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8. 知的財産権の出願・登録
なし

調査内容

《性　別》 □男　□女

《PE発症時 年齢》 _____ 歳

《精神科 疾患名》 _____.

《その他の合併疾患名》
□糖尿病 □高脂血症 □高血圧 （□喫煙）
□その他（　　）

《PE発症時の内服薬・注射薬》

向精神薬
■抗精神病薬
・フェノチアジン系 □クロルプロマジン □レボメプロマジン
□その他
（　　）
・ブチロフェノン系 □ハロペリドール □ブロムペリドール
□その他
（　　）
・ベンズアミド系 □スルピリド □その他
（　　）
■その他 □リスペリドン □その他
（　　）
□抗不安薬（　　）
□抗うつ薬（　　）
□抗躁薬（　　）
□睡眠導入薬（　　）
□その他（　　）
その他の薬剤（　　）

調査内容(続き)

《精神科入院からPE発症までの日数》 _____ 日

《PE発症時の重症度》 □心肺停止　□ショック　□非ショック

《30日後の転帰》 □生　□死（PE発症から死亡までの日数:
_____日）

《30日以内に死亡した場合の死因》 □肺塞栓症　□それ以外
（死因:　　）

《PEの危険因子》 □3日以上の臥床状態　□悪性疾患　□麻痺のある脳血管障害
□手術後1ヶ月以内　□骨折後1ヶ月以内　□外傷後1ヶ月以内
□出産後1ヶ月以内
□妊娠中　□肥満（BMI≧25%）　□活動中の感染症　□脱 水
□歩行障害
□中心静脈カテーテル留置　□慢性心不全　□慢性呼吸不全
□抗リン脂質抗体症候群　□プロテインC欠乏症　□プロテインS欠乏症　□アンチトロンビン欠乏症

《PE発症時の状況》 □身体拘束　□薬物による活動性低下
□精神科疾患による活動性低下

《その他のPEの危険因子と思われる因子や状況》

_____.

《PEの予防》 □予防なし　□弾性ストッキング　□間欠的空気圧迫法
□抗凝固療法　□その他の予防(　　)

新潟県中越地震被災者の慢性期静脈血栓に対する対照検査

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研究要旨
新潟県中越地震1年後の DVT が震災の影響であるか否かを検証するために対照地域検査を行った。新潟県中越地震被災地とよく似た新潟県阿賀町を対照地域とした。対象者は新潟県中越地震被災地で集めたのと同様に集めた一般住民327 人を対象とし下肢静脈エコー検査と血液検査を行った。対象となった 327 人のうち6人(1.8%)にヒラメ静脈に血栓を認め、そのうち4人(1.2%)に浮遊血栓を認めた。また対照地域住民の右ヒラメ静脈最大径は5.7±1.7mm、左ヒラメ静脈最大径は5.9±1.9mm であった。D ダイマーについては基準値の2倍である2.0μg/ml 以上は5人であったが全員血栓を認めなかった。1年後の被災地では1260 人のうち92 人(7.3%)に血栓を認め、特に浮遊血栓は35 人(2.8%)に認め対照地域よりも有意に高かった(p<0.0001)。被災者の右ヒラメ静脈最大径の平均は7.8±1.9mm(n=1501)、左ヒラメ静脈最大径 7.1±1.9mm(n=1491)であり、それぞれ対照地域よりも有意に大であった (p<0.00000001)。したがって中越地震1年後に見つかった DVT は地震と関連あることが確認された。一方、日本人でもヒラメ静脈血栓の頻度は低くない可能性が示唆された。

1．研究目的

新潟県中越地震発生から1年経っても被災地では多数の深部静脈血栓症 (DVT) が見つかることを報告してきたが、この被災地で見つかる DVT が震災の影響であるか否かを検証するために新潟大学、新潟県及び新潟県医師会と共同で2006 年3月に対照地域検査を行った。

2．研究方法

新潟県中越地震被災地とよく似た環境である山間部豪雪地帯で新潟県中越地震の震央から約 100km 離れ、新潟県と福島県の県境に位置する新潟県阿賀町を対照地域とした。対象者は新潟県中越地震被災地で集めたのと同様に阿賀町の広報や地域の保健師などにより「下肢に症状のある方、足の病気が心配な方、症状は無いが検査して欲しい方などは検査に来てください無料で検査します」と呼びかけていただき募集した。そこで集まった阿賀町の一般住民 327 人(男女比 1:2、平均年齢 63±13 才)を対象とし、アンケート調査と 2006 年 3 月 9 日と 12 日に下肢静脈エコー検査および採血を行った。エコー機器は医療機器メー

