Table 2. Clinical features of patients

Patient No.	Age years	Gender	Risk factors	Embolic source	NIHSS score on admission	Duration of UFH up to the day of platelet nadir, days	Dose of UFH ($\times 10^3$ units)		Platelet count ($\times 10^3/\mu l$)	
							before nadir	total	baseline	nadir
Definite HIT										
1	64	M	HT	AF	15	9	88	88	247	122
2	69	F	HT	AF	17	9	106	106	245	119
Suspected HIT										
3	56	M	HL, smoking	NBTE[1]	18	37[3]	375	431	237	111
4	94	F	HT	AF	7	21[3]	130	130	165	67
Clinically suspected HIT										
5	72	F	DM	MVR, AF	18	9	80	80	185	88
6	58	M	smoking	none	16	2	30	30	220	82
7	70	M	HT, DM, smoking, alcohol	LV dyskinesis	9	7	102	102	244	146
8	80	F	DM, HL, smoking, alcohol	AF	17	8[3]	10	176	153	86

HT = Hypertension; AF = atrial fibrillation; HL = hyperlipidemia; NBTE = nonbacterial thrombotic endocarditis; AMI = acut myocardial infarction; DM = diabetes mellitus; MVR = mitral valve replacement (prosthetic valve); LV = left ventricle.

[1] Identified by autopsy examination.

[2] In patient No. 3, argatroban was administered for 14 days after the initial thrombocytopenic event according to diagnosis o clinical HIT, although anti-PF4/heparin Abs by ELISA assayed on day 24 was negative.

[3] UFH was discontinued and restarted several days later in these 3 patients.

ficity for clinical HIT differs between the two assays; that of SRA was reported to be 100% [17], and that of ELISA was reported to be 54.8 and 86% [17, 24]. Thus, in the present study, patients who were positive by both ELISA and SRA were regarded as having definite HIT, while those with a positive finding only by ELISA were regarded as having suspected HIT. Neither clinical scoring system was exactly compatible with the final diagnosis; a patient with a high probability of HIT according to the 4Ts system was not serologically positive, and 2 patients with a very high score according to the system proposed by Pouplard et al. were finally diagnosed as having suspected, not definite, HIT. Such discrepancies suggest that it is difficult to diagnose HIT based solely on clinical information, especially given the effects of alternate anticoagulation therapy. These discrepancies also suggest limitations in the serological assays, including a different specificity between ELISA and SRA, as well as technical issues, including the timing of blood sampling and cutoff values.

The estimated incidence of both clinically and serologically ascertained HIT in the present study was somewhat low compared to that reported in neurological patients by Harbrecht et al. [12] (2.5% by ELISA and 1.0% by an additional functional assay for heparin-induced platelet activation) and that reported in surgical patients receiving UFH (1–5%) [26]. The dose of UFH appears to be a determinant for HIT occurrence, since UFH is more likely to result in HIT when given in therapeutic rather than in prophylactic doses [27]. In the present study, the total dose of UFH in the 4 patients with definite or suspected HIT (118×10^3 units in median) was much lower than the dose given the patients with positive HIT antibodies reported by Harbrecht et al. [12] ($366 \pm 224 \times 10^3$ units). While ethnic difference may be another determinant, studies dealing with HIT in the Asian population are rare.

All patients who were diagnosed as having definite HIT developed HIT during the typical time period (5–10 days) [28], and most of the remaining 6 patients developed HIT during the period or later. Only 1 patient (No. 6) developed thrombocytopenia rapidly (24 h after the initiation of heparin); other than rapid-onset HIT, no other underlying etiology for the thrombocytopenia

Duration of UFH up to the day of assays, days	Therapy after suspicion of HIT		Thrombotic complication	mRS score on discharge
	cessation of UFH	alternative anticoagulation		
10	yes	argatroban	none	3
8	yes	argatroban	none	5
28	yes	argatroban[2]	DVT, PE, AMI, recurrent stroke	dead
19	yes	argatroban	none	dead
9	yes	–	recurrent stroke	5
3	yes	–	none	5
7	yes	–	none	4
9	no	–	DVT	5

could be identified [28]. However, the serological assay done at day 3 was negative for anti-PF4/heparin Abs.

The present study is unique in that the initial neurological severity and the subacute outcome of the stroke patients having HIT were determined. The admission NIHSS score (median, 16.5) was quite high, partly because most of our HIT patients had cardioembolic stroke, which is generally severer than other ischemic stroke subtypes. The poor outcome of the present patients appeared to be mainly due to the initial stroke severity and partly due to the associated thrombotic complications. HIT thrombosis syndrome occurred in approximately 25–50% of HIT patients who were managed by heparin cessation alone [29–31]. Intravenous argatroban has the potential to decrease the occurrence of thrombotic events related to HIT [31–33]. Argatroban was used for all of our patients who had definite or suspected HIT; this might prevent them from having additional thrombotic events, except for 1 patient (No. 3) with possible cancer-associated pseudo-HIT syndrome [22]. Adenocarcinoma is an important cause of venous and arterial thrombosis associated with thrombocytopenia; disseminated intravascular coagulation is the probable mechanism of these events. Thus, there can be rapid recurrence of thrombocytopenia within hours or days of discontinuing UFH. In the present patient No. 3, recurrent stroke occurred 3 days after discontinuing UFH.

An essential problem regarding the present theme is that recommendation for UFH differs among guidelines [6–8]. Urgent anticoagulation by UFH with the goal of preventing early recurrent stroke is not recommended in a recent guideline from the American Heart Association/American Stroke Association [6] based on trials including the International Stroke Trial [34]. According to a Cochrane review, although anticoagulant therapy, including UFH, was associated with about 9 fewer recurrent ischemic strokes per 1,000 patients treated (OR = 0.76; 95% CI = 0.65–0.88), it was also associated with a similar-sized 9 per 1,000 increase in symptomatic intracranial hemorrhages (OR = 2.52; 95% CI = 1.92–3.30) [35]. Nevertheless, UFH has frequently been given for acute ischemic stroke [13, 14, 36]. In contrast, another guideline suggests the effectiveness of low-dose UFH in reducing DVT for acute stroke patients [7]. In the Seventh American College of Chest Physicians Conference, prophylactic low-dose subcutaneous UFH or low-molecular-weight heparins or heparinoids were also recommended for acute ischemic stroke patients with restricted mobility (grade 1A) [8]. An overview analysis reviewed the results of 10 trials that evaluated heparin in 1,047 patients with acute ischemic stroke, and found an 80% reduction in DVT and a 58% reduction in PE [37]. In the International Stroke Trial, there was a significant reduction in the frequency of fatal or nonfatal PE, from 0.8 to 0.5%, among those treated with subcutaneous UFH [34].

This study had some limitations. First, HIT antibodies were measured only in 10 patients who were suspected of having HIT. Therefore, HIT may have been underdiagnosed. Second, ELISA was done immediately after being requested in most cases, and the ELISA results affected the therapeutic strategy, including the use of argatroban, which could have affected the clinical outcome. On the other hand, SRA was performed later to confirm the diagnosis of HIT; hence, the SRA results did not affect the therapeutic strategy. Third, venous ultrasound was not done in all of the patients; therefore, subclinical DVT, which is the typical thrombotic complication associated with HIT, may have been underdiagnosed.

In conclusion, HIT is uncommon, but not rare during or after UFH treatment for acute ischemic stroke. UFH should be stopped and alternative anticoagulation should be considered in acute stroke patients receiving UFH who

are clinically suspected of having HIT, especially in patients who develop thrombocytopenia or thromboembolic events between days 5 and 10 of the start of heparin therapy, which is the highest risk window for HIT. The combination of a clinical scoring system and serological tests for detecting HIT antibodies is useful for diagnosing HIT. However, at present, functional assays such as SRA can be performed with high-quality control in only a few institutes.

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Takotsubo Cardiomyopathy in Acute Ischemic Stroke

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Objective: Takotsubo cardiomyopathy, which is characterized by transient left ventricular apical ballooning, is a known complication of subarachnoid hemorrhage. The aim of this study was to identify the clinical characteristics of acute ischemic stroke patients who experienced development of takotsubo cardiomyopathy.

