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Ｈ．知的財産権の出願・登録状況

１．特許取得

なし

２．実用新案登録

なし

３．その他

なし

（別添５）

III.　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
な　し							

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Miyata T	Guest editorial: current understanding of thrombosis and hemostasis - from bench to bedside.	Int J Hematol	95(4)	331-332	2012
Kita T, Banno F, Yanamoto H, Nakajo Y, Iihara K, Miyata T	Large infarct and high mortality by cerebral ischemia in mice carrying the factor V Leiden mutation.	J Thromb Haemost	10(7)	1453-1455	2012
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宮田敏行	遺伝子多型と抗凝固・抗血小板薬	Modern Physician	32(6)	749-752	2012
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宮田敏行、松本雅則	ADAMTS13 と von Willebrand 因子	カレントテラピー	31(3)	94	2013

IV.　研究成果の刊行物・別刷

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Guest editorial: current understanding of thrombosis and hemostasis—from bench to bedside

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Introduction

In this "Progress in Hematology", I would like to offer four excellent invited reviews in the field of thrombosis and hemostasis. On July 23–27, 2011, the XXIII Congress of the International Society on Thrombosis and Haemostasis was held in Kyoto, even after the unprecedented earthquake and tsunami on March 11, followed by the nuclear power plant accident. The Local Organizing Committee hosted more than 160 invited guest speakers and more than 3,000 oral and poster presentations from 75 countries. On this occasion, I had the privilege to organize the "Progress in Hematology" in the fields of thrombosis and hemostasis.

Thrombosis sometimes causes life-threatening events. It is a major healthcare problem and a social burden. Platelet activation and thrombin generation are two key events for thrombosis, which result in the occlusion of the blood vessels and the subsequent ischemic damage to the tissues. Enormous efforts to uncover the detailed molecular mechanisms of thrombus formation have occurred over the last two decades, but the goal is still far. We need to achieve the accumulation of more comprehensive basic, as well as clinical, knowledge of thrombosis. In this regard, basic research in blood coagulation has remained very important for clinical thrombosis. For many years, warfarin and aspirin have been utilized as oral antithrombotic drugs for patients with thrombotic complications and proven to be very effective for the prevention of thrombosis. Heparin is also widely used in clinical settings. In the basic research area, most of the proteins involved in the blood coagulation have been resolved mainly by crystallography. All of the genes involved in the blood coagulation and its regulation have already been knocked-out in mice, and phenotypes of these mice have been examined intensively in vivo. These progresses link the clinical and the basic approaches to thrombosis. However, there are still mysteries between them, because we do not have enough tools to perfectly regulate thrombosis in vivo. In this "Progress in Hematology", we have four excellent articles on (1) anticoagulant protein C, (2) polyphosphate, a new substance of coagulation initiator and amplifier, (3) von Willebrand factor, and (4) transglutaminase factor XIII.

The first review, by Griffin et al. [1], describes the anticoagulant and cytoprotective functions of activated protein C (APC) and how APC is beneficial for the disease states. APC works as an anticoagulant protein through the proteolytic degradation of activated factor V and VIII, and thereby downregulates the coagulation reactions resulting in the suppression of thrombin generation. APC has another beneficial function, i.e., a cytoprotective function. APC binds to endothelial protein C receptor EPCR through its Gla-domain and activates protease-activated receptor-1 (PAR-1) through proteolytic cleavage, resulting in generation of the cytoprotective signal. Currently, the APC-EPCR-PAR-1 system is a well-recognized agent for anti-apoptotic activity, anti-inflammatory activity, endothelial or epithelial barrier protection, and alteration of gene expression profiles. Finally, this review introduces the remarkable neuroprotective effects of APC in vivo using a murine ischemic stroke model.

The review by Morrissey [2] describes novel functions of inorganic polyphosphate in blood coagulation. Polyphosphate, in chains of tens to hundreds of phosphate residues, is abundantly stored in microbes, as well as platelets, and is released by a variety of stimuli. In

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platelets, polyphosphate is stored in dense granules. This polymer provides a polyanionic scaffold to assemble macromolecules including coagulation factors. Biochemical experiments indicated that polyphosphate can initiate the contact phase of coagulation, potentiate the blood coagulation cascade, and modulate the fibrinolytic system. In particular, polyphosphate could most likely be the long-sought physiologic activator of factor XII probably released from platelets. In this regard, polyphosphate, by intravenous injection or release from platelets, can induce lethal pulmonary embolism in wildtype mice, but factor XII-deficient mice survived. These in vivo studies demonstrate the physiologic roles of polyphosphate, and polyphosphate is now established as a new modulator of blood coagulation.

The review by Denis and Lenting [3] describes a multimeric plasma protein, von Willebrand factor (VWF). VWF is involved in both hemorrhage and thrombosis. Congenital deficiency in VWF causes a bleeding disorder known as von Willebrand disease. The biological activity of VWF is regulated by the shear force and the multimer size, with the longest being the most active in binding platelets. High molecular weight VWF multimers in blood can be lost by the passage through aortic valve stenosis, and this would result in bleeding. In contrast, epidemiological studies suggested that the increased plasma levels of VWF are a risk for cardiovascular diseases. Unusually large VWF multimers, due to the absence of VWF cleaving protease ADAMTS13, can cause a life-threatening disease, thrombotic thrombocytopenic purpura. In a mouse model, VWF deficiency can protect the focal cerebral ischemia. This review summarizes the various thrombotic disorders related to VWF.

Finally, the review by Ichinose [4] describes recent progress of coagulation factor XIII, a transglutaminase that can crosslink fibrin monomers to make a firm and stable hemostatic plug. Factor XIII consists of two catalytic A subunits and two non-catalytic B subunits and mice lacking these genes have been developed and intensively studied. From the analyses of these mice, the mechanisms of clot retraction have been revealed, and the active involvement of factor XIII in pathogen entrapment through cross-linking of bacteria to fibrin fibers has also been recognized. Congenital deficiency of factor XIII is a rare bleeding disorder. Female patients show recurrent miscarriage. Most of the genetic mutations are found in the A subunit. Congenital deficiency of the B subunit was also described. Acquired deficiency of factor XIII due to anti-factor XIII autoantibody is a rare bleeding disorder. A nationwide study for acquired factor XIII deficiency has started in Japan and patients with factor XIII deficiency are now being identified. Accumulation of the clinical information on these patients will contribute to future improvements of the treatment.

Acknowledgments I would like to thank the authors who took time from their busy schedule to create the excellent reviews and worked on a tight schedule to make the deadline. I thank Dr. Walter Kisiel for the English editing.

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Large infarct and high mortality by cerebral ischemia in mice carrying the factor V Leiden mutation

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Factor V Leiden (FVL) mutation (R506Q mutation) is an established risk factor for venous thromboembolism due to a hypercoagulable state through the resistance to activated protein C [1]. The FVL mutation also exerts a modest effect on arterial ischemic diseases. It is associated with ischemic stroke in children [2] and in young adults [3] and appears to be weakly associated with ischemic stroke in the general adult population [4]. A mouse model carrying a targeted homologous mutation at R504 to Q in FV has been developed [5] and studied under various stimulations or pathophysiologic conditions [6–8]. However, the role of the FVL mutation in ischemic stroke has not been confirmed using the mouse model. In this study, we applied brain ischemia-reperfusion injury in FVL mice using the three-vessel occlusion technique [9]. This technique produces a constant infarcted lesion limited to within the neocortex with small variances and does not require intraluminal thread insertion, which might activate the coagulation system during ischemia.

