lupus anticoagulant, anticardiolipin antibody, and anti-β2-glycoprotein-I antibody [28].

2.3 DNA sequencing of protein S, protein C, and antithrombin genes

We sequenced the entire coding region of protein S, protein C, and antithrombin genes in 18 patients with DVT. The method of direct sequencing using the 96-capillary 3730xl DNA Analyzer (Applied Biosystems Japan, Tokyo, Japan) has been described previously [20, 29]. We have adopted the numbering standards of the Nomenclature Working Group, wherein the A of the ATG of the initiator Met codon is denoted as nucleotide +1, and the initial Met residue is denoted as amino acid +1 [30].

3 Results

3.1 DVT history of enrolled patients

We enrolled 18 Japanese symptomatic DVT patients in this study, and only one patient had previous DVT event. All patients were negative for the antiphospholipid syndrome. Thirteen patients were primiparous and five were multiparous. One patient without genetic mutation had a history of miscarriage. One patient without genetic mutation had a history of first trimester artificial abortion that was also complicated with DVT at the time. As an additional risk factor, two out of 13 DVT patients without genetic mutation showed hyperemesis, but all five patients with genetic mutation did not show hyperemesis. Other risk factors such as bed rest, preeclampsia, multiple pregnancy, and preterm labor were not observed in all 18 patients. One patient without genetic mutation had the travelers' thrombosis in the first trimester. One patient without genetic mutation showed paradoxical embolism after DVT postpartum.

3.2 Identification of genetic mutation in DVT patients

We sequenced the coding regions of the protein S, protein C, and antithrombin genes in the 18 DVT patients and identified missense mutations in the protein S gene in four cases, and in the protein C gene in one case, but not in the antithrombin gene (Table 1). Two patients, cases 1 and 2, had the K196E mutation in the protein S gene; this is the most popular thrombophilic mutation in the Japanese population [19, 21, 24]. These two patients had protein S anticoagulant activity above 50% (Table 1). Case 3 had a missense mutation, L446P, in the protein S gene. Case 4 had two missense mutations, D79Y and T630I, in the protein S gene with very low anticoagulant activity of 4%, with family history of DVT in her father. The protein S anticoagulant activities during pregnancy in cases 2, 3, and 4 were decreased to 25, <20, and <1%, respectively. Case 5 had the C147Y mutation in the protein C gene with 45% amidolytic activity. Her protein C activity did not change during pregnancy (Table 1). None of the 18 patients with DVT had nonsynonymous mutations in the antithrombin gene. All patients were not obese with body mass index between 18 and 24. Case 1, 2, and 3 had term vaginal delivery; however, case 4 and 5 had cesarean section due to other obstetric indication.

3.3 Onset of DVT in patients with genetic mutation

Table 2 shows the onset of the DVT events in patients with or without genetic mutation. DVT was found in all five patients with genetic mutations in their first and second trimesters, but not in postpartum. In 13 patients without genetic mutations, DVT events occurred in postpartum for four patients and in the first and second trimesters for nine patients. Two out of four patients without genetic mutation underwent cesarean section. Thus, DVT in pregnant patients with genetic mutation tended to occur in the first and second trimesters and not postpartum.

4 Discussion

Although the relationship between DVT and genetic mutations in protein S, protein C, and antithrombin genes is well established, the clinical courses of DVT patients with genetic mutation among Japanese women during pregnancy and postpartum have not been well characterized. Recent genetic analysis of inherited thrombophilia revealed ethnic differences in DVT between Caucasians and Asians [19, 21], suggesting that the study of venous thromboembolism within individual ethnic populations is highly valuable [12]. It has been established that Caucasians have factor V Leiden mutation and prothrombin G20210A mutation as genetic risk factors for DVT, whereas Japanese do not carry them [10, 11]. However, Japanese have the K196E mutation in the protein S gene as a genetic risk for DVT [19, 21, 22]. The study of DVT in a Japanese population without factor V Leiden mutation or prothrombin G20210A mutation may reveal different clinical characteristics and give rise to hitherto unrecognized issues. In particular, sub-group analyses, such as DVT during pregnancy and postpartum, would be valuable. In the present study, we enrolled 18 pregnant Japanese women with DVT and found that five out of 18 patients (28% patients) had genetic mutations in the protein S or protein C gene. None carried mutations in the antithrombin gene.

The question of when DVT events occur in pregnant women with genetic mutations has been debated. Studies of

Table 1 Nonsynonymous mutations identified in protein S and protein C genes in patients ($n = 18$) with DVT during pregnancy and postpartum

Patient	cDNA[a]	Region	Amino acid change	Protein S[b] or protein C[c] activity (%)	Protein S[b] or protein C[c] activity, during pregnancy (%)	Age	Gravida	Parity	Body mass index	Family history	Other complications of pregnancy	Onset of DVT (weeks of gestation)	Delivery mode	Recurrence of DVT	Complication of PTE (weeks of gestation)
Protein S gene															
Case 1	c.586	Exon 6	K196E	57[b]	n.d.	30	1	1	18.6	None	None	27	TVD	None	27
Case 2	c.586	Exon 6	K196E	68[b]	25[b]	27	0	0	20.3	None	None	10	TVD	None	None
Case 3	c.1337	Exon 12	L446P	13[b,d]	<20[b]	30	0	0	18.8	None	None	27	TVD	None	None
Case 4	c.235	Exon 3	D79Y	4[b]	<1[b]	35	0	0	22.5	Father	None	6	C/S	None	None
	c.1889	Exon 15	T630I												
Protein C gene															
Case 5	c.440	Exon 6	C147Y	45[c]	57[c]	28	0	0	24.2	None	None	20	C/S	None	None

TVD term vaginal delivery, *C/S* cesarean section, *PTE* pulmonary thromboembolism

[a] Position from A of initial ATG in cDNA

[b] Protein S anticoagulant activity

[c] Protein C amidolytic activity

[d] Protein S activity was obtained under warfarin treatment

pregnant Caucasian women have reported a 3- to 12-times higher risk of thrombosis postpartum than during pregnancy [3, 4]. On the other hand, a large retrospective study found that events were twice as likely during pregnancy as postpartum [5]. In our new study, we found that Japanese patients with genetic mutations manifested DVT events in their first two trimesters (Table 2). In particular, pregnant Japanese patients with genetic mutation had no DVT events postpartum. Although this trend went against previous findings [3, 4], it was consistent with the results that there were twice as many DVT events during pregnancy as postpartum [5]. DVT onset at the early stage of pregnancy in patients with genetic mutation might be reasonable, since genetic mutation accelerates DVT onset, and patients with mutation might have DVT events in their early stage of pregnancy.

In the present study, we enrolled 18 pregnant Japanese women with DVT and found that four out of 18 patients (22% patients) had genetic mutations in the protein S gene. A previous study on thrombophilia activity screening in Japanese patients with DVT reported a high prevalence of protein S deficiency [31], and this was later confirmed by genetic analysis [19]. Taken together with these previous findings, our study reinforced the theory that protein S deficiency is an important risk factor for DVT in Japanese. This observation was in stark contrast to the case in Caucasians, in whom factor V Leiden and prothrombin G20210A mutations are involved in almost 50% of all DVT cases in pregnant women [8]. It is well known that the level of protein S activity was decreased immediately after pregnancy [25]. Therefore, predisposed thrombophilia should be considered in the care of patients with pregnancy-related complications, and antithrombotic prophylactic therapy might be applicable for those patients. Also, it might be good for women of child-bearing years to know their own thrombophilic nature.

A previous study reported on DNA sequence analyses of the protein S, protein C, and antithrombin genes in 173 Japanese DVT patients [20]. In this study, 55 patients (accounting for 32% of total patients) had nonsynonymous mutations in one of three genes. Among the three genes, mutations in the protein S gene were predominant, being found in 29 patients (17% of the total). Among various nonsynonymous mutations in the protein S gene, the K196E mutation was most prevalent. It was found in one out of 55–70 Japanese individuals, from analyses of general Japanese populations [19, 21, 22, 24]. In our study, we sequenced three genes in 18 patients with pregnancy-related thrombosis and identified missense mutations in five patients (accounting for 28% of the patients). Among five patients, four (22% of the total) had missense mutations in the protein S gene, which reconfirmed the predominance of inherited protein S deficiency in Japanese patients with

Table 2 Onset of DVT according to trimester of pregnancy and postpartum, and according to delivery mode

	Onset of DVT				Delivery mode			Complication of PTE
	First trimester	Second trimester	Third trimester	Postpartum period	Artificial abortion	Term vaginal delivery	Term cesarean section	
Patients with genetic mutation								
Protein S ($n = 4$)	2	2	0	0	0	3	1	1
Protein C ($n = 1$)	0	1	0	0	0	0	1	0
Total ($n = 5$)	2	3	0	0	0	3	2	1[a]
Patients without genetic mutation								
Total ($n = 13$)	4	5	0	4[c]	1[d]	8	4	1[b]

PTE pulmonary thromboembolism

[a] PTE events with genetic mutation occurred during the second trimester

[b] PTE events in the patients without genetic mutation occurred postpartum after cesarean section

[c] Two out of 4 patients without genetic mutation underwent cesarean section

[d] First trimester

DVT. Two of these patients had K196E mutation. Thus, K196E mutation in the protein S gene would be a genetic risk for not only DVT in general, but also for pregnancy-related DVT.

There are limitations to the present study. This was a small-scale retrospective study with 18 patients. We performed genetic analysis in those patients and identified five patients with genetic mutation. To understand the DVT risk in pregnant Japanese patients with inherited or acquired thrombophilia, we will have to recruit patients consecutively and perform thrombophilic screening, including genetic analysis, in the future evaluation.

