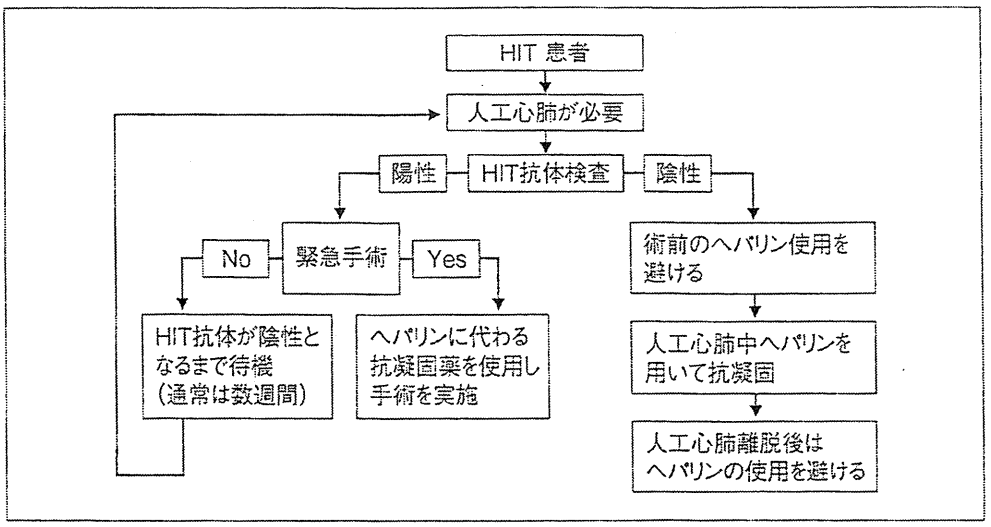

図 2　HIT 患者における人工心肺使用手術の抗凝固療法選択のアルゴリズム
[Warkentin TE et al(ed) : Heparin-Induced Thrombocytopenia, 4th ed, Informa Healthcare, New York, p496, 2007 より引用]

謝辞：HIT に対するアルガトロバンの医師主導治験を，国立循環器病研究センターをはじめ，日本全国にわたる施設の先生方のご指導，ご協力によってなしえたことに，心から御礼申しあげます．

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経皮的冠インターベンション(PCI)における ヘパリン起因性血小板減少症(HIT)

—岩手県・岩手医科大学循環器医療センター：伊藤智範

ヘパリン起因性血小板減少症(HIT)の発症機序

伊藤智範CCU室長

ヘパリンは血栓症の予防や治療，体外循環など，さまざまな領域で頻用されている抗凝固薬だが，副作用の1つとしてヘパリン起因性血小板減少症(HIT：heparin-induced thrombocytopenia)の発症が知られている．

血液中に投与されたヘパリンは血小板第4因子（PF4）と複合体を形成し，自己抗体である抗PF4／ヘパリン抗体の産生を誘導するが，HITはその抗体によって引き起こされることがわかっている．HIT抗体の存在下でヘパリンが投与されると，血小板が活性化されマイクロパーティクルを放出し，トロンビンを生成して血栓塞栓などを引き起こすというのがHITの発症機序である．

HITは，適切な治療を行わなければ，発症患者の30～50％に血栓塞栓症が生じ，その死亡率は10～20％に及ぶ．たとえば，ステント血栓症を離脱できず，救命の必要性から経皮的心肺補助法（PCPS：percutaneous cardiopulmonary support）を導入するまでに至ったが，さらに回路内に血栓を形成したことで，ようやくHITを疑い始めたという症例もある．こうした致死的な状況に陥る前に，HITを疑うことが重要である．

PCI施行中におけるHITの疑い

経皮的冠インターベンション(PCI：percutaneous coronary intervention)施行中に生じるHITの割合は決して多くはない．しかし，それゆえにHITを疑うタイミングが難しいともいえる．わが国ではHITの診断基準として確立されたものはないが，通常，ヘパリン投与中ないし投与後に，血小板数が前値と比べて30～50％以上減少し，4Ts'スコアが4点以上*あれば，HITの疑いが強いと考えられている（図1）．しかし，我々が行うPCIはほとんどが緊急のものであり，施行中に臨床上理解できない血栓症の発現をみた段階で，迅速な判断が求められる．*(「臨床のあゆみ」89号P20参照)

PCI施行中における臨床上理解できない血栓症とは，十分なヘパリン投与を行い，活性化全血凝固時間（ACT：activated coagulation time）が治療域の300秒前後にあり，血管内超音波でステントの拡張が確認できるにもかかわらず血栓症が生じた場合である（図2）．それを認めた段階で我々はまずHITを疑う．

その他，PCI施行中以外でもHITを疑う状況はいくつもある（表）．一方で，HIT以外に血小板

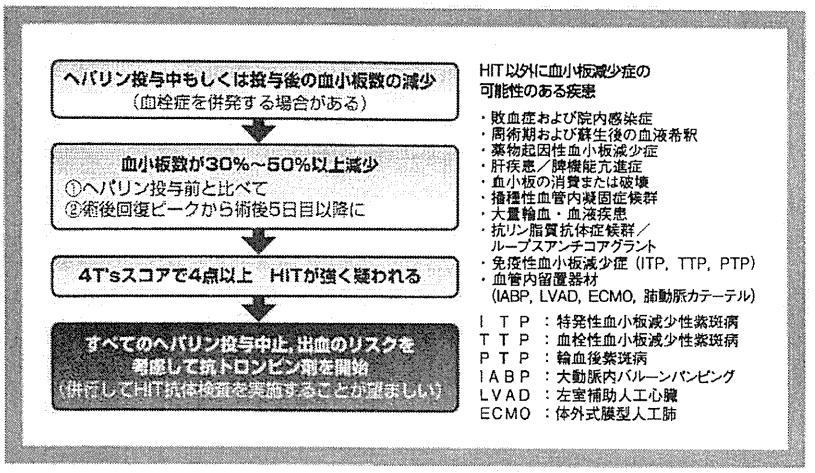


図1　HITの診断と治療

監修：国立循環器病研究センター輸血管理室　宮田茂樹
Napolitano LM et al：Crit. Care Med.，2006；34（12）：2898-2911．より一部改変

減少症を起こす病態・疾患（図1-右）もあり，これらが集中治療領域においてHITか否かの判断を紛らわしくする要因になっている．

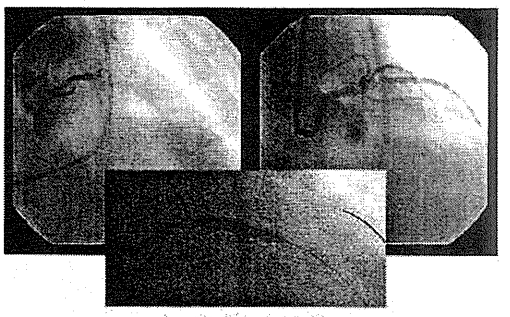
HIT治療の流れ

PCI施行中にHITを疑った場合の手順を示す．

①ただちにヘパリン投与を中止し，選択的抗トロンビン剤であるアルガトロバン製剤（ノバスタン®）を点滴静注する．

②ヘパリンロックなど少量でもヘパリンの使用を中止する．また，ヘパリンコーティングしている医療器具（特にスワンガンツカテーテルなど）の使用中止や，それらを乗せていたワゴンや手術台なども可能な限り入れ替える．医療器具については，事前にメーカーにヘパリンコーティングの有無を確認しておくのが望ましい．

③HIT抗体測定を行い，臨床的診断と血清学的診断の両面から診断を行う．ただし，抗体の結果が判明するまで時間がかかるため，PCI施行中は臨床症状と血小板数減少でHITを疑うのが現実的である．



血栓吸引後のPOBA

図2　亜急性ステント血栓症発症時の左主幹部閉塞

表　HITを疑う主な状況

- 中心静脈栄養時のヘパリン使用
- ヘパリンコーティング製品の使用
- 各種検査時でのライン確保時のヘパリンロック
- 心房細動でのヘパリン投与
- 血液浄化装置の回路内血栓症
- ステント留置例でのステント血栓症
- カテーテル後の発熱
- 深部静脈血栓形成

④PCI終了後は患者をCCUに移し，血小板数が回復するまでアルガトロバン製剤の投与を続ける．PCI終了後は，活性化部分トロンボプラスチン時間（APTT：activated partial thromboplastin time）が投与前値の約2倍（製品添付文書では1.5～3倍）となるよう投与量を調節する．また，HITではトロンビン活性が亢進することから，凝固線溶系マーカーであるトロンビンアンチトロンビンⅢ複合体（TAT：thrombin anti-thrombinⅢ complex）をモニタリングするのも1つの方法である．

臨床的な勘を磨く

PCIにおいてヘパリンは欠かすことのできない抗凝固薬である．だからこそ，施行中に血栓症が生じればヘパリンの追加投与を考えても不思議はない．ましてや，ヘパリンが血栓症を誘導しているという逆説的な発想はなかなか思いつかないだろう．しかし，そこで「待てよ，何かおかしい」と勘を働かせてほしいのである．その臨床的な勘こそが，HITの診断・治療へとつながり，重篤化の回避につながるのである．臨床的な勘とは，日常診療の中で例外的な事例に出合ったときにいろいろな可能性を考慮するというトレーニングを重ねることで磨かれていくと考える．

最後に，PCIにおいてHITが疑われる場合は，迷わずヘパリンからアルガトロバン製剤に切り替えることをおすすめする．我々の経験では，アルガトロバン投与中に出血性合併症を生じたことはなく，また，国内の研究でも発症が少ないと報告されていることからも，使いやすい薬剤であるといえる．

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Causes of Thrombocytopenia in Chronic Hepatitis C Viral Infection

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Abstract

We retrospectively studied 89 patients with chronic hepatitis C virus (HCV) infection, including 50 chronic hepatitis (CH) cases, 18 liver cirrhosis (LC) cases, and 21 LC with hepatocellular carcinoma (LC + HCC) cases, with regard to various factors related with thrombocytopenia. The platelet count decreased with the stage advancement of liver diseases. Multiple regression analysis revealed that splenomegaly and von Willebrand factor (vWF) were explanatory variables that correlated with thrombocytopenia. Splenomegaly appears to be the most responsible factor, although there are a considerable number of thrombocytopenic cases without splenomegaly, suggesting other factors may also be responsible. The vWF level is inversely correlated with the platelet count. Soluble thrombomodulin, a marker of endothelial dysfunction, increases with the advancement of liver fibrosis. It is positively correlated with vWF and inversely with the platelet count. Our present results imply that vascular endothelial dysfunction is also involved in thrombocytopenia during chronic HCV infection.

