料に対する研究を含み、その試料提供者や関係者の人権および利益の保護の取り扱いについて十分配慮する。
計画の対象とする個人の人権の保護：適切なインフォームドコンセント、身体的安全性およびプライバシー保護など、試料提供者の尊厳および人権を尊重する。試料提供者に対して研究内容および資料提供者や関係者の人権保護および利益の保護の取り扱いについての説明用文書を用いたインフォームドコンセントによって文書により同意を得る。

C. 研究結果

自己抗体のエピトープ別解析については自己免疫疾患患者の血清やIgGをファージディスプレイ法によりスクリーニングし、エピトープ別に自己抗体の定量化する系を確立した。即ち、エピトープを含むペプチドをアイソトープラベルして抗原とし、患者血清やIgGを用いて免疫沈降することにより自己抗体を定量化する系を構築した。また血栓形成能解析システムT-TASを用いた個人別血栓形成能評価を行い、患者検体を簡便に測定する系を確立した。これらの結果より、今回の診断基準の作成のみならず、今後必要となる治療のモニターに有用な情報が得られた。

D. 考察

新規自己抗体を探索する方法として、ファージディスプレイ法は有効な手段の１つと考えられるが、我々は、この方法を用いて、自己抗体を含む多数の蛋白相互作用を解析してきた。本方法は自己抗体エピトープについての詳細な情報を得ることが可能であることより、これらの知見をもとにHIT患者の血清中の新たな抗体価測定法を確立することは今後の本疾患の診断の精度向上につながると考えられる。さらにフローの系を用いた血栓形成能との関連の検討は、本疾患の病態解明に有用である可能性が高いと思われる。

E. 結論

ヘパリン起因性血小板減少症の診断基準作成に必要な新たな検査方法の確立をめざし、HIT抗体のエピトープ別抗体価測定法や個体の血栓形成能の新規評価方法の基礎的検討を行った。HIT診断の精度向上や発症リスクの予測に貢献すると思われる。

F. 健康危険情報

特になし

G. 研究発表

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2. 学会発表　なし

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得　なし

2. 実用新案登録　なし

3. その他　なし

Ⅲ.　研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表

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Ⅳ.　研究成果の刊行物・別刷

bjh research paper

Prospective multicentre cohort study of heparin-induced thrombocytopenia in acute ischaemic stroke patients

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Summary

Acute ischaemic stroke patients sometimes receive heparin for treatment and/or prophylaxis of thromboembolic complications. This study was designed to elucidate the incidence and clinical features of heparin-induced thrombocytopenia (HIT) in acute stroke patients treated with heparin. We conducted a prospective multicentre cohort study of 267 patients who were admitted to three stroke centres within 7 d after stroke onset. We examined clinical data until discharge and collected blood samples on days 1 and 14 of hospitalization to test anti-platelet factor 4/heparin antibodies (anti-PF4/H Abs) using an enzyme-linked immunosorbent assay (ELISA); platelet-activating antibodies were identified by serotonin-release assay (SRA). Patients with a 4Ts score ≥4 points, positive-ELISA, and positive-SRA were diagnosed as definite HIT. Heparin was administered to 172 patients (64·4%: heparin group). Anti-PF4/H Abs were detected by ELISA in 22 cases (12·8%) in the heparin group. Seven patients had 4Ts ≥ 4 points. Among them, three patients (1·7% overall) were also positive by both ELISA and SRA. National Institutes of Health Stroke Scale score on admission was high (range, 16–23) and in-hospital mortality was very high (66·7%) in definite HIT patients. In this study, the incidence of definite HIT in acute ischaemic stroke patients treated with heparin was 1·7% (95% confidence interval: 0·4–5·0). The clinical severity and outcome of definite HIT were unfavourable.

Keywords: acute stroke care, anticoagulation, heparin, platelet, thrombocytopenia.

Immune-mediated heparin-induced thrombocytopenia (HIT), which is caused by platelet-activating IgG antibodies that recognize platelet factor 4 bound to heparin (anti-PF4/heparin Abs), is a relatively common side effect of heparin therapy and presents a strong risk factor for thromboembolic events associated with high mortality and morbidity (Warkentin, 2007a). Prospective studies in Western countries have shown that the prevalence of HIT is 0·3–5% of patients treated with unfractionated heparin (UFH), which varies depending on the clinical settings (Warkentin *et al*, 1995, 2000; Kappers-Klunne

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et al, 1997). Thrombotic complications occur in approximately one-third to one-half of HIT patients (Warkentin, 2007a). On the other hand, some studies of UFH therapy for acute stroke reported no cases of HIT (Toth & Voll, 2002; Camerlingo *et al*, 2005). To elucidate the prevalence of HIT in acute ischaemic stroke patients who were treated with heparin, we organized a prospective multicentre cohort study that included systematic collection of blood for detection of the antibodies that cause HIT.

Some clinical guidelines do not recommend prescribing heparin in acute ischaemic stroke, and others recommend it mainly for the prevention of deep vein thrombosis (DVT) and pulmonary embolism (PE) (Albers *et al*, 2004; Cardiovascular Disease Educational and Research Trust, 2006; Adams *et al*, 2007). At the participating stroke centres in our study, in addition to the prevention of DVT and PE, UFH is given during the acute phase of ischaemic stroke to the following: patients with emboligenic heart disease or superimposed thrombi on the carotid plaque to prevent embolic complications; patients with particular stroke aetiologies, including cerebral arterial dissection and vasculitis; and patients with embolic stroke of unknown origin until the presence of heart disease is excluded by the results of prolonged electrocardiography and transesophageal echocardiography (Caplan, 2003).