カーの無償提供協力をしていただいた。下肢静脈エコー検査は7.5MHz以上のリニア型プローベを用いて膝窩静脈を含めた下腿静脈のみを検査した。血栓の有無は輝度の高い血栓エコーの有無またはプローベの圧迫による静脈虚脱の有無で判断した。またヒラメ静脈の短軸像において最大径を超音波の走査線方向（縦軸）で計測し、左右のうち最大のものを最大径とした。採血ではフィブリンモノマーコンプレックスとDダイマーを検査した。血液検査は検査会場で採血した後で血漿を遠心分離して冷蔵保存しBML(株)に回収してもらい後日 BMLで測定を行った。

3．研究結果

2005年12月22日に新潟県で風雪による大規模な停電が起きた（新潟大停電）。その際に新潟県阿賀町では最大48時間の停電のあった地域が存在していた。アンケート調査の結果、検査を受けた方のうち38人が停電の際に寒さと灯りが無いために乗用車やバスに避難していたことが判明した。この38人については新潟県中越地震後の車中泊避難と似た環境におかれた可能性があるために対照検査対象から除外した。また対象となった327人のうち162人は65才未満であった。6人(1.8%)にヒラメ静脈に血栓を認め、そのうち4人(1.2%)に浮遊血栓を認めた。血栓のあった6人のうち1人のみ65才未満であり、その他は65才以上であった。65才未満の血栓有病率は0.6%、65才以上の血栓有病率は3.0%であった。また血栓があった6人のうちで6ヵ月前に外傷の既往1人(浮遊血栓)、過去にDVTの既往が1人（壁在血栓）あった。したがって病気やDVT既往もなく健常な方で血栓があったのは4人(1.2%)であった。また対照地域住民では血栓有りも含めて右ヒラメ静脈最大径は5.7±1.7mm、左ヒラメ静脈最大径は5.9±1.9mmであった。Dダイマーについては基準値の2倍である2.0μg/ml以上は5人であったが全員血栓を認めなかった。逆に血栓を認めた方のDダイマー値は0.78±0.39μg/mlであった。

4．考察

被災地の長岡市、小千谷市、十日町市において2005年9月30日から2006年1月17日に行った検査では対象者1260人のうち92人(7.3%)に血栓を認め、特に浮遊血栓は35人(2.8%)に認めた。これらの血栓頻度は対照地域よりも有意に高かった($p<0.0001$)。また被災者のうち65才未満の血栓陽性率は6.6%、65才以上の血栓陽性率は12.9%であった。したがって対照地域に比べて被災地では65才未満で10倍、65才以上で3倍の血栓陽性率であった。これは震災後に若年者に負担が大きかったことを示唆していると思われた。また新潟県中越地震被災者の右ヒラメ静脈最大径の平均は7.8±1.9mm(n=1501)、左ヒラメ静脈最大径 7.1±1.9mm(n=1491)であり、それぞれ対照地域よりも有意に大であ

った（$p<0.00000001$）。Dダイマーに関して地震1年後の被災者においては基準値よりも2倍であった方のうち45.5%に血栓を認め、基準値の2倍以下では7.7%であったことからDダイマーと血栓との間に関連が認められている。しかし対照地検査ではDダイマーと血栓の有無との間に関連は認められなかった。これは被災地での血栓が少なくとも1年以内に発生したものであるのに対し、対照地では1年以上経過している慢性血栓が多いためではないかと考えられた。