Keywords

thrombocytopenia, hepatitis C, splenomegaly, von Willebrand factor, thrombomodulin

Introduction

The platelet count is known to decrease in proportion to the advancement of the stage of liver disease in chronic hepatitis C virus (HCV) infection. Liver biopsy is the golden standard for evaluating the stage of fibrosis in HCV patients. It is, however, a considerably invasive procedure and more simple, noninvasive laboratory methods capable of predicting the stage of fibrosis would be of great help in clinical settings. A strong correlation between liver fibrosis and thrombocytopenia has been noted in a number of papers, and the platelet count is presently used as an index for fibrosis staging.[1,2]

Thrombocytopenia in liver fibrosis can be attributed to (1) platelet destruction/sequestration by the spleen, (2) the decreased production of platelets, and (3) platelet consumption. Based on several papers that have reported a strong correlation between spleen size and thrombocytopenia,[3-5] platelet destruction/sequestration as a result of splenomegaly caused by portal hypertension has been considered to be the most important determinant. On the other hand, a splenectomy or portal vein shunting does not necessarily normalize the platelet count,[6,7] suggesting that factors other than splenomegaly are also operative in reducing the platelet count during liver fibrosis.

Since thrombopoietin (TPO), which facilitates the proliferation and differentiation of the megakaryocytic lineage (resulting in the production of platelets), is released from the liver, some reports have suggested that inadequate TPO production is at least partly responsible for thrombocytopenia in liver fibrosis.[4,8-11] The expression level of c-mpl, the TPO receptor, is also reportedly low in patients with liver cirrhosis.[12] On the other hand, some reports have argued against a correlation between thrombocytopenia and hepatic TPO production,[13] and whether decreased thrombopoiesis contributes to thrombocytopenia during liver fibrosis awaits further elucidation.

As for platelet consumption, several hypotheses related to von Willebrand factor (vWF) have been proposed.

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Inflammatory changes that accompany chronic HCV hepatitis and liver cirrhosis may lead to an increase in the vWF level, resulting in platelet consumption.[14-16] The impaired hepatic production of ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13) and its activity, which cleaves vWF, may also lead to an increase in ultra-large multimers of vWF, resulting in platelet microthrombi formation[17] and consumptive thrombocytopenia, similar to the conditions observed with thrombotic thrombocytopenic purpura (TTP).

In the present study, we evaluated various factors, including spleen size, TPO, vWF, and ADAMTS13, as well as general hematological, biochemical, and coagulation parameters in patients with chronic HCV infection, with fibrosis stages ranging from a relative early phase to an advanced stage of liver cirrhosis and attempted to determine the contributing power of each factor to thrombocytopenia using a multiple regression analysis and other analytical methods.

Materials and Methods

Patients

Our study was made up of 89 patients with chronic HCV infection who had been referred to the outpatient clinic of the University of Yamanashi Hospital. Based on the chronic HCV staging, 50 patients had chronic hepatitis (CH), 18 patients had liver cirrhosis (LC), and 21 patients had liver cirrhosis plus hepatocellular carcinoma (LC + HCC). The diagnosis of chronic hepatitis and LC was made by an expert in hepatology (M.S.), fundamentally using the score of Fib-4.[18] Fib-4 was calculated according to the formula of (age × AST/(platelets × $ALT^{0.5}$)), and the cases exceeding the score of 3.25 were diagnosed as LC. The study was endorsed by the Institutional Review Board of the University of Yamanashi (No. 224), and all the patients gave their written informed consent prior to participation in the study. Blood was withdrawn from the ante-cubital vein; after laboratory measurements for diagnostic purposes, the residual samples were used to assess various factors that may be involved in thrombocytopenia.

Methods

A complete blood count (CBC) analysis was performed using an SE-3000 (Sysmex Co, Kobe, Japan). Serum and plasma anticoagulated with citrate were obtained by centrifugation of the whole blood within 2 hours of blood collection and were stored at −80°C until measurement.

Repeated freezing was avoided as much as possible; when necessary, the thawing of the frozen serum and plasma was performed at 37°C. Biochemical parameters, including albumin, alanine aminotransferase (ALT), and total bilirubin, were measured using a BM-2000 (JEOL Ltd, Tokyo, Japan). The vWF antigen level was determined using STA-LIA kits (Roche Diagnostics K.K., Tokyo Japan), and the ADAMTS13 antigen level was determined using ADAMTS13 ELISA kits (Mitsubishi Chemical Medience Co, Tokyo, Japan).[19] ADAMTS13 activity was measured using the ADAMTS13 Activity Kit (Kainos Laboratories, Tokyo, Japan),[20] thrombomodulin (TM) was measured using TM (MKI) EIA kits (Mitsubishi Kagaku Iatron Co Ltd), and TPO was measured using Human TPO Immunoassay kits (R&D Systems, Minneapolis). For coagulation and fibrinolysis testing, an LPIA A-700 (Mitsubishi Chemical Medience Co) was used to measure the prothrombin time, with results expressed as international normalized ratio (PT-INR), D-dimers, and tissue plasminogen activator inhibitor (t-PAI). The spleen size was determined using computed tomography (CT), magnetic resonance imaging (MRI), or ultrasonographic measurements. The splenic index (SI $^{1/4}$(long axis/2) × short axis) was calculated, and spleens with an SI value higher than 20 were considered to exhibit splenomegaly.

Statistical Analysis

The analysis of the biochemical parameters, coagulation/fibrinolysis factors, SI index, and so on, and the multiple regression analysis were preformed using the data analysis software STAT FLEX, Ver. 4.1 (Artec, Ltd, Osaka, Japan).

Results

Patient Background

The numbers and sex (male/female) of patients at each stage was 50 patients (37/13) with CH, 18 patients (7/11) with LC, and 21 patients (9/12) with LC + HCC. The patient age tended to increase with the progression of the fibrosis stages: 57.4 ± 10.6 years among patients with CH, 65.3 ± 9.5 years among patients with LC, and 70.0 ± 9.1 years among patients with LC + HCC. The CBC profile, biochemical parameters, coagulation/fibrinolysis factors, and inflammation markers are summarized according to each stage in Table 1.

Platelet Count

The platelet count decreased with the progression of fibrosis staging, and significant differences in the platelet count were observed between the stages: 182.8 ± 47.5 × 10^3/μL among patients with CH, 85.9 ± 33.6 × 10^3/μL among patients with LC, and 66.7 ± 25.2 × 10^3/μL among patients with LC + HCC (Table 1 and Figure 1).

Liver Function

Prothrombin time–international normalized ratio (PT-INR), which represents the overall coagulation capacity of the extrinsic pathway, is a good marker for hepatic protein synthesis. Prothrombin time–international normalized ratio was positively correlated with the progression of the fibrosis stages; 1.03 ± 0.05 among patients with CH, 1.18 ± 0.16 among patients with LC, and 1.22 ± 1.13 among patients with LC + HCC; significant differences were observed among the stages ($P < .01$; Table 1). A negative correlation was seen between PT-INR and the platelet count ($r = -.71$,

Table 1. Clinical Characteristics of Patients With Chronic HCV Infection

Variable	CH	LC	LC + HCC
Platelet count ($\times 10^3/\mu L$)	182.4 ± 48.7[a,b]	91.3 ± 33.0	65.7 ± 23.1[c]
Splenic index (SI)	14.7 ± 4.2[a,b]	23.4 ± 9.5	24.2 ± 7.3
PT-INR	1.03 ± 0.05[a,b]	1.18 ± 0.16	1.22 ± 0.13
Albumin, g/dL	4.3 ± 0.4[a,b]	3.6 ± 0.7	3.5 ± 0.4
Total bilirubin, mg/dL	0.5 ± 0.2[a,b]	0.7 ± 0.4	0.88 ± 0.33
Thrombopoietin, pg/mL	42.5 ± 33.1[d]	39.9 ± 37.8	70.3 ± 68.7
von Willebrand factor, %	153.4 ± 52.6[a,b]	208.7 ± 83.1	243.6 ± 65.3
Thrombomodulin, U/mL	16.0 ± 5.7[a,b]	22.9 ± 8.8	25.8 ± 7.5
ADAMTS13 antigen, %	103.2 ± 32.9[a]	138.6 ± 58.1	107.6 ± 31.2
ADAMTS13 activity, %	97.2 ± 28.8[a]	123.0 ± 43.2	102.1 ± 27.5
vWF/ADAMTS13 activity	1.7 ± 0.7[b]	2.0 ± 1.3	2.7 ± 1.5
D-Dimer, μg/mL	0.44 ± 0.28[b,c]	0.72 ± 0.60	0.87 ± 0.82
PAI-1, ng/mL	18.9 ± 6.5	19.8 ± 7.1	19.1 ± 10.5
CRP, mg/dL	0.11 ± 0.02	0.11 ± 0.02	0.13 ± 0.05

Abbreviations: CH, chronic hepatitis; LC, liver cirrhosis; LC + HCC, LC complicated with hepatocellular carcinoma; PT-INR, prothrombin time–international normalized ratio; vWF, von Willebrand factor; CRP, C-reactive protein; PAI-1, plasminogen activiator inhibitor 1; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13.