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Male young adult mice (7–18 weeks old) of wild-type $Fv^{+/+}$, heterozygous $Fv^{Q/+}$ and homozygous $Fv^{Q/Q}$ (Jackson Labs, Bar Harbor, ME, USA) were used for the experiments. All animal procedures were approved by the Animal Care and Use Committees of the National Cerebral and Cardiovascular Center. Temporary focal ischemia was induced using the three-vessel occlusion technique as described previously [9]. Briefly, we electrocauterized the distal M1 portion of the left middle cerebral artery, peripheral to the perforating arteries of the basal ganglia, and made temporal occlusions on the bilateral common carotid arteries for 15 min using vascular clips. After 24 h, neurological deficits were assessed using a scoring scale (from 0 to 4) as described [10,11], and the brains were excised and stained with 2, 3, 5-triphenyl tetrazolium chloride. The infarct and total hemispheric volumes were measured as described [10]. The infarct volume was adjusted for edema by dividing the volume by the edema index (left hemisphere volume/right hemisphere volume) [10,11]. The regional cerebral blood flow (rCBF) at the penumbra-like area of the left hemisphere was monitored using a laser-Doppler blood flowmeter TBF-LN1 (Unique Medical, Tokyo, Japan) [9,10]. In separate experimental groups the survival of the mice was monitored for 7 days after ischemia and graphed using a Kaplan–Meyer plot. Data were analyzed by the one-way ANOVA test followed by the post hoc Bonferroni's multiple comparison test. Survival rates were analyzed by the Mantel-Cox log-rank test. Data were expressed as the means ± standard deviation. P-values < 0.05 were considered significant.

Infarct volumes 24 h after ischemia in $Fv^{Q/+}$ and $Fv^{Q/Q}$ mice were significantly larger than those in $Fv^{+/+}$ mice (15.9 ± 5.3 mm^3 in $Fv^{+/+}$, n = 11; 26.7 ± 4.8 mm^3 in $Fv^{Q/+}$, n = 12; 29.9 ± 4.1 mm^3 in $Fv^{Q/Q}$ mice, n = 8; $Fv^{+/+}$ vs. $Fv^{Q/+}$, $P < 0.001$; $Fv^{+/+}$ vs. $Fv^{Q/Q}$, $P < 0.001$) (Fig. 1A,B). The edema index (1.06 ± 0.03 in $Fv^{+/+}$, 1.05 ± 0.02 in $Fv^{Q/+}$, 1.04 ± 0.04 in $Fv^{Q/Q}$) and the neurological deficit score (2.82 ± 0.40 in $Fv^{+/+}$, 2.58 ± 0.67 in $Fv^{Q/+}$, 2.75 ± 0.46 in $Fv^{Q/Q}$) were not different among the groups. The rCBF during ischemia in $Fv^{Q/Q}$ mice (9.9 ± 1.2%, n = 7) was more severely decreased than in either $Fv^{+/+}$ (21.2 ± 0.5%, n = 7) or $Fv^{Q/+}$ mice (19.5 ± 2.0%, n = 7) ($P < 0.001$) (Fig. 1C). In every group, the rCBF was recovered to the preischemic normal level immediately after removal of the vascular clips, ensuring sufficient reperfusion of the left middle cerebral artery territory. $Fv^{Q/Q}$ mice started to die from day 2, and only two out of seven mice survived for 7 days (Fig. 1D). $Fv^{Q/+}$ mice started to die from day 4, and only two mice survived. Two $Fv^{+/+}$ mice died on day 7, and the remaining five survived. The 7-day survival in $Fv^{Q/+}$ and $Fv^{Q/Q}$ mice was significantly lower than that in $Fv^{+/+}$ mice ($P < 0.05$).

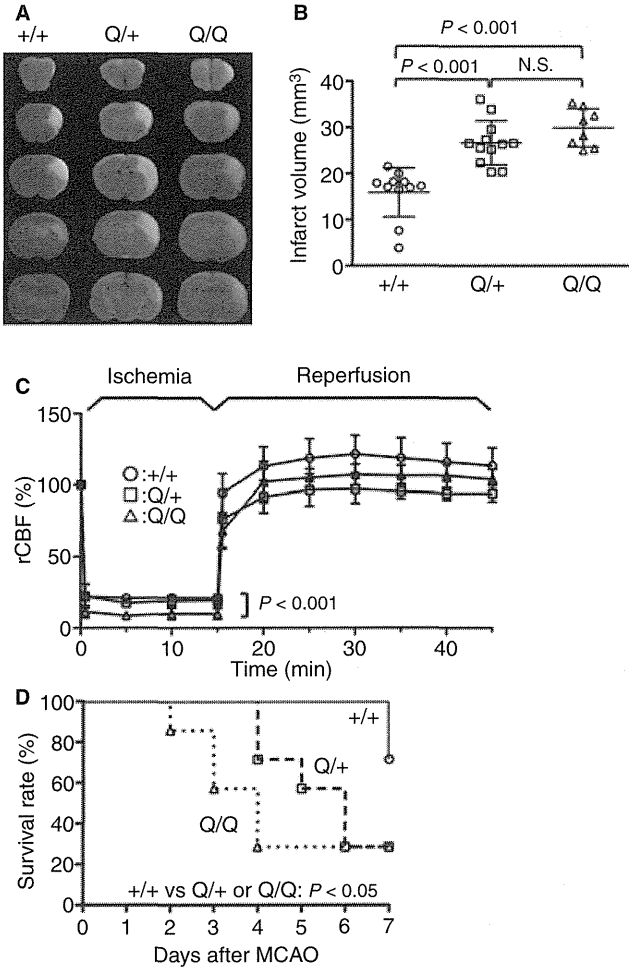

Fig. 1. Effects of factor V Leiden (FVL) on focal ischemia. (A) Representative images of coronal sections of $Fv^{+/+}$, $Fv^{Q/+}$ and $Fv^{Q/Q}$ mouse brains. *Red* areas represent vital brain tissue and *white* areas represent cerebral infarction. (B) Infarct volumes of $Fv^{+/+}$ (○), $Fv^{Q/+}$ (□) and $Fv^{Q/Q}$ (△) mice. *Bars* represent the means ± standard deviation. N.S., not significantly different ($P > 0.05$). (C) rCBF of $Fv^{+/+}$, $Fv^{Q/+}$ and $Fv^{Q/Q}$ mice during 15-min ischemia and 30-min reperfusion. The rCBFs were expressed as percentages of the baseline flow obtained before middle cerebral artery occlusion. Data are the means ± standard deviation of seven mice per group. (D) Seven-day survival of $Fv^{+/+}$, $Fv^{Q/+}$ and $Fv^{Q/Q}$ mice after temporary ischemia (n = 7/group).

We demonstrated that both $Fv^{Q/+}$ and $Fv^{Q/Q}$ mice showed increased infarct volumes and decreased long-term survival compared with $Fv^{+/+}$ mice after the temporary focal ischemia-reperfusion stress. Although the three-vessel occlusion technique does not activate the coagulation system during ischemia, the infarct lesion was larger in $Fv^{Q/+}$ or $Fv^{Q/Q}$ mice than in $Fv^{+/+}$ mice. The reperfusion process after transient cerebral ischemia is known to induce many cellular events, including the loss of normal permeability in the blood brain barrier, which deteriorates cerebral metabolism, increases cerebral damage [12], and induces ischemic cerebral damage-related secondary activation of the coagulation system with subsequent thrombus formation around the infarct lesion [13]. The hypercoagulable state of FVL, which can enhance reperfusion injury after ischemia, is considered to be the primary cause of both the enlargement of infarct lesions and the vulnerability of the brain after ischemic stroke. The other possible pathophysiology is that FVL may have affected some of the intrinsic vascular and/or neuronal protection systems.

The findings that both $Fv^{Q/+}$ and $Fv^{Q/Q}$ mice showed larger infarct volumes and lower survival rates than $Fv^{+/+}$ mice after temporary focal ischemia-reperfusion stress support a direct causal relationship between the FVL mutation and increased susceptibility to ischemic stroke in young adult individuals.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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Different bleeding risk in type 2A and 2M von Willebrand disease: a 2-year prospective study in 107 patients: a rebuttal

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See also Castaman G, Baronciani L, Canciani MT, Federici AB. Different bleeding risk in type 2A and 2M von Willebrand disease: a 2-year prospective study in 107 patients: a reply to a rebuttal. This issue, pp 1458–60.

