlation Coefficients in LOOCV When Interpolating Hematological Data by Spline of Several Algorithms

Case	WBC			RBC			MCV		
	TP	BH	CU	TP	BH	CU	TP	BH	CU
1	0.052	0.053	0.186	0.426	0.426	0.915	0.425	0.403	0.942
2	0.561	0.558	0.772	0.580	0.579	0.862	0.887	0.885	0.971
3	0.228	0.227	0.352	0.641	0.639	0.799	0.987	0.988	0.991
4	0.753	0.752	0.610	0.799	0.798	0.850	0.824	0.824	0.973
5	0.388	0.387	0.145	0.858	0.859	0.897	0.930	0.931	0.960
6	0.014	0.017	0.841	0.633	0.629	0.836	0.949	0.948	0.981
7	0.310	0.308	0.137	0.744	0.744	0.792	0.992	0.992	0.979
8	0.414	0.419	0.371	0.218	0.215	0.792	0.942	0.941	0.995
9	0.203	0.203	0.530	0.405	0.404	0.763	0.903	0.903	0.973
10	0.161	0.161	0.186	0.696	0.696	0.836	0.935	0.935	0.971

TP; thin-plate, BH; biharmonic, CU; cubic.

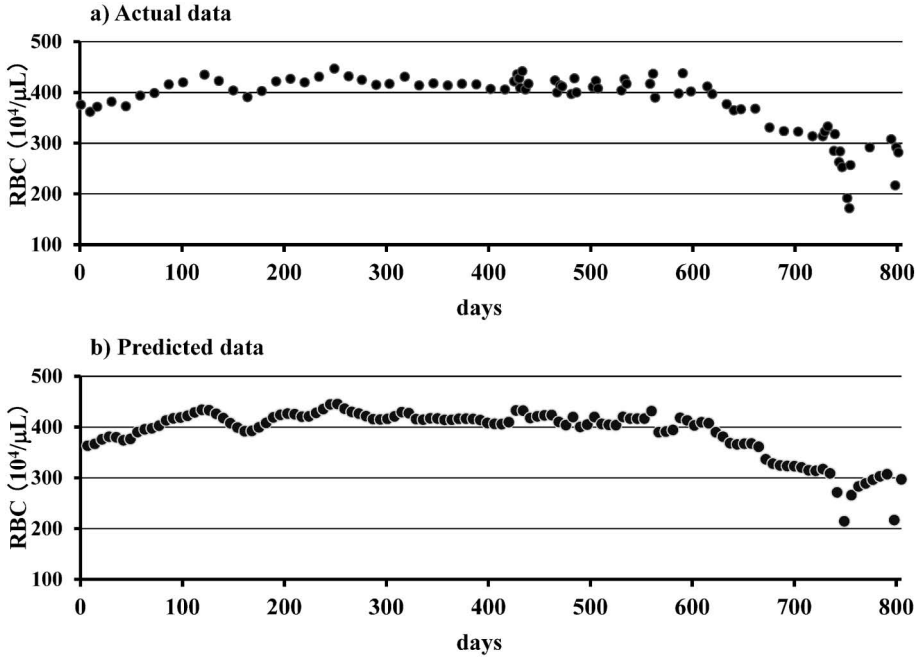

Fig. 2. Time Course of RBC Data Measured and Predicted by Cubic Spline Interpolation in Case 1
The prediction was made every 7 days.

shown in Figs. 6 and 7. The periodicity of RBC was matched with the medication timing until 4th LDC course, as for Δ_i of RBC; however, the cycles gradually became longer than the medication times. By contrast, no such phenomenon was seen for the Δ_i of MCV. During MTD, just after the administration of mFOLFOX6, rapid reduction of Δ_i of RBC was observed. Although weak, this phenomenon was observed also in the Δ_i of MCV (Fig. 7).

Δ_i also showed the same features in other cases; the fluctuation was very small in two cases (cases 2 and 6), but periodicity could be found.