In conclusion, we identified inherited thrombophilia in pregnant Japanese women with DVT and found protein S deficiency to be a predominant cause of thrombophilia. By DNA sequence analysis, we found two patients with a K196E mutation in the protein S gene that is prevalent in the Japanese population. Since pregnant women showed reduced protein S levels, a diagnosis of protein S deficiency based on its activity has an intrinsic limitation. Since the onset of DVT tends to occur at an early stage during pregnancy, the genetic analysis might be an alternative diagnostic tool.

Acknowledgments We thank Dr. Toshiyuki Sakata for the activity assay, Ms. Junko Ishikawa, Ms. Kanako Sakashita, Ms. Yukiko Sato, and Dr. Shigenori Honda for DNA sequencing, and Dr. Yoshihiro J. Ono for critical reading of the manuscript. This study was supported by a Grant-in-Aid from the Ministry of Health, Labor, and Welfare of Japan; by the Ministry of Education, Culture, Sports, Science, and Technology of Japan and by the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO) of Japan.

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Original Article

Association of Platelet Aggregation with Lipid Levels in the Japanese Population: the Suita Study

Sachika Kameda[1], Toshiyuki Sakata[1], Yoshihiro Kokubo[2], Mana Mitsuguro[1], Akira Okamoto[1], Michitaka Sano[1], and Toshiyuki Miyata[3]

[1]Laboratory of Clinical Chemistry, National Cerebral and Cardiovascular Center, Osaka, Japan
[2]Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, Osaka, Japan
[3]Department of Molecular Pathogenesis, Research Institute, National Cerebral and Cardiovascular Center, Osaka, Japan

Aim: Platelets play a pivotal role in atherothrombotic diseases. Platelet aggregability induced by agonists has great interindividual variability; however, the factors influencing platelet aggregability variation have not been characterized in Asia.

Methods: To examine the confounding factors influencing platelet counts and responsiveness to agonists, we measured the platelet counts and platelet aggregability induced by 1.7 μM adenosine diphosphate (ADP) or 1.7 μg/mL collagen using a light transmittance aggregometer in the Japanese general population without medication or cardiovascular disease (387 men and 550 women) in the Suita Study.

Results: Platelet counts were negatively correlated with age in both men and women (Spearman's rank correlation coefficient: $r_s = -0.230$ and -0.227; $p < 0.01$, respectively). In women, platelet counts were correlated negatively with the high-density lipoprotein (HDL) cholesterol level and positively with the low-density lipoprotein (LDL) cholesterol/HDL cholesterol (L/H) ratio ($r_s = -0.135$ and 0.119; $p < 0.01$, respectively). In women, platelet aggregabilities by ADP and collagen were correlated with age ($r_s = 0.118$ and 0.143; $p < 0.01$, respectively), and collagen-induced platelet aggregability was correlated with the LDL cholesterol level, the L/H ratio, and the non-HDL cholesterol level ($r_s = 0.167$, 0.172, and 0.185; $p < 0.01$, respectively). Even after adjustment for age, systolic blood pressure, body mass index, and current smoking and drinking, the association of platelet counts with the L/H ratio in women and associations of collagen-induced platelet aggregability with the L/H ratio and the non-HDL cholesterol level remained.

Conclusion: Examination of platelet counts and platelet aggregability induced by ADP and collagen revealed gender, age and lipid levels as factors influencing inter-individual variability.

J Atheroscler Thromb, 2011; 18:560-567.

Key words; LDL cholesterol, Lipid, Platelet aggregation, Platelet count

Introduction

Platelet thrombi form at the site of vascular injury or the site of a ruptured atherosclerotic plaque. Platelets contribute pivotally to atherothrombotic disease such as myocardial infarction and stroke; therefore, the suppression of platelet aggregability using anti-platelet drugs is widely recognized as a therapeutic means to prevent cardiovascular events, and these drugs show evidence of event prevention[1].

It is generally accepted that the response of platelets to agonists has large inter-individual variability within the population[2-7]. This interindividual responsiveness has a high degree of heritability[2, 4-6, 8-13]. In addition, increased platelet aggregability has been shown in women[14, 15]. Specifically, women showed higher platelet aggregability in response to collagen, adenosine diphosphate (ADP), arachidonic acid, and

Address for correspondence: Sachika Kameda, Laboratory of Clinical Chemistry, National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan
E-mail: skameda@hsp.ncvc.go.jp
Received: July 8, 2010
Accepted for publication: January 11, 2011

epinephrine in whole blood and platelet-rich plasma than in men[16]. Smoking is a common environmental factor that increases platelet function[17, 18]. In the context of this research, population-based research is helpful in providing information on the confounding factors for platelet responsiveness to agonists; however, these studies are very limited due to the difficulty of platelet aggregability measurement in a community setting. In the large population-based sample of the Framingham Heart Study, platelet responsiveness to agonists was associated with age, body mass index, triglyceride level, high-density lipoprotein (HDL) cholesterol, and diabetes[2]. In this study, higher fibrinogen levels were associated with increased epinephrine-induced aggregation and a tendency to word ADP-induced aggregation[8]. Evidence suggests that increased platelet reactivity could identify individuals at risk for atherothrombotic diseases; however, large cohort studies, including the Northwick Park Heart Study and the Caerphilly Prospective Study, did not show an association of platelet aggregability with cardiovascular events[3, 19].

Studies on the variability of platelet responsiveness to agonists have been mainly performed in the Caucasian population; studies in the Asian population are very limited. Since 1989, we have conducted the Suita Study, an epidemiological study of cerebrovascular and cardiovascular diseases, in a general urban population cohort in Japan[20-22]. The present study was undertaken to clarify the factors influencing the inter-individual variability of platelet responsiveness to agonists in a Japanese urban general population. This is a first step in unraveling systematically the complex interindividual variability of platelet responsiveness in our population.

Methods

Study Population

The study population of the Suita Study was based on samples randomly selected from 12,200 Japanese residents of Suita[20-22]. The participants had been visiting the National Cerebral and Cardiovascular Center every 2 years since 1989 for regular health checkups. Participants attended the National Cerebral and Cardiovascular Center from November, 2005 to December, 2007. A physician or nurse administered questionnaires covering medications, personal habits, and the personal history of cardiovascular diseases. Some cohort members of the study population were excluded from the study because they met one or more of the following criteria: past or present history of cardiovascular disease, failure to fast for at least 10 hours before venipuncture or missing data, age less than 39 years or more than 70 years, or use of any medications. After these exclusions, 937 individuals (men: 387, women: 550) remained in the study. Informed consent was obtained from all subjects. This study was approved by the Institutional Review Board of the National Cerebral and Cardiovascular Center.

Laboratory Measurements

Fasting (≥10 hours) blood samples for the platelet aggregation test were collected between 9 and 10 am from an antecubital vein through a needle into disposable, siliconized, evacuated plastic tubes containing 0.1 vol of 3.13% trisodium citrate, and blood collected in a second tube was used. The samples were centrifuged at 1,100 rpm for 10 minutes at room temperature within 1 hour of collection to obtain platelet-rich plasma. Platelet aggregation was measured using native platelet-rich plasma[23] by a single operator on a PA-200 platelet aggregometer (Kowa Company, Japan) using techniques based on the method of Born[24]. Incubation time was 5 minutes at 37℃, the stir bar speed was 1200 rpm, and sample run time was 7 minutes after addition of agonists. The agonists used were 1.7 μM ADP (Arkray Factory Inc., Japan) or 1.7 μg/mL equine-tendon-derived collagen (Arkray Factory)[25]. Percent platelet aggregation was expressed as the maximal percentage change in light transmission relative to that of platelet-poor plasma.

Glucose, total cholesterol, HDL cholesterol, and triglycerides were measured by enzymatic methods. Low-density lipoprotein (LDL) cholesterol was estimated using the Friedewald formula[26]. The LDL cholesterol /HDL cholesterol (L/H) ratio was obtained by dividing LDL cholesterol by HDL cholesterol. Non-HDL cholesterol was obtained by subtracting HDL cholesterol from total cholesterol. The subjects were classified as current smokers if they smoked at least one cigarette per day, and as non-smokers if they had never smoked or had stopped smoking. Similarly, subjects were classified as alcohol non-drinkers if they had never drunk or had drunk only in the past. Blood pressure (BP) was measured three times with subjects in a sitting position after 5 minutes of rest. Systolic BP (SBP) and diastolic BP (DBP) were taken to be the average of the second and third measurements recorded at least 1 minute apart by well-trained doctors. We measured height and weight in a fasting state. Body mass index was calculated as weight (kg) divided by the square of the height (m^2).

Statistical Analysis

For a comparison between gender groups, the

Table 1. Characteristics of study population by sex

	Men $n=387$	Women $n=550$	p value
Age, years	58.3 (7.1)	57.1 (7.1)	0.006
Systolic BP, mmHg	123.1 (17.3)	115.6 (17.0)	<0.001
Diastolic BP, mmHg	79.8 (11.2)	71.8 (10.6)	<0.001
Body mass index, kg/m^2	23.3 (2.6)	21.8 (2.9)	<0.001
Total cholesterol, mg/dL	200.6 (30.4)	218.8 (33.9)	<0.001
HDL cholesterol, mg/dL	57.1 (14.6)	67.1 (15.1)	<0.001
LDL cholesterol, mg/dL	120.4 (29.2)	134.4 (31.4)	<0.001
L/H ratio	2.23 (0.79)	2.11 (0.74)	0.015
non-HDL cholesterol, mg/dL	143.5 (31.4)	151.8 (34.9)	0.003
Platelet count, $\times 10^3/\mu$L	247 (62)	256 (60)	0.011
ADP-induced platelet aggregation, %	68.2 (12.0)	72.2 (10.6)	<0.001
Collagen-induced platelet aggregation, %	77.4 (9.1)	79.5 (8.4)	<0.001
Current smoking, %	33.7	20.7	<0.001
Current drinking, %	66.1	39.7	<0.001

Values are the means (standard deviation) or percent. BP, blood pressure; ADP, adenosine diphosphate; L/H ratio, LDL cholesterol/HDL cholesterol ratio.