以上から今回の検討により新潟県中越地震1年後に見つかったDVTは地震と関連あることが確認された。すなわち大地震では被災者にDVTが起きやすいことが明らかになった。その原因としてヒラメ静脈が対照地域よりも有意に拡張していたことから、車中泊避難や避難所における窮屈な姿勢による静脈うっ滞が関係していることが示唆された。さらに被災地では対照地域よりも若年者に血栓が多く発生していることから、若年者でもDVTの危険が低くないことを示している。また日本人では欧米の白人に比してDVTの頻度が低いことが報告されてきた。一方、今回の対照地域検査結果では日本人でも慢性を含めたヒラメ静脈血栓の頻度は低くない可能性が示唆され、頻度も欧米と同じ程度である可能性が示唆された。慢性化した血栓も急激な慢性反復性(acute on chronic)のDVTを惹起して致死性の肺塞栓を起こすことも報告されていることから看過することができないと考えられ、今後豪雪山間部以外の地域での一般住民における下腿静脈血栓の頻度調査が必要であると思われた。

5．結論

新潟県中越地震1年後に見つかったDVTは地震と関連あることが確認された。すなわち大地震では被災者にDVTが起きやすいことが明らかになった。その原因としてヒラメ静脈が対照地域よりも有意に拡張していたことから、車中泊避難や避難所における窮屈な姿勢による静脈うっ滞が関係していることが示唆された。

（謝辞）
この対照地域検査は新潟県及び新潟県医師会からの予算を使用して行われ、検査で得られたデータの解析については国立病院機構新潟病院の援助と厚生労働省科学研究費を使用して行いました。また関係した多くの先生方に感謝申し上げます。

6．健康危険情報

なし

7．研究発表

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別添６

研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表

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研究成果の刊行物・別冊

Rheumatology 2006;45:851–854
Advance Access publication 17 January 2006

doi:10.1093/rheumatology/kel010

Concise Report

Two types of autoantibody-mediated thrombocytopenia in patients with systemic lupus erythematosus

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Objectives. **To determine whether autoantibodies to two platelet-specific antigens, glycoprotein IIb/IIIa (GPIIb/IIIa) and thrombopoietin receptor (TPOR), contribute to thrombocytopenia in patients with systemic lupus erythematosus (SLE).**
Methods. **Circulating B cells producing anti-GPIIb/IIIa antibodies and serum anti-TPOR antibodies were measured in 32 SLE patients with thrombocytopenia, 30 SLE patients without thrombocytopenia, 92 patients with idiopathic thrombocytopenia and 60 healthy controls. The megakaryocyte density in bone-marrow smears from all the patients with thrombocytopenia was evaluated.**
Results. **Anti-GPIIb/IIIa and anti-TPOR antibody responses were more frequent in SLE patients with thrombocytopenia than in those without thrombocytopenia (88 *vs* 17%, $P<0.0001$; and 22% *vs* 0%, $P=0.01$, respectively). The frequencies of these platelet-related antibodies were comparable between SLE patients with thrombocytopenia and patients with idiopathic thrombocytopenia. Twenty-nine (91%) SLE patients with thrombocytopenia had either anti-GPIIb/IIIa or anti-TPOR antibody, and six had both. In SLE patients with thrombocytopenia, the anti-TPOR-positive patients had significantly higher frequencies of megakaryocytic hypoplasia and poorer therapeutic responses to corticosteroids and intravenous immunoglobulin than did the anti-TPOR-negative patients, most of whom had the anti-GPIIb/IIIa antibody alone.**
Conclusions. **Anti-GPIIb/IIIa and anti-TPOR antibodies are major factors contributing to SLE-associated thrombocytopenia, but the clinical presentations associated with these autoantibodies are different.**

KEY WORDS: Autoantibodies, Glycoprotein IIb/IIIa, Systemic lupus erythematosus, Thrombocytopenia, Thrombopoietin receptor.

Thrombocytopenia is a major haematological complication in patients with systemic lupus erythematosus (SLE) [1]. The pathogenesis of thrombocytopenia in SLE patients is heterogeneous, but the most common mechanism is believed to be increased platelet clearance mediated by anti-platelet autoantibodies, which is analogous to the mechanism seen in patients with idiopathic thrombocytopenic purpura (ITP) [1]. Other potential mechanisms include thrombotic thrombocytopenic purpura, disseminated intravascular coagulation, haemophagocytic syndrome, antiphospholipid syndrome and impaired thrombopoiesis. Anti-platelet autoantibodies in ITP patients preferentially recognize platelet surface glycoproteins (GP), and the most common target is GPIIb/IIIa [2]. A recent study by Michel *et al.* [3] showed that anti-GPIIb/IIIa antibodies also play a primary role in SLE-associated thrombocytopenia. On the other hand, we recently identified autoantibodies to thrombopoietin receptor (TPOR), also called c-Mpl, which is clinically associated with thrombocytopenia in SLE patients and inhibits thrombopoietin (TPO)-dependent megakaryogenesis *in vitro* [4]. In this study, the roles of these two types of autoantibody responses in SLE-associated thrombocytopenia were evaluated.