In a previous study of 137 stroke patients who were treated with UFH, 21 patients (15·3%) developed thrombocytopenia (≥40% fall in platelet counts) during or after heparin therapy, and five of these 21 patients had an additional ischaemic stroke (Ramirez-Lassepas *et al*, 1984). A recent study of 200 neurological patients treated with UFH for at least 5 d, including 102 patients with cerebrovascular disorders, demonstrated that 41 patients (20·5%) had anti-PF4/heparin Abs and 5 (2·5%) developed HIT, when the serological diagnosis was made from the presence of antibodies detected by an enzyme-linked immunosorbent assay (ELISA) (Harbrecht *et al*, 2004).

Only a few studies have investigated the prevalence of HIT in acute stroke patients receiving UFH, especially in the Asian population (Kawano *et al*, 2008). In our previous retrospective report of acute ischaemic stroke patients who were treated with UFH, 0·5% of the patients developed HIT diagnosed by both the clinical scoring systems and the serological assays, including ^{14}C-serotonin release assay (SRA) (Kawano *et al*, 2008). However, our retrospective study assessing the prevalence of HIT was limited by the fact that was that antibodies were not assayed in all patients. This limitation may cause an under diagnosis of HIT.

Thus, we performed this prospective multicentre cohort study in 267 patients to determine a more accurate incidence of HIT in patients with acute ischaemic stroke and to elucidate the clinical features of HIT.

Methods

Study design

A prospective multicentre cohort study.

Subjects and settings

This study was conducted in three Japanese stroke centres at the then National Cardiovascular Centre (currently the National Cerebral and Cardiovascular Centre, Osaka), Research Institute for Brain and Blood Vessels Akita (Akita), and Kumamoto University (Kumamoto). Between October 2006 and May 2007, all consecutive patients who met the following criteria were enrolled. Eligible patients were 20 years of age or older and admitted within 7 d after the onset of acute ischaemic stroke, including cerebral infarction and transient ischaemic attack. Patients were excluded for any of the following: (i) active infectious endocarditis, (ii) urgent neurosurgery or cardiovascular surgery would be required, (iii) chronic thrombocytopenia (defined as a platelet count $<100 \times 10^9$/l for more than 30 d), (iv) haematopoietic malignancy and (v) an ongoing need for an anticancer-drug treatment. The study was approved by the research ethics committee of each centre. Heparin therapy was provided to a number of patients depending on the physician's decision (mainly considering the type of stroke and/or the patient's clinical status as described in the Introduction.)

Evaluation

The following patient characteristics were obtained: age, sex, height, body weight, body-mass index, modified Rankin Scale (mRS) score (van Swieten *et al*, 1988) before stroke onset, vascular risk factors (hypertension, diabetes mellitus, dyslipidaemia, current and past smoking habits, drinking habit, including occasional drinking), past history (autoimmune disease, haemodialysis, renal dysfunction, angina, myocardial infarction, cerebral infarction, transient ischaemic attack, pulmonary thromboembolism, extremity gangrene, amputation of an extremity, angiography, heparin exposure, surgical procedure and HIT), platelet counts, antiplatelet/anticoagulant drug use and blood transfusions. The timing and period of heparin administration (including heparin flushes), changes in platelet count, and alternative anticoagulant therapy for HIT (if given) were also examined. Other risk factors for stroke, such as emboligenic heart diseases including atrial fibrillation, were assessed based on the criteria from the Trial of Org 10172 in Acute Stroke Treatment (TOAST) study (Adams *et al*, 1993). Based on the neurological, radiological, cardiological and haematological profiles, the stroke subtype was determined according to the TOAST subtype classification system by a consensus of stroke neurologists. The neurological severity of each patient was assessed by an experienced stroke neurologist according to the National Institutes of Health Stroke Scale (NIHSS) score (Lyden *et al*, 1994) on admission and discharge, and at 3 months after onset. Patient global outcome was also assessed with mRS (van Swieten *et al*, 1988).

Clinical evaluation. The clinical probability of HIT was assessed using the 4Ts scoring system (Warkentin & Heddle, 2003), which is composed of four clinical features that are

given scores of 0, 1, or 2; magnitude of thrombocytopenia; timing of platelet count fall (in relation to heparin therapy); thrombosis or other sequelae; and presence of other explanations for thrombocytopenia. The case reports of the patients, filled out by their physicians, were assessed independently in a blinded fashion by the external Data Assessment Committee, which consisted of two stroke neurologists, according to the 4Ts scoring system after the patient follow-up was completed. If the judgment was not concordant between the two stroke neurologists, they discussed the cases to reach a final consensus and decision. Based on the 4Ts score, the estimated pretest probabilities of HIT were categorized into three groups: low (0–3), intermediate (4–5) and high (6–8) scores. We diagnosed the patients with an intermediate or a high score as 'potential HIT' and those with a low score as 'clinical non-HIT'. These objective assessments for the clinical probability of HIT were done after the patient follow-up was completed as described above, so that no results influenced clinical management. Therefore, some patients were ultimately diagnosed as HIT even though the physicians in charge did not suspect HIT as described in details in the Results section.

Serological evaluation. Blood samples were collected from all patients on the first (to the third) and 14th (±4) hospital days to be tested for anti-PF4/heparin Abs using ELISA (Asserachrom HPIA; Diagnostica Stago, Asnieres, France). The assays were performed in a blinded fashion after patient follow-up was completed. ELISA was performed according to the manufacturer's instructions. The titres of the samples were expressed as values of optical density (OD). The result was considered positive when the titre was greater than the cut-off value, which was determined using the reference control for each kit. To confirm the diagnosis of HIT, SRA was measured for all patients with a positive ELISA and/or ≥4 points in the 4Ts scoring system (n = 29). In addition, samples from 39 patients selected randomly from among all the patients were tested by SRA as a control. Samples were measured as described elsewhere at the Platelet Immunology Laboratory, McMaster University (Hamilton, ON, Canada) blinded to all clinical, platelet count and serological data (Warkentin *et al*, 1992). Any sample that produced ≥10% mean serotonin release with <10% release in the presence of high heparin (at a final concentration of 100 u/ml) and the anti-FcγRIIa monoclonal antibody (IV.3) was considered SRA-positive.