[a] $P < .01$ versus LC.

[b] $P < .01$ versus LC + HCC.

[c] $P < .05$ versus LC.

[d] $P < .05$ versus LC + HCC.

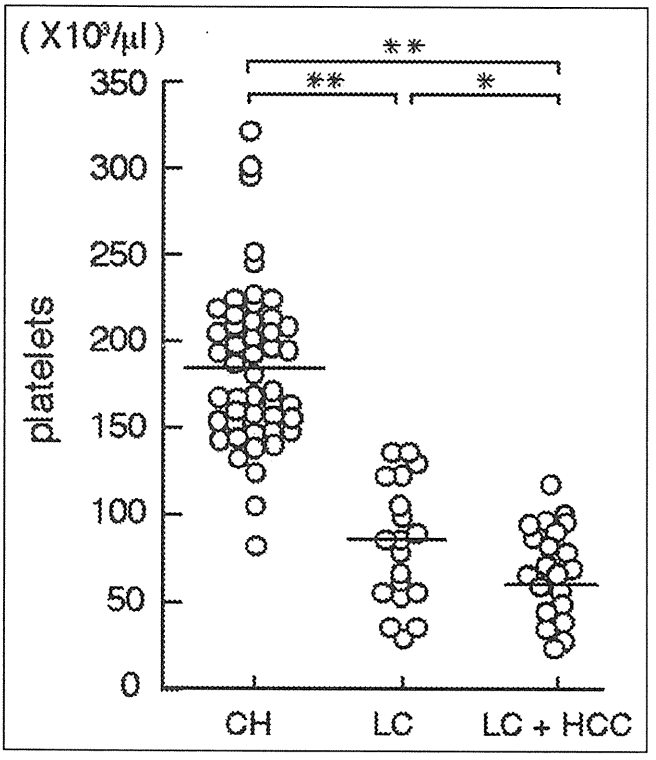

Figure 1. Platelet counts in chronic hepatitis C virus (HCV) infection patients with chronic hepatitis (CH), liver cirrhosis (LC), and liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC). The open circles represent each individual patient, and the mean of each patient group is indicated by the horizontal line. Statistical differences are indicated with *P < .05 and **P < .01.

$P < .0001$), suggesting that the platelet count decreases with impaired liver function in patients with chronic HCV infection (Figure 2). A positive correlation ($r = .59$, $P < .0001$) was observed between the platelet count and the albumin level, which represents hepatic protein synthesis, and an inverse correlation was observed between the platelet count and the total bilirubin level, the elevation of which represents a fibrosis-related impairment in bile secretion ($r = -.49$, $P < .0001$). Of these markers of liver function, the coefficient value of PT-INR exceeded those of the others. A negative correlation was also observed between PT-INR and the albumin level ($r = -.73$, $P < 0.0001$), and a positive correlation was observed between PT-INR and the total bilirubin level ($r = .76$, $P < .0001$).

Splenomegaly

Splenomegaly is considered to be one of the major causes of thrombocytopenia during chronic HCV infection. The SI was 14.7 ± 4.2 among the patients with CH, 23.7 ± 9.2 among the patient with LC, and 25.3 ± 8.2 among the patients with LC + HCC. These values were significantly different ($P < .01$; Table 1), suggesting that splenomegaly increases in size with the progression of the fibrosis stage. A negative correlation was observed between the platelet count and the splenomegaly ($r = -.65$, $P < .0001$; Figure 3), confirming the previous notion that the thrombocytopenia was attributable to splenomegaly in proportion to the stage of progression. On the other hand, of the 33 patients with a platelet count of less than $100 \times 10^3/\mu L$, 8 patients had no significant splenomegaly, while 25 patients had splenomegaly with SI values higher than 20. These findings imply that there are some cases of thrombocytopenia that are unexplainable by splenomegaly.

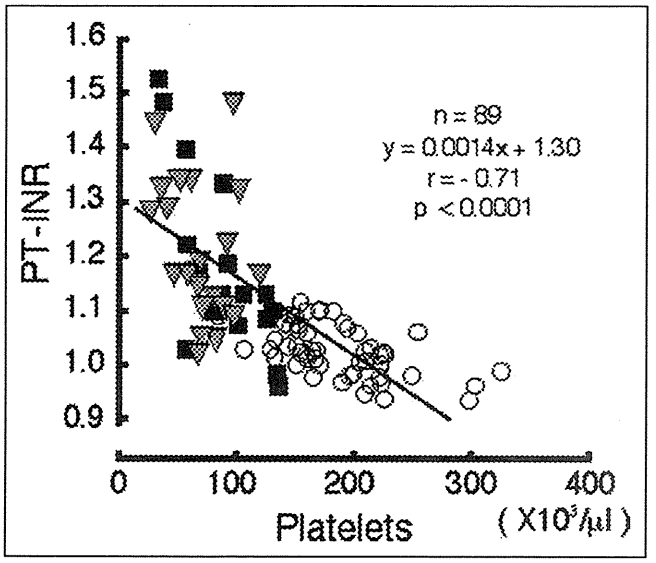

Figure 2. Scattergram showing the correlation between prothrombin time–international normalized ratio (PT-INR) and the platelet count ($r = -.71$, $P < .0001$). The line represents the linear regression of PT-INR versus platelet count. The symbols represent the patients as follows: open circle, chronic hepatitis (CH); square, liver cirrhosis (LC); and triangle, liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC).

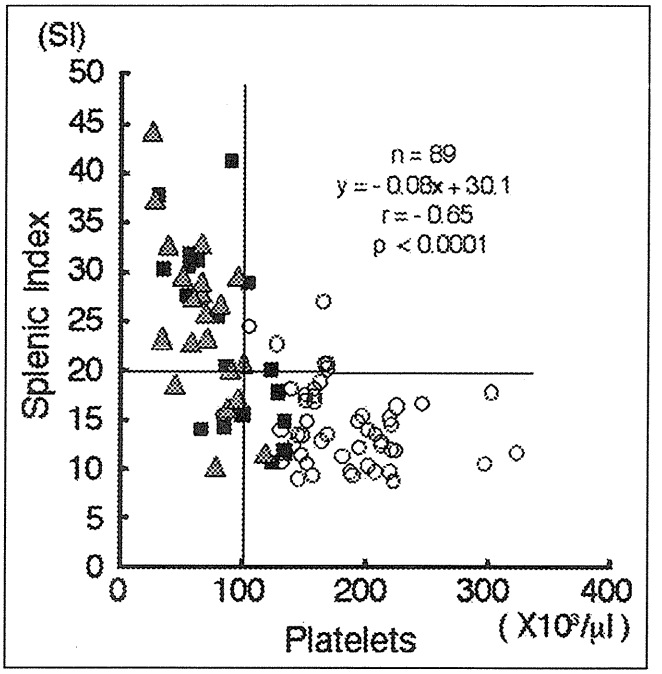

Figure 3. Scattergram showing the correlation between the platelet count and the splenic index (SI; $r = -.65$, $P < .0001$). The horizontal and vertical lines denote the cutoff values used to define splenomegaly, with SI values higher than 20 and thrombocytopenia with a platelet count less than 100×10^3 cells/μL, respectively. The symbols represent the patients as follows: open circle, chronic hepatitis (CH); square, liver cirrhosis (LC); and triangle, liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC).

Thrombopoietin

The TPO concentrations were 42.5 ± 33.1 pg/mL among patients with CH, 39.9 ± 37.8 pg/mL among patients with LC, and 70.3 ± 68.7 pg/mL among patients with LC + HCC, respectively; no significant differences were observed among the stages (Table 1). No correlation between the platelet count and the TPO concentration was seen ($r = -.23$, $P < .0475$).

von Willebrand factor Antigen

The vWF antigen values were 153.4% ± 52.6% among patients with CH, 208.7% ± 83.1% among patients with LC, and 243.6% ± 65.3% among patients with LC + HCC. These values were significantly different ($P < .01$; Table 1), suggesting that vWF antigen increases with the progression of the stage of chronic HCV infection. A negative correlation was observed between the platelet count and the vWF antigen value ($r = -.54$, $P < .0001$; Figure 4).

Thrombomodulin

Thrombomodulin is often used as a marker of endothelial cell damage. As expected, the TM values were 16.0 ± 5.7 U/mL among patients with CH, 22.9 ± 8.8 U/mL among patients with LC, and 25.8 ± 7.5 U/mL among patients with LC + HCC. These values were significantly different ($P < .01$; Table 1). A negative correlation was observed between the platelet count and the TM value ($r = -.51$, $P < .0001$; Figure 5A), and a positive correlation was observed between the vWF antigen value and the TM value ($r = .48$, $P < .0001$; Figure 5B).