We were very interested to read the recent report by Castaman *et al.* [1] in this journal. This study described an increased bleeding risk in patients with type 2A von Willebrand disease (VWD) as compared with those with type 2M VWD. This is an important finding, and should provide support for the ongoing diagnostic differentiation of type 2A and type 2M VWD. The exact frequencies of these two forms of VWD are unknown, although a recent geographically global analysis indicated much variability in their perceived incidence [2], with type 2A and type 2M VWD, respectively, reported as comprising 5.5–82.6% and 0–56.3% (Fig. 1A) of all cases of type 2 VWD. Interestingly, there was a strong negative correlation between their relative 'incidence' (see Fig. 1B), such that laboratories identifying more cases of type 2A VWD reported fewer cases of type 2M VWD, and vice versa. However, most centers reported far more cases of type 2A VWD than of type 2M VWD, and only a few centers identified type 2M VWD as comprising a high proportion of type 2 VWD cases. These data do not reflect a variation in the frequencies of type 2A and type 2M VWD according to geography, but rather a differential ability of laboratories to appropriately identify type 2M VWD, as well as diagnostic biases related to perceptions of VWD classification. Indeed, as reported by Castaman *et al.* [1], 'VWD2M is often difficult to diagnose accurately because of its phenotype which can be difficult to differentiate from VWD1 or VWD2A unless a full range of phenotypic evaluations is carried out'. According to our regional external quality assurance (EQA) experience, type 2M VWD is typically misidentified as type 1 or type 2A VWD, because of laboratories either using only limited von Willebrand factor (VWF) test panels or misinterpreting their own test data [3].

According to the latest ISTH VWF Scientific Standardization Committee (SSC) classification, as used by Castaman *et al.* [1], type 2A reflects 'decreased VWF-dependent platelet

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it is clear that allogeneic T cells in the transplant and in donor lymphocyte infusions can bring about a graft-versus-leukemia effect. Evidence for the importance of an autologous T-cell response against AML has come from the tumor vaccination field. Vaccination against the leukemia-associated antigens[2] Wilms tumor protein 1 (WT1),[3-6] PR1 (derived from proteinase 3),[4] and receptor for hyaluronic acid–mediated motility (RHAMM)[7] can bring about clinical antileukemic effects in AML. The clinical response was generally correlated with the T-cell responses elicited.[3,4,6] Loss of clinical response has been reported to be associated with decrease or loss of specific T-cell immunity.

But can an antileukemic immune response be elicited in patients not receiving immunotherapy? The answer comes from a vast body of work, demonstrating that, contrary to general belief, certain chemotherapeutic agents can augment immune responses against tumors.[8] Chemotherapy thus not only has direct cytotoxic effects on cancerous cells, but can also boost the immunity against them by different mechanisms, including stimulating tumor antigen presentation by dendritic cells to cytotoxic T lymphocytes. This is particularly true of anthracyclines, still the mainstay of treatment of AML, which have been demonstrated to be a prototype of immunogenic chemotherapy.[9] It was already known for a while that the antitumoral effect of doxorubicin in certain animal models was strongly reduced if the immune system was not functioning properly.

In the case of *NPM1*^{mut} AML, especially if it is also *FLT3-ITD*^{neg}, the autologous T-cell response induced by the mutated NPM1 could bring about a significant antileukemic effect directly after chemotherapy (figure panels A and C). At this stage, the number of leukemic cells would significantly be reduced, the immune response could be strengthened, and the stimulated anti-NPM1^{mut} cytotoxic T lymphocytes could mount a final attack against the remaining leukemic cells. This could account for the cures seen with chemotherapy alone in *NPM1*^{mut} AML. But not all patients with *NPM1*^{mut}*FLT3-ITD*^{neg} AML are cured by chemotherapy alone. The findings by Greiner et al theoretically suggest the possibility that postremission immunotherapy directed against NPM1^{mut} could induce cures and/or longer-lasting remissions in this type of AML and maybe even in *NPM1*^{mut}*FLT3-ITD*^{pos} AML, especially if there is molecular evidence of residual disease.

An additional potential advantage of the T-cell immune response directed against certain leukemia antigens is that it may also be directed against the leukemic stem cells.[2] Leukemic stem cells are relatively resistant to chemotherapy,[10] accounting at least in part for the (minimal) residual disease persisting after cytotoxic treatment in a majority of AML cases (figure panel B). The chemotherapy resistance of minimal residual disease has led to the development of another type of postremission treatment, that is, immunotherapy, to try to definitively cure AML patients (figure panel D). NPM1^{mut}, a leukemia-specific antigen,[2] is expressed in leukemic stem cells,[11] making those cells vulnerable to immune eradication, as discussed above.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Fuchs et al, page 1157

A second hit for TMA

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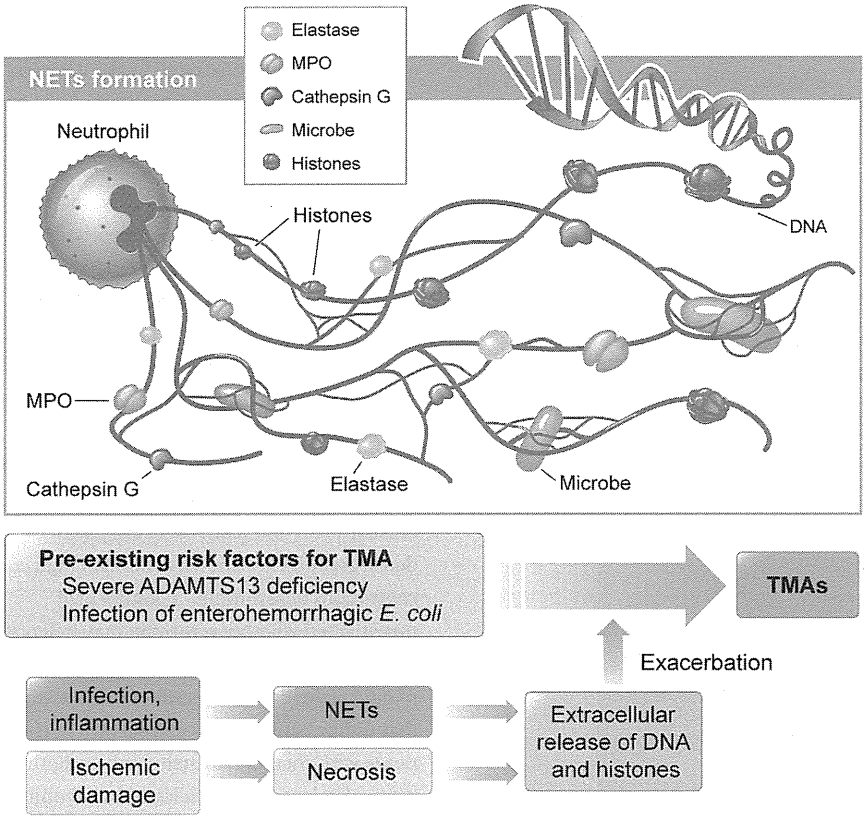
In this issue of *Blood*, Fuchs and colleagues provide evidence that circulating DNA and histones, presumably released from neutrophils, would be the second hit for development of thrombotic microangiopathies (TMAs), a group of life-threatening disorders characterized by thrombi in the microvasculature resulting in thrombocytopenia, microangiopathic hemolysis, and organ dysfunction.[1]

TMA includes thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). TTP is caused by a severe deficiency of von Willebrand factor cleaving protease, ADAMTS13, because of autoantibodies or genetic mutations. HUS is caused by infection with Shiga-toxin–producing *Escherichia coli* and is typically associated with bloody diarrhea. Atypical HUS, which has a link with defective complement regulation, is also present. Other conditions such as cancer, bone marrow transplantations, and lupus can present with features of TMA. Although patients with congenital TTP show severe ADAMTS13 deficiency, some patients may remain asymptomatic for many years.[2] An infection often precedes acute TMA.[3]

The innate immune response plays a crucial role for defense against invading microbes. Neutrophils, the most abundant leukocytes, are early responding cells that migrate in large numbers to sites of infection and release



Neutrophils release nucleosomes, a complex of DNA and histones, in response to infection or inflammatory stimuli. Neutrophil extracellular traps (NETs) are composed of nucleosomes decorated with granular components including myeloperoxidase (MPO), neutrophil elastase, and cathepsin G.[4] NETs bind and kill microbes.[4] NETs also immobilize platelets[10] and erythrocytes. Histones are known to stimulate thrombosis and to cause cytotoxicity in mice.[5-7] Once patients with pre-existing risk factors for thrombotic microangiopathy (TMA) are infected, DNA and histones, in conjunction with granular proteins, are released and acute TMAs would be induced. Another possible origin of extracellular DNA and histones is necrotic tissue released after ischemic damage. Professional illustration by Kenneth X. Probst.

nuclear chromatin associated with nuclear histones and granular antimicrobial proteins after cell death to form neutrophil extracellular traps (NETs; see figure).[4] Microbes bind to NETs and are subsequently killed by antimicrobial proteins. Extracellular DNA and histones have recently been shown to have prothrombotic characteristics.[5,6] Histones cause thrombocytopenia, promote thrombosis, and contribute to organ damage and death.[7,8] In most cases, in vivo studies demonstrating the prothrombotic characteristics of histones have been performed in the mouse or baboon model.