SD of Δ_i Table 3 and Fig. 8 show the SD of Δ_i of RBC (SD-RBC) and Δ_i of MCV (SD-MCV). SD-RBC in the MTD period was larger than in the LDC period in all cases. SD-RBC showed significant differences between LDC and MTD periods. Five cases had larger SD-MCV in the MTD period than in the LDC

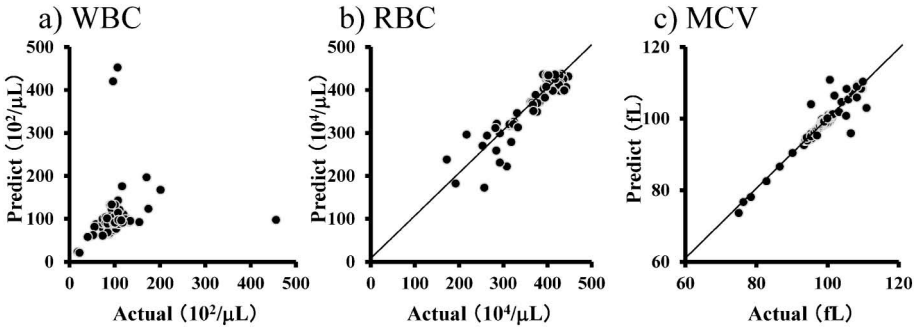

Fig. 3. Leave-one-out Cross-validation Plots of Hematological Data Measured and Predicted by Cubic Spline in Case 1
Actual data are plotted on horizontal axis, and predicted data are plotted on vertical axis.

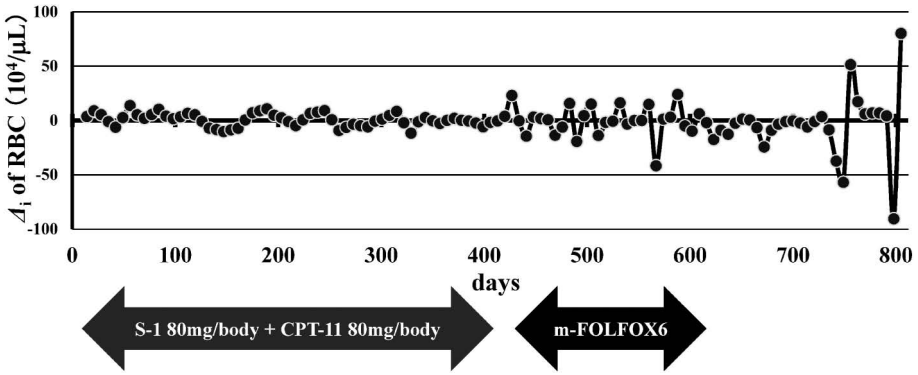

Fig. 4. Time Course of Δ_i in RBC in Case 1
Arrows at the bottom indicate the chemotherapeutical period. Patients took S-1 for 21 consecutive days, and then rested for 7 days. CPT-11 was injected on day 1 and 15 in every course.

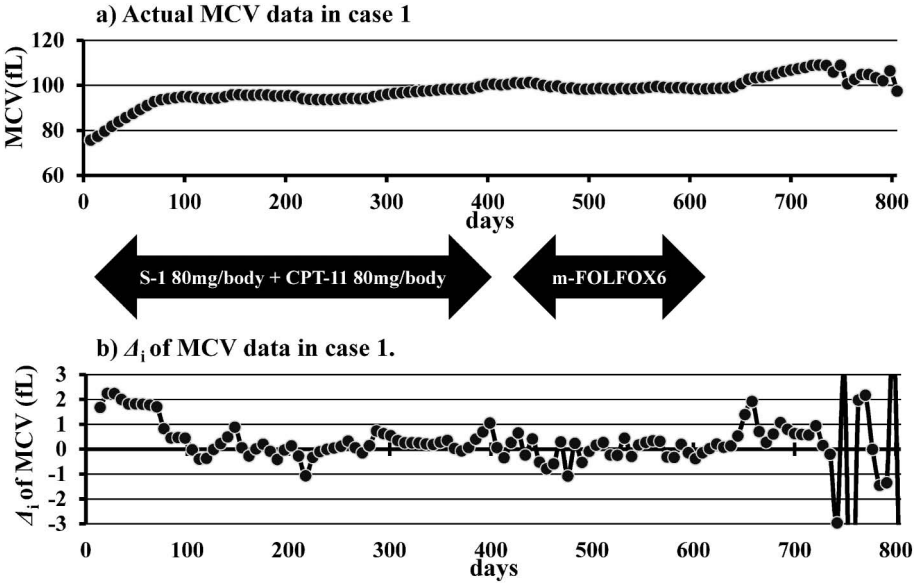

Fig. 5. Time Course of Interpolated Data and Δ_i in MCV in Case 1
Arrows between figures indicate the chemotherapeutical period. Patients took S-1 for 21 consecutive days, and then rested for 7 days. CPT-11 was injected on day 1 and 15 in every course.