Methods: Seven patients who were diagnosed as having takotsubo cardiomyopathy based on their electrocardiographic and echocardiographic findings were studied. They were selected from among 569 consecutive patients who were admitted to our stroke center within 24 hours after onset of acute ischemic stroke. The findings of nine previously published cases were also reviewed.

Results: All seven patients were women, and six were 75 years or older. The initial National Institutes of Health Stroke Scale score ranged from 3 to 28. The culprit infarcts included or were close to the insular cortex in six patients and were located extensively in the vertebrobasilar arterial territory in the other patient. Abnormal findings on electrocardiographic monitoring appeared within 10 hours after stroke onset in five patients and at 6 and 12 days, respectively, in the other two patients. The cardiomyopathy was symptomatic in only two patients. Plasma brain natriuretic peptide levels exceeded the upper normal limit by 10-fold in all patients. The previously published cases were mostly women and had mainly vertebrobasilar stroke.

Interpretation: Takotsubo cardiomyopathy is not a rare complication of acute ischemic stroke. It most often occurred soon after stroke onset and was commonly asymptomatic. Female sex and insular damage were predominant features of the stroke patients who experienced development of takotsubo cardiomyopathy.

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Takotsubo cardiomyopathy (apical ballooning syndrome, ampulla cardiomyopathy, stress cardiomyopathy) is characterized by transient left ventricular apical ballooning that resembles a Japanese octopus catcher pot (takotsubo) with a short narrow neck and round bottom.[1,2] The typical electrocardiogram (ECG) abnormalities are ST elevation in the acute phase followed by giant negative T waves, most frequently in leads V3 and V4.[3,4] The abnormality of left ventricular wall motion sometimes mimics acute coronary syndrome, but it extends beyond a single epicardial vascular distribution and improves within weeks in most cases. Profound psychological stresses, including earthquakes, gambling loss, and estrangement from family, trigger this unique syndrome.[5,6] Although the precise cause of this syndrome is still unknown, possible mechanisms include catecholamine-induced myocardial stunning, ischemia-mediated stunning caused by multivessel epicardial or microvascular spasm, and myocarditis.[3,4] In particular, frequent occurrence of preceding psychological events suggests a key role of catecholamines in triggering the syndrome. Wall motion abnormalities have been observed in conditions associated with catecholamine surge, including a pheochromocytoma.[7] In a recent classification of cardiomyopathies advocated by the American Heart Association, takotsubo cardiomyopathy was ranked as a primary acquired cardiomyopathy.[8]

Takotsubo cardiomyopathy is a well-known complication of subarachnoid hemorrhage (SAH); massive catecholamine release caused by the hemorrhage may trigger the cardiomyopathy.[9,10] On the other hand, the occurrence of this complication in patients with acute ischemic stroke has not been systematically examined.

The goal of this study was to identify the clinical characteristics of acute ischemic stroke patients who experienced development of takotsubo cardiomyopathy.

Patients and Methods

Between February 2005 and October 2006, 569 patients were admitted to our stroke center within 24 hours after onset of acute ischemic stroke. All patients underwent ECG

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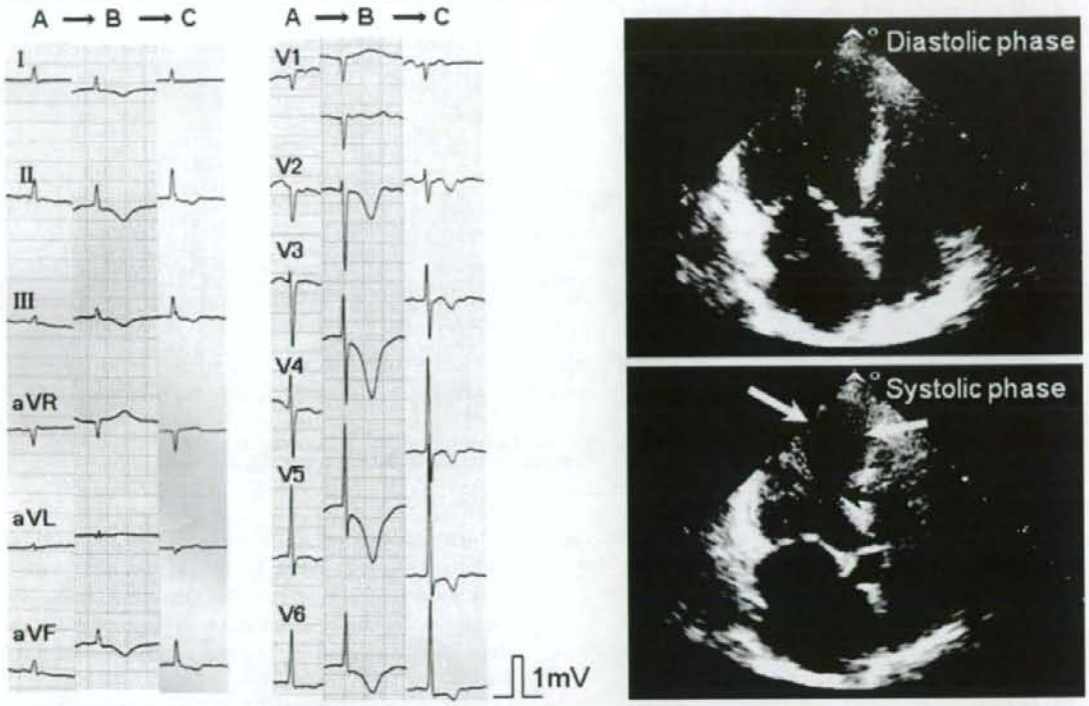

Fig 1. Typical electrocardiogram and echocardiogram in takotsubo cardiomyopathy. (left panels) Electrocardiographic (ECG) changes in Patient 2 on admission (A), *at 4 days* (B), *and at 3 weeks* (C). *(right panels) Four-chamber view of ECG of Patient 3 showing balloon-like asynergy of the apical region* (white arrow) *and hypercontraction of the basal segment* (black arrows).

monitoring during the initial several days they were hospitalized in our stroke care unit (for at least 24 hours); longer ECG monitoring was performed if necessary. The criteria used to identify patients experiencing development of takotsubo cardiomyopathy during the initial days of hospitalization included: (1) appearance of abnormal ECG findings, including ST-segment elevation and negative giant T waves during the initial days of hospitalization that were absent on admission (Fig 1A); and (2) "balloon-like asynergy," which involves circumferential hypokinesis, akinesis, or dyskinesis of mid and apical segments of left ventricle with normal or hypercontraction of the basal segment on echocardiogram, whereas ECG showed the abnormal findings (see Fig 1B). Patients who satisfied both criteria were prospectively enrolled.

The following underlying risk factors were examined: sex, age, history of cardiopulmonary disease and stroke, hypertension (blood pressure $\geq$ 140/90mm Hg before stroke or history of antihypertensive medication), diabetes mellitus (fasting blood glucose $\geq$ 126mg/dl, positive 75gm oral glucose tolerance test, or history of antidiabetic medication), hypercholesterolemia (serum total cholesterol $\geq$ 220mg/dl or history of antihypercholesterolemic medication), current or previous smoking habit, and alcohol consumption of 2 or more drinks per day.

Ischemic stroke was defined as focal neurological symptoms for which diffusion-weighted magnetic resonance imaging (MRI), or computed tomography if MRI was contraindicated, demonstrated corresponding infarcts. Cephalocervical arterial lesions were assessed using magnetic resonance angiography, unless contraindicated, and ultrasound for all patients. Based on neurological, radiological, and cardiological findings, the stroke subtype was determined by trained vascular neurologists according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) subtype classification system.[11] Neurological deficits were evaluated using the National Institutes of Health Stroke Scale score on admission and at discharge around 3 weeks after stroke onset. The grade of disability was assessed using the modified Rankin Scale score at discharge.

In addition to the ECG and echocardiogram examinations, creatine kinase and its myocardial band (MB) subtype, troponin-T, and brain natriuretic peptide (BNP) levels were measured in the blood as indicators of cardiac changes. Cardiac MRI and coronary angiography were performed if needed.