At Bern University Hospital and the University of Bern, plasma samples from TMA patients of different clinical categories have been collected and stored more than 10 years. Retrospectively, Fuchs and colleagues used these samples to investigate the possible risk factors to develop TMAs.[1] First, they found elevated plasma levels of DNA, nucleosomes, lactate dehydrogenase (LDH), myeloperoxidase (MPO), and S100A8/A9 in acute TMA patients. LDH, a cytoplasmic enzyme, is a marker of tissue damage. MPO is abundantly stored in granules of neutrophils. S100A8/A9, present in the cytosol of neutrophils and monocytes, is a marker of inflammation. In clinical remission, plasma levels of DNA, LDH, MPO, and S100A8/A9 were decreased. Importantly, the great reduction of plasma levels of DNA and MPO was concomitant with the increase in platelet counts and plasma ADAMTS13 levels in acute TTP patients with remission, indicating correlation of DNA and MPO levels in disease state. Severe ADAMTS13 deficiency per se does not lead to an increase in plasma DNA or MPO levels, while DNA and MPO are elevated only during disease flare-up. These findings indicated that extracellular DNA and histone levels during acute TMA could increase the risk for developing TMAs and provide a second hit that triggers acute disease in patients at risk for TMA. Disease pathogenesis sometimes involves additional unknown genetic factors and/or environmental triggers. For example, mice lacking the ADAMTS13 gene are predisposed to acute TMA, but Shiga-toxin is needed to induce the acute disease.[9]

What is the triggering event for extracellular DNA and histones in TMA patients? Recent studies showed infection as the most commonly identified etiologic factor for TMA,[3] and LPS can induce NET formation through platelet TLR4.[10] Therefore, it is conceivable that release of DNA and histones from neutrophils is caused by a preceding infection. What is the origin of extracellular DNA and histones? Beside NETs, necrotic tissue after ischemic damage could be a source of these 2 compounds (see figure). What are the natures of DNA and histones? Circulating DNA is likely fragmented by endogenous DNases[4] and histones are cleaved by activated protein C[7] and/or other proteases. Therefore, tools that degrade and inactivate prothrombotic DNA and histones would seemingly be promising candidates for preventing TMAs and other thrombotic complications. Finally, this is a retrospective study; thus, whether nucleosomes, DNA, or histones contribute directly to the clinical manifestation of acute TMAs remains to be determined. Nevertheless, evidence that extracellular DNA and histones are elevated in patients with acute TMA and decreased in remission concomitant with the increase in platelet counts advances the understanding of the pathogenesis of acute TMAs.

Conflict-of-interest disclosure: T.M. is an inventor of the ADAMTS13 assay method, which is related to its patent. X.P.F. declares no competing financial interests. ■

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● ● ● VASCULAR BIOLOGY

Comment on Cloutier et al, page 1334

The functional dissonance of platelets

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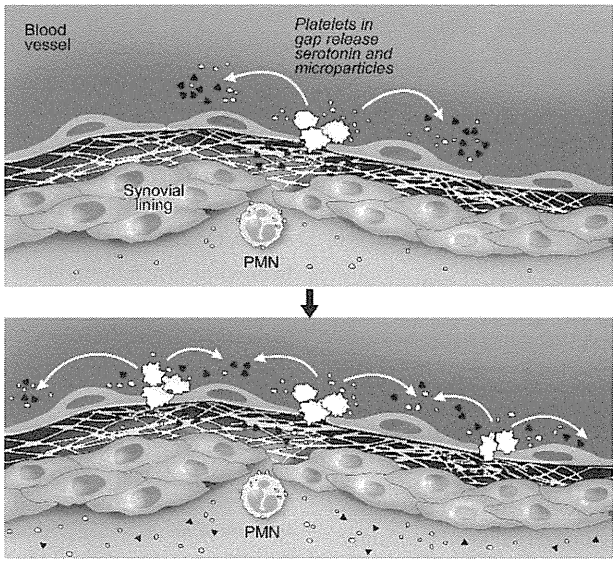
In this issue of *Blood*, Cloutier et al answer a long-standing but unappreciated question about the influence of platelets on vascular permeability.[1]



Proposed pathway for the formation of gaps and amplification of the vasculature permeability by platelets during arthritis. (Top panel) Gaps between endothelial cells in arthritic joints are formed. The GPVI-expressing platelets are activated by the leakage during disease. Note that the precise anatomic location of platelet activation, and the route by which microparticles enter the joint remains speculative. Δ indicates serotonin and ○, platelet microparticle. Professional illustration by Steve Moskowitz, Advanced Medical Graphics. See Figure 7 in the article by Cloutier et al that begins on page 1334.

The role of platelets in responding to vascular injury and the prevention blood loss is very well understood. Cloutier and colleagues have now used fluorescent microspheres, 2-photon microscopy, and genetically modified mice to convincingly demonstrate that in addition to their role in hemostasis, platelets can also promote vascular leakage. More importantly, using a model of rheumatoid arthritis, they present data that uncoupled vascular permeability from inflammation, and their data suggest that platelets can increase vascular permeability directly, through the release of serotonin (see figure).

The concept of platelet-induced vascular permeability is not new; more than 40 years ago Nachman et al reported that platelet granule extracts were capable of inducing vascular permeability.[2] However, despite efforts to identify the molecular components responsible for this activity,[3] the molecular mechanism whereby platelets promote vascular permeability have remained elusive.

Nevertheless, over the years circumstantial evidence has supported the notion that platelets can induce vascular leakage.[4,5] Here, Cloutier et al present convincing evidence that endothelial gap formation in arthritic vessels depends on the presence of platelets, and that this activity is independent of the inflammation normally seen in rheumatoid arthritis. Specifically, animals treated with platelet-depleting antibodies showed significantly less vascular leakage than controls after the onset of arthritis, as demonstrated by the direct injection of fluorescent microspheres. In addition, inducing inflammation with injections of IL-1β did not change the outcome of platelet-depleting antibody treatment on vascular permeability, suggesting that platelets and not inflammation were the cause of increased vascular permeability in rheumatoid arthritis.

Interestingly, the size of microspheres that gained access to arthritic joints in this study appeared to be limited to 0.45 μm to 0.84 μm, a range that is very similar to serotonin-induced endothelial gaps seen in vessels in the cremaster muscle identified by electron microscopy (0.1-0.8 μm).[6] Because platelet-dense granules are known to contain high concentrations of serotonin,[7] Cloutier et al investigated whether serotonin in platelets was associated with vascular permeability in rheumatoid arthritis. These studies demonstrate that unlike patients with osteoarthritis, patients with rheumatoid arthritis have both more platelet microparticles and serotonin in their synovial fluid. They also found that direct injection of serotonin into healthy mice induced vascular leakage reminiscent of arthritic animals. These data suggest that platelet-derived serotonin was important in endothelial gap formation. To test this hypothesis, Cloutier et al took advantage of mice deficient in the serotonin transporter (SERT), which enables platelets to take up and store serotonin.[8] Using the SERT-deficient mice, Cloutier et al convincingly demonstrated that mice with low levels of serotonin in their platelets had significantly reduced fluorescent microspheres accumulation in their joints during arthritis development. Finally, Fluoxetine, a psychiatric drug that inhibits the uptake of serotonin, significantly reduced vascular leak in their rheumatoid arthritis mouse model. This observation is consistent with a previous report by Sacre et al on the efficacious effect of Fluoxetine in rheumatoid arthritis but may offer a different mechanism.[9]

Overall, this novel finding that platelet-induced vascular permeability is mediated via serotonin in rheumatoid arthritis signifies a change in the view about how platelets can affect vascular integrity. More importantly, understanding this new pathway may generate new treatment options for diseases such as rheumatoid arthritis.