ADAMTS13

A decrease in ADAMTS13 activity is known to result in an increase in ultra-large vWF multimers, which is associated with a low platelet count. The ADAMTS13 activities were 97.2% ± 28.8% among patients with CH, 123.0% ± 43.2% among patients with LC, and 102.1% ± 27.5% among patients with LC + HCC. No significant differences were observed among the stages (Table 1). Neither a correlation between ADAMTS13 activity and the platelet count ($r = -.22$, $P < .0825$; Figure 6) nor a correlation between ADAMTS13 activity and the VWF antigen value was observed ($r = -.02$, $P < .8746$).

Multiple Regression Analysis

With the parameters measured in this study, we performed a multiple regression analysis using thrombocytopenia as the target index. As a result, splenomegaly, PT-INR, and the vWF antigen value were extracted as factors that were significantly responsible for thrombocytopenia (Table 2, panel A). Since splenomegaly is related to the impairment of liver function,

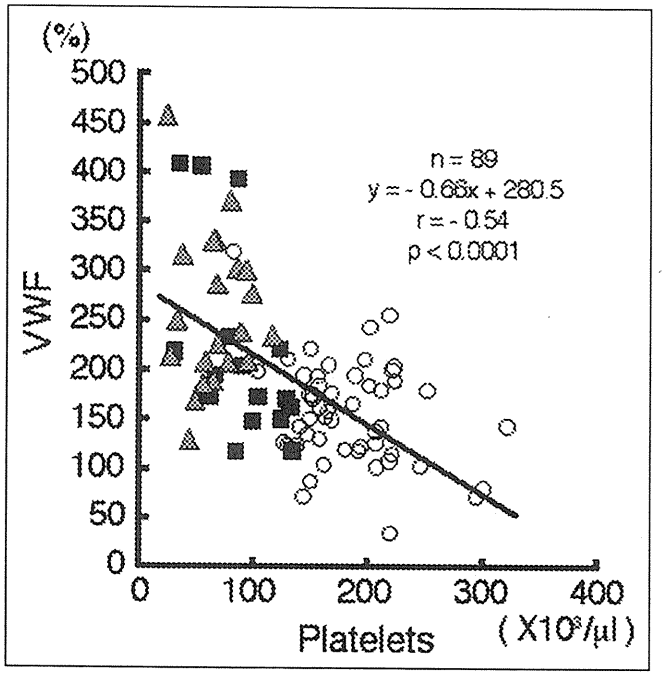

Figure 4. Scattergram showing the correlation between the von Willebrand factor (vWF) antigen value and the platelet count ($r = -.54$, $P < .0001$). The line represents the linear regression of vWF antigen versus the platelet count. The symbols represent the patients as follows: open circle, chronic hepatitis (CH); square, liver cirrhosis (LC); and triangle, liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC).

with which the PT level is assumed to be associated, we also performed an analysis without PT-INR, revealing splenomegaly and the vWF antigen value as significant factors (Table 2, panel B). We further performed a stratified analysis, in which the patients were subdivided into 4 groups, based on 2 parameters: splenomegaly (+, SI > 20) or (−) and thrombocytopenia (+, platelet count < 100 × 10^3 cells/μL) or (−). In the splenomegaly (+) group, significant differences in spleen size (SI), liver function, and the vWF antigen value were observed between the thrombocytopenia (+) group and (−) group (Table 3). Taking into consideration the fact that impaired liver function is related to splenomegaly and that these 2 factors may be evaluated as one in this group, splenomegaly appears to be the major determinant of thrombocytopenia. However, among the 55 cases without splenomegaly, a significant increase in the vWF antigen value and PT-INR was observed in the thrombocytopenia (+) group compared with the (−) group, while no difference in spleen size or platelet count was observed. Thus, in the group of patients without splenomegaly, some factors related to the vWF antigen increase or liver function impairment may play a role in inducing thrombocytopenia.

Other Markers

Fibrinolysis markers such as D-dimer and PAI-1 were evaluated in reference to thrombocytopenia. However, no correlation was

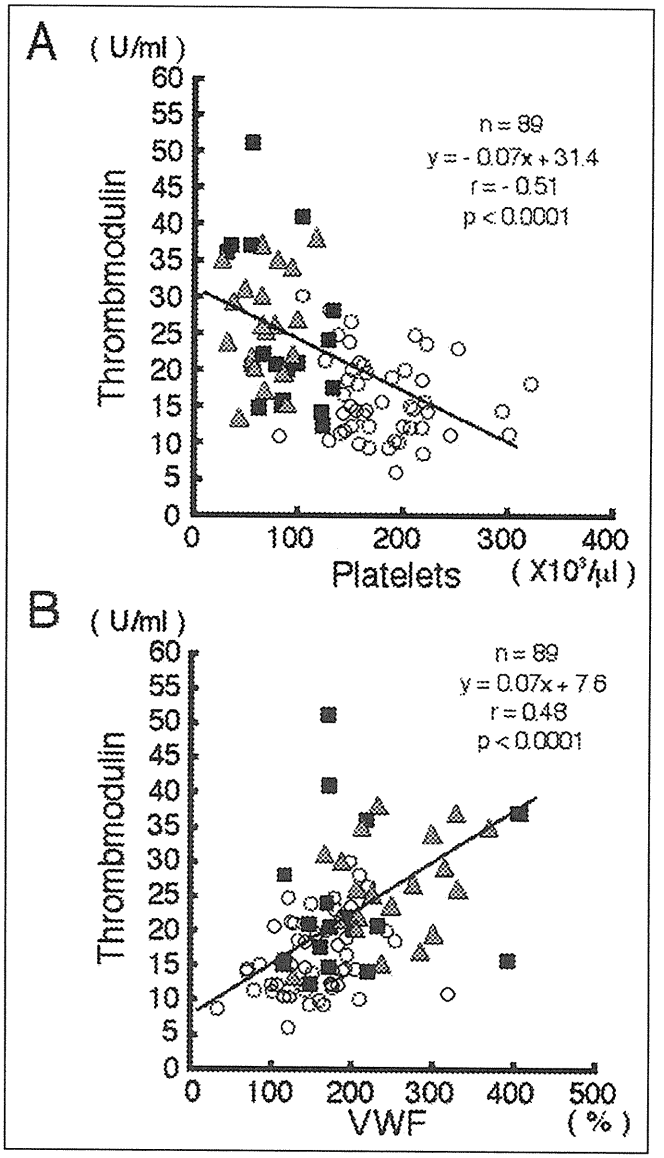

Figure 5. Scattergram showing the correlation between thrombomodulin (TM) and the platelet count ($r = -.51$, $P < .0001$) (A) and between TM and the von Willebrand factor (vWF) antigen value ($r = .48$, $P < .0001$) (B). The line represents the linear regression. The symbols represent the patients as follows: open circle, chronic hepatitis (CH); square, liver cirrhosis (LC); and triangle, liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC).

observed between these markers and the platelet count in patients with chronic HCV infection. Furthermore, no correlation was observed between the platelet count and the CRP level, which is often used a marker of systemic inflammation.

Discussion

Thrombocytopenia in chronic HCV infection may be caused by platelet destruction/sequestration, the decreased production of platelets, or platelet consumption. In this study, we sought to

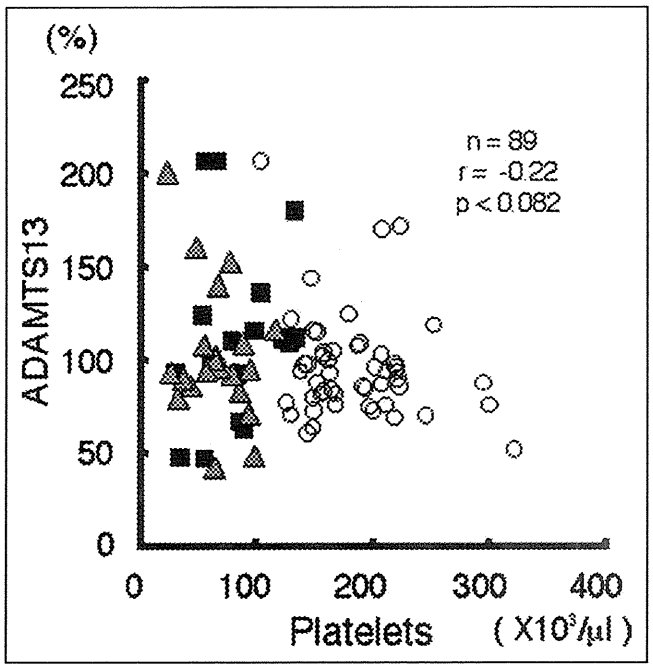

Figure 6. Scattergram showing the correlation between ADAMTS13 activity and the platelet count ($r = -.22$, $P < .0825$). The symbols represent the patients as follows: open circle, chronic hepatitis (CH); square, liver cirrhosis (LC); and triangle, liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC).

evaluate the roles of various factors that may contribute to thrombocytopenia.

Platelet destruction/sequestration by splenomegaly induced by liver fibrosis is a major cause of thrombocytopenia. In this study, examining 89 patients infected with HCV, splenomegaly, impaired liver function as represented by PT, and the vWF antigen value were correlated well with thrombocytopenia, and a multiple regression analysis also extracted these 3 parameters as explanatory variables for thrombocytopenia, confirming the results of previous reports.[4,14-16] Since splenomegaly and PT are partially dependent, we removed PT from the analysis to extract other underlying factors; only splenomegaly and the vWF antigen value were identified as major determinants of thrombocytopenia, with splenomegaly exhibiting the stronger dependence. On the other hand, a splenectomy or the shunting of the portal veins does not necessarily correct the low platelet count,[6,21] suggesting that some mechanism other than splenomegaly is responsible for thrombocytopenia in liver fibrosis induced by HCV infection. In our stratified analysis of 55 cases without splenomegaly (SI < 20), significant differences in the vWF antigen value and PT were observed between the thrombocytopenia (+) group and the thrombocytopenia (−) group, although no significant difference in spleen size was noted. These findings suggest that even among cases without splenomegaly, thrombocytopenia is induced by some other factors that may be related to an elevation in the vWF antigen level or the impairment of liver function.