Diagnosis

Based on the results of both the 4Ts clinical score and the serological assays, patients were categorized into four groups as follows: (i) definite HIT (4Ts score ≥4 points with positive results in both ELISA and SRA), (ii) possible HIT (4Ts score ≥4 points with positive result in either ELISA or SRA) and (iii) clinically suspected HIT (4Ts score ≥4 points with negative results in both ELISA and SRA), seropositive status (4Ts score <4 points with positive in both ELISA and SRA). The remaining patients were categorized as HIT unlikely.

Statistical analysis

The variables between the groups of patients treated with and without heparin were compared using Fisher's exact test and the Wilcoxon test. For NIHSS, the change, NIHSS score at discharge minus that at admission, was also determined. Statistical analyses were performed using SAS software version 9.1 (SAS Institute Inc, Cary, NC, USA).

Results

Patient characteristics

A total of 267 patients (mean age 71·7 years; 66·2% men), who were admitted to three stroke centres within 7 d after stroke onset during a 6-month period, were enrolled. Intravenous UFH was administered to 172 patients (64·4%: heparin group) (Fig 1). Male gender, atrial fibrillation, previous ischaemic heart disease, history of surgery using UFH, and history of intra-arterial catheter procedures were significantly more common in patients treated with than without UFH (Table IA). In regard to stroke subtype, large artery atherosclerosis and cardioembolism were more frequent in patients treated with UFH, and small vessel occlusion was more frequent in those without UFH treatment. There was no significant difference in the history of antiplatelet drug use before admission between the patients treated with (66 cases, 38·4%) and without UFH (32 cases, 33·7%) (P = 0·508) (Table IA). Both the NIHSS score at discharge (median, 2 vs. 1, P = 0·020) and mRS at 3 months after stroke onset (median, 2 vs. 1, P < 0·001) were higher in patients treated with UFH (Table IB).

The incidence of HIT

Anti-PF4/heparin Abs were detected at any time point in 22 patients (12·8%) in the heparin group and in 3 (3·2%) of 95 patients who did not receive intravenous UFH respectively (Fig 1), and the difference was significant (P = 0·008). Seven patients (4·1%) were diagnosed as having potential HIT according to the 4Ts score (≥4 points). All seven patients had intermediate scores. Among them, three showed positive results in both ELISA and SRA, to give an incidence of definite HIT of 1·7% [95% confidence interval (CI): 0·4–5·0]. Possible HIT, clinically suspected HIT, and seropositive status were 0%, 2·3% (n = 4), and 2·3% (n = 4), respectively (Fig 1). Of the 95 patients with a positive ELISA who did not receive heparin within 3 months before admission and/or during hospitalization, three were SRA-negative. The OD values of anti-PF4/heparin Abs detected by ELISA seemed a little higher in definite HIT patients than the seropositive status group, although statistical analysis was not performed because of the

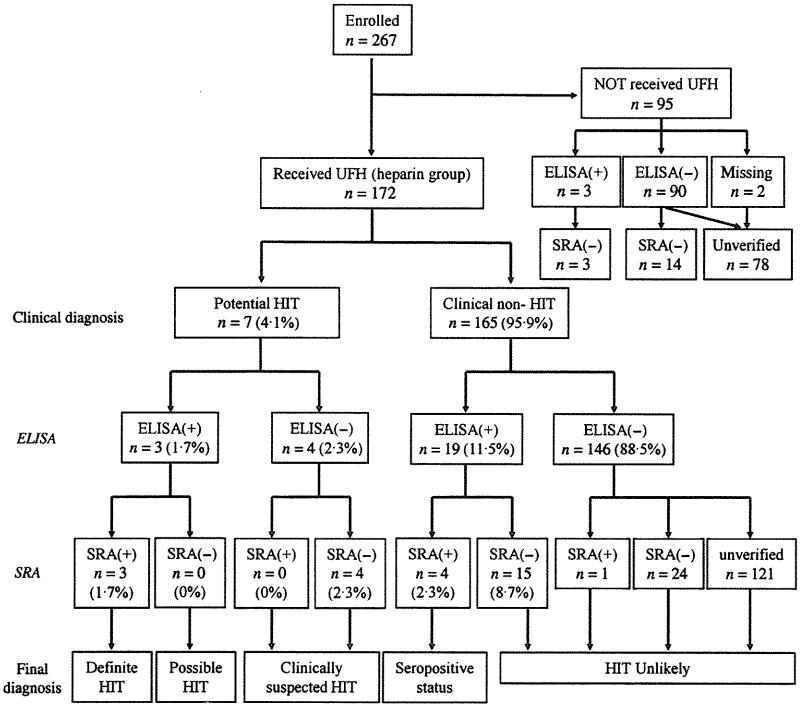

Fig 1. Flow chart for diagnosis of heparin-induced thrombocytopenia. HIT, heparin-induced thrombocytopenia; UFH, unfractionated heparin; ELISA, enzyme-linked immunosorbent assay; SRA, serotonin-release assay.

small sample size (Table II). OD values in ELISA did not correlate with the mean percentage release in SRA (Fig 2). However, the proportion of samples with positive-SRA to those with negative-SRA was greater in the samples with ≥1·5 OD value in ELISA as compared to those with <1·5 OD value. The prevalence of positive-ELISA was not significantly different between patients who received UFH for five or more days (15·9%) and for <5 d (11·4%).

Clinical course and the treatment of definite HIT patients

Only one (Case 3) of three definite HIT patients was suspected of having HIT by the treating physician. This patient had atrial fibrillation and an infarct in the right anterior and middle cerebral arteries. The admission NIHSS score was 17 (Table II). The patient's platelet count decreased from 156 to 99×10^9/l (approximately a 37% fall) in the typical HIT window (5–10 d) and recovered to 227×10^9/l soon after stopping heparin administration on day 7 due to the suspicion of HIT. The patient had a further fall in platelet count, from 227 to 99×10^9/l (approximately a 56% fall), after day 10 with a high OD value (2·086) in ELISA and a weak positive SRA (11% release) (Table II). The patient died due to deterioration from an underlying stroke. The very weak SRA, which was performed during the second platelet count fall, argues somewhat against this patient having HIT. However, HIT antibodies sometimes become weaker very quickly (Warkentin & Kelton, 2001; Greinacher *et al*, 2009), and so it is possible that the SRA would have been stronger during the first platelet count fall.