Mann-Whitney U test was used. The association between the platelet count or level of platelet aggregations and the analyzed parameters was assessed by Spearman correlation analysis. We used ANCOVA to investigate whether plasma levels of total cholesterol, LDL cholesterol and HDL cholesterol were positively and independently associated with the platelet count or level of platelet aggregation. We performed adjustments for age, body mass index, SBP, and lifestyle factors (current smoking and drinking) for each gender. Differences of $p<0.05$ were considered to be significant. All analyses were performed with SAS statistical software (release 8.2; SAS Institute Inc.).

Results

Characteristics of Populations

After exclusion of individuals with cardiovascular disease and medications, 937 individuals (men: 387, women: 550), aged from 40 to 69 years, were eligible (**Table 1**). Mean ages (standard deviations, SD) of men and women were 58.3 (7.1) and 57.1 (7.1), respectively. SBP, DBP, body mass index, and habits of smoking and drinking were higher in men than in women (**Table 1**). Total cholesterol, HDL cholesterol, LDL cholesterol, and non-HDL cholesterol were higher in women than in men. We calculated the L/H ratio as a new parameter of the lipid profile. This ratio was higher in men than in women. Platelet counts were higher in women than in men. Platelet aggregabilities induced by ADP and collagen were both higher in women than in men (**Table 1**).

Correlates of Platelet Counts and Platelet Aggregation in Response to ADP and Collagen with Age and Other Covariates

In men, the correlations between platelet count and ADP- and collagen-induced aggregations were $r_s=0.050$ and $r_s=0.022$, respectively. The correlation between ADP- and collagen-induced aggregations was $r_s=0.559$. In women, these correlations were $r_s=0.086$, $r_s=0.051$, and $r_s=0.590$, respectively.

Spearman's rank correlation coefficients of the platelet count, ADP- or collagen-induced aggregation with age and other factors are listed in **Table 2**. Platelet counts were negatively correlated with age in both sexes. Platelet counts were negatively correlated with HDL cholesterol and positively correlated with the L/H ratio in women. ADP- and collagen-induced aggregation was correlated with age in women. Collagen-induced aggregability was correlated with LDL cholesterol, the L/H ratio, and non-HDL cholesterol in women. Smoking was correlated with platelet counts and ADP- and collagen-induced aggregation in both sexes. Drinking was correlated with platelet counts in both sexes.

Age-Related Changes of Platelet Counts and Platelet Aggregation in Response to ADP and Collagen

Platelet counts and platelet aggregabilities induced by ADP and collagen are shown according to the decade of life and sex in **Table 3**. Platelet counts

Table 2. Correlation of platelet count and platelet aggregation with age and other covariates

	Men (n=387)			Women (n=550)		
	Platelet count	ADP-induced platelet aggregation	Collagen-induced platelet aggregation	Platelet count	ADP-induced platelet aggregation	Collagen-induced platelet aggregation
Age, years	−0.230**	−0.059	0.029	−0.227**	0.118**	0.143**
Systolic BP, mm Hg	−0.070	0.005	0.066	0.064	0.027	0.027
Diastolic BP, mm Hg	−0.045	−0.004	0.046	0.062	0.002	0.017
Body mass index, kg/m^2	−0.050	−0.062	−0.019	0.106*	0.058	0.034
Total cholesterol, mg/dL	0.092	0.044	0.033	0.013	0.050	0.137*
HDL cholesterol, mg/dL	−0.004	−0.057	−0.127*	−0.135**	−0.091*	−0.101*
LDL cholesterol, mg/dL	0.072	0.044	0.054	0.026	0.070	0.167**
L /H ratio	0.051	0.068	0.092	0.119**	0.101*	0.172**
non-HDL cholesterol, mg/dL	0.104*	0.047	0.076	0.054	0.087*	0.185**
Glucose, mg/dL	−0.038	−0.030	0.055	0.002	0.061	0.094*
Hemoglobin A1c,%	0.102*	−0.002	0.077	0.105*	0.055	0.047
Current smoking	0.145**	0.109*	0.146**	0.212**	0.237**	0.220**
Current drinking	0.191**	0.098	0.072	0.131**	0.105*	0.080

Data indicate Spearman's rank correlation coefficient. *p<0.05, **p<0.01, BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ADP, adenosine diphosphate; L/H ratio, LDL cholesterol/HDL cholesterol ratio.

Table 3. Age-related changes of platelet counts and ADP- and collagen-induced platelet aggregation by sex

	Age group, years	40-49	50-59	60-69
Men	Numbers of individuals	62	138	187
	Platelet count, ×10^3/μL	261.0 (8.1)	256.0 (5.3)	235.9 (4.6)**
	ADP-induced platelet aggregation, %	68.0 (1.6)	69.2 (1.0)	67.6 (0.9)
	Collagen-induced platelet aggregation, %	76.3 (1.2)	77.1 (0.8)	78.0 (0.7)
Women	Numbers of individuals	99	234	217
	Platelet count, ×10^3/μL	287.1 (5.9)	257.4 (3.8)**	241.4 (4.0)**
	ADP-induced platelet aggregation, %	71.9 (1.1)	71.8 (0.7)	73.0 (0.7)
	Collagen-induced platelet aggregation, %	77.7 (0.9)	79.5 (0.5)*	80.5 (0.6)**

*p<0.05, **p<0.01 compared with 40-49 age-group in the same sex. Values are the means (standard errors).

decreased in individuals aged 60-69 compared to aged 40-49 in both sexes. ADP-induced aggregability in individuals aged 60-69 was not different from aged 40-49 in both sexes; however, collagen-induced aggregability in individuals aged 50-59 and 60-69 was higher than aged 40-49 in women, but not men.

Multivariate Analysis of Lipid Levels According to the Quartile Rank of Platelet Counts or Platelet Aggregation

We divided platelet counts and platelet aggregability induced by ADP or collagen into quadripartite rank by sex and compared lipid levels among the quartiles after adjustment for age, SBP, body mass index, and lifestyle (current smoking and drinking).

In men, increased total cholesterol and an increased ratio of LDL cholesterol to HDL cholesterol were observed in the highest (Q4) platelet-count quartile (**Table 4**). Non-HDL cholesterol was associated with the platelet count (p for trend, 0.042). In women, the L/H ratio was associated with the platelet count (p for trend, 0.037).

In analysis of the quadripartite rank of ADP-induced platelet aggregability, a weak increment of HDL cholesterol in the Q2 rank was observed in women, but no other parameters showed significant differences among quartiles (**Table 5**).

In contrast to ADP-induced platelet aggregability, LDL cholesterol and non-HDL cholesterol and the L/H ratio in women were increased in the highest (Q4) quartile of collagen-induced platelet aggregability (**Table 6**). The L/H ratio and non-HDL choles-

Table 4. Lipid levels according to quadripartite rank of platelet counts by sex

	Rank	Q1	Q2	Q3	Q4	*p* for trend
Men	Platelet count, $\times 10^3/\mu L$	39-210	211-244	245-283	284-824	
	Total cholesterol, mg/dL	194.3 (3.2)	202.5 (3.1)	200.2 (3.1)	205.5 (3.1)**	0.085
	HDL cholesterol, mg/dL	57.3 (1.4)	56.6 (1.4)	58.4 (1.4)	56.3 (1.4)	0.679
	LDL cholesterol, mg/dL	115.0 (3.0)	122.7 (2.9)	120.0 (2.9)	124.0 (2.9)	0.155
	L/H ratio	2.13 (0.08)	2.30 (0.07)	2.16 (0.07)	2.35 (0.07)*	0.118
	non-HDL cholesterol, mg/dL	137.0 (3.2)	145.9 (3.1)	141.8 (3.1)	149.2 (3.1)	0.042
Women	Platelet count, $\times 10^3/\mu L$	75-210	211-244	245-283	284-569	
	Total cholesterol, mg/dL	218.0 (3.0)	217.1 (2.9)	217.4 (2.8)	222.5 (2.9)	0.514
	HDL cholesterol, mg/dL	69.3 (1.2)	67.6 (1.2)	65.4 (1.2)*	66.1 (1.2)	0.114
	LDL cholesterol, mg/dL	132.7 (2.7)	133.3 (2.6)	133.4 (2.6)	138.0 (2.6)	0.487
	L/H ratio	2.00 (0.06)	2.08 (0.06)	2.16 (0.06)	2.23 (0.06)*	0.037
	non-HDL cholesterol, mg/dL	148.6 (2.9)	149.5 (2.8)	152.1 (2.8)	156.4 (2.9)	0.231

Values are the means (standard errors) adjusted for age, systolic blood pressure, body mass index, and lifestyle factors (current smoking and drinking). *$p<0.05$, **$p<0.01$, compared with Q1. HDL, high-density lipoprotein; LDL, low-density lipoprotein; L/H ratio, LDL cholesterol/HDL cholesterol ratio.