Materials and methods

Patients and controls

We studied 32 patients with SLE who had thrombocytopenia (mean platelet count 23×10^9/l, range $5–57\times10^9$/l) and were followed at Keio University Hospital between 1997 and 2004. The inclusion criteria were as follows: (i) requirement for treatment because of a significant bleeding tendency; (ii) pretreatment bone marrow films were available; and (iii) exclusion of clinically apparent conditions that can cause thrombocytopenia, i.e. disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, haemophagocytic syndrome and drug-induced thrombocytopenia. The control subjects were 30 SLE patients who had never been thrombocytopenic and 60 healthy individuals. All the SLE patients satisfied the American College of Rheumatology preliminary criteria [5], and three with thrombocytopenia and four without thrombocytopenia additionally satisfied the Sapporo criteria for antiphospholipid syndrome [6]. We also examined 92 patients with idiopathic thrombocytopenia, defined as thrombocytopenia ($<100\times10^9$/l) that is not accompanied by morphological evidence of dysplasia in the bone marrow and cannot be attributed to other

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Received 17 October 2005; revised version accepted 8 December 2005.

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primary diseases or conditions. Blood samples were obtained after the patients and control subjects had given their written informed consent, as approved by the Keio University Institutional Review Board.

Clinical findings

The demographic and clinical features of each SLE patient were evaluated at the time of blood collection. Thirty-seven clinical and laboratory findings were recorded; these were the individual items included in the American College of Rheumatology preliminary classification criteria [5] and the SLE Disease Activity Index (SLEDAI) [7]. All SLE patients with thrombocytopenia received moderate- to high-dose oral corticosteroids (>40 mg/day; $n=26$) or methylprednisolone pulse therapy (1 g/day for 3 days; $n=6$), and eight of them simultaneously received intravenous immunoglobulin (IVIG; 0.4 g/day for 3–5 days) and/or platelet transfusion. During the course of the disease, 19 patients who required surgical or invasive procedure received IVIG without increase in the corticosteroid dosage or the initiation of immunosuppressant. A therapeutic response was defined as a platelet count $>100\times10^9$/l in association with these therapies. The efficacy of the corticosteroid treatment was assessed 3 months afterwards, when the potential influence of IVIG or platelet transfusion could be ignored; the efficacy of IVIG was assessed at 1 week.

Autoantibody analysis

Anti-double-stranded DNA antibody was measured by the Farr assay, and anti-Sm and anti-SSA antibodies were identified using an RNA immunoprecipitation assay [8]. IgG anti-cardiolipin antibodies were measured with an enzyme-linked immunosorbent assay (ELISA) kit (MBL, Nagano, Japan).

Anti-GPIIb/IIIa antibody-producing B cells

The anti-GPIIb/IIIa antibody response was evaluated by detecting peripheral blood B cells secreting IgG anti-GPIIb/IIIa antibodies. For this, we used an enzyme-linked immunospot assay, which is a sensitive and specific method for evaluating the presence or absence of autoantibody-mediated thrombocytopenia [9]. Briefly, peripheral blood mononuclear cells (10^5/well) were cultured in pentaplicate on GPIIb/IIIa-coated 96-well microplates at 37°C for 4 h, and subsequently incubated with alkaline phosphatase-conjugated goat anti-human IgG. Finally, the anti-GPIIb/IIIa antibodies that bound to the membrane were visualized as spots by incubation with a substrate. The frequency of circulating anti-GPIIb/IIIa antibody-producing B cells was calculated as the number per 10^5 peripheral blood mononuclear cells. The cut-off value was defined as 2.0 [9].

Anti-TPOR antibody

Serum anti-TPOR antibody was detected by ELISA using a recombinant protein encoding the entire extracellular domain of human TPOR as the antigen, as described before [4]. Antibody units were calculated from the optical density at 450 nm, using a standard curve obtained from serial concentrations of rabbit anti-human TPOR polyclonal antibodies (Kirin Brewery, Takasaki, Japan), and the cut-off value was defined as 18.0 units [4].