The other two patients (Cases 1 and 2) that ultimately met the criteria for definite HIT in this study were not suspected of having HIT by their physicians. One patient (Case 1) experienced a stroke of other determined aetiology due to arterial dissection in the intracranial left vertebral artery. The admission NIHSS score was 23 (Table II). The patient had bilateral cerebellar and brain stem infarcts. UFH was administrated for 7 d, and UFH flushes for intravascular catheter were continued for an additional 4 d. The patient showed a 52·0% decrease in platelet count, from 331 to 107×10^9/l, that began on day 5 of heparin with relatively high values in SRA (63·9% release) and ELISA (2·271 OD value) (Table II). Death occurred from stroke on day 11. The other patient (Case 2) with a previous history of recent transient ischaemic attacks had a cardioembolic stroke due to atrial fibrillation 9 d after urgent hemiarch replacement due to aortic dissection. The admission NIHSS score was 16. The patient's platelet count declined from 436 to 286×10^9/l (a drop of approximately 34%) during the typical HIT window of days 5–10 with relatively high values in SRA (51·6% release) and ELISA (1·725 OD value); although the platelet count evolution may be explained by a platelet count profile of post-cardiovascular surgery with cardiopulmonary bypass overshooting around postoperative day 14 and returning gradually to the baseline (Table II). The patient was dependent at discharge and at 3-month follow-up.

Table I. (A) Demographic data of patients treated or not with unfractionated heparin (UFH) and (B) clinical data of patients treated or not with UFH.

	With UFH (n = 172; 64·4%)	Without UFH (n = 95; 35·6%)	*P*-value
(A)			
Age (years), median (range)	71 (23–98)	73 (42–93)	0·515
Male gender (%)	122 (70·9)	53 (55·8)	0·015
Weight (kg)	60·1 ± 12·2	59·4 ± 11·6	0·673
BMI (kg/m^2)	23·3 ± 3·8	23·4 ± 3·7	0·936
HTN (%)	133 (77·3)	74 (77·9)	1·000
DM (%)	55 (32·0)	30 (31·6)	1·000
CRF (%)	17 (9·9)	5 (5·3)	0·247
HD (%)	3 (1·7)	0 (0)	0·555
Atrial fibrillation (%)	59 (34·3)	11 (11·6)	<0·001
Smoking (%)	78 (45·3)	37 (38·9)	0·303
Drinking (≥2 cups) (%)	49 (28·5)	21 (22·1)	0·249
Previous IHD (%)	33 (19·2)	5 (5·3)	0·002
Previous CVD (%)	51 (29·7)	28 (29·5)	1·000
Previous PTE (%)	0	0	–
Previous DVT (%)	4 (2·3)	1 (1·1)	0·658
History of heparin use within 3 months (%)	6 (3·5)	0 (0)	0·180
History of surgery using heparin	33 (19·2)	3 (3·2)	<0·001
History of intra-arterial catheter procedure (%)	43 (25·0)	8 (8·4)	<0·001
History of warfarin use (%)	18 (10·5)	5 (5·3)	0·176
History of antiplatelet agency use (%)	66 (38·4)	32 (33·7)	0·508
Stroke subtype			
TIA (%)	9 (5·2)	20 (21·1)	<0·001
Stroke (%)	163 (94·8)	75 (78·9)	
LAA (%)	38 (23·3)	5 (6·7)	
CE (%)	64 (39·3)	5 (6·7)	<0·001
SV (%)	26 (16·0)	48 (64·0)	
OT + UD (%)	35 (21·5)	17 (22·7)	
Platelet count (×10^9/l)	222 (103–583)	230 (119–483)	0·670
NIHSS score on admission, median (range)	5 (0–32)	3 (0–20)	<0·001
(B)			
Treatment during the hospital stay			
Warfarin use (%)	70 (40·7)	9 (9·5)	<0·001
Antiplatelet agency use (%)	105 (61·0)	84 (88·4)	<0·001
Cessation of heparin (%)	142 (82·6)	0	<0·001
Alternative anticoagulation (%)	67 (39·0)	37 (38·9)	1·000
Intra-arterial catheter procedure during the hospital stay (%)	70 (40·7)	0 (0)	<0·001
Surgery with heparin use during the hospital stay	7 (4·1)	0 (0)	0·053
Thromboembolic vents or death	25 (14·5)	4 (4·2)	0·012
Recurrence of ischaemic stroke	12 (7·0)	2 (2·1)	
Thromboembolic events during catheter	4 (2·3)	0	
Other thromboembolism	7 (4·1)	2 (2·1)	
React of heparin infusion	1 (0·6)	0	
Death	5 (2·9)	0	
NIHSS score at discharge, median (range)	2 (0–42)	1 (0–20)	–
NIHSS change, discharge-admission (range)	−2 (−21 to 19)	−1 (−8 to 9)	0·020
mRS at discharge, mean (median)	2 (0–6)	1 (0–5)	0·002
mRS at 3 months, median (range)	2 (0–6)	1 (0–5)	<0·001

BMI, body mass index; HTN, hypertension; DM, diabetes mellitus; CRF, chronic renal failure; HD, haemodyalysis; IHD, ischaemic heart disease; CVD, cerebrovascular disease; PTE, pulmonary thromboembolism; DVT, deep vein thrombosis; TIA, transient ischaemic attack; LAA, large artery atherosclerosis; CE, cardioembolism; SV, small vessel occlusion; OT, stroke with alternative aetiology; UD, stroke of undetermined aetiology; UFH, unfractionated heparin; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin scale.