In addition, all available published case reports on cardiac dysfunction occurring in acute ischemic stroke patients that was attributable to takotsubo cardiomyopathy were identified in the PubMed database using the following search terms: "takotsubo cardiomyopathy," "stress-induced cardiomyopathy," "apical ballooning," "broken heart syndrome," "myocardial stunning," or "cardiac dysfunction"; and "ischemic

Table 1. **Underlying Characteristics and Stroke Features for Seven Patients**

Patient No.	Sex	Age, yr	History of Cardiopulmonary Disease	History of Stroke	Vascular Risk Factors	Stroke Subtype	Arterial Lesion	Major Neurological Signs	Admission NIHSS	NIHSS at Discharge	mRS at Discharge
1	F	78	Pulmonary tuberculosis	None	HT, smoking, drinking	Other (aotogenic embolism)	None	Hemiparesis, aphasia	9	3	2
2	F	90	AF, AS, CHF	None	Smoking	Cardiogenic embolism	R ICA occlusion	Hemiparesis, USN, anosognosia	15	14	4
3	F	78	AF	Ischemic stroke	None	Cardiogenic embolism	None	Hemiparesis, aphasia	19	17	5
4	F	75	AF, MSR, CHF	None	HT, HC	Cardiogenic embolism	None	Hemiparesis	3	0	0
5	F	82	Pulmonary tuberculosis	None	HT, HC	Other	None	Hemiparesis	8	8	4
6	F	80	None	None	HT, DM	Other	None	Hemiparesis	6	19	5
7	F	47	PFO	None	None	Other	BA occlusion (dissection)	Tetraparesis locked-in	28	25	5

NIHSS = National Institutes of Health Stroke Scale; mRS = modified Rankin Scale; HT = hypertension; AF = atrial fibrillation; AS = aortic stenosis; CHF = congestive heart failure; ICA = internal carotid artery; USN = unilateral spatial neglect; MSR = mitral stenosis with regurgitation; HC = hypercholesterolemia; DM = diabetes mellitus; PFO = patent foramen ovale; BA = basilar artery.

stroke" or "cerebral infarction". The Japanese literature was also searched using the Web of Japana Centra Revuo Medicina with the following terms: "takotsubo," and "brain infarction" or "artery occlusion".

Results

Analyses of the Seven Patients Admitted to Our Stroke Center

Of the 569 patients studied, 7 (1.2%) were diagnosed as having takotsubo cardiomyopathy. All patients were women and 75 years or older, except for a 47-year-old woman (Patient 7; Table 1). Six patients had histories of cardiopulmonary disease. One (Patient 3) had a previous history of a mild ischemic stroke in the left parietal lobe. Five patients had one or more vascular risk factors.

Four patients (Patients 1–4) experienced development of an infarct in a unilateral hemisphere including the insular cortex caused by cardiogenic or aortogenic

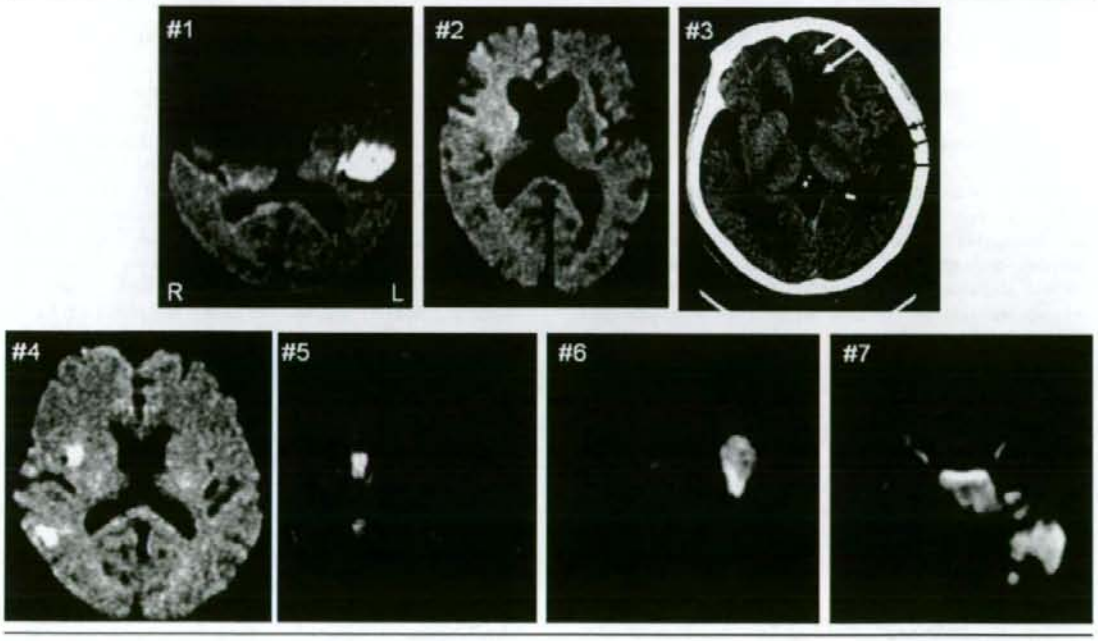

Fig 2. Distribution of brain infarcts for the seven patients.

Table 2. Characteristics of Cardiomyopathy for Seven Patients

Patient No.	Cardiopulmonary Symptom	Electrocardiography				Echocardiography	
		Appearance of Change*	ST Elevation	Abnormal Q	Negative Giant T	% FS	Apical Wall Motion
1	None	5 hours	Present	Present	Present	40	Severe hypokinesis
2	Chest pain	9.5 hours	Absent	Absent	Present	12	Akinesis
3	None	8 hours	Present	Absent	Present	34	Severe hypokinesis
4	Respiratory discomfort	2.5 hours	Present	Absent	Present	30	Akinesis
5	None	5 hours	Present	Present	Present	27	Akinesis
6	None	6 days	Present	Present	Present	45	Akinesis
7	Unclear (because of locked-in syndrome)	12 days	Absent	Absent	Present	21	Severe hypokinesis

*Time interval between stroke onset and electrocardiographic change.
NYHA = New York Heart Association; FS = fractional shortening; CK = creatine kinase (reference value: 45-163U/L), CK-MB = creatine kinase myocardial band (<0.23U/L): Troponin-T (<0.25ng/ml); BNP = brain natriuretic peptide (<20.0pg/ml).

embolism; one of these patients (Patient 2) had an embolic occlusion of the right internal carotid artery (see Table 1; Fig 2). Two patients (Patients 5 and 6) had a basal ganglia infarct that gradually expanded close to the insular area and caused neurological deterioration; their diagnosis may have been branch atheromatous disease. The remaining young patient (Patient 7) experienced development of bilateral cerebellar and pontine infarcts with basilar artery occlusion caused by dissection. All seven patients had motor paresis, three (Patients 1–3) had cortical signs, and one (Patient 7) had a locked-in syndrome. During the first several days, all patients received intravenous anticoagulation using unfractionated heparin (Patients 1–4) or argatroban (Patients 5–7), primarily to prevent embolic events from the hypokinetic cardiac wall caused by the cardiomyopathy. During their hospitalization, no patients had stroke recurrence. Five patients needed a wheelchair for daily living at the time of discharge, corresponding to a modified Rankin Scale score of 4 or 5.

The cardiomyopathy was symptomatic in only two patients (Patients 2 and 4) (Table 2). ECG changes appeared within 10 hours after stroke onset in five patients (Patients 1–5), and at 6 and 12 days, respectively, in the other two patients (Fig 3). In all seven patients, echocardiography showed localized left ventricular hypokinesis around the apical area. In six patients who received follow-up echocardiography (except for Patient 3), the hypokinesis improved within a month. For two of three patients who underwent cardiac MRI (Patients 4 and 5), unusual hypokinesis or akinesis was identified on cine image; the remaining patient (Patient 1) had normal MRI findings 3 weeks after stroke onset, when her ECG and echocardiography were also normal. For two patients who underwent coronary angiography (Patients 1 and 5), the coronary arteries were not stenotic. Of the cardiac enzymes, only the BNP level showed a marked increase (>10-fold increase of the upper normal limit). For one patient (Patient 5), virus titers were measured twice on days 12 and 33; no virus titers including echoviruses and coxsackie viruses were elevated. Except for two patients who had pre-existing valvular disease and heart failure (Patients 2 and 4), none had heart failure at discharge.