Evaluation of bone-marrow megakaryocyte density

Bone-marrow films from all the patients with thrombocytopenia were available. The proportion of megakaryocytes to the total number of nucleated cells was evaluated from Wright–Giemsa-stained bone-marrow smears. At least 1000 nucleated cells were counted for each sample. A proportion of megakaryocytes that was $\leq$0.2% was regarded as a decrease and one of $>$1.0% as an increase.

Statistical analysis

All continuous results were expressed as the mean ± S.D. Comparisons to determine statistical significance between two groups were performed using Fisher's exact test or unpaired Student's *t*-test, as appropriate.

Results

Anti-GPIIb/IIIa and anti-TPOR antibody responses in SLE patients with thrombocytopenia

Anti-GPIIb/IIIa antibody-producing B cells and anti-TPOR antibody levels in SLE patients with thrombocytopenia were significantly higher than in SLE patients without thrombocytopenia or healthy controls, but were comparable to those in patients with idiopathic thrombocytopenia (Fig. 1). When all the subjects were stratified above or below the cut-off, an anti-GPIIb/IIIa antibody response was detected in 28 (88%) SLE patients with thrombocytopenia, but in five (17%) without thrombocytopenia ($P<0.0001$). Anti-TPOR antibody was detected exclusively in SLE patients with thrombocytopenia and in those with idiopathic thrombocytopenia, and its frequency was significantly higher in SLE patients with thrombocytopenia than in SLE patients without it (22 *vs* 0%, $P=0.01$). The respective frequencies of anti-GPIIb/IIIa and anti-TPOR antibodies in SLE patients with thrombocytopenia were comparable to those in patients with idiopathic thrombocytopenia (86 and 10%). Finally, 29 (91%) of the SLE patients with thrombocytopenia produced either anti-GPIIb/IIIa or anti-TPOR antibody, and six of these patients produced both.

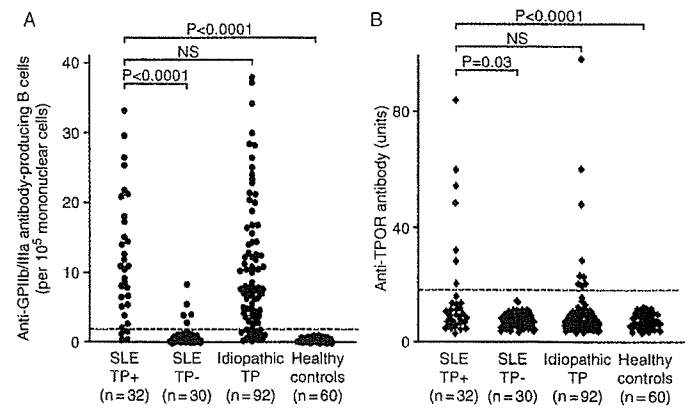

FIG. 1. Circulating anti-GPIIb/IIIa antibody-producing B cells (A) and serum anti-TPOR antibody (B) in 32 SLE patients with thrombocytopenia (TP), 30 SLE patients without thrombocytopenia, 92 patients with idiopathic thrombocytopenia and 60 healthy controls. A broken line indicates a cut-off level (2.0 for anti-GPIIb/IIIa antibody-producing B cells and 18.0 for anti-TPOR antibody). Levels were compared between SLE patients with thrombocytopenia and other groups using the unpaired *t*-test. NS, not significant ($P>0.05$).

Megakaryocyte density in association with autoantibody status

Examination of the bone marrow from SLE patients with thrombocytopenia revealed that eight (25%), 17 (53%) and seven (22%) patients had increased, normal and decreased bone megakaryocytes, respectively. A similar distribution was observed in the 92 patients with idiopathic thrombocytopenia; i.e. 20, 66 and 14% had increased, normal and decreased bone megakaryocytes, respectively. The status of anti-GPIIb/IIIa and anti-TPOR antibodies was compared with the bone marrow megakaryocyte density of patients with SLE and thrombocytopenia and those with idiopathic thrombocytopenia. Seven SLE patients who had anti-TPOR antibody had a significantly higher frequency of megakaryocytic hypoplasia than 25 patients who did not (86 *vs* 4%, $P<0.0001$; Table 1), and this association appeared to be independent of anti-GPIIb/IIIa antibody production. In contrast, none of the SLE patients who produced anti-GPIIb/IIIa antibody but not anti-TPOR antibody had megakaryocytic hypoplasia. Similarly, in patients with idiopathic thrombocytopenia, megakaryocytic hypoplasia was significantly more frequent in the nine patients with anti-TPOR antibody than in the 83 without this antibody (79 *vs* 7%, $P<0.0001$).