Table 5. Lipid levels according to quadripartite rank of ADP-induced platelet aggregation by sex

	Rank	Q1	Q2	Q3	Q4	*p* for trend
Men	ADP-induced platelet aggregation, %	23-62	63-71	72-77	78-93	
	Total cholesterol, mg/dL	200.6 (3.2)	198.3 (3.4)	202.3 (2.9)	200.8 (3.0)	0.859
	HDL cholesterol, mg/dL	57.3 (1.4)	57.6 (1.5)	57.8 (1.3)	55.9 (1.3)	0.758
	LDL cholesterol, mg/dL	119.1 (3.0)	117.8 (3.2)	123.4 (2.7)	120.4 (2.9)	0.577
	L/H ratio	2.18 (0.08)	2.19 (0.08)	2.25 (0.07)	2.29 (0.07)	0.669
	non-HDL cholesterol, mg/dL	143.3 (3.2)	140.7 (3.5)	144.5 (2.9)	144.8 (3.1)	0.818
Women	ADP-induced platelet aggregation, %	29-62	63-71	72-77	78-96	
	Total cholesterol, mg/dL	217.5 (3.1)	219.8 (2.9)	218.7 (2.7)	219.1 (2.9)	0.957
	HDL cholesterol, mg/dL	66.1 (1.3)	69.7 (1.2)*	67.1 (1.1)	65.4 (1.2)	0.072
	LDL cholesterol, mg/dL	134.0 (2.8)	133.0 (2.6)	135.3 (2.4)	134.9 (2.6)	0.927
	L/H ratio	2.12 (0.06)	2.04 (0.06)	2.13 (0.05)	2.18 (0.06)	0.377
	non-HDL cholesterol, mg/dL	151.3 (3.0)	150.1 (2.9)	151.5 (2.7)	153.7 (2.8)	0.841

Values are the means (standard errors) adjusted for age, systolic blood pressure, body mass index, and lifestyle factors (current smoking and drinking). *$p<0.05$, compared with Q1. HDL, high-density lipoprotein; LDL, low-density lipoprotein; L/H ratio, LDL cholesterol/HDL cholesterol ratio.

terol in women were associated with collagen-induced platelet aggregability (*p* for trend; 0.005 and 0.036, respectively).

Discussion

In the present study, we found gender differences in the platelet count and platelet aggregability and revealed the correlation of these parameters with some lipid levels. The interindividual variability of the platelet count and platelet responsiveness to ADP and collagen appeared to be partly explained by gender, age and lipid levels.

We found in the present study that women had higher platelet counts and platelet aggregability in response to ADP and collagen than men. These results were consistent with previous findings that women show higher platelet responsiveness to agonists in whole blood and platelet-rich plasma than men[16]. This gender difference in the platelet aggregability may be related to marked changes of the lipid profile in postmenopausal women. Both total cholesterol and

Table 6. Lipid levels according to quadripartite rank of collagen-induced platelet aggregation by sex

	Rank	Q1	Q2	Q3	Q4	*p* for trend
Men	Collagen-induced platelet aggregation, %	8-73	74-78	79-82	83-95	
	Total cholesterol, mg/dL	196.9 (3.4)	202.6 (3.2)	202.1 (3.0)	200.5 (2.9)	0.619
	HDL cholesterol, mg/dL	58.8 (1.5)	58.9 (1.4)	57.1 (1.3)	54.7 (1.2)*	0.088
	LDL cholesterol, mg/dL	115.6 (3.2)	122.6 (3.0)	121.2 (2.9)	121.4 (2.7)	0.400
	L/H ratio	2.11 (0.08)	2.22 (0.08)	2.25 (0.07)	2.32 (0.07)	0.296
	non-HDL cholesterol, mg/dL	138.1 (3.4)	143.7 (3.2)	145.0 (3.1)	145.8 (2.9)	0.342
Women	Collagen-induced platelet aggregation, %	7-73	74-78	79-82	83-97	
	Total cholesterol, mg/dL	212.7 (3.1)	218.9 (2.9)	221.2 (2.7)*	220.7 (2.8)	0.179
	HDL cholesterol, mg/dL	68.3 (1.3)	68.7 (1.2)	65.8 (1.1)	66.0 (1.2)	0.223
	LDL cholesterol, mg/dL	127.9 (2.9)	133.3 (2.6)	137.8 (2.4)*	136.5 (2.5)*	0.050
	L/H ratio	1.97 (0.06)	2.03 (0.06)	2.23 (0.05)*	2.20 (0.06)*	0.005
	non-HDL cholesterol, mg/dL	144.5 (3.1)	150.2 (2.9)	155.4 (2.6)**	154.7 (2.8)*	0.036

Values are the means (standard errors) adjusted for age, systolic blood pressure, body mass index, and lifestyle factors (current smoking and drinking). *$p<0.05$, **$p<0.01$, compared with Q1. HDL, high-density lipoprotein; LDL, low-density lipoprotein; L/H ratio, LDL cholesterol/HDL cholesterol ratio.

LDL cholesterol are markedly increased in postmenopausal women[20], and hypercholesterolemia is associated with hyperaggregability. In the present study, the mean age of women was 57.1 year old and thus most were postmenopausal. Furthermore, we found that platelet counts were negatively correlated with age and positively correlated with smoking in both men and women. We also found that smoking correlated with platelet aggregability which was not in agreement with the Framingham Heart Study[2]. The discrepancy in terms of smoking between the two studies is not clear; however, it might have been caused by the difference in the frequency of smokers.

Beside cellular interactions of platelets with other blood cells and vascular cells, interactions of platelets with lipoproteins seem to be quite important, and circulating lipoproteins in blood directly or indirectly influence platelet properties[27]. LDL is an atherogenic lipoprotein and increases platelet activation. Platelets are directly associated with LDL in blood[28]. LDL is modified to oxidative LDL. Oxidative LDL induces platelet activation followed by quick changes in shape and aggregation contributing to thrombus formation after plaque rupture. In contrast with LDL, HDL particles have several antiatherogenic activities, including anti-inflammatory, antithrombotic, antioxidative, and vasodilatory properties[29]. Lowering LDL cholesterol or raising HDL cholesterol therapy has well-established benefits in the primary and secondary prevention of atherothrombotic diseases[30-33]. Actually, platelet aggregation evaluated with a thrombus area on the aorta in an *ex vivo* superfusion chamber under 1,000 s^{-1} has been inversely correlated with HDL cholesterol levels[34]. Infusion of reconstituted HDL to humans showed a transient inhibition of platelet aggregation induced by arachidonic acid and collagen[35]. These findings also suggested that HDL has antiplatelet actions. In our study, HDL cholesterol was negatively associated with collagen-induced platelet aggregation. This negative association was also observed in the Framingham Heart Study[2].

In the present study, collagen-induced platelet aggregability was associated with the L/H ratio in women, even after adjustment for age, systolic blood pressure, body mass index, and current smoking and drinking. Recently, the L/H ratio has been considered to be a clinically useful marker, because it is more closely associated with the occurrence of cardiovascular events than the levels of LDL cholesterol or HDL cholesterol[36]. Therefore, our findings suggest that increased collagen-induced platelet aggregation in women is potentially associated with early atherosclerotic conditions.

There have been few studies on the prediction of cardiovascular events by platelet tests. Two small studies have suggested that platelet aggregability assessed by a light transmittance aggregometer could be predictive of cardiovascular events[37, 38]; however, the Northwick Park Heart Study, a large cohort study consisting of 740 men followed up for 10.1 years, found no association of ADP-induced aggregation with ischemic heart disease events[19]. In the Caerphilly Prospective Study, consisting of 2000 elderly men followed up for 10 years, the aggregative response to ADP in platelet-

rich plasma, that to ADP in whole blood measured using an impedance method, and platelet aggregation induced in whole blood by high-shear flow did not show an association with myocardial infarction[3].

In this study, we found that the platelet count and platelet aggregation are affected by factors such as gender, age, and lipid levels in the Japanese population. Furthermore, increased platelet aggregation by collagen in women is closely associated with the LDL-C/HDL-C ratio and LDL-C as risk factors for atherosclerotic disease. Therefore, this study offers modest support for the hypothesis that increased platelet aggregation by collagen even within the normal range might be associated with atherosclerosis in middle-aged women. However, future studies are necessary to establish whether platelet aggregation by collagen is a useful marker to predict coronary events and mortality. We are now following the occurrence of cardiovascular disease events in the Suita Study. Moreover, the response of platelets to agonist may have inter-individual variability within the population that is partly due to genetics. We are now genotyping the DNA polymorphisms of the study participants using a candidate gene approach.

Acknowledgements

This study was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), a Grant-in-Aid from the Ministry of Health, Labor, and Welfare of Japan, the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and Suzuken Memorial Foundation. We would like to express our deepest gratitude to Drs. Yasushi Kotani and Katsuyuki Kawanishi, all members of Suita City Health Center, and Suita Medical Association. We also thank all researchers and staff of the Division of Preventive Cardiology for performing the medical examinations. We also thank *Satsuki-Junyukai*, the volunteers who administered the Suita study.