Literature Review

To the best of our knowledge, nine patients have been reported as developing an unusual cardiomyopathy during acute ischemic stroke that was attributable to takotsubo cardiomyopathy: two were English case reports (Cases 8 and 9),[12,13] three were Japanese case reports (Cases 10–12),[14–16] and four were Japanese conference abstracts (Cases 13–16) (Table 3).[17–19] Of these nine patients, seven were women, and the patients' ages ranged from 60 to 85 years. The location of brain infarcts or arterial lesions was described in seven patients; four had a vertebrobasilar stroke (Patients 11 and 14–16), including a patient with a definite basilar artery occlusion that recanalized after intraarterial thrombolysis (Patient 11). Of the four patients whose cardiopulmonary symptoms could be assessed, one was asymptomatic (Patient 9). ECG changes appeared within 6 days after stroke onset in all patients. In two patients, the initial ECG already documented abnormalities (Patients 11 and 12); thus, the cardiomyopathy may have developed before the stroke.

Table 2. Continued

Peak Titer of Myocardial Markers				Coronary Angiography	NYHA	
CK (U/L)	CK-MB (U/L)	Troponin-T (ng/ml)	BNP (pg/ml)		On Admission	At Discharge
183	Undone	0.01	1,182.3	No stenosis	I	I
118	Undone	0.01	651.6	Undone	IV	III
133	26	0.3	undone	Undone	I	I
53	11	0.05	374.7	Undone	IV	IV
308	20	Undone	605.7	No stenosis	I	I
67	5	Undone	265.5	Undone	I	I
120	14	0.1	1065.7	Undone	I	I

Discussion

This is the first study to present a series of acute ischemic stroke patients who experienced development of takotsubo cardiomyopathy. The major findings included: (1) 1.2% of the consecutive inpatients with acute ischemic stroke experienced development of takotsubo cardiomyopathy; (2) all patients with takotsubo cardiomyopathy in our center and most of the previously reported patients were women, and they were generally elderly; (3) the preceding stroke was generally severe, and the culprit infarcts mainly included or were close to the insular cortex in most of our cases, whereas the vertebrobasilar arterial territory was affected in one of our cases and in some of the previously reported cases; (4) ECG changes often appeared within 10 hours after stroke onset, although most of the ECG changes were not associated with cardiac symptoms; and (5) marked plasma BNP level increase was indicative of cardiomyopathy.

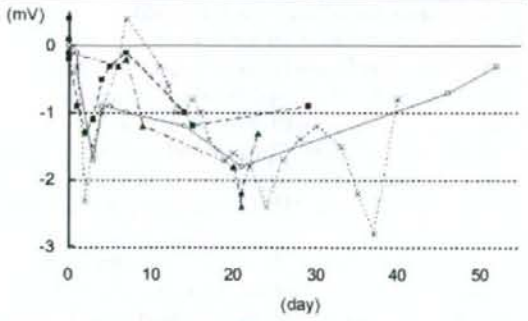

Fig 3. T-wave amplitude changes in V4 of Patients 2 (circles), *4* (squares), *5* (×), *and 6* (triangles). *X = Pt. No. 5.*

Although the precise incidence of takotsubo cardiomyopathy is unknown, it has been estimated to occur in 1.5 to 2.2% of patients with acute coronary syndrome.[3] Dysfunction of left ventricular regional wall motion occurs in approximately 18% of SAH patients,[20] and takotsubo cardiomyopathy was identified on ECG and imaging studies in 8 of 661 (1.2%) SAH patients.[10] At least one patient with intracerebral hemorrhage was reported to have experienced development of takotsubo cardiomyopathy.[21] This incidence of takotsubo cardiomyopathy in ischemic stroke patients (1.2%) suggests that the cardiomyopathy is as important a complication of ischemic stroke as of SAH. The main reasons for underestimating takotsubo cardiomyopathy in acute ischemic stroke appear to be that this syndrome is reversible and sometimes asymptomatic, and some stroke patients have communication problems and difficulty in complaining about cardiac symptoms. A relatively high frequency of reports dealing with takotsubo cardiomyopathy from Japan may be because of a racial difference in the incidence of this disease. A high female predominance of takotsubo cardiomyopathy has been reported in most studies and reviews, and the patients were generally elderly.[3,4,10,22] The hormonal changes that occur in postmenopausal women may be related to the causative mechanism.

Electrical stimulation of the rat insular cortex triggers tachycardia, bradycardia, heart block leading to escape rhythms, and asystole.[23,24] Intraoperative insular stimulation in epileptic patients often produces brady-

Table 3. Literature Review

Patient No.	First Author (publication year)	Sex	Age, yr	Site of Infarct	Arterial Lesion	Neurological Signs
8	Wang (1997)[12]	F	65	R parietotemporal area	ND	Hemiparesis
9	Sadamatsu (2000)[13]	M	65	ND	ND	ND
10	Nakamura (2003)[14]	F	81	L putamen–corona radiata	ND	Hemiparesis
11	Ueno (2006)[15]	F	61	Vertebrobasilar (multiple)	BA occlusion	Tetraparesis
12	Matsui (2007)[16]	F	85	L anterior cerebral artery territory	ND	Hemiparesis
13	Kato (1998)[17]	F	75	ND	ND	ND
14	Kamizuma (2001)[18]	F	60	R cerebellum	ND	Ataxia
15	Sugie (2004)[19]	F	78	ND	Possible BA occlusion	Coma
16	Sugie (2004)[19]	M	74	BA territory	Possible BA occlusion	Coma

[a]Time interval between stroke onset and electrocardiographic (ECG) change.
[b]The stenoses did not correspond to the regional wall-motion abnormality.
NYHA = New York Heart Association; ND = not described; RCA = right coronary artery; LAD = left anterior descending artery; BA = basilar artery.

cardia and depressor responses.[25] Patients with an insular infarct (particularly right sided) have been reported to show decreased heart rate variability, and an increased incidence of complex arrhythmias and sudden death.[26] Thus, the insular cortex appears to play a major role in autonomic control of cardiac activity. The predilection for infarct location at or close to the insular cortex in our patients suggests that insular ischemia is strongly associated with takotsubo cardiomyopathy in acute stroke. The medulla is also known to be the center of autonomic modulation of cardiovascular activity. The nucleus ambiguus, the nucleus tractus solitarius, the dorsal motor nucleus of the vagus, and the rostral ventrolateral medulla control the cardiovascular system through the afferent pathway from baroreceptors and the efferent pathway to the heart, vessels, and adrenal glands. Thus, extensive brainstem ischemia may induce autonomic disturbances and cause takotsubo cardiomyopathy.