Table 2. Multiple Regression Analysis of Variables Associated With Thrombocytopenia

A. Analysis with PT, splenic index, and vWF antigen		
Variable	*t*	*P*
PT-INR	4.662	.000013
Splenic index (SI)	3.079	.002905
vWF antigen, %	2.410	.018406
B. Analysis with splenic index and vWF antigen		
Splenic index (SI)	6.413	<.00001
vWF antigen, %	3.484	.00078

Abbreviation: PT-INR, prothrombin time–international normalized ratio; vWF, von Willebrand factor.

The impaired production of platelets, that is, impaired thrombopoiesis, may be partially responsible for thrombocytopenia in patients infected with HCV. Thrombopoietin is a major cytokine that stimulates the proliferation and differentiation of the megakaryocytic lineage, with resultant platelet production.[22,23] Since TPO is produced by the liver, impaired liver function in chronic HCV infection may lead to a low level of TPO in the blood, which cannot maintain normal thrombopoiesis in the bone marrow and the peripheral platelet count.[24] In agreement with this hypothesis, a negative correlation between the blood TPO level and the progression of the stage of liver disease has been reported in patients with HCV infection.[25] On the other hand, a previous study hypothesized that the total blood TPO is maintained at a certain level, irrespective of liver function, and that the blood TPO level is inversely related to the platelet count since TPO binding to its receptors on platelets and megakaryocytes tends to lower the blood TPO level.[26] Based on this hypothesis, it follows that the blood TPO is elevated in proportion to the severity of thrombocytopenia in patients with chronic HCV infection. In the present study, we found that no correlation existed between the platelet count and the blood TPO level, although the blood TPO level tended to be elevated in the LC + HCC group. A multiple regression analysis did not recognize TPO as an explanatory factor, and it is likely that TPO contributes minimally to thrombocytopenia in patients with chronic HCV infection. Consistently, recent reports have proposed that TPO produced by stromal cells in the bone marrow acts locally on megakaryocytes[27,28] and that the blood TPO level does not reflect thrombopoiesis in the bone marrow.[29]

Other hypotheses have also been presented in relation to vWF-induced platelet consumption, which accounts for the thrombocytopenia in patients infected with HCV. von Willebrand factor associates with Glycoprotein IB (GPIB) molecules on the platelet membrane and leads to platelet adhesion/aggregation at sites of vascular damage. If the blood vWF level is increased in patients with liver fibrosis, its interaction with platelets may lead to the increased consumption of platelets, resulting in thrombocytopenia.[14-16,30] von Willebrand factor antigen has been reported to increase significantly with the progression of the stage of liver fibrosis,[30] and vWF production

Table 3. Stratified Analysis of Patients with Thrombocytopenia With or Without Splenomegaly

Splenomegaly	−	−	+	+
Platelet	$>10^5/\mu L$	$<10^5/\mu L$	$>10^5/\mu L$	$<10^5/\mu L$
n	47	8	8	25
Splenic index (SI)	13.5 ± 2.9	15.4 ± 2.5	23.3 ± 3.4	29.3 ± 6.1[a]
Platelet ($\times 10^3/\mu L$)	182.5 ± 47.1	79.4 ± 17.5[b]	140.5 ± 29.1	61.2 ± 21.7[c]
von Willebrand factor, %	152.7 ± 48.2	202.8 ± 75.3[d]	179.1 ± 29.2	268.7 ± 88.6[e]
Thrombomodulin, U/mL	18.0 ± 9.9	18.7 ± 5.2	20.5 ± 10.2	27.4 ± 9.1
PT-INR	1.03 ± 0.06	1.12 ± 0.04[f]	1.07 ± 0.05	1.26 ± 0.15[c]

Abbreviation: PT-INR, prothrombin time–international normalized ratio.
[a] $P < .05$ versus splenomegaly (+) plus platelet > $10^5/\mu L$.
[b] $P < .01$ versus splenomegaly (−) plus platelet > $10^5/\mu L$.
[c] $P < .01$ versus splenomegaly (+) plus platelet > $10^5/\mu L$.
[d] $P < .05$ versus splenomegaly (−) plus platelet > $10^5/\mu L$.
[e] $P < .01$ versus splenomegaly (+) plus platelet > $10^5/\mu L$.
[f] $P < .01$ versus splenomegaly (−) plus platelet > $10^5/\mu L$.

has been postulated to be facilitated by the remodeling of the liver tissue or endotoxic damage to the hepatocytes[31] or extrahepatic organs, such as the spleen.[32] Recent reports on vWF regulation in patients with chronic HCV infection have focused on ADAMTS13 activity, which cleaves the vWF multimers. An elevated vWF antigen level in patients with chronic HCV infection may reflect a proportional decrease in ADAMTS13 activity and the existence of ultra-large vWF multimers, which are apt to react with platelets.[33] possibly leading to thrombocytopenia. In accordance with this notion, a recent report has demonstrated a correlation between the platelet count and ADAMTS13 activity in patients with advanced stages of liver fibrosis, including HCV infection.[17]

Contrary to our expectation, we were unable to observe a significant correlation between ADAMTS13 antigen/activity (data not shown) and the platelet count in this study, and no significant differences in these ADAMTS13-related parameters were observed among the stages of liver fibrosis. The discrepancy between our study and previous reports, particularly that of Uemura,[17] appears to be attributable to the overall severity of liver fibrosis in patients evaluated in each study. The report of Uemura et al deals with a number of patients with considerably advanced stages of liver fibrosis, such as those complicated with ascites. The patient profile in terms of Child's classification corresponded to 33 cases of CH, 35 cases of LC Child A, 33 cases of Child B, and 41 cases of Child C, with mean ADAMTS13 activities of 87%, 79%, 63%, and 31%, respectively. A clear difference in the ADAMTS13 activities was observed among the stages of liver fibrosis, with the lowest level observed with Child C. On the other hand, the patients in our study all attended our outpatient clinic on a regular basis, and the overall severity of liver fibrosis was far less than that of the series reported by Uemura, which was comprised of 50 cases of CH, 23 cases of LC Child A, 13 cases of Child B, and 3 cases of Child C; the ADAMTS13 activities were 95.9%, 119.5%, 92%, and 81%, respectively. Of note, the ADAMTS13 activities of even our Child B and C groups were fairly well retained (81%-92%), although these patients exhibited considerably severe thrombocytopenia. Recent reports on the pathogenesis of TTP have demonstrated that severe ADAMTS13 activity of less than 3% is required to increase ultra-large vWF multimers, resulting in thrombocytopenia.[34-36] It is also now known that a simple deficiency in ADAMTS13 does not lead to overt TTP.[37] Furthermore, hepatic stellate cells have been reported to possibly be a key factor in the reduction of plasma ADAMTS13 activities in rats with liver injury.[38] Taken together with the findings of these previous reports, our findings that thrombocytopenia occurs in the apparent absence of clear changes in ADAMTS13 activity suggests that ADAMTS13 changes may not be heavily involved in thrombocytopenia during chronic HCV infection.

Stellate cells appear to play an important role in liver fibrosis, and it is well known that the number of hepatic stellate cells is increased in cirrhotic liver in humans as well as rats. Furthermore, the current study revealed a relation between the increased production of ADAMTS13 and the enhanced plasma ADAMTS13 activity in a rat model of steatohepatitis during the process of liver fibrosis, where hepatic stellate cells are known to proliferate, suggesting that hepatic stellate cells in the liver play a significant role in the regulation of plasma ADAMTS13 activity.[39] Thus, it is speculated that stellate cells remain functional until the very last stage of liver fibrosis,[25] and the level of ADAMTS13, which is produced by stellate cells, may be maintained until the most advanced stage of liver cirrhosis,[40] which agrees well with our findings and those of a previous report[30] in which little difference in the ADAMTS13 level was noted among the different stages of liver fibrosis. The regulatory mechanism responsible for ADAMTS13 production by hepatic stellate cells in advanced cirrhosis and the inactivation of ADAMTS13 in humans requires further elucidation.

The vWF antigen value increased significantly with the progression of the stage of liver fibrosis in this study, in agreement with the results of a previous report.[30] Since the vWF value was not correlated with that of ADAMTS13, the increase in the vWF antigen value is likely due to its release from activated endothelial cells. von Willebrand factor antigen is negatively

correlated with the platelet count; in the multiple regression analysis, it was extracted as an explanatory variable for thrombocytopenia, although the P value was significance less than that for splenomegaly. Thus, an elevation in the vWF antigen value may be partly responsible for the thrombocytopenia in patients with chronic HCV infection. However, the reference value for vWF antigen is considerably wide among healthy individuals, and further studies are needed to determine whether the difference in the vWF antigen value according to the stage of fibrosis is related to thrombocytopenia and to elucidate the possible mechanism.