Table II. Clinical features of HIT patients.

Pt	Age (years)	Gender	Past history	Stroke subtype	4Ts score	ELISA (OD)	SRA (mean % release)	Platelet count ($\times 10^9$/l) Baseline	Nadir	Duration of UFH (day)	Duration of UFH up to the day of platelet nadir, days	Thrombotic complication	NIHSS on admission	mRS on discharge
Definite HIT														
1	62	Male	CI, HTN	Other	4	+(2·271)	+(63·9)	331	107	11	7	None	23	Dead
2	64	Female	CI, HTN, AF	CE	5	+(1·725)	+(51·6)	436	286	18	10	None	16	4
3	88	Female	AF	CE	5	+(2·086)	+(11·0)	156	99	7	15	None	17	Dead
Clinically suspected HIT														
4	67	Male	HTN, DM, AF, CRF	CE	4	−(0·138)	−(<1)	281	210	14	7	DVT	7	4
5	82	Male	CI, HTN, AF	CR	4	−(0·052)	−(<1)	137	27	1	4	None	10	4
6	66	Male	MI, HTN	CE	4	−(0·102)	−(<1)	583	225	13	17	None	12	1
7	69	Female	HTN, AF	CE	5	−(0·091)	−(<1)	297	120	23	6	RI	7	4
Seropositive status														
8	70	Female	HTN, AF	CE	0	+(1·666)*	+(53·2)	141	123	4	NA†	None	13	2
9	59	Female	HTN, AF, AID	CE	0	+(1·505)	+(76·8)	163	158	18	NA†	None	15	4
10	87	Male	IHD, HTN, AF	CE	0	+(0·977)	+(13·3)	200	150	13	NA†	None	8	5
11	90	Female	HTN, AF	CE	2	+(2·378)	+(28·8)	235	210	9	NA†	IHD	29	5

ELISA, enzyme-linked immunosorbent assay; SRA, serotonin-release assay; OD, optical density; CI, cerebral infarction; IHD, ischaemic heart disease; HTN, hypertension; DM, diabetes mellitus; AF, atrial fibrillation; CRF, chronic renal failure; MI, myocardial infarction within 4 weeks; AID, autoimmune disease; RI, renal infarction; DVT, deep vein thrombosis; other, stroke of other determined aetiology; CE, cardioembolism; NA, not applicable.

*ELISA was negative (OD: 0·079) in the sample drawn 7 d after admission, when SRA was positive. ELISA was positive (OD: 1·666) in the sample obtained 1 week later.

†Patient did not demonstrate thrombocytopenia.

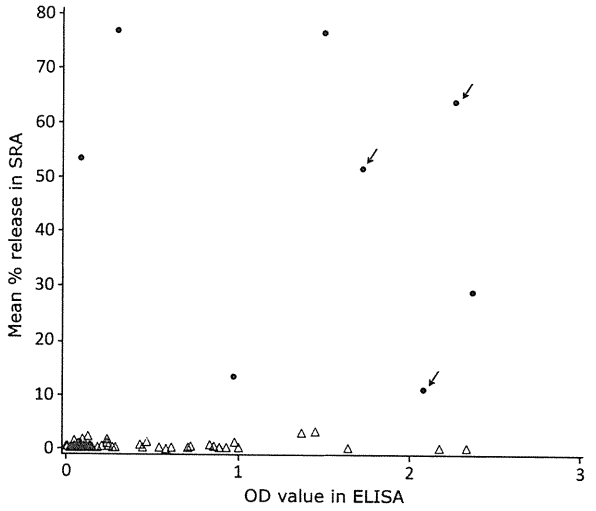

Fig 2. The correlation of optical density (OD) values for anti-platelet factor 4/heparin antibodies detected by enzyme-linked immunosorbent assay (ELISA) and mean percentage release by serotonin-release assay (SRA). These values showed poor correlation. Arrows indicate the data points of the three patients who met the criteria for definite HIT. •, SRA-positive cases, including one patient classed as 'HIT unlikely': OD = 0·298, and mean percentage release = 76·74; △, SRA-negative cases.

None of the patients in this study met the diagnosis of rapid or delayed onset HIT. None of the patients classified as definite HIT received treatment with alternative anticoagulants, such as thrombin inhibitors, nor did the patients develop additional thromboembolic events.

Discussion

HIT should be recognized as a clinicopathological syndrome because none of the currently available HIT diagnostic tools have sufficient sensitivity and specificity to be used as the primary or only tool to diagnose HIT. Thus, both clinical and serological diagnoses are crucial. In this prospective study, clinical probability was assessed using the 4Ts scoring system, which is a popular method, by two independent stroke neurologists who were blinded from the results of serological assays. As a result, 4·1% of the acute stroke patients treated with heparin were suspected clinically of having HIT with ≥4 points in the 4Ts scoring system. Among them, 1·7% (95% CI: 0·4–5·0) had platelet activating antibodies against the complexes of PF4 and heparin detected by ELISA and SRA, supporting the diagnosis of definite HIT. All of these definite HIT patients had intermediate scores in the 4Ts as well as four clinically suspected HIT cases, as shown in Table II. Thus, it was very difficult to distinguish HIT patients from non-HIT patients through clinical information alone. This may possible explain why only one among three definite HIT cases was suspected of having HIT by the treating physicians.

Our results were similar to those reported in other studies of patients with ischaemic stroke (Ramirez-Lassepas *et al*, 1984; Harbrecht *et al*, 2004) and the frequency of definite HIT was less than in surgical patients (Kappers-Klunne *et al*, 1997; Warkentin, 2007b). For two of the three definite HIT patients reported here, one had a possible alternative aetiology that could explain her platelet count fall (Case 2) and the other had a weak positive-SRA (Case 1) as described in detail in the Result section. Thus, we cannot exclude the possibility that these two patients might not have had HIT. If we exclude these patients, the incidence of HIT could be as low as 0·6%. However, this result was compatible with our previous retrospective study of the same patient population (the incident of HIT was 0·5%) (Kawano *et al*, 2008). Therefore, we can conclude that the incidence of HIT in acute stroke patients treated with UFH seems to be approximately 0·5–1·7%. These results emphasize that HIT diagnosis should be considered in the management of acute ischaemic stroke.