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A Survey of the Clinical Course and Management of Japanese Patients Deficient in Natural Anticoagulants

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Abstract

Few data are available on the clinical course of Japanese patients deficient in natural anticoagulants (antithrombin (AT), protein C, and protein S). We conducted a nationwide survey to reveal the clinical course of these patients. Questionnaires were sent to 321 council members of the Japanese Society on Thrombosis and Hemostasis, Japanese Society for Vascular Surgery, and Japanese Society of Phlebology. A total of 103 responses were obtained and data of 183 patients were collected. Of 183 patients, 142 (78%) experienced at least one episode of venous thromboembolism (VTE). The first VTE occurred before the age of 40 years in 71 patients (45%). Venous thromboembolism recurred in 15 (39%) patients with AT deficiency and 19 (18%) patients with other deficiencies. These findings suggest that half of the first episodes of VTE in patients deficient in natural anticoagulants occur before middle age and the risk of VTE recurrence is high in patients with AT deficiency.

Keywords

anticoagulants, thrombophilia, venous thromboembolism

Introduction

Patients with inherited thrombophilia, that is, those deficient in natural anticoagulants, such as antithrombin (AT), protein C (PC), or protein S (PS), and polymorphisms of coagulation factors, such as factor V Leiden (FVL) or prothrombin G20210A (PT G20210A), have an increased risk of venous thromboembolism (VTE). Inherited thrombophilia is detected in at least 30% to 40% patients with VTE and is a concern in patients with early-onset thrombotic events, a tendency toward recurrent thrombotic events, a family history of VTE, thrombosis at unusual sites, or idiopathic thrombosis.[1] Although deficiencies in AT, PC, and PS are well-known hereditary risk factors for VTE, they are very rare, presenting in much less than 1% of the general population,[2] and account for 5% to 10% of cases of VTE in Western countries.[3,4] On the other hand, in Western countries, FVL and PT G20210A are more frequently found in the general population compared with deficiencies of natural anticoagulants. The estimated prevalence of FVL and PT G20210A is 5% and 2%, respectively, in whites and FVL is present in 12% to 20% of patients with VTE in Western countries.[5,6] Several studies have reported the clinical course of subjects with inherited thrombophilia in Western countries,[7-11] revealing that deficiencies of natural anticoagulants cause a 5- to 10-fold increase in VTE and the annual incidence of VTE is greater than 1%. On the other hand, FVL and PT G20210A are associated with a lower increase (2- to 5-fold) in VTE and the annual incidence of VTE is less than 0.5%. These data are essential to establish adequate management of patients with inherited thrombophilia. The results of studies in Western countries, however, may not apply to the Japanese because the frequencies of carriers of inherited thrombophilia in the Japanese are quite different from those in whites. For example, the

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Table 1. Questionnaire Regarding the Status of Japanese Patients Deficient in Natural Anticoagulants (AT, PC, and PS; Originally Written in Japanese)

Return form in reply-paid envelope	
Please clearly check the correct response or write an alternative answer where appropriate	
Are you currently taking care of patients with inherited thrombophilia (eg, AT deficiency, PC deficiency, or PS deficiency)?	
No/Yes, Institute/Name/e-mail address (OPT)	
If you answered "Yes" to the above question, please answer the following questions.	
1. Diagnosis	AT deficiency/PC deficiency/PS deficiency
2. Age at diagnosis (years)	0-9/10-19/20-29/30-39/40-49/50-59/60+
3. Sex	Male/Female
4. Laboratory data (if applicable)	AT antigen/AT activity/PC antigen/PC activity/PS antigen/ PS activity
5. Family history	Yes/No
6. Prescription of VKA	No/Yes if "Yes", dose and PT-INR
7. Occurrence of VTE	None/Once/Twice or more
If you answered "Once" for question 7, please answer question 8, and if you answered "Twice or more" please answer questions 8 and 9.	
8. About the first episode of VTE	
1. Age at occurrence of VTE (years)	0-9/10-19/20-29/30-39/40-49/50-59/60+
2. Location of VTE	
3. Risk factor for VTE	idiopathic/provoked if "provoked", please specify
4. Prescription of VKA at the occurrence	No/Yes if "Yes", dose and PT-INR
5. Anticoagulants other than VKA at the occurrence	No/Yes if "Yes", name of anticoagulants
9. About the recurrence of VTE	
1. Age at recurrence of VTE (years)	0-9/10-19/20-29/30-39/40-49/50-59/60+
2. Location of VTE	
3. Risk factor for VTE	idiopathic/provoked if "provoked", please specify
4. Prescription of VKA at the recurrence	No/Yes if "Yes", dose and PT-INR
5. Anticoagulants other than VKA at the recurrence	No/Yes if "Yes", name of anticoagulants

Abbreviations: AT, antithrombin; PC, protein C; OPT, optional; PS, protein S; VKA, vitamin K antagonist; VTE, venous thromboembolism; PT-INR, prothrombin time-international normalized ratio.

frequency of PS deficiency is as high as 2.04% in the Japanese,[12] whereas the frequency of PS deficiency is less common in whites.[13] Although carriers of FVL or PT G20210A are not rare in whites, they neither has been detected in the Japanese.[14-18] Therefore, it is necessary to clarify the clinical course of Japanese patients with inherited thrombophilia, but few data are available. To investigate this issue, we conducted a nationwide survey of Japanese patients deficient in natural anticoagulants.

Materials and Methods

Questionnaires were sent to 321 council members of the Japanese Society on Thrombosis and Hemostasis, Japanese Society for Vascular Surgery, and Japanese Society of Phlebology in April 2009. Physicians who belong to these societies should be specialists of VTE with detailed knowledge of inherited thrombophilia and were assumed to be involved in the management of patients deficient in natural anticoagulants in their institutes. The society members included hematologists, cardiologists, physicians of respiratory medicine, and vascular surgeons. The questionnaire comprised mainly multiple choice questions regarding diagnosis of inherited thrombophilia, family history, oral vitamin K antagonists (VKA) prescription, occurrence of the first episode of VTE, and VTE recurrence. Reply-paid envelopes were used for return of data (Table 1). This study was approved by the Institutional Ethical Review Board of Keio University School of Medicine.

Results

Response

Of 321 physicians, 103 (32%) replied to the questionnaires and 33 of them were taking care of patients deficient in natural anticoagulants. Among the 33 physicians, 23 were doing clinical practice in University hospitals, 10 in nonuniversity teaching hospitals, 17 were vascular surgeons, 13 were hematologists, 2 were neurologists, and 1 was a pediatrician.

Patient Characteristics

Detailed information of the clinical course and management was obtained for 183 patients deficient in natural anticoagulants: 50 patients (male to female ratio: 17:33) with AT deficiency, 62 patients (29:33) with PC deficiency, 59 patients (24:35) with PS deficiency, and 12 patients (8:4) with combined deficiencies. Of 17 male patients and 33 female patients, 10 (59%) and 20 (61%) patients, respectively, were diagnosed with AT deficiency before the age of 40 years, whereas 2 (12%) male and 5 (15%) female patients were diagnosed at the age of 60 years or above. Of 29 male patients and 33 female patients, 12 (41%) and 14 (42%), respectively, were diagnosed with PC deficiency before the age of 40 years, whereas 5 (17%) and 7 (21%) patients were diagnosed at the age of 60 years or above. Of 24 male patients and 35 female patients, 11 (46%) and 17 (49%) patients, respectively, were diagnosed with PS

Table 2. General Characteristics of the Patients Based on the Responses to the Questionnaire

Type of Deficiency	AT, n = 50	PC, n = 62	PS, n = 59	Combined, n = 12
Male:Female	17:33	29:33	24:35	8:4
Age at diagnosis (years)			Male: female	
0-39	10:20	12:14	11:17	2:1
40-59	5:8	12:12	9:8	3:3
60+	2:5	5:7	4:10	3:0

deficiency before the age of 40 years, whereas 4 (17%) and 10 (29%) were diagnosed at the age of 60 years or above. Of 8 males and 4 females, 2 (25%) and 1 (25%), respectively, were diagnosed with combined deficiencies before the age of 40 years, whereas 3 (38%) male and 0 (0%) female patients were diagnosed at the age of 60 years or above (Table 2). There were 4 patients with combined AT + PC deficiencies, 3 patients with AT + PS deficiencies, and 5 patients with PC + PS deficiencies.