Because takotsubo cardiomyopathy sometimes causes congestive heart failure, cardioembolism,[27] and sudden death, screening using ECG monitoring appears to be important, particularly in elderly female patients with insular or vertebrobasilar infarcts. Ischemic myocardial markers in patients with takotsubo cardiomyopathy, including creatine kinase, its MB subtype, and troponin-T, are generally within normal limits.[2,28] On the other hand, BNP levels have been reported to increase to an average of more than 500pg/ml.[28] BNP is known to be related to left ventricular systolic and diastolic dysfunction[29]; thus, sudden changes in left ventricular function caused by takotsubo cardiomyopathy may accelerate BNP production. With respect to other noninvasive diagnostic procedures, ^{123}I-metaiodobenzylguanidine myocardial scintigraphy documents ventricular asynergy caused by cardiac sympathetic hyperactivity.[30] On cardiac MRI, takotsubo cardiomyopathy shows left ventricular apical ballooning on cine image and no delay of gadolinium enhancement.[31] Measurement of virus titers appears to be available for differentiating takotsubo cardiomyopathy from viral myocarditis.[32] Although myocarditis would be one of the mechanisms of takotsubo cardiomyopathy, recent reviews do not support this possibility because several studies could not demonstrate biopsy evidence or typical cardiac MRI findings that were compatible with myocarditis.[3,4]

The features of the commonly seen "stunning" after SAH appear to be somewhat different from those of this cardiomyopathy after ischemic stroke. The largest difference is distribution of regional wall-motion abnormality. After SAH, the basal and mid-ventricular segments are frequently affected, and there was relative sparing of the apical segments.[33,34] SAH-induced cardiomyopathy is most commonly associated with diffuse T-wave inversions and left ventricular dysfunction at hospital arrival with gradual improvement. In addition, an increased

Table 3. Continued

Cardiopulmonary Symptom	Appearance of ECG Change[a]	Apical Wall Motion on Echocardiogram	Coronary Angiography	Admission NYHA	NYHA at Discharge
Dyspnea	Several minutes	Severe hypokinesis	Untested	I	(dead)
Asymptomatic	3 days	Akinesis	Stenosis of RCA and LAD[b]	I	I
Chest pain	5 hours	Akinesis	No stenosis	I	I
Unclear (because of coma)	On admission	Hypokinesis	Untested	ND	I
Respiratory discomfort	On admission	Akinesis	No stenosis	ND	ND
ND	3 days	Akinesis	No stenosis	ND	ND
ND	6 hours	Hypokinesis	ND	ND	ND
Unclear (because of coma)	6 days	Severe hypokinesis	ND	ND	ND
Unclear (because of coma)	6 days	Severe hypokinesis	ND	ND	ND

troponin level is an available marker.[34–36] These differences suggest different severity of cardiac damage or different mechanism of cardiomyopathy between patients with SAH and those with ischemic stroke.

This study had several limitations. Because most of the consecutive stroke patients underwent ECG monitoring only for the initial several days, development of cardiomyopathy during the later phase might have been overlooked. Because most of the patients with cardiomyopathy did not undergo coronary angiography, primarily because of its invasiveness, the effect of coronary artery lesions on cardiomyopathy might have been underestimated. Among patients who already showed abnormal findings on the initial ECG right after hospital arrival and who were thus excluded from this study, there might have been some patients who experienced development of takotsubo cardiomyopathy between stroke onset and hospital arrival. Finally, data were lacking for some of the reported cases.

In conclusion, takotsubo cardiomyopathy was not a rare complication of acute ischemic stroke and was often asymptomatic. Female sex and insular damage were predominant features of the patients having takotsubo cardiomyopathy. Long-term ECG monitoring, as well as repeated ultrasound examinations once specific abnormalities are identified on ECG, can be used to promptly detect and then appropriately manage this unique complication.

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A NOTE ON PARAMETERIZATIONS IN PHARMACOKINETIC COMPARTMENT MODELS

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In pharmacokinetics, compartment models often play an important role in the description of the concentration of the drug in the blood over time after its administration to a subject. In the inference in the compartment models in pharmacokinetics, unlike that of the usual nonlinear regression models, parameterizations with respect to the parameters related to pharmacokinetic indices are frequently applied, for some reasons of the constraint that the parameter takes only the positive value, facilitation of the compartment models, or interpretation of the relationships between the parameters and the demographic or physiological individual attributes. However, in practice no attention is paid to checking if the utilized parameterization is valid. In this article, the relative curvature measure that enables us to assess the intrinsic and parameter-effects nonlinearity in the model is applied for checking it from the latter nonlinearity. From a viewpoint of the relative curvature measure, we reconsider several well-known examples and demonstrate the parameterization checking.

1. Introduction

The relation between the dose of the drug that is medicated to a patient and the usefulness of the drug in treating a disease of the patient is described by sharing a role with pharmacodynamics and pharmacokinetics. The former is concerned with "what does the drug do to the body?", and on the other hand, the latter is concerned with "what does the body do to the drug ?". In this article we focus on the pharmacokinetics. Pharmacokinetics is the study of the time course of drug disposition in the human body, including absorption, distribution, metabolism and excretion (Gibaldi & Perrier, 1982). Drug disposition over time in the human body is studied primarily by sampling the blood to construct a concentration-time profile in the blood circulation, and is often described by a compartment model that contains the parameters directly or indirectly related to the pharmacokinetic indices, e.g., the rate constant, the volume of distribution ($\mathcal{V}$), the clearance (Cl), the area under the blood drug concentration-time curve (AUC), the highest blood drug concentration observed following administration of an extravascular dose ($C_{\max}$), and the time at which the highest blood drug concentration occurs following administration of the extravascular dose ($t_{\max}$), in the nonlinear form. Therefore, in order to understand the individual pharamcokinetics, it is required to appropriately fit the com-

Key Words and Phrases: curvature, parameter-effects nonlinearity, intrinsic nonlinearity, reparameterization, linearized approximation.

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partment model to the individual data. Statistical inference in the compartment model can be considered in the framework of nonlinear regression problems and be usually based on a linear approximation with respect to the model parameter (Bates & Watts, 1988; Seber & Wild, 1989; Davidian & Giltinan, 1995). Then, pharmacokinetists or pharmacokinetic investigators frequently parameterize the compartment models; for example, the compartment model is log-parameterized for the reason of the constraint that the parameter takes only the positive value or because for the population pharmacokinetic analysis the parameters, e.g., V and Cl, often have the log-normal distribution; the compartment model is parameterized from the rate constants into the parameters that are not directly related to pharmacokinetics indices for the purpose of facilitating the model; the compartment model is parameterized from the rate constants into e.g., V, Cl, or the ratio (V/Cl), in order to make it simple to interpret the relationships between the parameters and the demographic or physiological individual attributes such as age, race, smoking status, and renal function. In practice pharmacokinetists or pharmacokinetic investigators (possibly even biostatisticians) have little doubt or believe that these utilized parameterizations are valid, and there are neither attempt or approaches to check them, though some parameterizations can lead us to the wrong inferential results based on the linear approximation, such as the biased or inprecise parameter estimates, estimated standard errors, or confidence intervals. Thus, in this article, an approach for applying the relative curvature measure to the parameterization checking is presented. In Section 2, a brief outline of statistical inference in the compartment models is given, and the formula for the relative curvature measure is provided. In section 3, the applications to several well-known pharmacokinetic data sets are illustrated.

2. The relative curvature measure

2.1 Statistical inference in compartment models

In a pharmacokinetic experiment, a drug is administered to a subject, concentrations in the blood are observed at n timepoints, and then a compartment model is often fitted to the observed blood drug concentration data. Statistical inference in the compartment model can be conducted in the standard nonlinear regression model framework, and the model is given by

$$\boldsymbol{Y} = \boldsymbol{f}(t;\boldsymbol{\beta}) + \boldsymbol{\varepsilon}, \tag{1}$$

where $\boldsymbol{Y}$ is the $n \times 1$ random vector of the blood drug concentrations given by $\boldsymbol{Y} = (Y_1, \ldots, Y_n)^{\mathrm{T}}$, $\boldsymbol{f}(t;\boldsymbol{\beta}) = (f(t_1;\boldsymbol{\beta}), \ldots, f(t_n;\boldsymbol{\beta}))^{\mathrm{T}}$; t is the time when the blood drug concentrations are observed and $\boldsymbol{\beta}$ is the $p \times 1$ vector of the parameters related to pharmacokinetics, $\boldsymbol{\varepsilon} = (\varepsilon_1, \ldots, \varepsilon_n)^{\mathrm{T}}$, and $\boldsymbol{\varepsilon}$ is assumed to be n dimensional multivariate normal distributed with mean vector $\mathbf{0}$ and $n \times n$ variance-covariance matrix $\sigma^2\boldsymbol{V}$ as $\mathrm{MN}_n(\mathbf{0}, \sigma^2\boldsymbol{V})$; σ is the scale parameter. Here, if the blood drug concentration data have no correlation and heteroscedasticity, $\boldsymbol{V} = \boldsymbol{I}_n$, where $\boldsymbol{I}_n$ is the $n \times n$ identity matrix. But it is noted that sometimes, the blood drug concentration data observed after administration has a degree of heteroscedasticity, depending on the method or instrument used for an assay.