Thrombomodulin is expressed on vascular endothelial cells and acts to regulate coagulation pathways by interacting with thrombin, producing activated protein C. Since TM is cleaved and released into the circulation during inflammatory processes, it is used as a marker of endothelial cell damage. Of particular interest is that this marker can be used to predict endothelial damage in the liver, independent of systemic circulation.[41] In this study, we found that the TM level increased with the progression of the stage of liver fibrosis, in the absence of an elevation in the CRP level, representing systemic inflammation. In chronic HCV infection, inflammatory processes in the liver are assumed to play a role in fibrotic changes, and our findings suggest that TM can be a good marker in predicting endothelial damage, that is, the process of inflammation and fibrosis in the liver. Although a negative correlation was observed between TM and the platelet count, this factor was not extracted as an explanatory variable in the multiple regression analysis. We found a good correlation between the vWF antigen value and TM ($r = .48$, $P < .0001$; Figure 4B), suggesting that both parameters may represent endothelial dysfunction induced by inflammatory changes in the liver. Thrombomodulin might not have been extracted as an explanatory variable in the multiple regression analysis as a result of its close association with vWF antigen. Our notion is in good agreement with a previous report that in liver damage induced by HBV and HCV infection in children, vWF antigen and TM can predict endothelial cell function.[42] Taken together, our findings, along with those of the previous report, suggest that TM can serve as a marker for inflammatory changes in the liver in patients with chronic HCV infection. Furthermore, thrombocytopenia in this disorder may be related to endothelial dysfunction revealed by its activation, that is, vWF release, and its damage, that is TM cleavage. This hypothesis seems consistent with previous results indicating that TM and vWF change in parallel as endothelial dysfunction markers.[43]

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Polymorphisms and mutations of *ADAMTS13* in the Japanese population and estimation of the number of patients with Upshaw–Schulman syndrome

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Upshaw–Schulman syndrome (USS), also called hereditary thrombotic thrombocytopenic purpura, is an autosomal recessive disease characterized by thrombocytopenia and microangiopathic hemolytic anemia. USS is associated with hereditary severe deficiency of plasma ADAMTS13 activity; patients with USS have homozygous or compound heterozygous mutations in the *ADAMTS13* gene [1–5]. ADAMTS13 is a plasma metalloprotease that regulates platelet aggregation through the cleavage of von Willebrand factor (VWF) multimers. ADAMTS13-deficient plasma derived from patients with USS contains unusually large VWF multimers, which can induce unwanted hyperaggregation of platelets and microvascular thrombi. In this study, we analyzed the relationship between genetic variation of *ADAMTS13* and plasma ADAMTS13 activity in the Japanese general population. In addition, on the basis of the data obtained via our genetic analysis, we estimated the number of patients with USS in Japan.

The population examined is based on the Suita Study [6], an epidemiologic study consisting of randomly selected Japanese residents of Suita City, which is located in the second largest urban area in Japan. Our study protocol was approved by the ethical review committee of the National Cerebral and Cardiovascular Center, and only subjects who provided written informed consent for genetic analyses were included.

To identify common polymorphisms in the population, we first sequenced all 29 exons and exon–intron boundaries of *ADAMTS13* using 346 consecutive subjects, by means of previously described methods [2]. We identified 25 polymorphisms with allele frequencies of the respective minor allele > 0.01, including two in the promoter region, 10 in the exons, and 13 in the introns. Of these, six were missense single-nucleotide polymorphisms (SNPs): p.T339R (c.1016C > G), p.Q448E (c.1342C > G), p.P475S (c.1423C > T), p.P618A (c.1852C > G), p.S903L (c.2708C > T), and p.G1181R (c.3541G > A). Next, we performed TaqMan genotyping assays (Applied Biosystems, Tokyo, Japan) for the missense SNPs, using 3616 subjects whose plasma ADAMTS13 activities had been measured with the FRETS-VWF73 assay [7]. Allele frequencies for the minor alleles were 0.027 for p.T339R, 0.192 for p.Q448E, 0.050 for p.P475S, 0.027 for p.P618A, 0.048 for p.S903L, and 0.022 for p.G1181R. The observed genotypes did not deviate significantly from Hardy–Weinberg equilibrium. The p.T339R and p.P618A SNPs were in absolute linkage disequilibrium (r^2 = 0.97), whereas the other missense SNPs were not strongly linked (r^2 < 0.11).

The p.Q448E and p.P475S SNPs, but not the other missense SNPs, were significantly associated with plasma ADAMTS13 activity (Fig. 1A). The ADAMTS13 activity (97% ± 25% in men, 111% ± 28% in women, mean ± standard deviation) of p.Q448E heterozygotes (QE) and minor allele homozygotes (EE) was slightly but significantly higher than that of major allele homozygotes (QQ) (91% ± 24% in men, 104% ± 26% in women). In contrast, the ADAMTS13 activity (79% ± 20% in men, 92% ± 24% in women) of p.P475S heterozygotes (PS) and minor allele homozygotes (SS) was significantly lower than that of major allele homozygotes (PP) (94% ± 24% in men, 108% ± 27% in women). The difference in activity was consistent with the observation that the recombinant ADAMTS13-P475S mutant has approximately 70% of the activity of wild-type ADAMTS13 [8]. It is interesting that p.P618A was not associated with plasma ADAMTS13 activity in the present study, whereas the conditioned medium of HEK293 cells expressing the A618 variant showed lower levels of activity (27%) and antigen (14%) than the wild type [9]. PolyPhen-2, a program that predicts damaging missense mutations [10], identified p.T339R and p.P618A as 'possibly damaging' and 'probably damaging', respectively, whereas the other four SNPs were predicted to be 'benign'.

As the *ADAMTS13* locus is near (130–190 kb) the *ABO* locus on chromosome 9q34, we compared the frequencies of the SNPs among ABO blood group genotypes (Fig. 1B). The relative frequencies of p.T339R minor allele homozygotes and

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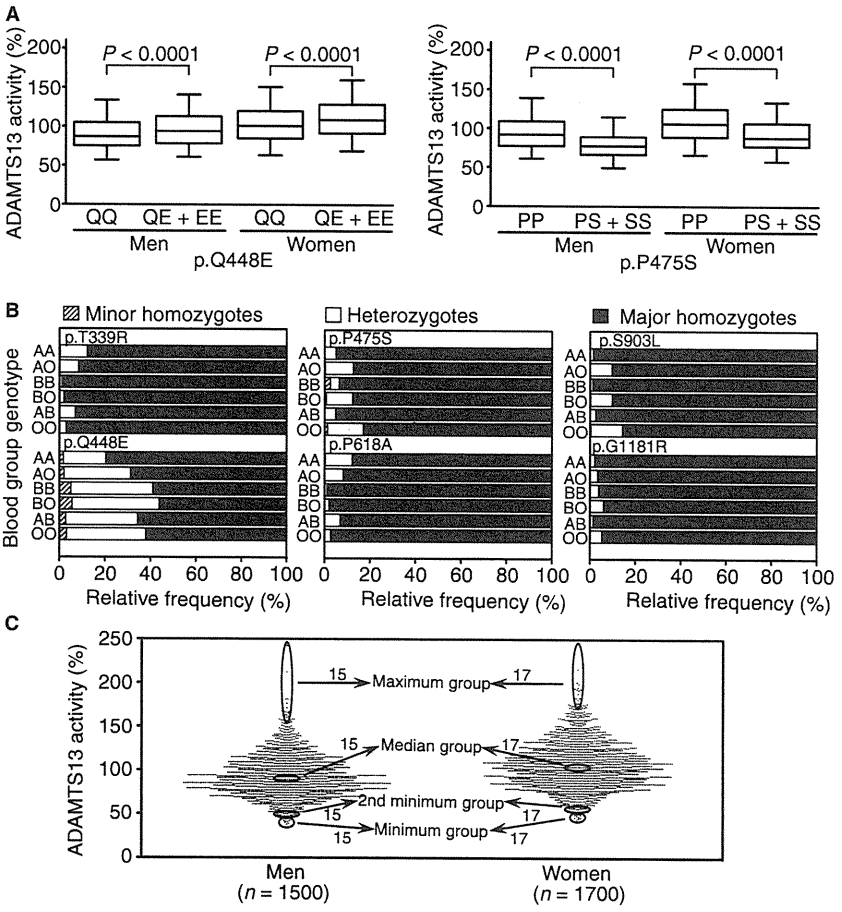

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		Min. (n = 32)	2nd min. (n = 32)	Med (n = 32)	Max. (n = 32)
Average activity		47.1%	53.1%	97.6%	183%
Common SNP	p.T339R	2	1	3	0
	p.Q448E	8 (1)	8	8	16
	p.P475S	9 (2)	6	2	1
	p.P618A	2	1	3	0
	p.S903L	2	3	5	3
	p.G1181R	2	4	1	1
Rare mutation		7	3	2	2

Fig. 1. *ADAMTS13* variation in a Japanese general population. (A) Box-and-whisker plot (5th–95th percentiles) of plasma ADAMTS13 activity in each genotype of p.Q448E and p.P475S. *P*, Kruskal–Wallis test. (B) The relative frequency of minor homozygotes, heterozygotes and major homozygotes for each genetic polymorphism. (C) Scatter dot plot of plasma ADAMTS13 activity for men and women. On the basis of these activity measurements, 128 subjects were selected for sequencing of *ADAMTS13*. (D) The numbers of minor allele carriers in each group. One in eight p.Q448E carriers and two in nine p.P475S carriers in the minimum group were homozygotes for the respective minor alleles.

heterozygotes were higher for AA, AO and AB than for BB, BO and OO, suggesting that p.T339R is associated with the blood group A allele. The p.P618A SNP, which is tightly associated with p.T339R, exhibited the same pattern. The p.P475S and p.S903L SNPs tended to be associated with the blood group O allele.