Another major finding was that the clinical severity and outcome of acute stroke patients who were diagnosed as having definite HIT were unfavourable. In particular, the in-hospital mortality of definite HIT was very high (66·7%). Previous reports also indicated that mortality was high in HIT patients (Warkentin *et al*, 1995, 2000; Kappers-Klunne *et al*, 1997). The present study is unique in that initial neurological severity and clinical outcomes of stroke patients with HIT were determined. The NIHSS score on admission (median, 17) in definite HIT was quite high, and the outcome at 90 d was poor. However, the poor outcome of those patients appeared to be mainly due to the severity of the initial stroke rather than HIT. Although clinical severity and outcome of patients treated with UFH were unfavourable compared to those without UFH, the patients with UFH intrinsically might be at high risk of thromboembolic complications because those patients more frequently had systemic atherosclerotic changes or embolic sources. In fact, stroke subtypes were distributed differently between patients with and without UFH in our study. Hoh *et al* (2005) reported significantly less favourable outcomes, including new thromboembolic episodes and deaths in patients with subarachnoid haemorrhage who developed HIT compared to those without HIT. They found that more patients with HIT showed a poorer Fisher Grade than those without HIT, although the diagnosis of HIT was based on clinical criteria, and serological examinations were not mandatory in the study (Hoh *et al*, 2005). It should be considered that serious neurological conditions might be vulnerable to HIT.

In the present study, four of 165 clinical non-HIT patients were positive by both ELISA and SRA. None of these patients demonstrated thrombocytopenia, nor did they die. A thromboembolic event occurred in one patient who developed an ischaemic heart event. Previous reports suggested that high OD values in ELISA and/or strong-positive SRA results were associated with a high degree of diagnostic accuracy for HIT (Warkentin *et al*, 1995, 2008; Lo *et al*, 2007). However, despite high OD values (≥1·5 units) in ELISA (Cases 8, 9, 11) or strong-positive (≥50% serotonin release) SRA results (Cases 8, 9), these patients did not develop HIT (Table II). One of the clinical non-HIT patients was ELISA-negative but SRA-positive and did not

develop any thrombocytopenia, thromboembolic event, or death. Furthermore, three of 95 patients without UFH were positive only by ELISA. In the present study, we blindly evaluated anti-PF4/heparin Abs in all clinical HIT and clinical non-HIT patients. Even if the results of anti-PF4/heparin Abs were positive, all patients with positive results would not always demonstrate HIT, and some of the positive results might not be pathological findings. Therefore, we should be aware of false negative and false positive results in both serological tests, and that diagnosis by the detection of anti-PF4/heparin Abs alone (even with a high OD value in ELISA and/or a strong-positive SRA result) can result in an overdiagnosis of HIT.

This study had some limitations. First, none of the patients underwent venous ultrasound; therefore, subclinical DVT, which is the typical thrombotic complication associated with HIT, may have been underdiagnosed. Second, the dose of UFH could be a determinant for the occurrence of HIT, as stoichiometrically optimal ratios of PF4:heparin influence immunization (Greinacher *et al*, 2008; Warkentin *et al*, 2010). However, in the present study, the dose and blood levels of UFH were not investigated.

In conclusion, the incidence of definite HIT in acute ischaemic stroke patients treated with UFH was 1·7% (95% CI: 0·4–5·0). HIT should be recognized as a clinicopathological syndrome in which both the clinical profile consistent with HIT and the results of serological tests should be carefully considered for HIT diagnosis. The clinical severity and outcome of acute stroke patients who were diagnosed as having definite HIT were unfavourable.

Author contribution

The study concept and design by H. Kawano, H. Yamamoto, S. Miyata, M. Izumi, and T. Hirano; writing by H. Kawano, H. Yamamoto, and S. Miyata; data collection by H. Kawano, H. Yamamoto, N. Toratani, M. Izumi, and T. Hirano; blinded independent assessments of the 4Ts score by S. Sato and S. Okamoto; ELISA assay by S. Miyata and I. Kakutani; SRA assay by Jo-AI. Sheppard and TE. Warkentin; analysis and interpretation of data by H. Kawano, H. Yamamoto, S. Miyata, and A. Kada; drafting of the manuscript by H. Kawano, H. Yamamoto, and S. Miyata; critical revision of the manuscript for important intellectual content by K. Toyoda, K. Nagatsuka, H. Naritomi, TE. Warkentin, and K. Minematsu; study supervision by M. Uchino and K. Minematsu.

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Argatroban therapy for heparin-induced thrombocytopenia during pregnancy in a woman with hereditary antithrombin deficiency

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Abstract

A 33-year-old woman developed deep venous thrombosis at 7 gestational weeks (GW). Heparin-induced thrombocytopenia was evident at 9 GW during unfractionated heparin infusion. Immediately, anticoagulation therapies together with antithrombin (AT) infusion were commenced with the use of argatroban from 9 GW, and fondaparinux was substituted for argatroban after 24 GW. The patient had hereditary AT deficiency type I determined by laboratory findings and results of genomic DNA analysis. The pregnancy ended in full-term vaginal delivery of a healthy male without adverse effects of the anticoagulation therapies. This was the first report of a pregnant woman who developed heparin-induced thrombocytopenia caused by heparin therapy for deep venous thrombosis due to AT deficiency.

Key words: antithrombin deficiency, argatroban, fondaparinux, heparin-induced thrombocytopenia.