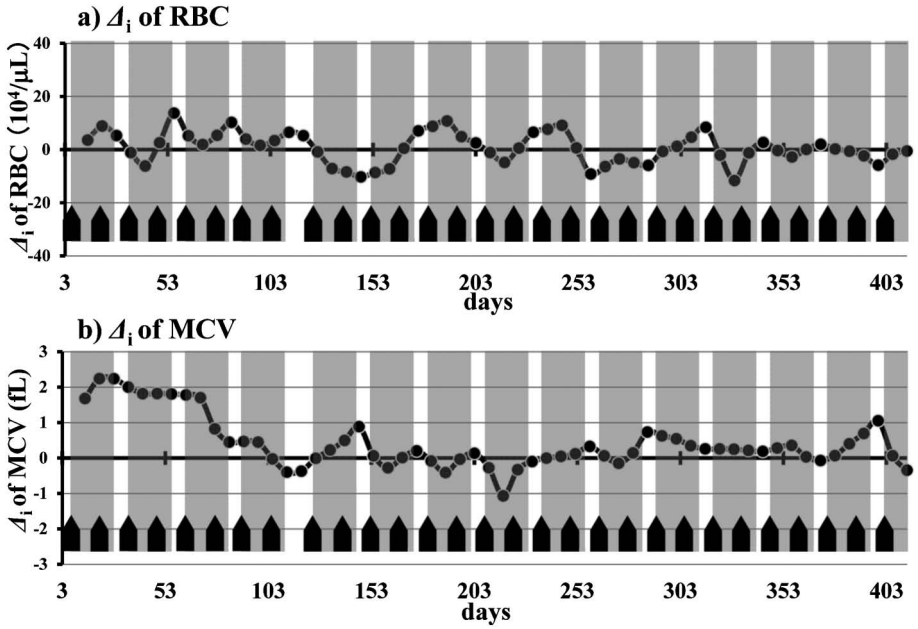

Fig. 6. The Actually Performed LDC Medication Schedule and Δ_i in Case 1
Arrows indicate the administration of CPT-11. Shading in the figures are the periods of S-1 medication.

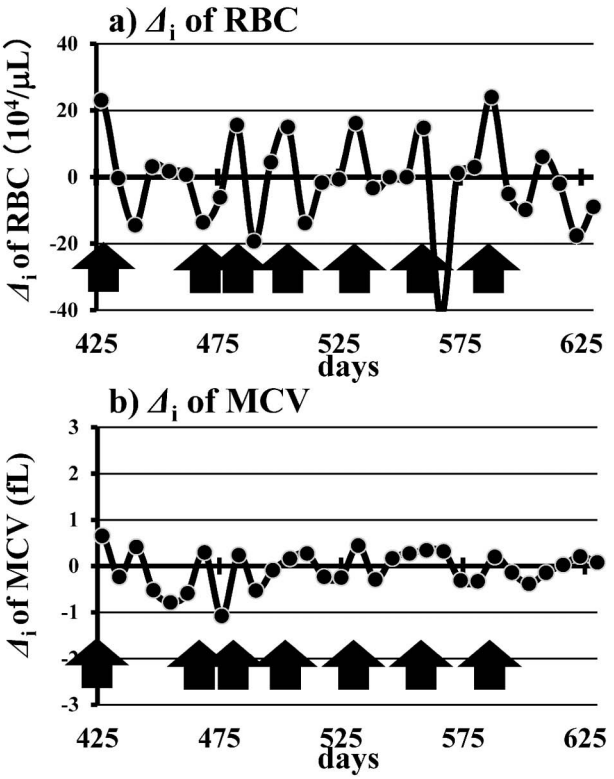

Fig. 7. The Actually performed MTD Medication Schedule and Δ_i in Case 1
Arrows indicate the administration of m-FOLFOX6.