We then utilized the plasma ADAMTS13 activity data to estimate the frequency of hereditary ADAMTS13 deficiency. In the population, 3200 DNA samples (1500 men and 1700 women) were available, from a quantitative standpoint, for sequencing of *ADAMTS13*. We selected 128 subjects according to their plasma ADAMTS13 activity (Fig. 1C): 32 subjects of the 'minimum' group (average activity, 47.1%), consisting of 15 men and 17 women with the lowest activities in each gender; 32 subjects of the 'second minimum' group (53.1%), consisting of 15 men and 17 women with the second lowest activities; 32 subjects of the 'median' group (97.6%), consisting of 15 men and 17 women with median activities; and 32 subjects of the 'maximum' group (183%), consisting of 15 men and 17 women with the highest activities. Each group corresponds to 1% of the population examined. All DNA samples from the four groups were subjected to *ADAMTS13* sequencing, which

revealed that 70 individuals had at least one of the six missense SNPs described above (Fig. 1D). Of these, only p.P475S showed a significant difference in minor allele frequency among four groups (P = 0.028, chi-square test). In addition, 14 individuals had rare non-synonymous mutations: seven (p.F324L, p.F418L, p.I673F, p.Q773X, p.Y1074AfsX46, p.R1095Q, and p.S1314L) in the 'minimum' group; three (p.I380T, p.Y1074AfsX46, and p.R1274C) in the 'second minimum' group; two (p.Q723K and p.N1321S) in the 'median' group; and two (p.L19F and p.R268Q) in the 'maximum' group. Of these, p.I673F (c.2017A > T) and p.Y1074AfsX46 (c.3220delTACC) had been identified as causative mutations in patients with USS [11,12]. All of the others were newly identified mutations.

To estimate the number of individuals with a hereditary ADAMTS13 deficiency, we generated several hypotheses: (i) as two individuals in each of the 'median' and 'maximum' groups had rare mutations, two of every 32 people should have a mutation that does not cause a functional defect of ADAMTS13; (ii) thus, five (= 7 – 2) individuals in the 'minimum' group and one (= 3 – 2) individual in the 'second minimum' group should be the heterozygotes carrying a mutation with a functional defect; (iii) other than these six (= 5 + 1) individuals in the 'minimum' and 'second minimum' groups, no individual should have any mutations that confer a functional defect. These hypotheses were consistent with a prediction based on PolyPhen-2: the p.S1314L, p.I380T, p.Q723K, p.N1321S, p.L19F and p.R268Q mutations are 'benign', p.I673F and p.R1274C are 'possibly damaging', and the others are 'probably damaging'. According to the hypotheses, we estimated that six of 3200 individuals were heterozygotes for ADAMTS13 deficiency. This estimation suggested that ~ 1 individual in 1.1×10^6 (= 6/3200 × 6/3200 × 1/4) should be a homozygote or a compound heterozygote for ADAMTS13 deficiency. In Japan, which has a population of approximately 1.3×10^8, ~ 110 individuals may have hereditary ADAMTS13 deficiency or USS. If we adjusted our estimate of ADAMTS13 deficiency from 6/3200 to 7/3200 or 5/3200, the number of patients would be 160 or 80, respectively. The validity of these calculation procedures was confirmed by StaGen Co., Ltd (Chiba, Japan), a company specializing in genetics, statistics, and data analysis.

In conclusion, this study demonstrated that, in the Japanese general population, there are six common missense SNPs: p.T339R, p.Q448E, p.P475S, p.P618A, p.S903L, and p.G1181R. Of these, p.Q448E and p.P475S are significantly associated with plasma ADAMTS13 activity. Allele frequencies of these SNPs correlate with ABO blood group. Finally, we estimated the number of patients with USS in Japan, yielding a figure that corresponds to approximately three times the number of patients already diagnosed as having this condition. Because of insufficient sample sizes, we may have underestimated the prevalence of USS. Further studies are needed to obtain more reliable conclusions.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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CASE REPORT

Successful fertility management of a patient with factor V deficiency: planned transfusion of fresh frozen plasma under infertility treatment

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Objective: To report the case of a patient with factor V deficiency who achieved pregnancy with the planned transfusion of fresh frozen plasma (FFP) while monitoring follicle development and ovulation induction using gonadotropin.
Design: Case report.
Setting: University hospital.
Patient(s): A 28-year-old nulliparous female.
Intervention(s): Medical management including infertility treatment.
Main Outcome Measure(s): Clinical follow-up.
Result(s): A patient with factor V deficiency experienced repeated ovulation-related hemoperitoneum following the withdrawal of oral contraceptive pills. The monitoring of follicle development and ovulation induction using gonadotropin followed by FFP transfusion was useful to avoid hemoperitoneum. Pregnancy was achieved within a relatively short period using intrauterine insemination.
Conclusion(s): Planned prophylactic FFP administration and intervention with infertility treatment might be useful to minimize the risk of ovulation-related hemoperitoneum in patients with factor V deficiency. (Fertil Steril® 2011;95:2124.e5–e7. ©2011 by American Society for Reproductive Medicine.)

Key Words: FFP, FV deficiency, hemoperitoneum

Congenital factor V (FV) deficiency is a rare bleeding disorder caused by homogenous or compound heterozygous mutations of the FV gene. It is an inherited autosomal recessive trait, and more than 60 mutations associated with FV deficiency have been reported (1). No precise epidemiologic data exist for congenital FV deficiency, but its prevalence has been estimated to be 1 in 1,000,000 persons (2). Patients with FV deficiency experience bleeding, mainly into the skin, mucous membranes, joints, and muscles (3).

In the field of gynecology, menorrhagia is one of the most common bleeding symptoms in females with inherited bleeding disorders, including FV deficiency (1). In those with normal hemostatic function, ovulation-related hemoperitoneum may be of little clinical consequence. However, more serious bleeding episodes have been described in female patients with von Willebrand disease (4, 5), hemophilia A (6), congenital afibrinogenemia (7, 8), factor X deficiency (9), and factor XIII deficiency (10). We report the case of an FV-deficient patient who desired to become pregnant and was treated with the prophylactic administration of fresh frozen plasma (FFP) accompanied with anaphylactic reactions against repeated occurrences of ovulation-related hemoperitoneum.

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CASE

The patient's coagulation defect was diagnosed at 3 months of age because of an intracranial hemorrhage. Her plasma FV activity was 0.7%, and molecular analysis revealed compound heterozygous defects. She had repeated bleeding into the skin, muscles, and joints and was treated with FFP. She had experienced menorrhagia since menarche at the age of 13 years. However, she needed neither FFP administration nor the routine use of oral contraceptives. At the age of 18 years, she first experienced intraabdominal bleeding after ovulation. Oral contraceptive pills were routinely administered starting at the age of 22 years, and successfully prevented the relapse

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of ovulation-related hemoperitoneum. At 28 years of age, she wanted to become pregnant and discontinued oral contraceptive pills. Transvaginal ultrasound showed a leading follicle of 16 mm in diameter on the thirty-third day of menstruation. She had acute abdominal pain the next day. Ultrasonographic examination showed a hemorrhagic cyst in the right ovary and a moderate amount of free fluid in the pelvis. A blood test showed a prothrombin time of 47.4 seconds (17% of control), an activated partial thromboplastin time of 145.3 seconds (18% of control), and a fall in hemoglobin level from 13.5 to 10.4 g/dL. FFP was administered for 2 days, and her symptoms improved. Because of previous allergic reactions to FFP, 100 mg of hydrocortisone sodium succinate (Solu-Cortef; Pfizer Japan, Tokyo, Japan) was administered intravenously as premedication. Because the prophylactic FFP administration was necessary for every ovulation period, controlled ovarian stimulation using gonadotropin (Gonapure; ASKA Pharmaceutical, Tokyo, Japan) and intrauterine insemination was performed to achieve pregnancy. The follicle development was monitored with transvaginal ultrasound every other day, and 5,000 IU of human chorionic gonadotropin (Gonatropin; ASKA Pharmaceutical) was administered intramuscularly in the morning of the day before intrauterine insemination when a leading follicle reached >18 mm in diameter; 10 IU of FFP with 100 mg of hydrocortisone sodium succinate was administered after hCG. Intrauterine insemination was performed at approximately 9:00 AM the next day. It was confirmed with transvaginal ultrasound 1 or 2 days after ovulation that no hemorrhagic cysts or no intraabdominal bleeding occurred. Pregnancy was achieved in the fifth treatment cycle with a similar protocol, which resulted in a 9-week spontaneous complete abortion, and neither FFP nor dilatation and curettage was needed. On-going pregnancy was achieved in the third treatment cycle after miscarriage. The course of the pregnancy was uneventful until 30 weeks' gestation. The patient then had severe lower abdominal pain at 31 weeks' gestation. An emergency Cesarean section, accompanied by FFP administration, was performed under general anesthesia. Blood loss during surgery was 1065 g. The infant was born with a weight of 1642 g (1- and 5-min American Pediatric Gross Assessment Record scores were 4 and 7, respectively); 10 IU of FFP was administered daily for 4 consecutive days after the operation. The patient's postoperative course was uneventful, and the patient was discharged on the seventh day after the birth of a healthy boy.

DISCUSSION

Severe FV deficiency is characterized by FV levels <10% and represents the phenotypic expression of mutations in a homozygous or combined heterozygous state (11). Patients who are homozygous have a median FV activity of <0.01 U/mL (range < 0.01–0.05). All ultimately experience bleeding, mainly into the skin and mucous membranes (44%), joints and muscles (23%), and genitourinary (19%) and gastrointestinal tracts (6%) (3). The mainstay of treatment for bleeding episodes including menorrhagia is FFP, because no FV-specific concentrate is available (11).