For example, high performance liquid chromatography often exhibits Poisson-like heterogeneous variance, and a radioactive counting device has constant coefficient of variation variance (Aarons et al., 1987; Beal & Sheiner, 1988; Davidian & Carroll, 1987; Davidian & Giltinan, 1995; Sheiner & Beal, 1985; Giltinan & Ruppert, 1989; Oberg & Davidian, 2000; Yuh et al., 1994). Furthermore, the blood drug concentration data could be correlated, though it is so difficult to identify its structure for the individual blood drug concentrations. In these cases, $\boldsymbol{V}$ can be rewritten as $\boldsymbol{V}(\boldsymbol{\beta}, \boldsymbol{\alpha}, \boldsymbol{\theta})$; $\boldsymbol{\alpha}$ is the $s \times 1$ vector of correlation parameters and $\boldsymbol{\theta}$ is the $q \times 1$ vector of variance parameters, and for example, be provided by

$$\boldsymbol{V}(\boldsymbol{\beta}, \boldsymbol{\alpha}, \boldsymbol{\theta}) = \boldsymbol{G}^{1/2}(\boldsymbol{\beta}, \boldsymbol{\theta}) \boldsymbol{\Gamma}(\boldsymbol{\alpha}) \boldsymbol{G}^{1/2}(\boldsymbol{\beta}, \boldsymbol{\theta}),$$

where $\boldsymbol{G}(\boldsymbol{\beta}, \boldsymbol{\theta})$ denotes the $n \times n$ diagonal matrix of a variance function, g, given by

$$\boldsymbol{G}(\boldsymbol{\beta}, \boldsymbol{\theta}) = \operatorname{diag}\left[g^2(t_1; \boldsymbol{\beta}, \boldsymbol{\theta}), \ldots, g^2(t_n; \boldsymbol{\beta}, \boldsymbol{\theta})\right].$$

That is to say, the variance function specifies the heteroscedasticity of the blood drug concentration data. $\boldsymbol{\Gamma}(\boldsymbol{\alpha})$ denotes the $n \times n$ correlation matrix between the blood drug concentration data. For example, $\boldsymbol{\Gamma}$ may be given by specification of the autoregressive correlation matrix of order one

$$\Gamma(\boldsymbol{\alpha}) = \begin{bmatrix} 1 & \alpha & \alpha^2 & \cdots & \alpha^{n-1} \\ & 1 & \alpha & \cdots & \alpha^{n-2} \\ & & \ddots & \ddots & \vdots \\ & & & \ddots & \alpha \\ & & & & 1 \end{bmatrix}.$$

The parameters $\boldsymbol{\beta}$, $\boldsymbol{\alpha}$, $\boldsymbol{\theta}$, and σ in Eq.(1) are estimated by the generalized least squares with the maximum pseudo log-likelihood (GLS-MPL). The GLS-MPL algorithm can be characterized by the following scheme (for the details, see Davidian & Giltinan (1995) and Vonesh & Chinchilli (1997)):

1. Estimate $\boldsymbol{\beta}$ by a preliminary estimator $\tilde{\boldsymbol{\beta}}$, e.g. the ordinary least squares estimator.
2. Given a preliminary estimator $\tilde{\boldsymbol{\beta}}$ for $\boldsymbol{\beta}$, estimate $\boldsymbol{\alpha}$, $\boldsymbol{\theta}$, and σ by maximizing with respect to them, the pseudo log-likelihood given by

$$l(\tilde{\boldsymbol{\beta}}, \boldsymbol{\alpha}, \boldsymbol{\theta}, \sigma; \boldsymbol{y}|t) = -\frac{n}{2}\ln(2\pi) - \frac{1}{2}\left|\ln(\sigma^2 \boldsymbol{V}(\tilde{\boldsymbol{\beta}}, \boldsymbol{\alpha}, \boldsymbol{\theta}))\right| - \frac{1}{2\sigma^2}\left\{\boldsymbol{y} - \boldsymbol{f}(t; \tilde{\boldsymbol{\beta}})\right\}^{\mathrm{T}} \boldsymbol{V}^{-1}(\tilde{\boldsymbol{\beta}}, \boldsymbol{\alpha}, \boldsymbol{\theta}))\left\{\boldsymbol{y} - \boldsymbol{f}(t; \tilde{\boldsymbol{\beta}})\right\}, \quad (2)$$

   where $\boldsymbol{y}$ is the blood drug concentration observed for $\boldsymbol{Y}$. That is to say, the maximization of Eq.(2) corresponds to maximizing the normal log-likelihood evaluated at $\tilde{\boldsymbol{\beta}}$.
3. Using the estimators of $\boldsymbol{\alpha}$, $\boldsymbol{\theta}$, and σ from step 2, reestimate $\boldsymbol{\beta}$ by the generalized least squares. Treating the resulting estimator as a new preliminary estimator, return to step 2.

2.2 The formula for the relative curvature measure

The estimation of the parameter $\boldsymbol{\beta}$, in particular, is carried out using the linear approximation about the parameter estimator $\hat{\boldsymbol{\beta}}$:

$$\boldsymbol{f}(t;\boldsymbol{\beta}) \approx \boldsymbol{f}(t;\hat{\boldsymbol{\beta}}) + \dot{\mathbf{F}}(\boldsymbol{\beta} - \hat{\boldsymbol{\beta}}), \tag{3}$$

where $\dot{\mathbf{F}}$ is the $n \times p$ first derivative matrix with respect to $\boldsymbol{\beta}$ evaluated at $\hat{\boldsymbol{\beta}}$, given by $\dot{\mathbf{F}} = \Delta_{\boldsymbol{\beta}} \boldsymbol{f}(t;\boldsymbol{\beta})|_{\boldsymbol{\beta}=\hat{\boldsymbol{\beta}}} = \left[\left(\frac{\partial f(t_j;\boldsymbol{\beta})}{\partial \beta_k} \right) \Big|_{\beta_k = \hat{\beta}_k} \right]$, $j = 1, \ldots, n,\ k = 1, \ldots, p$. Inference based on the linear approximation in Eq.(3) is valid in the situation of a large number of observed concentrations for a given subject, on the premises that the compartment model function is not misspecified and the behavior about $\hat{\boldsymbol{\beta}}$ is approximately or locally close to that of the linear model. However, in pharmacokinetic experiments, the number of measurements of the blood drug concentration obtained from one subject is limited because of ethical restrictions, and thus we could not utilize methods appropriate for the large number of measurements. In order to understand the pharmacokinetics in the human body on the basis of a compartment model, it is important to quantitatively check if this model can be regarded as close to linear or locally linear for the small number of measurements, that is, if inference based on the linear approximation can be valid. Therefore we focus on the Bates-Watts' relative curvature measure (Bates & Watts, 1980), and apply it to check if the linear approximation is satisfactory and furthermore to check the validity of the parameterizations (Bates & Watss, 1981, 1988; Daimon & Goto, 2007a; Ratkowsky, 1983). However, since the relative curvature measure is defined for the homogenous variance and no correlation structure (that is, $\boldsymbol{V} = \boldsymbol{I}_n$, where $\boldsymbol{I}_n$ is the $n \times n$ identity matrix) in Eq.(1), we must present an approach for making it possible to apply the relative curvature measure in the situation where the blood drug concentrations are heteroscedastic or correlated. If $\boldsymbol{V}$ is known or estimated, we can apply a transformation to remove the dependence in Eq.(1):

$$\boldsymbol{L}^{-1}\boldsymbol{Y} = \boldsymbol{L}^{-1}\boldsymbol{f}(t;\boldsymbol{\beta}) + \boldsymbol{L}^{-1}\boldsymbol{\varepsilon},$$

to get

$$\boldsymbol{Y}^* = \boldsymbol{f}^*(t;\boldsymbol{\beta}) + \boldsymbol{\varepsilon}^*, \tag{4}$$