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Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

Guidelines for the Diagnosis, Treatment and Prevention of Pulmonary Thromboembolism and Deep Vein Thrombosis (JCS 2009)

– Digest Version –

JCS Joint Working Group

Table of Contents

(*Circ J* 2011; **75:** 1258–1281)

Abbreviations Used in the Guidelines

$AaDO_2$: alveolar-arterial oxygen difference
ACE: angiotensin converting enzyme
APTT: activated partial thromboplastin time
BNP: brain natriuretic peptide
CABG: coronary artery bypass grafting
CDT: catheter-directed thrombolysis
CT: computed tomography
CTEPH: chronic thromboembolic pulmonary hypertension
CTR: cardiothoracic ratio
DBP: diastolic blood pressure
DVT: deep vein thrombosis
HIT: heparin-induced thrombocytopenia
HLA: human leukocyte antigen
HOT: home oxygen therapy
ICU: intensive care unit
INR: international normalized ratio
IPC: intermittent pneumatic compression
IVC: inferior vena cava
MDCT: multi-detector CT
MHLW: Ministry of Health, Labour and Welfare
MRA: magnetic resonance angiography
MRV: magnetic resonance venography
MSCT: multi-slice CT
NHI: National Health Insurance
NO: nitric oxide
NYHA: New York Heart Association
$PaCO_2$: partial pressure of arterial carbon dioxide
PaO_2: partial pressure of arterial oxygen
PCPS: percutaneous cardiopulmonary support
PE: pulmonary embolism
PEA: pulmonary thromboendarterectomy
PEEP: positive end-expiratory pressure
PG: prostaglandin
PH: pulmonary hypertension
PT: prothrombin time
PTCA: percutaneous transluminal coronary angioplasty
PTE: pulmonary thromboembolism
SBP: systolic blood pressure
SpO_2: peripheral oxygen saturation
UK: urokinase
VTE: venous thromboembolism

Released online March 25, 2011
Mailing address: Scientific Committee of the Japanese Circulation Society, 8th Floor CUBE OIKE Bldg., 599 Bano-cho, Karasuma Aneyakoji, Nakagyo-ku, Kyoto 604-8172, Japan. E-mail: meeting@j-circ.or.jp
This English language document is a revised digest version of Guidelines for the Diagnosis, Treatment and Prevention of Pulmonary Thromboembolism and Deep Vein Thrombosis reported at the Japanese Circulation Society Joint Working Groups performed in 2008. (website: http://www.j-circ.or.jp/guideline/pdf/JCS2009_andod_h.pdf)
Joint Working Groups: The Japanese Circulation Society, The Japan Radiological Society, The Japanese Association for Thoracic Surgery, The Japanese Society for Vascular Surgery, The Japanese Society on Thrombosis and Hemostasis, The Japanese Respiratory Society, The Japanese Society of Phlebology, The Japanese Society for Cardiovascular Surgery, The Japanese College of Cardiology
ISSN-1346-9843 doi:10.1253/circj.CJ-88-0010

Introduction to the Revised Guidelines

The Japanese Circulation Society (JCS) has already provided guidelines for the diagnosis and treatment of major cardiovascular diseases. The JCS decided to revise the Guidelines for the Diagnosis, Treatment and Prevention of Pulmonary Thromboembolism and Deep Vein Thrombosis, which were first completed in April 2004, to include new advanced techniques of diagnosis and treatment of these conditions that have been developed since publication of the first guidelines. As was the case for the previous guidelines, the Working Groups for the present guidelines consisted of cardiologists and cardiovascular surgeons who have been involved in research on the diagnosis, treatment and prevention of pulmonary thromboembolism (PTE).

Although the etiology and pathology of PTE have yet to be completely determined, it is known that deep vein thrombosis (DVT) plays an important role in the development of PTE. PTE may thus be considered a complication of DVT, and these conditions are regarded as a single disease entity that should be called venous thromboembolism (VTE). Treatment for PTE differs significantly depending on whether the condition is acute or chronic. Although acute PTE is an emergent condition especially prevalent in Europe and the United States, it is becoming increasingly prevalent in Japan as well because of Westernization of Japanese lifestyle, the rapid increase in population of the elderly, increased recognition of this disease, and advancement of diagnostic techniques. Acute PTE has received much media attention as an economy-class syndrome and an unexpected secondary disaster following earthquakes. It is also a postoperative complication that should be careful monitored for in patients with prolonged bed rest following gastrointestinal surgery, gynecologic treatment, or orthopedic surgery. Patients with acute PTE require prompt diagnosis and appropriate treatment. Patients with acute PTE often respond well to thrombolytic therapy and anticoagulation therapy, and new drugs have been approved and are available for this patient population. Patients with large amounts of thrombus or circulatory collapse can be treated effectively with catheterization and surgery. Inferior vena cava (IVC) filters are used to prevent PTE, and the use of non-permanent filters (also referred to as temporal filters and removable filters) has become increasingly common. Chronic PTE associated with pulmonary hypertension (PH) is a serious condition that causes right heart failure and respiratory failure and does not respond well to conventional medical treatment. However, its prognosis has been improved by new drugs efficacious in the treatment of PH. Pulmonary thromboendarterectomy (PEA) with cardiopulmonary bypass and deep hypothermic intermittent circulatory arrest is performed as radical treatment of PTE, and has significantly improved the outcome of surgery, clinical symptoms, and cardiopulmonary hemodynamics as well as the QOL of patients with central type of chronic PTE. Prevention of perioperative VTE by physical therapy is quite important, and new drugs have become available for this purpose.

The Working Groups revised the present guidelines, placing emphasis on currently available evidence whenever possible, but it should be noted that the present guidelines include up-to-date information that may be utilized by cardiologists, cardiovascular surgeons, and other surgeons involved in surgical treatment of VTE in the clinical setting as guidance for diagnosis and treatment of this disease. It should also be noted that determination of treatment by attending physicians based on the specific conditions and circumstances of their patients should take precedence over the guidelines, and that the present guidelines provide no grounds for argument in cases of legal prosecution. The guidelines may be revised in the future to include description of newer methods of diagnosis and treatment of VTE.

We hope that the guidelines will aid physicians in the diagnosis, treatment, and prevention of VTE.

In the present guidelines, levels of recommendation are rated according to the following classification as used in other guidelines for the Diagnosis and Treatment of Cardiovascular Diseases.

Class I: Conditions for which there is evidence for and/or general agreement that the procedure or treatment is useful and effective.

Class II: Conditions for which there is conflicting evidence and/or a divergence of opinion regarding the usefulness/efficacy of a procedure or treatment.

Class IIa: The weight of evidence/opinion is in favor of usefulness/efficacy.

Class IIb: Usefulness/efficacy is less well established by evidence/opinion.

Class III: Conditions for which there is general agreement that a procedure/treatment is neither useful nor indicated and may be harmful.

I General Descriptions

1. Acute Pulmonary Thromboembolism

1 Epidemiology

PTE becoming prevalent in Japan, and should no longer be considered a rare condition. In 2006, PTE occurred in 7,864 patients in Japan. The number of patients has increased 2.25-fold in the past decade,[1] and the incidence of this condition is estimated to be 62 cases/million population. Since the incidence of PTE in the United States is about 500 cases/million population, that in Japan in 2006 is about one-eighth that in the United States.

The incidences of perioperative PTE in Japan were 4.41, 4.76, 3.62, and 2.79 cases/10,000 surgeries in 2002, 2003, 2004, and 2005, respectively. The incidence began to decrease in 2004 when the guidelines for the prevention of PTE were published and healthcare costs for preventive treatment began to be covered by the National Health Insurance (NHI) of Japan.[2]

In Japan, acute PTE develops more frequently in females than in males. The most common age of onset is in the sixth and seventh decades.[3]

2 Risk Factors

Major risk factors for PTE are listed in **Table 1**. Virchow's triad, ie, the presence of (1) interrupted blood flow, (2) endo-

Table 1. Risk Factors for Pulmonary Thromboembolism

	Acquired factors	Congenital factors
Interrupted blood flow	Prolonged bed rest Obesity Pregnancy Cardiopulmonary disease (eg, congestive heart failure, chronic cor pulmonale) General anesthesia Anesthesia of the lower limbs Plaster bandage of the lower limbs Varicose veins in the lower limbs	
Endothelial dysfunction	Surgeries Trauma, fractures Central venous catheterization Catheter test/intervention Vasculitis Antiphospholipid syndrome Hyperhomocysteinemia	Hyperhomocysteinemia
Hypercoagulability	Malignant tumors Pregnancy Surgeries, trauma, fractures Burns Drugs (eg, oral contraceptives, estrogens) Infections Nephrotic syndrome Inflammatory bowel disease Myeloproliferative disorders, polycythemia Paroxysmal nocturnal hemoglobinuria Antiphospholipid syndrome Dehydration	Antithrombin deficiency Protein C deficiency Protein S deficiency Abnormal plasminogen Abnormal fibrinogen Increase in tissue plasminogen activator inhibitor Abnormal thrombomodulin Activated protein C resistance (Factor V Leiden)* Prothrombin gene mutation (G20210A)*

*Not observed in Japanese population.

Table 2. Classification of Clinical Severity of Acute Pulmonary Thromboembolism

	Hemodynamics	Right heart overload observed on echocardiography
Cardiac arrest Collapse	Cardiac arrest or circulatory collapse	Present
Massive	Unstable Shock or hypotension (defined as a systolic blood pressure of <90 mmHg last ≥15 minutes or a decrease in blood pressure by ≥40 mmHg, regardless of the presence/absence of new onset of arrhythmia, dehydration or sepsis)	Present
Submassive	Stable (absence of the above findings)	Present
Non-massive	Stable (absence of the above findings)	Absent

thelial dysfunction, and (3) hypercoagulability, is a very important set of factors that affect susceptibility to thrombus formation.

3 Conditions of Onset

Acute PTE often occurs when patients stand up or begin walking or during micturition or defecation after resting.[3,4] Since the major source of emboli is thrombi in veins of the lower limbs or intrapelvic veins, it is believed that muscle contraction in the lower limbs increases venous return, with the muscles acting in pump-like fashion to push blood, resulting in release of thrombi that cause PTE.