After ovulation, the follicles may form hemorrhagic ovarian cysts, which cause a hemoperitoneum if rupture occurs (12). This ovulation-related hemoperitoneum has been seldom described in inherited bleeding disorders, although life-threatening bleeding episodes might occur. Girolami et al. (13) reviewed patients with rare coagulation disorders who had ovulation-related hemoperitoneum as a bleeding manifestation. Only three cases, including the present case, have been reported regarding treatment for ovulation-related hemoperitoneum in FV deficiency (Table 1) (13, 14). The

TABLE 1
Prevention of ovulation-related hemoperitoneum in patients with factor V deficiency.

Case	Year	Other symptoms	Age at onset	Details on hemoperitoneum	Management	Pregnancy and delivery
Girolami et al. (13)	2008	Recurrent epistaxis and menorrhagia	18	Laparotomy with FFP and WB transfusion	OC for 21 y	No attempted pregnancy
Coppola et al. (14)	2010	Easy bruising, epistaxis and menorrhagia	18	FFP and RBC transfusion; laparotomy with partial resection of the left ovary	OC until 33 y of age	Spontaneous pregnancy; cesarean section with FFP and recombinant activated factor VII
Present case	2010	Bleeding into the skin, muscles, and joints and mild menorrhagia	18	FFP transfusion	OC until 28 y of age	Intervention with infertility treatment; cesarean section with FFP

Note: FFP = fresh frozen plasma; WB = whole blood; OC = oral contraceptive; RBC= red blood cell.

Iwase. Pregnancy attempt in FV deficiency. Fertil Steril 2011.

prevention of recurring ovulation-related hemoperitoneum is desirable in order to avoid life-threatening bleeding and loss of ovarian function because of surgery.

Combined oral contraceptive pill use has been widely accepted for its efficacy in managing of dysmenorrhea, menorrhagia, and metrorrhagia. Patients with menorrhagia owing to inherited bleeding disorders may also benefit from hormonal therapy (3). Moreover, hormonal suppression of ovulation with the use of the combined oral contraceptive pill has successfully prevented recurrence in patients with rare bleeding disorders, including FV deficiency (12, 13). However, a problem can arise if the woman wishes to suspend contraceptive treatment in order to conceive. In addition, pregnancy and delivery seem to represent a dangerous challenge for patients with severe FV deficiency. Moreover, it is possible that such factors may have been involved in a small number of pregnancies and deliveries reported previously (15, 16). In our case, the patient had been taking oral contraceptive pills for 6 years and wished to become pregnant. Therefore, we planned to perform the prophylactic administration of FFP during ovulation. For patients with homozygous deficiencies of factors X, V, XIII, and VII, afibrinogenemia, and dysfibrinogenemia, allergic reactions to plasma products were reported to occur in 2%–26% of treatment episodes (3). The administration of FFP with prophylaxis against allergic reactions every ovulation period brings physical burdens and high cost to the patient. Close monitoring for the follicle development and ovulation made it possible to prevent ovulation-related hemoperitoneum with a single dose of FFP; it also maximized the patient's chances of achieving pregnancy.

In summary, the planned pregnancy attempted with the hormonal control of ovulation and menstruation, the monitoring of follicle development, prophylactic FFP administration, and intervention with infertility treatment was found to be useful for minimizing the risk of ovulation-related hemoperitoneum in a patient with FV deficiency. This treatment approach might therefore be useful in the treatment of other similar cases.

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ORIGINAL ARTICLE

Epidemiology of primary immune thrombocytopenia in children and adults in Japan: a population-based study and literature review

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Abstract The epidemiology of primary immune thrombocytopenia (ITP) is not well-characterized in the general population. Most published studies, which have included relatively small numbers of ITP patients, have been conducted in England or Scandinavian countries. No epidemiologic data from Asian countries have been published. This study describes the epidemiology of ITP in a Japanese population. We analyzed the database registry of the Ministry of Health, Labour, and Welfare of Japan, and extracted newly diagnosed acute and chronic ITP patients with a platelet count of $<100 \times 10^9$/L. From 2004 to 2007, 7,774 cases of ITP were reported, giving an overall incidence of 2.16/100,000/year. The incidence differed greatly between males and females, being 1.72 and 2.58, respectively. The median age of the total affected population was 56 years old. In male patients, there was a striking preponderance of boys below 4 years and a very high peak among those aged 75–89 years. In female patients, the number of ITP patients appeared to show a trimodal distribution by age, with the first peak representing patients below 4 years, the second peak those aged 20–34 years, and the third peak those aged 50–89 years. In conclusion, the incidence of ITP in Japan is not markedly different from that of European countries studied to date. This population-based study reveals that, contrary to previously published studies, the maximum age-specific incidence is in the eighth decade.

Keywords ITP · Epidemiology · Incidence

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1 Introduction

Primary immune thrombocytopenia (ITP) is an autoimmune bleeding disorder in which antiplatelet autoantibodies bind to antigens on the surface of platelets and cause accelerated destruction [1]. Impaired platelet production may also contribute to the low platelet counts [2].

There are only limited data on the incidence of ITP [3–12]. Published studies describe a relatively small number of ITP patients.

We aim to update earlier estimates of the incidence of this disease, as these are essential in designing treatment trials and planning services for the patient population. In the present study, we report up-to-date estimates of the incidence of ITP using the large database of the Ministry of Health, Labour, and Welfare of Japan.

2 Materials and methods

2.1 Patients

The diagnosis of ITP was based on thrombocytopenia (platelet count less than 100×10^9/L), normal or increased

Table 1 Overall and sex-specific incidence of child and adult ITP

	Overall		Childhood		Adult	
	Number of patients	Incidence	Number of patients	Incidence	Number of patients	Incidence
Overall ITP	7,774	2.16	929	1.91	6,845	2.20
Male	3,043	1.72	505	2.01	2,538	1.68
Female	4,731	2.58	424	1.79	4,307	2.69

bone marrow megakaryocytes without morphologic evidence of dysplasia, and no secondary immune or non-immune disease that could account for the thrombocytopenic state [13]. Acute and chronic ITP patients were included in this analysis.

2.2 Health care system for intractable disease in Japan

In Japan, ITP is considered to be an intractable disease and treated as a specified disease. Specified diseases are subsidized partly or totally based on the severity of the disease by public expense. A physician who newly diagnoses and treats an ITP patient issues a medical certificate and an application form for the recognition of the specified disease, which certifies that the patient is suffering from ITP and describes detailed clinical information. The patient applies for the recognition of the specified disease to the Department of Health and Medical Care of the regional prefecture through the regional public health center. Before the application form is registered in the centralized database, the form undergoes a series of quality checks, such as the accuracy of diagnosis according to the criteria mentioned above. Thereafter, the data are sent from the Department of Health and Medical Care of the regional prefecture to the database of the Ministry of Health, Labour, and Welfare of Japan.

2.3 Data analyses

We analyzed the database for the years 2004–2007. The database includes details of patient characteristics, hemorrhagic symptoms, prescription information, and laboratory tests. Population information was obtained from the census of the Ministry of Internal Affairs and Communications. The population included in this study comprised an average of 90.0 million inhabitants, corresponding to 71% of the total Japanese population.

ITP patients were divided by age into those with child and adult ITP. Child ITP was that in patients below 14 years.

Comparisons of therapies and platelet counts were performed using the χ^2 test with Yates's correction. Significance was defined as a probability value of less than 0.05.

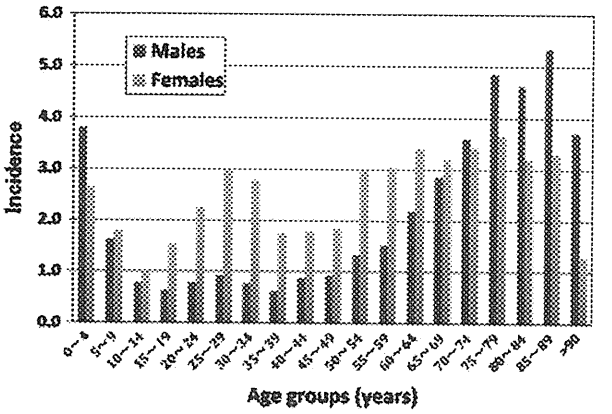

Fig. 1 Age/gender-specific incidence of ITP per 100,000 population

3 Results

3.1 Incidence

There were 7,774 patients in the database, and they consisted of 4,731 females (61%) and 3,043 males (39%) in the years 2004–2007 (Table 1). The annual incidence was 2.16 per 100,000. The incidence differed greatly for males and females. The incidence in females was 2.58, while that in males was 1.72. ITP patients were divided into those with child and adult ITP. The 7,774 patients consisted of 929 with child ITP (females 424; males 505), and 6,845 with adult ITP (females 4,307; males 2,538). The incidence of child ITP was 1.91 (females 1.79; males 2.01), and that of adult ITP was 2.20 (females 2.69; males 1.68).

3.2 Age and sex distribution

The median age of the entire population was 56 years (females 54 years; males 60 years). Figure 1 shows the age/gender-specific incidence, and Fig. 2 shows the age and sex distribution of ITP patients.

In male patients, there was a marked preponderance of boys among newborns and infants below 4 years, after which the incidence fell to a low level between 10 and 49 years old, thereafter rising gradually with increasing age. The highest peak of the incidence was among those aged 75–89 years (Fig. 1), but the peak of the number of patients moved to those aged 65–79 years (Fig. 2). Male