Clinical associations with anti-TPOR antibody

Additional clinical and laboratory findings for SLE patients with thrombocytopenia were compared based on the presence or absence of anti-TPOR antibody (Table 1). There was no significant difference in sex, age at examination, SLE-related clinical findings, lowest platelet count, autoantibody status, including anti-GPIIb/IIIa antibody-producing B cells, or SLEDAI between these two groups. A poor therapeutic response to corticosteroids was more prevalent in patients with anti-TPOR antibody than in those without, most of whom had anti-GPIIb/IIIa antibody alone ($P=0.0006$). Thus, the immunosuppressant use for thrombocytopenia was significantly more frequent in anti-TPOR-positive patients than in anti-TPOR-negative patients (71 *vs* 8%, $P=0.002$). Furthermore, all five anti-TPOR-positive patients who received IVIG were non-responders, while only one patient (10%) without this antibody showed a poor response to IVIG ($P=0.002$).

TABLE 1. Clinical and laboratory findings for SLE patients with thrombocytopenia who did or did not produce anti-TPOR antibody

Clinical and laboratory findings	Anti-TPOR-positive ($n=7$)	Anti-TPOR-negative ($n=25$)	P
Sex (% female)	86	88	NS
Age at examination (yr, mean ± S.D.)	44.0 ± 5.6	37.3 ± 15.0	NS
Malar rash (%)	43	36	NS
Discoid rash (%)	14	8	NS
Photosensitivity (%)	28	20	NS
Oral ulcers (%)	14	16	NS
Arthritis (%)	14	12	NS
Serositis (%)	14	8	NS
Renal disorder (%)	14	24	NS
Neurological disorder (%)	14	4	NS
Haemolytic anaemia (%)	0	4	NS
Leucopenia (%)	57	60	NS
Lowest platelet count ($\times 10^9$/l; mean ± S.D.)	20.7 ± 17.9	24.1 ± 12.1	NS
Anti-dsDNA antibody (%)	71	76	NS
Anti-Sm antibody (%)	14	4	NS
Anti-SSA antibody (%)	57	44	NS
Anti-cardiolipin antibody (%)	28	48	NS
Anti-GPIIb/IIIa antibody-producing B cells (/10^6 mononuclear cells, mean ± S.D.)	8.4 ± 8.1	12.8 ± 8.8	NS
Megakaryocytic hypoplasia (%)	86	4	<0.0001
Poor response to corticosteroids (%)	86	12	0.0006
Poor response to IVIG (n/n,%)	5/5 (100)	1/10 (10)	0.002
SLEDAI (mean ± S.D.)	9.0 ± 5.8	7.5 ± 7.3	NS

NS, not significant ($P \geq 0.05$).
dsDNA, double-stranded DNA; IVIG, intravenous immunoglobulin; SLEDAI, SLE disease activity index.

Discussion

Our findings demonstrate that both anti-GPIIb/IIIa and anti-TPOR antibodies are associated with thrombocytopenia in SLE patients, although the tests used were not necessarily comparable: antibody-secreting peripheral blood B cells were measured to detect the anti-GPIIb/IIIa antibody response while serum samples were used to detect the anti-TPOR antibody response. More than 90% of SLE patients with thrombocytopenia had at least one of these platelet-related autoantibodies, indicating that thrombocytopenia mediated by these two types of autoantibody is a dominant mechanism for SLE-associated thrombocytopenia, as for idiopathic thrombocytopenia.