4 Pathophysiology

Acute PTE is caused by abrupt blockage of pulmonary vessels by thrombi that has formed in the veins or the heart and has traveled through the blood stream. The source of emboli is the veins of the lower limbs or pelvis in more than 90% of cases. The main manifestations of acute PTE are sudden onset of PH and hypoxemia.[5,6] Since the mean pulmonary arterial pressure that can be generated by the right ventricle is 40 mmHg in individuals without cardiopulmonary disease,[7] when pulmonary arterial pressure exceeds 40 mmHg during the acute phase of PTE, physicians should suspect acute-on-chronic PTE, ie, acute exacerbation of chronic PTE due to the occurrence of acute PTE, or chronic PTE. Pulmonary infarction, which occurs as a hemorrhagic infarction, develops in about 10 to 15% of patients with acute PTE,[8,9] often as a result of occlusion of a peripheral pulmonary artery.

5 Severity Classification

Since the prognosis and rate of recurrence of acute PTE differ significantly by the presence or absence of echocardiographic findings of pressure overload in the right ventricle, the severity of acute PTE is commonly classified according to clinical signs/symptoms and echocardiographic findings,[10] as outlined in Table 2.

6 Prognosis and Clinical Course

According to available data in Japan, the mortality rate of

acute PTE is 14% overall, 30% among patients with cardiogenic shock (20% among those receiving thrombolytic therapy and 50% among those not receiving it), and 6% among patients without cardiogenic shock.[3] According to data in Europe and the United States, the mortality rate of acute PTE is as high as 30% when it is not diagnosed and treated promptly, but decreases to 2 to 8% when appropriate treatment is performed.[11,12] It is known that early diagnosis and appropriate treatment decrease the mortality rate substantially. Independent determinants of mortality of acute PTE include right ventricular dysfunction on echocardiography, advanced age (≥70 years), cancer, congestive heart failure, chronic obstructive pulmonary disease, hypotension, and tachypnea.[13]

In a follow-up study in Japan, PH developed in 3.7% in patients with acute PTE.[14] In the United States, it has been estimated that PH secondary to chronic PTE develops in 0.1 to 3.8% of patients with a history of acute PTE.[15–17]

2. Chronic Pulmonary Thromboembolism

1 Definition

Chronic PTE develops as a result of chronic occlusion of pulmonary arteries by organized thrombi. In Japan, chronic PTE is defined as abnormal pulmonary blood flow distribution and pulmonary circulation hemodynamics that persist for ≥6 months without substantial changes.[18] Some patients with chronic PTE exhibit clinical manifestations such as shortness of breath during exercise (exertional dyspnea) due to thrombotic occlusion of several pulmonary arteries, which causes PH. This condition is referred to as chronic thromboembolic pulmonary hypertension (CTEPH). CTEPH is classified by clinical course into two types, ie, recurrent CTEPH with a history of signs/symptoms suggestive of acute PTE, and latent CTEPH with progression of PH but without clear clinical findings of acute PTE. Patients with mild CTEPH are treated with medical treatment mainly consisting of anticoagulation therapy to prevent progression of disease, while patients with severe PH may also have right heart failure and a poor prognosis.[19,20] In 1998, the Ministry of Health, Labour and Welfare (MHLW; formery Ministry of Health and Welfare) of Japan termed CTEPH "idiopathic chronic PTE with PH" and designated it a specific disease for which healthcare costs are covered by public expenditure. In the present guidelines, however, the term CTEPH is used.

2 Epidemiology

The incidence of PTE, including acute and chronic PTE, in Japan is believed to be lower than in Europe and the United States. According to data in annual reports on pathologic autopsy cases, the incidence of acute PTE in Japan is about one-tenth that in the United States,[21] although these data are rather old. In the United States, it is estimated that acute PTE occurs in 0.5 to 0.6 million individuals each year, and that CTEPH occurs in about 0.1 to 0.5% of patients surviving the acute phase of PTE.[15,16] However, a recent report noted that CTEPH occurred in 3.8% of patients with a history of acute PTE.[17] Physicians should be aware of the risk of progression to CTEPH when treating patients with acute PTE.

In Japan, the Specific Disease Respiratory Failure Study Group of the MHLW established criteria for the diagnosis of CTEPH and conducted a nationwide survey in 1997.[22] The number of patients with CTEPH was estimated to be 450 (95% confidence interval: 360 to 530).[23,24] The MHLW then designated CTEPH a specific disease and has conducted an annual epidemiological survey of it. In 2006, a total of 800 patients with CTEPH were provided with medical care certificates for the treatment of a specific disease. Assessment of case reports on 520 of the 800 patients with CTEPH revealed that female patients were predominant, with a female to male ratio of 2.8:1, and that the mean age of patients was 62± 13 years. Female patients were predominant, especially among those over 40 years of age, with no gender difference observed in younger patients.[25]

3 Etiology

The mechanisms of onset of CTEPH are still uncertain. In Europe and the United States, CTEPH is considered a chronic condition occurring in patients with a history of acute PTE caused by DVT. In a nationwide survey in Japan, only 28% of patients with CTEPH had DVT.[24] Although some patients with CTEPH had known risk factors for DVT, such as coagulopathy (such as presence of antiphospholipid antibodies and deficiency of antithrombin, protein C, or protein S), heart disease, and malignant tumors, 43.9% of patients assessed had no apparent underlying conditions. Frequencies of human leukocyte antigen (HLA)-B*5201 and HLA-DPB1*0202 were high among patients with a particular type of CTEPH, and in patients carrying HLA-B*5201 and/or -DPB1*0202, the frequency of DVT was significantly lower than in other patients.[26] These findings suggest that there may be a different mechanism of onset of CTEPH in the Japanese population not shared by Western populations. Some patients with CTEPH may remain asymptomatic for several months to years following a period with findings suggestive of acute PTE. This asymptomatic period is referred to as a "honeymoon period".[15] Although the mechanism of latent progression of PH is unknown, several hypotheses, including repetition of latent thrombosis and progression of thrombosis in the pulmonary arteries, have been suggested. Recently, the involvement of small vessel disease in the pathogenesis of CTEPH has been hypothesized.[27]

4 Clinical Manifestations

Although there are no symptoms specific to CTEPH, almost all patients experience exertional dyspnea. Abrupt dyspnea and chest pain develop repeatedly in patients with repetitive CTEPH, while exertional dyspnea becomes severe over time in patients with latent CTEPH without apparent recurrence. Other symptoms such as chest pain, dry cough, and syncope, may develop as well, and bloody sputum and fever may develop in patients complicated by pulmonary bleeding and pulmonary infarction. Patients with right heart failure due to PH may exhibit abdominal distension, body weight gain, and edema of the lower legs.

5 Diagnosis

The diagnosis of CTEPH is made according to the criteria for diagnosis of idiopathic chronic PTE with PH described below. Contrast multi-slice computed tomography (termed multi-slice CT [MSCT] or multi-detector CT [MDCT]) is useful in the diagnosis and differential diagnosis of CTEPH. However, pulmonary angiography is required to determine whether surgery is indicated.

6 Prognosis

According to a report by Riedel et al and the results of a survey of CTEPH in Japan, the prognosis of patients with a mean pulmonary arterial pressure during the stable period of ≥30 mmHg is poor.[19,20] The prognosis of patients under-

Table 3. Risk Factors for Deep Vein Thrombosis

	Risk factors
Demographics/ environment	Elderly Prolonged sitting: During trips and during disasters
Pathology	Trauma: Leg fractures, leg palsy, spinal injuries Malignant tumors Congenital hypercoagulability: Coagulation inhibitor deficiencies Acquired hypercoagulability: Following surgery Heart failure Inflammatory bowel disease, antiphospholipid syndrome, vasculitis Varicose veins of lower limbs Dehydration/polycythemia Obesity, pregnancy, postpartum status Congenital iliac bands and webs, iliac compression by the iliac artery History of venous thromboembolism: Vein thrombosis, pulmonary thromboembolism
Treatment	Surgeries: Orthopedic surgery, neurosurgery, abdominal surgery Drugs: Female hormone, hemostatics, corticosteroids Catheter test/intervention Prolonged bed rest: Management of severe patients, postoperative patient management, patients with cerebrovascular disorders

going medical treatment has recently improved. However, since there are patients whose QOL and vital prognosis are improved significantly following surgery (PEA), accurate diagnosis and severity classification are essential in considering whether patients are indicated for surgery, including the determination whether lesions are surgically accessible and whether significant disorder of major organs is present.

7 Treatment

Since the prognosis of patients receiving medical treatment alone is poor, physicians should consider PEA when the organized thrombi are surgically accessible and no significant disorders are observed in other major organs.[15,16,18,28–34] In patients in whom the proximal ends of the organized thrombi are located in lobe arteries or main pulmonary arteries, significant improvement of pulmonary hemodynamics, QOL, and prognosis may be obtained after successful PEA.[30–34] It has also been reported that surgery may improve the pulmonary hemodynamics and QOL of patients in whom thrombi are located in segmental arteries.[32,34] Recent reports have noted that drugs for the treatment of pulmonary arterial hypertension are effective in the treatment of patients with CTEPH not indicated for surgery.[33–37]

3. Deep Vein Thrombosis

1 Definition

The veins of the extremities are classified into superficial veins that lie above the fascia and deep veins that lie under the fascia. Acute venous thrombosis is thus classified as DVT affecting deep veins and thrombophlebitis affecting superficial veins. The manifestations of DVT depend on the location of affected veins. The present guidelines mainly describe DVT in the veins of the pelvis and lower limbs.[38]

2 Epidemiology

At autopsy, DVT is observed in 24 to 60% of patients who died in hospital in Europe and the United States and 0.8% of those in Japan.[39–41] In an epidemiological survey, the Venous Disease Survey Committee of the Japanese Society of Phlebology in 1997 reported that DVT occurred in 506 patients per year,[42] while the number of new patients with DVT was estimated to be 14,674 patients/year,[1] ie, 12/100,000 population/year, in a questionnaire survey conducted by the Japanese Society of Pulmonary Embolism Research in 2006. These findings reflects the fact that the incidence of DVT has increased about 30-fold during the last decade. On the other hand, the annual incidence of DVT in Europe and the United States were calculated as 50/100,000 population/year on the basis of reports published between 1976 and 2000.[43] The incidence of DVT in Japan has increased rapidly to about one-fourth those in Europe and the United States.