where $\boldsymbol{V} = \boldsymbol{L}\boldsymbol{L}^{\mathrm{T}}$ is the Cholesky decomposition of $\boldsymbol{V}$ and $\boldsymbol{\varepsilon}^*$ have $\mathrm{MN}_n(\mathbf{0}, \sigma^2 \boldsymbol{I}_n)$. Therefore the Bates-Watts' relative curvature measure can be redefined for this equation Eq.(4) and be included in the curvature measure for exponential family nonlinear models that has been discussed in Wei (1998). For this case the relative curvature measure is given by

$$\gamma_h^{PE} = \rho \frac{\left\| \boldsymbol{h}^{\mathrm{T}} \ddot{\mathbf{F}}^{*PE} \boldsymbol{h} \right\|}{\left\| \dot{\mathbf{F}}^* \boldsymbol{h} \right\|^2} \text{ and } \gamma_h^{IN} = \rho \frac{\left\| \boldsymbol{h}^{\mathrm{T}} \ddot{\mathbf{F}}^{*IN} \boldsymbol{h} \right\|}{\left\| \dot{\mathbf{F}}^* \boldsymbol{h} \right\|^2}, \tag{5}$$

where γ_h^{PE} and γ_h^{IN} are parameter-effects curvature and intrinsic curvature, respectively, in a direction $\boldsymbol{h}$ of $\hat{\boldsymbol{\beta}}$, $\boldsymbol{h}$ is the $p \times 1$ vector of any direction of the line through the

vector of the parameter estimates $\hat{\boldsymbol{\beta}}$, given by $\boldsymbol{h} = (h_1, \ldots, h_p)^{\mathrm{T}}$, $\ddot{\mathbf{F}}^*$ is the $n \times p \times p$ array of second derivatives of $\boldsymbol{f}^*(t;\boldsymbol{\beta})$ with respect to $\boldsymbol{\beta}$ evaluated at $\hat{\boldsymbol{\beta}}$, given by $\ddot{\mathbf{F}}^* = \nabla_{\boldsymbol{\beta}} \boldsymbol{f}^*(t;\boldsymbol{\beta})\Big|_{\boldsymbol{\beta}=\hat{\boldsymbol{\beta}}} = \left[\left(\frac{\partial f^*(t_j;\boldsymbol{\beta})}{\partial \beta_k \partial \beta_l}\right)\Big|_{\boldsymbol{\beta}=\hat{\boldsymbol{\beta}}}\right]$, $j = 1, \ldots, n,\ k = 1, \ldots, p,\ l = 1, \ldots, p$; $\ddot{\mathbf{F}}^{*PE}$ and $\ddot{\mathbf{F}}^{*IN}$ denote the tangential and orthogonal arrays respectively, corresponding to $\ddot{\mathbf{F}}^*$ (For the details, see Appendix A), $\|\cdot\|$ denotes the norm of a vector, that is $\|\boldsymbol{\xi}\| = (\boldsymbol{\xi}^{\mathrm{T}}\boldsymbol{\xi})^{1/2}$ for any given vector $\boldsymbol{\xi}$; here, for example, $\left\|\boldsymbol{h}^{\mathrm{T}}\ddot{\mathbf{F}}^{*IN}\boldsymbol{h}\right\|$ is the $n \times 1$ vector, given by $\left\|\sum_{k=1}\sum_{l=1} \ddot{\boldsymbol{f}}^{*IN}_{kl} h_k h_l\right\|$, where $\ddot{\boldsymbol{f}}^{*IN}_{kl} = (\ddot{f}^{*IN}_{1kl}, \ldots, \ddot{f}^{*IN}_{nkl})^{\mathrm{T}}$, and ρ is used to make the curvature scale-invariant; $\rho = \hat{\sigma}\sqrt{p}$. The notation here follows Seber & Wild (1989).

Daimon & Goto (2007a) utilized the original relative curvature measure for the homoscedastic error in Eq.(1), and proposed only replacing $\hat{\sigma}$ by $\sqrt{\sum_{j=1}^{n}\left[g(t_j;\hat{\boldsymbol{\beta}},\hat{\boldsymbol{\theta}})\right]^{-2}(Y_j - f(t_j;\hat{\boldsymbol{\beta}}))^2 \Big/ (n-p)}$, to take only the heteroscedasticity into account. Their proposed approach is simple, but is not included in the curvature measure for the exponential family nonlinear models in Wei (1998).

The relative curvature measure in terms of the parameter-effects curvature and intrinsic curvature describes a local surface (called the expectation surface) on $\boldsymbol{\beta}$ of $f^*(t;\boldsymbol{\beta})$ (see Bates & Watts (1988) and Seber & Wild (1989)). The parameter-effects curvature assesses the nonlinearity depending on the model parameter. For a small parameter-effects curvature, straight parallel equispaced lines in the parameter space map onto ones in the expectation surface, as happens with the tangent plane. The parameter-effects curvature can be decreased by suitable parameterizations (Bates & Watts, 1981). On the other hand, the intrinsic curvature measures the degree of nonlinearity inherent in the model itself. In the case of a small intrinsic curvature, the expectation surface can be locally replaced by the tangent plane. However, this curvature cannot be decreased by parameterizations.

For an omnibus investigation of the nonlinearity underlying the compartment model, we utilize the root mean square (RMS) parameter-effects curvature measure $\gamma^{PE}_{\mathrm{RMS}}$ and RMS intrinsic curvature measure $\gamma^{IN}_{\mathrm{RMS}}$, which are calculated as root mean squares of γ^{PE}_h and γ^{IN}_h respectively over all directions $\boldsymbol{h}$. $\gamma^{PE}_{\mathrm{RMS}}$ takes larger values as the parameter-effects nonlinearity increases. On the other hand, $\gamma^{IN}_{\mathrm{RMS}}$ takes larger values as the intrinsic nonlinearity increases. Therefore each curvature measure can range from 0 (for linear models) to $+\infty$. However, in practice, a criterion will be needed to decide if the curvature is sufficiently small to ensure that the linear approximation is satisfactory. There is a rule of thumb that the linear approximation applied to the expectation surface is satisfactory if each value of the curvature measures is less than $1/\sqrt{F_{p,n-p,1-\alpha}}$ or $1/2\sqrt{F_{p,n-p,1-\alpha}}$, where $F_{p,n-p,1-\alpha}$ is the $100(1-\alpha)$ percentile of the F-distribution with degree of freedom given by $(p, n-p)$. For the rationale for this rule of thumb, see Bates & Watts (1980, 1988), Seber & Wild (1989), and Ratkowsky (1983).

3. Examples

3.1 Example 1: Theophylline data

As the first example, we consider the concentration-time profiles obtained from a study of the kinetics of anti-asthmatic agent theophylline reported in Boeckmann et al. (1994) and analyzed in Davidian & Giltinan (1995) and Pinheiro & Bates (2000). In this experiment, the drug was administered orally to 12 subjects, and serum concentrations were measured at $n = 11$ time points per subject over the subsequent 25 hours. A common compartment model for the kinetics of theophylline following oral administration is the one-compartment open model with first-order absorption and elimination. The serum concentration for subject $i(= 1, \ldots, 12)$ at time t is given by

$$f(t;\boldsymbol{\beta}_i) = \frac{F\mathrm{D}_i\kappa_{ai}}{\mathscr{V}_i(\kappa_{ai}-\kappa_{ei})}\left\{\exp(-\kappa_{ei}t) - \exp(-\kappa_{ai}t)\right\}, \tag{6}$$

where F is the fraction drug available, D is the dose on a per-weight basis, and $\boldsymbol{\beta}_i = (\mathscr{V}_i, \kappa_{ai}, \kappa_{ei})^{\mathrm{T}}$; $\mathscr{V}$ is the volume of distribution, κ_a is the absorption rate constant, and κ_e is the elimination rate constant. $\boldsymbol{\beta}_i$ is the subject-specific parameters to be estimated, and so we set the fraction of drug available $F \equiv 1$ in the analyses given here.