Activity and Antigen Level of Anticoagulants

The activities of AT or PC were measured in all patients with a diagnosis of AT or PC deficiency. Activity of AT was 48.0% ± 11.4% (n = 50; mean ± standard deviation [SD]) and activity of PC was 43.9% ± 15.0% (n = 62; mean ± SD). Levels of AT antigen were measured in 13 patients with AT deficiency; of them, 10 were diagnosed with AT deficiency type I and 3 were diagnosed with AT deficiency type II. Levels of PC antigen were measured in 32 patients with PC deficiency; of them, 24 were diagnosed with PC deficiency type I and 8 were diagnosed with PC deficiency type II. Activity of PS, measured in 36 of 59 patients with PS deficiency, was 30.5% ± 17.5% (n = 36; mean ± SD). Total PS antigen and/or free PS antigen levels, but not activity of PS, were measured in 23 patients. Both activity and antigen level were measured in 14 patients; 1 was diagnosed with PS deficiency type I and 13 were diagnosed with PS deficiency type II.

Sites of the First VTE Episode

Of 17 male and 33 female patients with AT deficiency, 12 and 26, respectively, had at least one episode of VTE. The first VTE episode was leg deep vein thrombosis (DVT) in 24 (male to female ratio: 6:18), pulmonary embolism (PE) in 4 (2:2), leg DVT + PE in 6 (4:2), and VTE at unusual sites in 4 (0:4; 1 portal vein thrombosis, 2 cerebral venous sinus thrombosis, and 1 leg DVT + cerebral venous sinus thrombosis). Of 29 males and 33 females with PC deficiency, 21 and 25, respectively, had at least one episode of VTE. The first VTE episode was leg DVT in 29 (male to female ratio: 16:13), PE in 6 (2:4), leg DVT + PE in 9 (3:6), and VTE at unusual sites (cerebral venous sinus thrombosis) in 2 (0:2). Of 24 males and 35 females with PS deficiency, 22 and 25, respectively, had at least one episode of VTE. The first VTE episode was leg DVT in 39 (male to female ratio: 18:21), PE in 1 (0:1), leg DVT + PE in 5 (4:1), and VTE at unusual sites in 2 (0:2; 1 leg DVT + mesenteric venous thrombosis and 1 cerebral venous sinus thrombosis). Of 8 males and 4 females with combined deficiencies, 7 and 4, respectively, had at least one episode of VTE. The first VTE episode was in the leg in 6 (male to female ratio: 4:2) and leg DVT + PE in 5 (3:2) (Table 3). In total, of the 183 patients deficient in natural anticoagulants, 142 (78%) experienced at least one episode of VTE. Further analysis was performed in these 142 patients.

Age at Occurrence and Predisposing Factors for First VTE Episode

Of the 12 first VTE episodes in male patients with AT deficiency, 8 (66%) occurred before the age of 40 years and 1 (8%) occurred at the age of 60 years or above. Of the 12 VTE episodes, 10 (83%) were idiopathic and the other 2 (17%) were with provoked VTE. Of the 21 first VTE episodes in male patients with PC deficiency, 9 (43%) occurred before the age of 40 years and 4 (19%) occurred at the age of 60 years or above. Of the 21 VTE episodes, 14 (67%) were idiopathic and 7 (33%) were with provoked VTE. Of the 22 first VTE episodes in male patients with PS deficiency, 10 (45%) occurred before the age of 40 years and 4 (18%) occurred at the age of 60 years or above. Of the 22 VTE episodes, 14 (64%) were idiopathic and 8 (36%) were with provoked VTE. Of the 7 first VTE episodes in male patients with combined deficiencies, 3 (43%) occurred before the age of 40 years and 2 (29%) occurred at the age of 60 years or above. Of the 7 VTE episodes, 6 (86%) were idiopathic and 1 (14%) was provoked VTE (Figure 1A).

Of the 26 first VTE episodes in female patients with AT deficiency, 15 (58%) occurred before the age of 40 years and 4 (15%) occurred at the age of 60 years or above. Of the 26 VTE episodes, 13 (50%) were idiopathic and 13 (50%) were with provoked VTE. Of the 9 provoked VTE that occurred before the age of 40 years, 8 (89%) were associated with pregnancy or parturition. Of the 25 first VTE episodes in female patients with PC deficiency, 12 (48%) occurred before the age of 40 years and 6 (24%) occurred at the age of 60 years or above. Of the 25 VTE episodes, 14 (56%) were idiopathic and 11 (44%) were provoked VTE. Three of the 6 patients with provoked VTE that occurred before the age of 40 years were associated with pregnancy or parturition. Of the 25 first VTE episodes in female patients with PS deficiency, 12 (48%) occurred before the age of 40 years and 9 (36%) occurred at the age of 60 years or above. Of the 25 VTE episodes, 14 (56%)

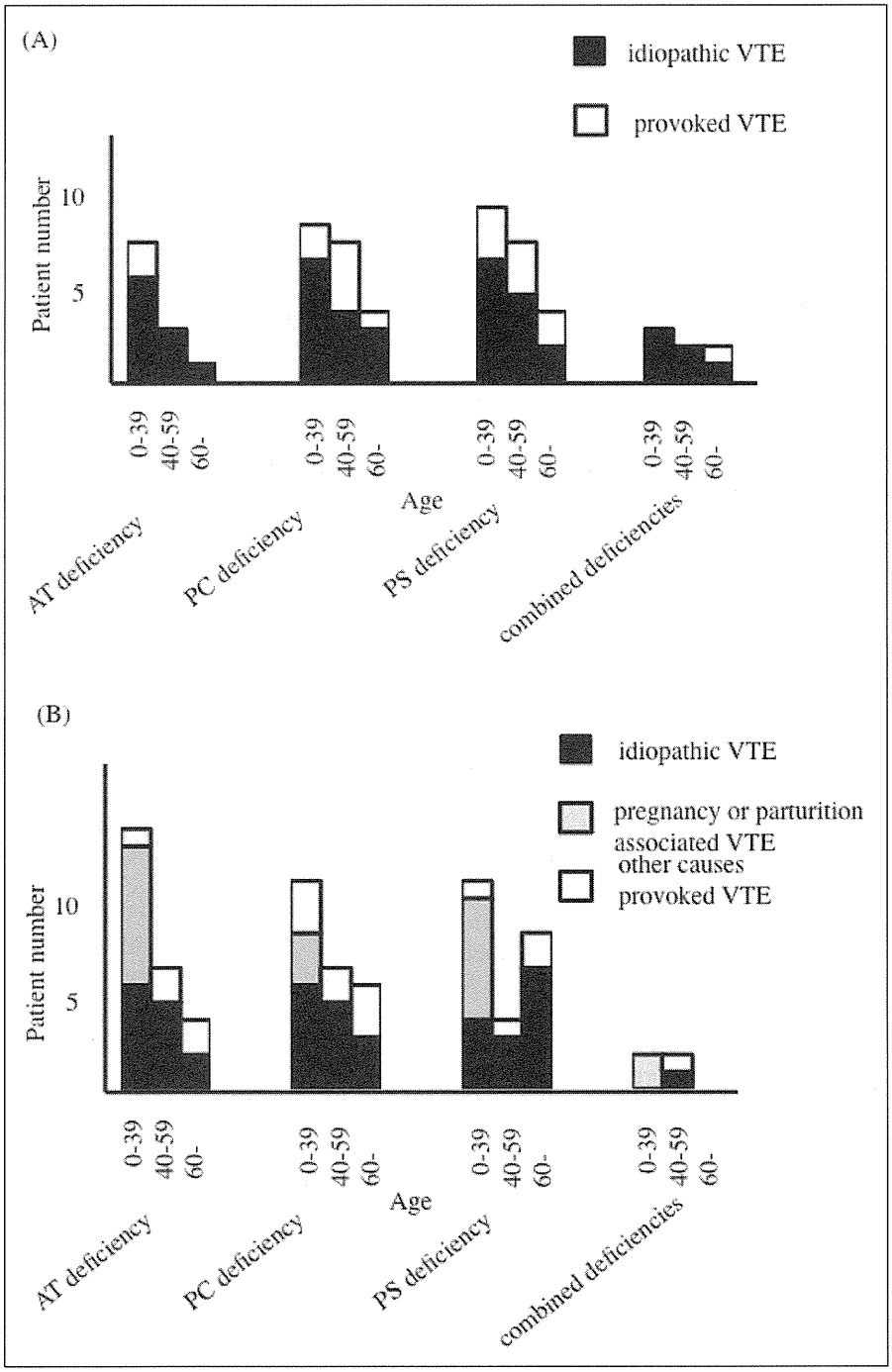

Figure 1. Age at occurrence and predisposing factors of the first episodes of venous thromboembolism (VTE) in male (A) or female (B) patients deficient in natural anticoagulants.