Introduction

Antithrombin (AT) plays a role in the regulation of hemostasis by inactivating thrombin and other activated coagulation factors. Women with hereditary AT deficiency have an increased risk of thromboembolism during pregnancy and puerperium, with an incidence of more than 70% in the absence of anticoagulant therapy.[1] Several clinicians have described the management of pregnant women with AT deficiency, and the administration of heparin together with AT replacement is usually standard in the literature.[1–3]

On the other hand, heparin-induced thrombocytopenia (HIT) is an adverse drug reaction caused by platelet-activating HIT-immunoglobulin (Ig)G that recognizes platelet factor 4/heparin (PF4/hep) complexes, which are capable of activating platelets via FcγIIa receptors.[4] Patients with HIT develop a wide spectrum of symptoms, ranging from a decrease in platelet counts to thromboembolic complications. The incidence of HIT is substantially less in pregnant women when compared with non-pregnant individuals.[5]

We encountered a pregnant woman who developed HIT caused by unfractionated heparin therapy for deep venous thrombosis (DVT) due to AT deficiency.

Case Report

A 33-year-old primigravida woman was hospitalized in another hospital due to pain and edema of the left leg at 7 gestational weeks (GW). A Doppler ultrasound examination revealed that the left common iliac vein and posterior tibial veins were extensively occluded by thrombus formation. She was diagnosed as having DVT. To avoid the development of pulmonary

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embolism, a temporary inferior vena cava filter was inserted and placed. Laboratory findings at 7–9 GW in the other hospital were as follows: AT activity 37.9%, AT antigen 13.6 mg/dL, D-dimer 51.6 μg/mL, fibrinogen/fibrin degradation products 95.6 μg/mL, thrombin-antithrombin complex 45.7 ng/mL, fibrinogen 363 mg/dL, proteins C activity 92% (antigen 100%) and protein S activity 33% (antigen 82%). Antiphospholipid antibodies, including lupus anticoagulant, anticardiolipin and β2GPI-dependent anticardiolipin antibodies, tested negative. The patient had no history of thromboembolic diseases. However, her mother had been diagnosed as having AT deficiency. A genomic DNA analysis at 20 GW confirmed that the patient had hereditary AT deficiency type I resulting from a single C to T substitution in the base of codon 359, mutating an Arg codon to a stop codon in Exon 5.[6] Anticoagulant therapies, including intravenous (i.v.) infusions of urokinase (24 000 units/day), unfractionated heparin (UFH) (20 000 units/day) and AT concentrate (3000 units/day) were commenced at 7 GW (Fig. 1). Ten days after the initiation of the therapies, the thrombus in her left common iliac vein became enlarged despite treatment. Meanwhile, the platelet count (273×10^9/L) decreased to 21×10^9/L 12 days after the initiation of the therapies. According to the 4T's score system for HIT diagnosis proposed by Warkentin, the patient had score 6, which is classified as a high risk of HIT.[7]

The development of HIT was strongly suspected at 9 GW. HIT antibodies in her serum were detected a few days after onset of HIT. Enzyme-linked immunosorbent assay (ELISA) measurements of two kinds of HIT antibodies to PF4/heparin complex were performed. The optical density value in IgG-specific PF4/hep ELISA was 1.489 (normal <0.400). PF4-dependent antibody of three immunoglobulin classes (IgG/IgA/IgM) against PF4/polyvinyl sulfonate (poly-ELISA) was found to be 2.764 (normal <0.612).

Consequently, heparin administration was immediately canceled and continuous i.v. injection of argatroban (2.5 μg/kg/min) was started at 9 GW with informed consent. In addition, decannulation of the temporary inferior vena cava filter was required, because it was coated by heparin. Three days after initiation of argatroban infusion the platelet count increased to 136×10^9/L, and to 265×10^9/L 7 days after. The patient was transferred to the Kobe University Hospital at 12 GW. Argatroban infusion (2.5–4.5 μg/kg/min) was maintained to achieve an adequate anticoagulation effect by serial monitoring of activated partial thromboplastin time (APTT). A Doppler ultrasound revealed a small streak thrombus in the left posterior tibial veins at 21 GW. Argatroban was discontinued, and prophylactic subcutaneous fondaparinux (2.5 mg/day) was started at 24 GW with informed consent. Vascular studies with Doppler

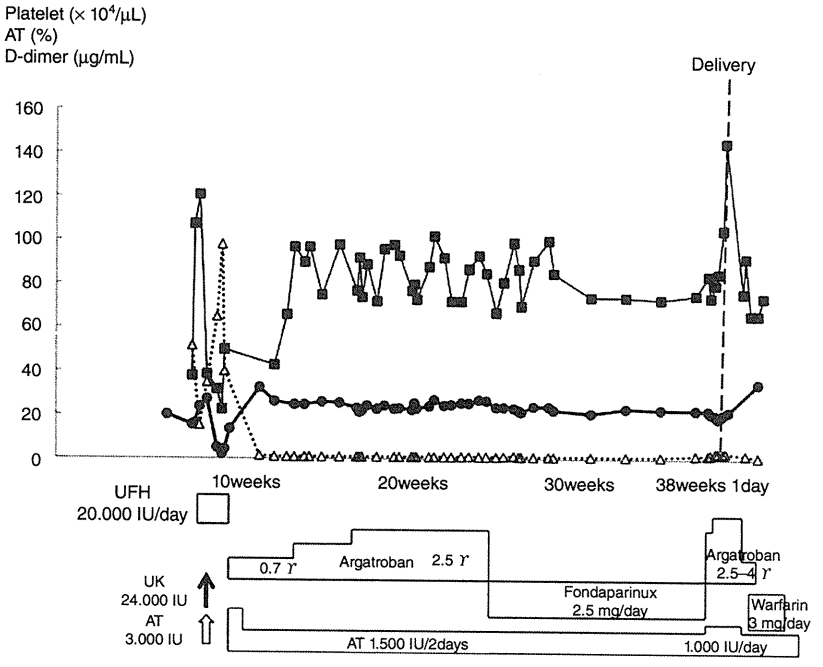

Figure 1 Clinical course of a patient. (●) Platelet count (×10⁴/μL), (■) antithrombin (AT) activity (%), (△) D-dimer (μg/mL). UFH, unfractionated heparin; UK, urokinase.

ultrasound showed complete disappearance of the DVT at 27 GW. After she was discharged at 28 GW, a therapy of fondaparinux self-injection was continued. APTT values were approximately 55 s during the period of argatroban use, and approximately 30 s during the period of fondaparinux use.