3 Etiology and Risk Factors

The major causes of venous thrombus are venous endothelial dysfunction, hypercoagulability, and interruption of venous blood flow.[44] The development of DVT involves these major causes as well as various other risk factors of various strengths[38,45] (**Table 3**). Most cases of DVT in the veins in the neck and upper limbs are iatrogenic, and caused by the placement of an intravenous line, pacemaker catheter, or hemodialysis shunt, and cases of thoracic outlet syndrome are also observed. DVT in the superior vena cava develops in patients with superior vena cava syndrome in whom DVT is typically caused by mechanical compression of this vein by mediastinal tumor. DVT in the IVC often develops as a result of extension of thrombus from veins in the pelvis or lower limb. Cases of IVC filter thrombosis and of Budd-Chiari syndrome are also observed. In the veins in the pelvis and lower limb, DVT may develop as a result of venous compression due to congenital iliac bands and webs in the pelvis, iliac compression by the iliac artery, insertion or placement of a catheter into the femoral vein, or bed rest with limited leg movement. Cases of DVT in the lower legs are predominant.[45,46] DVT in the lower leg often develops in the veins of the soleus muscle,[38,47] which receive blood from the medial, central, and lateral parts of the lower leg,[48–51] and the vein running through the central portion of the soleus muscle is largest and the main location of DVT.[48]

4 Pathophysiology

Thrombus in a vein adheres to the venous wall over a period of several days after thrombus formation as a result of inflammatory changes, and then regresses due to organization. Although venous valves included within the lesion may be damaged, some valves maintain function.[38,45] Blood flow recovers after lysis or regression of thrombi during the acute phase. During the chronic phase, recovery of blood flow occurs after organization or recanalization of thrombi. Thrombi in the popliteal vein and the distal veins disappear almost completely in several days to several weeks, while thrombi in the proximal leg veins remain as fibrotic bands, although about 50% of such thrombi regress within one year after formation.[52] The central edge of thrombus can become embolic or a source of emboli. While white thrombi and mixed thrombi tend to adhere to the venous wall, red thrombi do not adhere to the venous wall tightly and are easily detached from the vascular wall and cause embolism.[53] Thrombus in veins in the pelvis and lower limbs are detached during movement of the hip and knee joints in the supine or sitting position. During walking, thrombi are detached from the wall as a result of calf muscle pump function.[49] Embolism often

occurs within one week after formation or progression of thrombus, but may recur depending on the amount of movement of the lower limbs and blood flow in the central edge of thrombus.[47,50] The severity of PTE correlates with the size of emboli and frequency of formation of emboli. Severe PTE is often caused by thrombus in leg veins above the popliteal vein, especially the femoral vein, but may be caused by thrombi in soleus veins.[47,49,50] Although the source of emboli is uncertain in 30 to 60% of patients with PTE,[38,45] autopsy has frequently revealed the presence of new and old sources of emboli in the veins of lower limbs.[50]

5 Typing and Staging of DVT

DVT in the pelvis and lower limb is classified into central type of DVT (iliac DVT and femoral DVT), which occurs in veins above the popliteal vein, and peripheral type of DVT (lower leg DVT), which occurs in popliteal vein and distal veins. In the present guidelines, DVT is also classified based on clinical signs/symptoms and severity of anomalous venous drainage into acute and chronic phases. Signs and symptoms of acute anomalous venous drainage include swelling, pain, and skin color change in patients with central type of DVT. In patients with iliac DVT with diffuse occlusion, venous necrosis due to poor arterial perfusion develops in the acute phase. It is of practical use to classify the severity of clinical signs/symptoms according to the presence/absence of painful swelling, painful swelling with discoloration (phlegmasia alba dolens [milk leg], phlegmasia cerulea dolens [blue leg]), and venous necrosis.[38,54] Although peripheral type of DVT typically causes pain, many patients are asymptomatic. Important findings of physical examination include the presence of thrombosed veins or tenderness on palpitation (direct findings) and hard lower leg muscles (indirect finding).[38,54] When DVT recurs during the chronic phase, the patient exhibits signs and symptoms characteristic both the acute and chronic phases of DVT.

6 Prognosis and Recurrence

The short-term prognosis of DVT in the pelvis and lower limb depends on the presence/absence and severity of acute anomalous venous drainage, acute PTE, and arterial embolism. Acute anomalous venous drainage often subsides within several months after onset. Acute PTE is the most serious condition,[45,55] and requires both primary and secondary prevention. In patients with arterial embolism, the presence/absence of patent foramen ovale must be confirmed.[56] The long-term prognosis of DVT depends on the presence/absence and severity of post-thrombotic syndrome, recurrent DVT, chronic PTE, and arterial embolism.[38,45,54] Post-thrombotic syndrome develops in about 40% of patient with central type of DVT,[57] and is caused by abnormal valves in perforating arteries and superficial veins. When DVT recurs, acute manifestations are observed, and the incidences of PTE and post-thrombotic syndrome are increased.[58] Early treatment improves the prognosis of patients with DVT. Patients with recurrent DVT following anticoagulation therapy must be assessed for thrombophilia.[59,60] To ensure prevention of recurrent DVT, patients must continue exercise and compression therapy as well as anticoagulation therapy for an appropriate length of time. The duration of anticoagulation therapy should be determined considering the reversibility of risk factors and whether the condition is idiopathic and/or permanent.[45,60]

II Descriptions of Individual Diseases

1. Acute Pulmonary Thromboembolism

1 Diagnosis

Accurate diagnosis of acute PTE is difficult, since no physical or laboratory findings are specific to acute PTE. Physicians should suspect acute PTE when the following non-specific findings are present. Acute PTE should be included in the differential diagnosis if patients have dyspnea that cannot be explained by other causes.

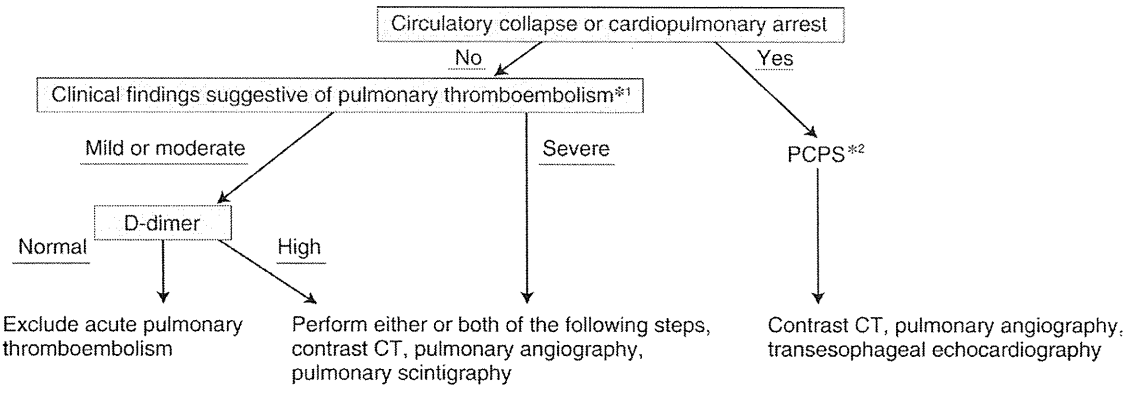

Figure 1. Steps in the diagnosis of acute pulmonary thromboembolism. Start heparin therapy when pulmonary embolism is suspected. Examine for deep vein thrombosis at once. *1Screen the patient with chest X-ray, ECG, arterial blood gas analysis, transthoracic echocardiography, and blood chemistry. *2When PCPS is not available, maintain circulation with cardiac compression and vasopressors. CT, computed tomography; PCPS, percutaneous cardiopulmonary support. Adapted from *Therapeutic Research* 2009; **30:** 744–747.