were idiopathic and 11 (44%) were provoked VTE. Of the 8 provoked VTE occurring before the age of 40 years, 7 (88%) were associated with pregnancy or parturition. Of the 4 first episodes of VTE in female patients with combined deficiencies, 2 (50%) occurred before the age of 40 years and none (0%) occurred at the age of 60 years or above. Of the 4 episodes of VTE, 1 (25%) was idiopathic and 3 (75%) were provoked VTE. Both cases (100%) of provoked VTE that occurred before the age of 40 years were associated with pregnancy or parturition (Figure 1B).

Prescription of Anticoagulants and VTE Recurrence

In 62 male patients with first episode of VTE, VTE recurred in 3 (38%) of 8 with AT deficiency, 1 (7%) of 14 with PC deficiency, none (0%) of 14 with PS deficiency, and none (0%)

Table 3. Sites of the First Episodes of VTE in Patients Deficient in Natural Anticoagulants

Type of Deficiency	AT, n = 38	PC, n = 46	PS, n = 47	Combined, n = 11
Male:Female	12:26	21:25	22:25	7:4
Sites of VTE				
Leg DVT	6:18	16:13	18:21	4:2
PE	2:2	2:4	0:1	0:0
Leg DVT + PE	4:2	3:6	4:1	3:2
Unusual sites	0:4	0:2	0:2	0:0

Abbreviations: AT, antithrombin; PC, protein C; PS, protein S; DVT, deep vein thrombosis; PE, pulmonary embolism; VTE, venous thromboembolism.

Table 4. Relationship Between Anticoagulant Therapy After First Episode of VTE in Male Patients Deficient in Natural Anticoagulants and Recurrence of VTE

Type of Deficiency	AT, n = 12	PC, n = 21	PS, n = 22	Combined, n = 7
Continued	8	14	14	4
Recurrence of VTE (%)	3 (38)	1 (7)	0 (0)	0 (0)
Discontinued	4	7	8	3
Recurrence of VTE (%)	4 (100)	4 (57)	4 (50)	1 (33)

Abbreviations: AT, antithrombin; PC, protein C; PS, protein S; VTE, venous thromboembolism.

Table 5. Relationship Between Anticoagulant Therapy After First Episode of Idiopathic VTE in Female Patients Deficient in Natural Anticoagulants and Recurrence of VTE

Type of Deficiency	AT, n = 13	PC, n = 14	PS, n = 14	Combined, n=1
Continued	9	7	10	1
Recurrence of VTE (%)	2 (22)	2 (29)	1 (10)	1 (100)
Discontinued	4	7	4	0
Recurrence of VTE (%)	4 (100)	0 (0)	1 (25)	0 (0)

Abbreviations: AT, antithrombin; PC, protein C; PS, protein S; VTE, venous thromboembolism.

of 4 with combined deficiencies who continued anticoagulant therapy after the first episode, while VTE recurred in 4 (100%) of 4 with AT deficiency, 4 (57%) of 7 with PC deficiency, 4 (50%) of 8 with PS deficiency, and 1 (33%) of 3 with combined deficiencies who had stopped anticoagulants (Table 4). In 42 female patients with first episode of idiopathic VTE, VTE recurred in 2 (22%) of 9 with AT deficiency, 2 (29%) of 7 with PC deficiency, 1 (10%) of 10 with PS deficiency, and 1 (100%) of 1 with combined deficiencies who continued anticoagulant therapy after the first episode, while VTE recurred in 4 (100%) of 4 with AT deficiency, none (0%) of 7 with PC deficiency, and 1 (25%) of 4 with PS deficiency who had stopped anticoagulants (Table 5). In 38 female patients with first episode of provoked, including pregnancy- or parturition-associated VTE, VTE recurred in 1 (13%) of 8 with

Table 6. Relationship Between Anticoagulant Therapy After First Episode of Provoked VTE in Female Patients Deficient in Natural Anticoagulants and Recurrence of VTE

Type of Deficiency	AT, n = 13	PC, n = 11	PS, n = 11	Combined, n = 3
Continued	8	5	6	1
Recurrence of VTE (%)	1 (13)	1 (20)	0 (0)	0 (0)
Discontinued	5	6	5	2
Recurrence of VTE (%)	1 (20)	0 (0)	2 (40)	1 (50)

Abbreviations: AT, antithrombin; PC, protein C; PS, protein S; VTE, venous thromboembolism.

AT deficiency, 1 (20%) of 5 with PC deficiency, none (0%) of 6 with PS deficiency, and none (0%) of 1 with combined deficiencies who continued anticoagulant therapy after the first episode, while VTE recurred in 1 (20%) of 5 with AT deficiency, none (0%) of 6 with PC deficiency, 2 (40%) of 5 with PS deficiency, and 1 (50%) of 2 with combined deficiencies who had stopped anticoagulants (Table 6). All 17 episodes of recurrent VTE in male patients were idiopathic; whereas in the female patients, 9 were idiopathic, 8 were provoked, and 4 were associated with pregnancy or parturition.

Discussion

Several case control studies have reported the incidence of inherited thrombophilia in Japanese patients with VTE. One study demonstrated that 113 Japanese patients are with VTE, 32 (28.3%) had an AT, PC, or PS deficiency,[19] and another study demonstrated that 13 (12%) of 108 patients with AT or PC deficiency had VTE.[20] Of 161 patients with VTE, 15 (9.3%) were carriers of the PS K196E mutation compared with 1.8% of the general population and this mutation is a confirmed genetic risk factor for VTE in the Japanese.[21] These studies demonstrated that deficiencies of natural anticoagulants are more common in Japanese patients with VTE than in whites with VTE, but the clinical course of Japanese patients deficient in natural anticoagulants is not clear. Therefore, we conducted a nationwide survey to elucidate the clinical course of these patients. Detailed information about the clinical course and management of 183 Japanese patients deficient in natural anticoagulants was obtained. Of the patients in our survey, 41 (22%) were diagnosed without previous history of VTE. Antigen and/or activity level of their natural anticoagulants are supposed to be measured because their relatives had been thought to be inherited thrombophilia. Rest of the patients in our survey had had at least one episode of VTE and 60% of the first VTE episodes in patients with AT deficiency occurred before the age of 40 years and approximately 45% of the first episodes of VTE in patients with PC or PS deficiency occurred before the age of 40 years. Approximately 60% of first VTE episodes were idiopathic and some of the first VTE episodes occurred at unusual sites, such as the mesenteric vein and cerebral sinus. Approximately 40% of patients with AT deficiency and 17% of patients with PC or PS deficiency with VTE experienced a recurrence.