In order to make it simple to interpret the relationships between the parameters and the demographic or physiological individual attributes or to understand the kinetics of theophylline, most pharmacokinetic investigators have more intrest on $\mathscr{V}$ and Cl, rather than κ_a and κ_e. Therefore, the usual parameterized function is often given in terms of $\boldsymbol{\beta}^* = (\beta_1^*, \beta_2^*, \beta_3^*)^{\mathrm{T}} = (\mathscr{V}_i, \kappa_{ai}, Cl_i)^{\mathrm{T}}$. The function is given by

$$f(t;\boldsymbol{\beta}_i^*) = \frac{F\mathrm{D}_i\kappa_{ai}}{\mathscr{V}_i(\kappa_{ai}-Cl_i/\mathscr{V}_i)}\left\{\exp\left(-\frac{Cl_i}{\mathscr{V}_i}t\right) - \exp(-\kappa_{ai}t)\right\}. \tag{7}$$

Furthermore, because each of these parameters in Eq.(7) is necessarily positive to be meaningful, we enforced this constraint on fitting Eq.(7) in parameterizing in terms of $\boldsymbol{\beta}_i^{**} = (\beta_{1i}^{**}, \beta_{2i}^{**}, \beta_{3i}^{**})^{\mathrm{T}} = (\ln\mathscr{V}_i, \ln\kappa_{ai}, \ln Cl_{ei})^{\mathrm{T}}$. Then, the function is given by

$$f(t;\boldsymbol{\beta}_i^{**}) = \frac{F\mathrm{D}_i\exp(\beta_{2i}^{**})}{\exp(\beta_{1i}^{**})\left\{\exp(\beta_{2i}^{**}) - \exp(\beta_{3i}^{**})/\exp(\beta_{1i}^{**})\right\}} \left\{\exp\left(-\frac{\exp(\beta_{3i}^{**})}{\exp(\beta_{1i}^{**})}t\right) - \exp(-\exp(\beta_{2i}^{**})t)\right\}. \tag{8}$$

The parameterization in Eq.(8) ensures non-negativity, and was adopted in Davidian & Giltinan (1995) and Pinheiro & Bates (2000), whereas the parameterizations in Eq.(6) and (7) have not been explicitly examined by the previous articles. First, let us consider the following model for the naive pooled data:

$$\boldsymbol{Y}_i = \boldsymbol{f}(t;\boldsymbol{\beta}_i^{**}) + \boldsymbol{\varepsilon}_i, \tag{9}$$

where $\boldsymbol{\beta}^{**} = (\ln\mathscr{V}, \ln\kappa_a, \ln Cl)^{\mathrm{T}}$, and $\boldsymbol{\varepsilon}_i$ is assumed to be $n = 11$ dimensional multivariate normal distributed with mean vector $\mathbf{0}$ and 11×11 variance-covariance matrix $\boldsymbol{I}_{11}$

Table 1: Results for the theophylline data.

Original parameterization: Eq.(6)	Estimate	Standard error
γ	0.485	0.024
κ_a	1.491	0.175
κ_e	0.080	0.009
σ	1.459	
γ_{RMS}^{PE}		0.219
γ_{RMS}^{IN}		0.072
Parameterization 1: Eq.(7)	**Estimate**	**Standard error**
β_1^*	0.485	0.024
β_2^*	1.491	0.175
β_3^*	0.039	0.003
σ	1.459	
γ_{RMS}^{PE}		0.184
γ_{RMS}^{IN}		0.072
Parameterization 2: Eq.(8)	**Estimate**	**Standard error**
β_1^{**}	−0.724	0.049
β_2^{**}	0.399	0.118
β_3^{**}	−3.248	0.074
σ	1.459	
γ_{RMS}^{PE}		0.129
γ_{RMS}^{IN}		0.072
Log-likelihood		−235.610

as $\mathrm{MN}_{11}(\mathbf{0}, \sigma^2 \boldsymbol{I}_{11})$. As discussed in Davidian & Giltinan (1995) and Pinheiro & Bates (2000), it is noted that fortunately, there are no need for the transformation of Eq.(4) since in Eq.(9) the variance homogeneity of the error, $\boldsymbol{\varepsilon}_i$, is assumed. The results are shown in Table 1.

As shown in Table 1, all the RMS values of the intrinsic and parameter effects curvature measures are smaller than $1/2\sqrt{F_{3,132-3,1-0.05}} = 0.305$. The linear approximation and the results of inference based on it could be quite reliable. The RMS value of the parameter-effects curvature measure for the parameterized function of Eq.(8) is the smallest, as compared with those of the other functions, and so this parameterization could be recommended. Of course, since the concentrations were measured at $n = 11$ time points per subject, it would be possible to fit the compartment model and to apply the relative curvature measure, to each subject's data, and in fact, Pinheiro & Bates (2000) have investigated it. The values of the relative curvature measures for each subject's data are plotted in Figure 1.

As shown in Figure 1, for all the subjects, the values of the RMS intrinsic curvature measure are smaller than $1/2\sqrt{F_{3,11-3,1-0.05}} = 0.248$, and the values of the RMS parameter-effects curvature measure for the parameterized function of Eq.(8) are the smallest, as compared with those of the other functions. However, for some subjects, subject

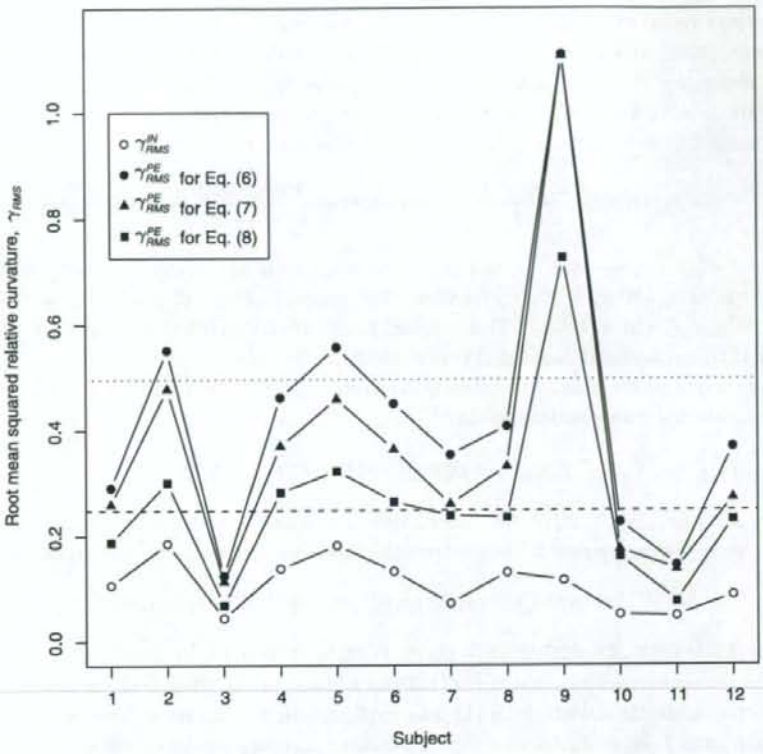

Figure 1: The values of the relative curvature measures for each subject's data; the broken line and the dotted line correspond to $1/2\sqrt{F_{3,11-3,1-0.05}} = 0.248$ and $1/\sqrt{F_{3,11-3,1-0.05}} = 0.496$, respectively.

No.2, 4, 5, 6, and 9, the values of the RMS parameter-effects curvatures for Eq.(8), exceed $1/2\sqrt{F_{3,11-3,1-0.05}} = 0.248$. Pinheiro & Bates (2000) have pointed out that subject No.9 had an unusually high absorption rate constant, κ_a, and our investigations for this subject also show that the linear approximation is all unreliable and the results, e.g., parameter estimates, estimated standard errors, and confidence intervals based on the linear approximation might be suspect. Furthermore, Pinheiro & Bates (2000) have commented that subject No.1 had an unusually low elimination rate constant. As shown in Figure 1, since the values of the RMS parameter-effects curvature measure are smaller than $1/\sqrt{F_{3,11-3,1-0.05}} = 0.496$, the reliability on their comment is supported from a point of view of the linear approximation.

3.2 Example 2: Indomethacin data

We consider a well-known data set from a study of indomethacin in six healthy