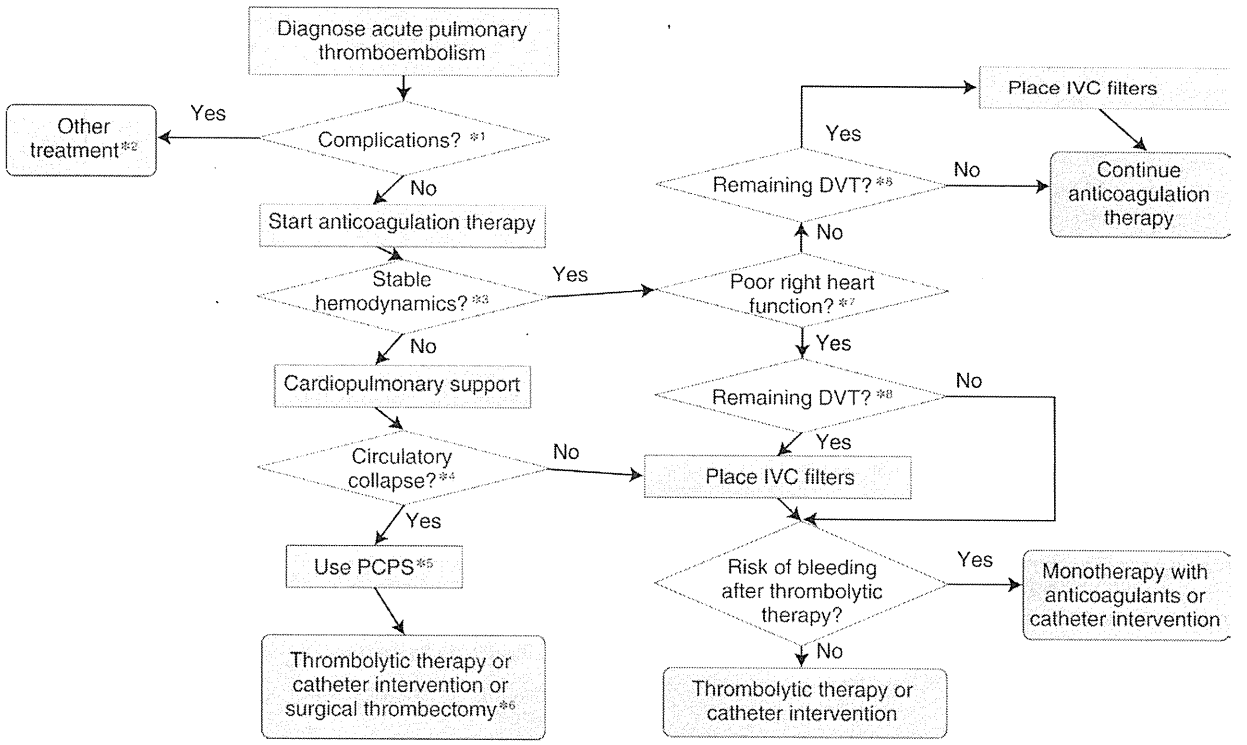

Figure 2. An example of a treatment algorithm for acute pulmonary thromboembolism. *1When risk of bleeding is high. *2Treat complications appropriately with available methods. *3Unstable hemodynamics consistent with shock or prolonged hypotension. *4Condition requiring cardiopulmonary resuscitation or prolonged severe shock. *5Consider PCPS according to hospital equipments and patient condition. *6Select appropriate treatment according to hospital equipments and patient condition. *7Evaluate based on right ventricular enlargement on echocardiography and severity of pulmonary hypertension. *8Presence/absence of DVT which may have serious effects if it releases emboli causing recurrent embolism. The above algorithm is an example. Each institution should select appropriate methods according to its healthcare resources. DVT, deep vein thrombosis; PCPS, percutaneous cardiopulmonary support; IVC, inferior vena cava.

(1) Symptoms

The absence of symptoms specific to acute PTE is a major reason for delay or lack of diagnosis of this disease. Its common and major symptoms are dyspnea and chest pain.[3,61–64] Typically, such symptoms develop when patients begin walking after bed rest, when they urinate or defecate, or when they change posture.

(2) Clinical Findings

Tachypnea and tachycardia are frequently present.[62,65] Shock and hypotension may develop as well. DVT may cause swelling of the lower legs and Homans' sign, etc.

(3) Examinations

Figure 1 illustrates the recommended steps in diagnosis. It should be noted that the flow chart reflects currently available techniques.[66]

[Levels of Recommendations]

1. MSCT, pulmonary angiography, pulmonary scintigraphy, arterial blood gas analysis, D-dimer: Class I
2. Transthoracic echocardiography, magnetic resonance angiography (MRA): Class IIa
3. Transesophageal echocardiography: Class IIb

2 Treatment

(1) Introduction

In the treatment of acute PTE, it should be noted that (1) prompt diagnosis and treatment are essential, since the prognosis after successful treatment during the acute phase is excellent, and that (2) after achievement of stable hemodynamics patients should be carefully followed for recurrence of PTE and should be treated promptly when DVT develop. The main component of treatment of PTE is pharmacological antithrombotic therapy, and anticoagulants and thrombolytics should be used appropriately based on the severity of the patient's condition. Patients with a high risk of bleeding should additionally be treated with non-permanent IVC filters and catheter intervention to support drug treatment, and percutaneous cardiopulmonary support (PCPS) and surgical thrombectomy should be performed for patients with severe condition. Physicians should also assess whether any DVT remains as soon as possible to consider whether IVC filters are indicated. **Figure 2** shows an example of an algorithm of treatment during the acute phase of PTE. It should be noted that this algorithm involves basic concepts and should be modified appropriately according to the condition of individual patients and hospital policies.

(2) Cardiopulmonary Management

The main pathological feature of acute PTE is acute cardio-

Table 4. Dose Adjustment Table for Unfractionated Heparin for Continuous Infusion*1

APTT (sec)	Bolus (units)	Hold (min)	Rate change (mL/hr)*2	Dose change (units/24 hr)	Repeat APTT
<50	5,000	0	+3	+2,880	6 hrs later
50 to 59	0	0	+3	+2,880	6 hrs later
60 to 85	0	0	0	0	Next morning
86 to 95	0	0	−2	−1,920	Next morning
96 to 120	0	30	−2	−1,920	6 hrs later
>120	0	60	−4	−3,840	6 hrs later

Unfractionated heparin should be administered intravenously as an initial bolus dose at 5,000 units, followed by continuous infusion at 1,400 units/hr. Six hours after the initial administration of unfractionated heparin, APTT should be determined for adjustment of the dose according to the above table.
*1Use this table for APTT reagents with a therapeutic range of 1.9 to 2.7 times the control.
*2When unfractionated heparin is administered at a concentration 40 units/mL.
APTT, activated partial thromboplastin time; Bolus, bolus dose for repeated administration; Hold, duration of suspension of continuous infusion; Rate change, change in infusion rate during continuous infusion; Dose change, change in dose during continuous infusion.
Adapted from Cruickshank MK, et al. A standard heparin nomogram for the management of heparin therapy. *Arch Intern Med* 1991; **151:** 333–337, with permission from American Medical Association. Added Dose change to this table.

Table 5. Contraindications to Thrombolytic Therapy

Absolute contraindications
- Active internal bleeding
- Recent spontaneous intracranial bleeding

Relative contraindications
- Major surgery, delivery, organ biopsy or puncture of non-compressible vessels within 10 days
- Ischemic stroke within 2 months
- Gastrointestinal bleeding within 10 days
- Severe trauma within 15 days
- Neurosurgery or ophthalmologic surgery within 1 month
- Uncontrolled severe hypertension (SBP >180 mmHg, DBP >110 mmHg)
- Recent cardiopulmonary resuscitation
- Platelet count <100,000/mm^3, prothrombin time <50%
- Pregnancy
- Bacterial endocarditis
- Diabetic hemorrhage retinopathy

SBP, systolic blood pressure; DBP, diastolic blood pressure.
Adapted from Guidelines on diagnosis and management of acute pulmonary embolism. *Eur Heart J* 2000; **21:** 1301–1336, with permission from Oxford University Press.

Table 6. Duration of Anticoagulation Therapy for Patients With Venous Thromboembolism

Types of risk factors	Duration of anticoagulation therapy
• Patients with reversible risk factors	3 months
• Idiopathic venous thromboembolism • Congenital coagulation disorder	At least 3 months (Determine the duration considering risks and benefits)
• Cancer patients • Patients with recurrent venous thromboembolism	Long term

pulmonary failure. Since mortality is particularly high immediately after the onset of PTE,[67] appropriate cardiopulmonary management is quite important.

1) Respiratory Management

Patients with acute PTE typically exhibit hypoxemia and hypocapnia (type I respiratory failure).[68] Oxygen therapy should be initiated for patients with an partial pressure of arterial oxygen (PaO_2) of ≤60 Torr (mmHg) (or a peripheral oxygen saturation [SpO_2] of ≤90%).

Nasal cannulas, an oxygen mask, or an oxygen mask with reservoir bag should be used as appropriate.

When oxygen therapy does not achieve a PaO_2 of ≥60 Torr (SpO_2 ≥90%), intubation and mechanical ventilation should be initiated.[69] During mechanical intubation, the tidal volume should be set at a low level, 7 mL/kg, to avoid increase in intrathoracic pressure.[10]

2) Circulatory Management

Although severity varies depending on the degree of occlusion in the pulmonary vascular bed, patients often exhibit PH, right heart overload, decreased right cardiac output, decreased left cardiac output, and/or shock. Theoretically, treatment should include drugs that have cardiotonic effects and widen the pulmonary artery.

There is no evidence to recommend volume loading. It has been pointed out that excessive volume loading in the right ventricle may compress the left ventricle and decrease left cardiac output.[70] Drug treatment (the drugs of first choice are dopamine and dobutamine;[71] norepinephrine[72] is effective in patients with hypotension; phosphodiesterase III inhibitors require further evaluation to accumulate clinical data), nitric oxide (NO) inhalation, and other appropriate treatment should be performed.

Patients with cardiopulmonary arrest and those not responding well to drug treatment (patients with progressive hypotension) should be treated promptly with PCPS and be considered for surgical thrombectomy.[73,74]

[Levels of Recommendations]
1. Respiratory management (oxygen therapy and low tidal volume ventilation): Class I
2. Circulatory management
   Volume loading: Class III
   Dopamine: Class IIa
   Dobutamine: Class IIa
   Norepinephrine: Class IIa
   Use of PCPS in severe patients: Class I