Table 3. SD-RBC and SD-MCV data

Case	SD-RBC		SD-MCV	
	LDC	MTD	LDC	MTD
1	16.43	28.66	0.775	0.711
2	5.81	13.41	0.735	0.400
3	12.15	22.90	0.612	1.130
4	13.30	17.96	1.078	0.809
5	7.65	9.12	0.404	0.703
6	6.46	12.88	0.718	0.693
7	14.65	22.81	0.573	0.581
8	14.43	18.89	1.037	1.284
9	18.84	19.72	1.678	0.645
10	8.75	18.98	0.479	0.581
ave.	11.85	18.53	0.810	0.754
s.d.	4.47	5.66	0.374	0.265

ave; average SD in differences, s.d.; standard deviation of S.D. values.

period. The SD-MCV average in the LDC period was larger than in the MTD period. SD-MCV showed no significant difference.

Attractor Plot Typical example for attractor plots of Δ_i in RBC are shown in Fig. 9. These three cases have a comparatively long observation period. The plots were attracted near zero both in MTD and LDC. However, it was observed that the plot concentrated on the narrower range in LDT.

DISCUSSION

The time course of hematological data in 10 patients with mCRC was examined. The administration dose of FOLFOX in this study was lower than the usual dose; however, anemia is sometimes observed in patients with a lower administration of FOLFOX. Most adverse effects are not observed in patients with a low-dose combination of S-1 and CPT-11; thus, FOLFOX in this study could be categorized for MTD. Moreover, one patient received combination therapy of biweekly oxaliplatin: 60 mg/m^2, and weekly 5-FU/LV: 500 mg, 25 mg/body. This was also judged to be MTD.

Study of the Algorithm of Spline Interpolation

In our previous report, many items in the blood test were interpolated by cubic spline and the prediction accuracy was validated by LOOCV. However, study of the algorithm was not performed since we treated a few cases in the report. In order to show clearly which algorithm is the most suitable, the LOOCV of spline interpolations with some available algorithms was studied. As seen in Table 2, the correlation coefficients of biharmonic and thin-plate splines were almost identical in all inspection items and patients. The values of cubic spline were superior to these of other algorithms, although they might be different when the correlation coefficient was very low (*cf.* WBC in cases 5, 7 and 8). In particular, higher correlation coefficients using cubic spline than other algorithms in RBC were obtained in cases 1 and 8. It was found that cubic spline was the best algorithm to interpolate the time course of hematological data in this study. The third-order polynomial equation is used as a basic algorithm of cubic spline interpolation. On the other hand, a boundary element method is used in both biharmonic and thin-plate spline interpolations. It can be thought that the third-order polynomial equation was better suited for approximation of RBC and MCV data. When considering each inspection item, the correlation coefficient was high in the order of MCV, RBC, and WBC. MCV, which has been used to diagnose anemia, was adopted because the values of MCV were more accurately interpolated than those of RBC. It was thought that monotonic changes such as MCV and RBC were well-predicted,

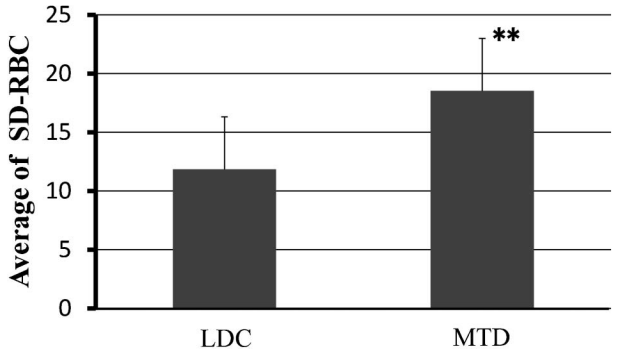

Fig. 8. Average SD-RBC during LDC and MTD
Error bars represent standard deviation of S.D. values. Welch *t*-test, ** $p<0.01$.