The patient was admitted for labor induction at 37 GW. During induced labor with oxytocin, argatroban (2.5–3.5 μg/kg/min to achieve APTT levels with 1.5–2.0 times control) was substituted for fondaparinux. To maintain more than 70% of plasma AT activity, she required 1500 units/day of AT concentrate. The patient delivered a 2800-g male infant with Apgar scores of 9 and 10 at 1 min and 5 min, respectively, and pH 7.331 in the umbilical artery. Blood loss at delivery was 838 mL. Six hours after the delivery, i.v. argatroban was restarted. Warfarin administration (3 mg/day) was started on the first postpartum day. The remainder of the patient's hospital course was uneventful, she and the newborn were discharged without morbidity 15 days later. A laboratory test revealed that the newborn had 14% of AT activity, but there has been no event so far.

Discussion

Several cases of women who developed HIT during their pregnancies have been reported.[8–11] In these reports, HIT in pregnancy usually occurred after UFH therapies for DVT. Only one report showed a cause of DVT in pregnancy as homozygous factor V Leiden mutations.[11] However, the others did not determine the causes of DVT in pregnancy. To the best of our knowledge, the present case is the first report of HIT in a pregnant woman with AT deficiency as the cause of DVT.

The clinical likelihood of HIT can be evaluated using the 4T's score system recently described by Warkentin.[7] This score system is based on four criteria (thrombocytopenia, timing of fall in platelet count, thrombosis, other cause for thrombocytopenia), and allows identification of three levels of HIT risks, that is, score 0–3 (low), 4–5 (intermediate), and 6–8 (high) before laboratory testing. The pretest probabilities of HIT were known to be 0% in low, 10.9% in intermediate and 80% in high-risk groups.[12] In our case, the patient had been treated with UFH and developed thrombocytopenia with platelet count nadir of 21×10^9/L (more than 90% fall). The onset of thrombocytopenia was 12 days after initiation of UFH. Progression of thrombosis was found, and no other cause for a decrease in platelet count was evident. In this situation, the 4T's score of this patient showed a high risk of HIT with score 6.

HIT results from the development of antibodies specific to heparin-modified PF4, which can be detected in the patient's blood by two categories of assays.[7] The first comprises immunoassays that are highly sensitive and well standardized. However, these assays detect IgG as well as IgA and IgM PF4-dependent antibodies, and their clinical specificity and positive predictive value are not very high.[13] As there is growing evidence that only antibodies of the IgG class are capable of inducing platelet activation,[14] it has been speculated that immunoassays with a monospecificity for IgG performed better than polyspecific assays. Bakchoul *et al.* reported that optical density value was associated with an increased probability of HIT.[15] The second category of biological tests was a functional assay, which was more specific for the diagnosis of HIT.[16] However, the functional assay is time-consuming, or not feasible in every laboratory, as it requires radioisotopes. In the present case, strong positive results were obtained by two different immunoassays.

Treatment modalities of HIT include withdrawal of UFH or low-molecular-weight heparin, and replacement with an alternative anticoagulant, including direct thrombin inhibitor (argatroban, lepirudin, bivalirudin) and fondaparinux.[17] Argatroban neither induces nor reacts to PF4/heparin antibodies. Argatroban administration is routinely initiated as a continuous i.v. infusion (2 μg/kg/min), the infusion rate can be titrated up or down to keep the APTT at 1.5 to 3 times the baseline value with a maximum infusion rate of 10 μg/kg/min.[18] Argatroban has a molecular weight of 526.66 Dalton, which can cross the placenta. Given to rats and rabbits, argatroban is found to have no embryo- or fetotoxicity. The US Food and Drug Administration (FDA) suggests that argatroban can be used in pregnancy only if clearly necessary. These points were addressed when informed consent of argatroban use was obtained. Only two cases of argatroban use during the second and third trimester in patients with suspected HIT have been reported. Young *et al.* first reported the administration of argatroban for the treatment of suspected HIT with portal vein thrombus after 33 GW.[19] The other report described use of argatroban for the treatment of DVT caused by flushing the central catheter with heparin.[20] Both reports showed uneventful vaginal deliveries of healthy newborns. In the present case, argatroban administration was started at 9 GW and maintained to 24 GW. For the first time, we used argatroban in the first trimester and used it for as long as 15 weeks.

It was reported that seven pregnant women with HIT used fondaparinux.[17] Fondaparinux had practical advantages over continuous administration of direct thrombin inhibitors. The advantages included once-daily subcutaneous administration, use on an outpatient basis, and no requirement for laboratory monitoring.[17,19] Recently, minimal transplacental passage of fondaparinux was reported *in vivo*.[21] In five pregnant women treated with fondaparinux for 1–101 days, the anti-factor Xa activities in the umbilical cord blood of newborns were found to be one-tenth of the maternal blood. This concentration was much lower than that required for anticoagulation effects. However, until large-scale studies confirm its safety, use of fondaparinux in pregnancy should be limited to patients either with severe allergic reactions to heparin or with HIT. The FDA suggests that use of fondaparinux in pregnancy is categorized as class B medication.

Previous reports described successful management of pregnant women with AT deficiency in which both heparin and AT infusions were carried out.[1–3] In our case, AT administration might not be necessary during the period when argatroban was used. We additionally administered AT for the prophylaxis of DVT, because this is the first pregnant case with DVT and AT deficiency followed by HIT.

Disclosure

None declared.

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