These findings are comparable with those of a recent prospective study in Europe, demonstrating that 58% of first VTE episodes in thrombophilic individuals were idiopathic and mean age of onset was around the age of 40 years.[9]

Women are at increased risk of VTE during pregnancy and the puerperium with an estimated incidence of 0.7 to 1.3 per 1000 pregnant women,[22,23] which is approximately 10 times higher than that in nonpregnant women of fertile age. Growing evidence suggests that pregnant women with inherited thrombophilia have a greater risk of VTE than pregnant women without thrombophilia. Administration of prophylactic anticoagulants during pregnancy and postpartum is a common practice for pregnant women with prior VTE and inherited thrombophilia. The management of pregnant women with no prior VTE, but inherited thrombophilia, is controversial, however, because the actual risk of VTE during pregnancy and parturition is uncertain. In a systematic review of 9 studies that estimated the risk of VTE in pregnant women with inherited thrombophilia, deficiencies of natural anticoagulants were associated with a moderately increased risk (AT deficiency: odds ratio [OR] 4.69, PC deficiency: OR 4.76, and PS deficiency: OR 3.19[24]). A case-control study of 119 women with first VTE episodes during pregnancy or parturition showed that the relative risk of VTE associated with AT, PC, or PS deficiency was increased by as much as 13-fold.[25] In a retrospective study of 72 000 pregnancies, the incidence of VTE was 1 in 113 pregnancies for those with PC deficiency, 1 in 2.8 pregnancies for those with AT deficiency type I, and 1 in 42 pregnancies for those with AT deficiency type II.[26] Considering these results, pregnant women with no prior VTE but AT deficiency should receive prophylactic anticoagulants, while pregnant women with no prior VTE but PC or PS deficiency should be under clinical surveillance or receive prophylactic anticoagulants antepartum and prophylactic anticoagulants postpartum based on the eighth The American College of Chest Physicians (ACCP) guideline.[27] Although the risk of VTE in Japanese pregnant women deficient in natural anticoagulants could not be estimated by our survey, approximately half of the first VTE episodes in female patients occurring before the age of 40 years were associated with pregnancy or parturition. These results suggest that pregnant Japanese as well as white women deficient in natural anticoagulants have an increased risk of VTE, and prophylactic anticoagulants might be beneficial even when the patient has had no prior VTE.

Recurrence of VTE and hemorrhagic complications during anticoagulant therapy are the major problems in the management of patients with VTE. Optimal duration of VKA therapy after the first VTE episode depends on the estimated risk of recurrence and the risk of hemorrhagic complications in patients with prolonged VKA, which is reported to be 1% to 3%.[28,29] A recent patient level meta-analysis demonstrated that the 5-year cumulative incidence of recurrent VTE after discontinuation of anticoagulants was 43.1% in men with idiopathic VTE, which was 2.2 times higher than in women with idiopathic VTE, but the risk of recurrence did not differ between men and women with provoked VTE.[30] The relationship between inherited thrombophilia and risk of recurrent VTE was not mentioned in that study. Two recent prospective cohort studies reported a similar risk of recurrence in patients with a first VTE episode regardless of whether they had a thrombophilic defect,[31,32] but these two studies included patients with an FVL or PT G20210A mutation as well as patients with AT, PC, or PS deficiency. On the other hand, a recent retrospective analysis involving only patients with AT, PC, or PS deficiency confirmed that relative risk of recurrent VTE was 1.4 for these patients compared with patients without natural anticoagulant deficiencies. The risk of recurrence increased after a first idiopathic VTE as well as with the concomitance of other thrombophilic defects.[33] A total of 34 patients deficient in natural anticoagulants experienced VTE recurrence and 22 episodes occurred after cessation of anticoagulant therapy in our survey. Recurrence of VTE seemed to be more frequent in patients with AT deficiency compared with patients with PC or PS deficiency. Although the optimal duration of anticoagulant therapy after first VTE episode for patients deficient in natural anticoagulants has not been established, based on the previous retrospective analysis and the results of our survey, long-term anticoagulants may be beneficial for male patients deficient in natural anticoagulants after the first VTE episode, and female patients after the first episode of idiopathic VTE, especially in patients with AT deficiency.

Our study has some limitations. All cases with AT or PC deficiency were diagnosed by the measurement of activity of AT or PC. In some cases, antigen levels were also measured to classify the deficiency as type I or type II. Activity of PS was measured in 61% of patients with PS deficiency and other patients with PS deficiency were diagnosed by the measurement of PS antigen only. A higher incidence of VTE in Japanese patients with AT deficiency type I than patients with AT deficiency type II[34] and a high prevalence of PS deficiency type II in the Japanese has been reported.[12] These previous reports led us to measure both antigen levels and activities of AT, PC, and PS in Japanese patients with suspected natural anticoagulant deficiencies, but our questionnaires revealed that all of them were measured in only a limited number of patients. All Japanese are basically covered by national health insurance. The measurement of activities of AT and PC and PS antigen is covered by insurance, but the measurement of activity of PS was not covered at the time of survey. This restriction made it difficult to measure both activities and antigen levels of anticoagulants in all patients with suspected natural anticoagulant deficiencies in clinical practice. We did not ask the cutoff value of the levels of natural anticoagulants in each institute and the variation of cutoff value might exist among institutes; however, the results of our survey suggested that cutoff value was 65% to 70% for the activity of AT and 60% to 65% for the activity of PC. Cutoff value for the activity of PS and PS antigen seemed to be 50% to 55% and 55% to 60%, respectively, except for pregnant women. We asked family history and genetic diagnosis in the questionnaires; however, genetic analysis of natural anticoagulants is not covered by the health insurance and response to the questionnaires about relatives or genetic diagnosis of patients' might conflict with the

privacy policy at some institutes. In addition, some patients were only recently diagnosed and attending physicians might have sufficient information about the patients' relatives, while others were diagnosed years ago and information about the patients' relatives might be uncertain because of changes in attending physicians. These limitations could result in insufficient information of family history or genetic diagnosis of patients. Patients with PS K196E might be included in patients with PS deficiency in our survey and it is interesting to compare the clinical course of these patients to those of other patients deficient in natural anticoagulants; however, we could not do it because of above reason. Furthermore, it cannot be completely denied that patients with secondary deficiency in natural anticoagulants might be included in our survey. Finally, most patients in our survey visited their physicians regularly. Because patients without medication or patients with no prior VTE might not continue to visit their physicians, most individuals deficient in natural anticoagulants and with no prior VTE could be excluded from this study. These might result in a high frequency of first or recurrent episodes of VTE and a high VKA prescription rate in patients with prior VTE.

In conclusion, our survey revealed useful information regarding the occurrence of VTE in Japanese patients deficient in natural anticoagulants and the clinical management of these patients. Most of the patients in our survey were followed-up by a vascular surgeon or hematologist. Societies of these specialists should collaborate to create a central registry for Japanese patients with deficiency in natural anticoagulants to determine the actual incidence of VTE in these patients and to establish guidelines for adequate management of these patients to prevent a first or recurrent VTE.

Appendix

Name and institute of the physicians who provided the information of patients deficient in natural anticoagulants are as follow (INPO): H Shikata (Kanazawa Medical University); M Yamazaki (Tokyo Women's Medical University); U Yatomi (Tokyo University); M Ieko (Hokkaido University); E Morishita (Kanazawa University); H Satokawa (Fukushima Medical University); K Okamoto and M Sakai (University of Occupational and Environmental Health, Japan); H Komai (Tokyo Medical University); M Uchiba (Kumamoto University); K Ijima (Tottori University); O Sato (Saitama Medical Center); T Koyama (Tokyo Medical Dental University); N Yamamoto (Hamamatsu Medical University); T Okamura (Kurume University); H Wada (Mie University); S Madoiwa (Jichi Medical University); K Ota, H Ishibashi, and T Yamada (Aichi Medical University); N Shirasugi (Aiseikai Aisei Hospital); M Yoshida (Hyogo Brain and Heart Center); N Morimoto (Jikeikai Kitami Central Hospital); S Sugiyama (Hiroshima Teishin Hospital); Y Shigekiyo (Tokushima Prefectural Hospital); N Nishikimi (Japanese Red Cross Nagoya Daiichi Hospital); K Niimi (KKR Tokai Hospital); K Naito (Hamamatsu Medical Center); S Matsumoto (National Hospital Organization Tokyo Medical Center); M Yasaka (National Hospital Organization Kyushu Medical Center); T Kawasaki (Osaka University); T Kojima (Nagoya University); K Yokoyama (Keio University).

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