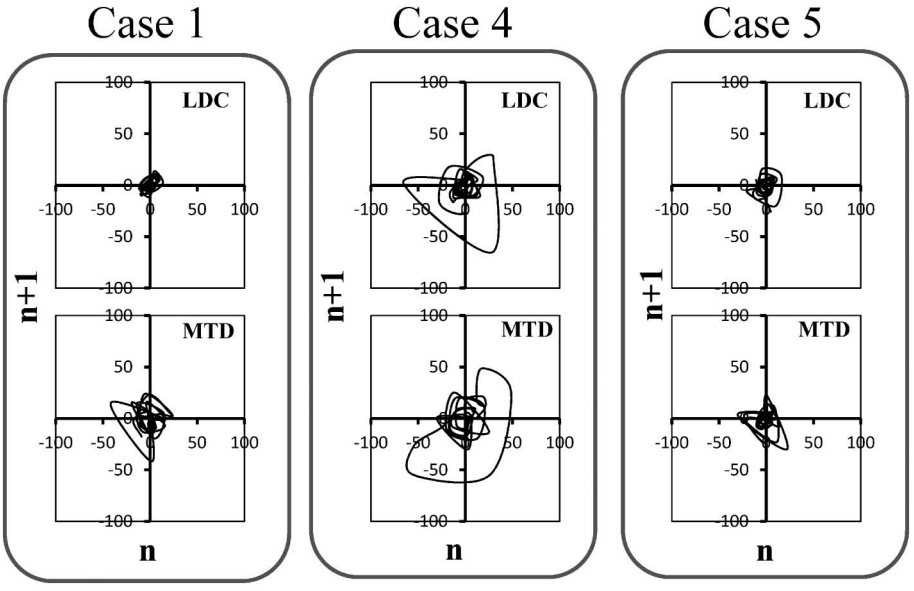

Fig. 9. Attractor Plots of Δ_i in RBC
Plots are the loci of the points which shift by time.

while data showing great deviation such as WBC were rather difficult to predict; therefore, bone-marrow function was investigated hereafter by using data of RBC and MCV.

Relation between Δ_i and Administration of Chemotherapy The tendency of the time course data was clarified by calculating Δ_i. Figure 4 shows the time course of Δ_i of RBC in case 1. During LDC, periodicity was observed with a slow, slight swing, whereas during MTD, large and frequent oscillation was observed. The periodicity of Δ_i of RBC was not in agreement with administration during LDC, but rather during MTD.

The periodicity during LDC was weak in two cases. The existence of periodicity may relate to effect of LDC to bone-marrow function. However, it remains unknown how periodicity is related with bone-marrow function. When more cases are accumulated, individual evaluation may clarify this link.

Evaluation of Bone-marrow Function Using SD of Δ_i and Attractor Plot of Δ_i in RBC In our previous report, it was discussed whether interpolated RBC is useful to evaluate bone-marrow function, and the attractor plot was used as an evaluation method; however, a phenomenon cannot be expressed numerically by only an attractor plot. In order to improve this problem, we introduced Δ_i, which indicates the change rate of hematological data, and the tendency was emphasized. Usually, a significant difference in SD is not identified, but was included because SD was treated as a parameter in this report. As shown in Fig. 8, MTD which markedly damaged bone-marrow function increased SD-RBC more than LDC. It is thought that SD-RBC was related with the effect of chemotherapeutic drugs, even if many factors affected bone-marrow function. This change, which is not easily visible by only actual measurement, could be identified by spline interpolation and computed SD-RBC.

On the other hand, there was no significant difference in SD-MCV. Oscillation was observed in Δ_i in MCV, although it was smaller than SD-RBC. No regular effect of chemotherapy on MCV was seen, although the difference may be dependent on other parameters.

Moreover, RBC data was evaluated by attractor plot of Δ_i in RBC as a time series analysis. See Fig. 9, narrow plot area means stable data. In short, it is considered that the effect of chemotherapy to bone-marrow is weak. Narrower plot area was observed in period of LDC than MTD. Actually, anemia appears hardly in LDC. Although it does not indicate whether the value is increasing or decreasing, attractor plot of Δ_i can clarify how much the values are vibrated. The meaning of attractor plot of Δ_i is similar with SD of Δ_i. However, attractor plot is expected to be a key which understands the tendency of laboratory data rather than SD. Temporary and wildly change of RBC was observed in the attractor plot of case 4. It is considered very important in clinical medication to observe such a period. Accordingly, attractor plot of Δ_i is better than SD of Δ_i in order to find out critical changes of hematological data as a function of time.

Prospects of Δ_i When bone-marrow suppression caused by chemotherapy is evaluated, it is difficult to identify the tendency using WBC, and it can be recognized from the results that WBC was incorrectly interpolated by cubic spline in this study. It is thought that slightly changing data could be well-predicted and were suited to identifying their tendency; however, MCV well-predicted by spline interpolation did not lead to useful parameters. Δ_i of RBC and SD-RBC seem to be promising factors to evaluate bone-marrow function. As described in the Introduction, the dosage of LDC is decided empirically since there is no marker to establish the optimal dose. Antiangiogenic efficacy appears to be optimized by LDC.[10] Some metronomic regimens can have surprisingly potent antitumor effects in preclinical models compared with the respective MTD regimens, despite being less toxic; however, a significant disadvantage is the absence of establishing the optimal dose and in monitoring therapeutic activity early during the course of treatment. It is considered that the curative effect is related with toxicity. Recently, it was reported that survival correlated with the occurrence and severity of a rash with the molecular target drug.[15,16] It is important to observe side effects carefully and to manage their symptoms, as this may enable patients to continue receiving therapy without dose interruption or discontinuation. There is a possibility that Δ_i of RBC and/or SD-RBC may contribute to monitoring untoward reactions and decision of medication. The relationship between the occurrence of adverse reactions and the parameters in patients with MTD administration is interesting. Moreover, observing attractor plot of Δ_i in RBC may become an approach to know the change in state of bone-marrow function

early.

CONCLUSION

In this study, hematological data of 10 patients with mCRC were interpolated. The correlation coefficients of cubic spline were obviously higher than those of other algorithms. Periodicity was observed during LDC in all cases but weak in 2 cases. SD-RBC during MTD was larger than during LDC in all cases. It may be useful to check on attractor plot of Δ_i in RBC together with SD-RBC in order to find out untoward reactions and decision of medication.

REFERENCES

1) Sotoishi N., Katsube T., Ogawa K., Yakou S., Takayama K., *J. Pharm. Pharm. Sci.*, **11**, 83–89, (2008).
2) De Boor C., ''A Practical Guide to Spline,'' Springer Verlag, New York, 2001.
3) Venook A., *The Oncologist*, **10**, 250–261, (2005).
4) Goldberg R. M., Sargent D. J., Morton R. F., Fuchs C. S., Ramanathan R. K., Williamson S. K., Findlay B. P., Pitot H. C., Alberts S. R., *J. Clin. Oncol.*, **22**, 23–30, (2004).
5) Colucci G., Gebbia V., Paoletti G., Giuliani F., Caruso M., Gebbia N., Carteni G., Agostara B., Pezzella G., Manzione L., Borsellino N., Misino A., Romito S., Durini E., Cordio S., Seri M. D., Lopez M., *J. Clin. Oncol.*, **23**, 4866–4875, (2005).
6) Saltz L. B., Clarke S., Diaz-Rubio E., Scheithauer W., Figer A., Wong R., Koski S., Lichinitser M., Yang T. S., Rivera F., Couture F., Sirzen F., Cassidy J., *J. Clin. Oncol.*, **26**, 2013–2019, (2008).
7) de Gramont A., Figer A., Seymour M., Homerin M., Hmissi A., Cassidy J., Boni C., Cortes-Funes H., Cervantes A., Freyer G., Papamichael D., Le Bail N., Louvet C., Hendler D., de Braud F., Wilson C., Morvan F., Bonetti A., *J. Clin. Oncol.*, **18**, 2938–2947, (2000).
8) Kerbel R. S., Kamen B. A., *Nature Reviews Cancer*, **4**; 423–436, (2004).
9) Gille J., Spieth K., Kaufmann R., Kaufmann R., *J. Dtsch. Dermatol. Ges.*, **3**, 26–32, (2005).
10) Shaked Y., Emmenegger U., Man S., Cervi D., Bertolini F., Ben-David Y., Kerbel R. S., *Blood*, **106**, 3058–3061, (2005).
11) Sandwell D. T., *Geophys. Res. Let.*, **14**, 139–142, (1987).
12) Wahba G., ''Spline Models for Observational Data,'' Society for Industrial and Applied Mathematics (SIAM), Philadelphia, 1990.
13) Takayama K., Obata Y., Morishita M., Nagai T., *Pharmazie*, **59**, 392–395, (2003).
14) Wolberg G., Alfy I., International Proceedings of the International Conference on Computer Graphics, June 1999, pp. 188–195.
15) Perez-Soler R., Chachoua A., Hammond L. A., Rowinsky E. K., Huberman M., Karp D., Rigas J., Clark G. M., Santavarbara P., Bonomi P., *J. Clin. Oncol.*, **22**, 3238–3247 (2004).
16) Wacker B., Nagrani T., Weinberg J., Witt K., Clark G., Cagnoni P. J., *Clin. Cancer Res.*, **13**, 3913–3921, (2007).