Interestingly, anti-GPIIb/IIIa and anti-TPOR antibodies were associated with different phenotypes of thrombocytopenia, in terms of bone-marrow megakaryocyte density and therapeutic responses to standard treatment regimens for immune thrombocytopenia. All the SLE patients with anti-GPIIb/IIIa antibody alone had normal or increased megakaryocyte density, whereas the anti-TPOR antibody was strongly associated with megakaryocytic hypoplasia. This different phenotype can be explained by the distinct biological effects of these antibodies: anti-GPIIb/IIIa antibody binds circulating platelets and facilitates Fcγ receptor-mediated clearance of opsonized platelets by reticuloendothelial phagocytes [2], whereas anti-TPOR antibody blocks TPO signalling, resulting in inhibition of megakaryogenesis in the bone marrow [4]. This different mode of action may also account for the lack of therapeutic response to IVIG of patients with anti-TPOR antibody. Since interactions between the Fc portion of the infused immunoglobulins and the Fc receptors on target cells are thought to be a primary action of IVIG [10], it is likely that IVIG has little effect on the TPO signal blockade through the variable region of the antibodies.

In summary, measurement of anti-GPIIb/IIIa anti-TPOR antibody responses is useful in distinguishing between subsets of patients with SLE and thrombocytopenia and predicting their therapeutic response.

Rheumatology

Key messages

- Anti-GPIIb/IIIa and anti-TPOR antibodies are major contributory factors to SLE-associated thrombocytopenia.
- Anti-TPOR antibody is associated with megakaryocytic hypoplasia and poor therapeutic responses to corticosteroids and intravenous immunoglobulin.

Acknowledgements

This work was supported by grants from the Japanese Ministry of Health and Welfare.

No conflict of interest has been declared by the authors.

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Journal of Thrombosis and Haemostasis, 4: 1936–1943

ORIGINAL ARTICLE

Preliminary laboratory based diagnostic criteria for immune thrombocytopenic purpura: evaluation by multi-center prospective study

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To cite this article: Kuwana M, Kurata Y, Fujimura K, Fujisawa K, Wada H, Nagasawa T, Nomura S, Kojima T, Yagi H, Ikeda Y. Preliminary laboratory based diagnostic criteria for immune thrombocytopenic purpura: evaluation by multi-center prospective study. *J Thromb Haemost* 2006; 4: 1936–43.

Summary. *Background:* We proposed diagnostic criteria for immune thrombocytopenic purpura (ITP) by modifying the existing guidelines for diagnosis of ITP and by incorporating laboratory tests found useful for predicting its diagnosis, for example erythrocyte count, leukocyte count, anti-GPIIb/IIIa antibody-producing B cells, platelet-associated anti-GPIIb/IIIa antibodies, percentage of reticulated platelets, and plasma thrombopoietin. *Objective and methods:* To validate our criteria, we conducted a multi-center prospective study involving 112 patients with thrombocytopenia and a morphologically normal peripheral blood film at the first visit. Each patient underwent a physical examination, routine laboratory tests, and specialized tests for the anti-GPIIb/IIIa antibody response and platelet turnover. *Results:* Ninety-one patients (81%) satisfied the proposed criteria at first visit. Clinical diagnosis was made by skilled hematologists > 6 months after the first visit; ITP was diagnosed in 88 patients and non-ITP disorders in 24. The proposed criteria had 98% sensitivity, 79% specificity, a 95% positive predictive value, and a 90% negative predictive value. A relatively low specificity appears to be attributed to a few patients who had both ITP and aplastic anemia or myelodysplastic syndrome. *Conclusions:* Our preliminary diagnostic criteria based on ITP-associated laboratory findings were useful for the differential diagnosis of ITP, but additional evaluations and modifications will be necessary to develop criteria that can be used routinely.

Keywords: clinical studies, immune thrombocytopenic purpura, platelet antibodies.

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Received 6 February 2006, accepted 8 June 2006

Introduction

Immune thrombocytopenic purpura (ITP) is an acquired hemorrhagic condition of accelerated platelet consumption caused by antiplatelet autoantibodies [1]. This condition is seen in patients with various diseases, such as systemic lupus erythematosus (SLE) and human immunodeficiency virus (HIV) infection, and can also occur without an underlying disease, in which case it is known as idiopathic or primary form of ITP. Currently, the diagnosis of ITP is principally based on the exclusion of other possible concurrent causes of thrombocytopenia [1,2]. According to the guidelines proposed by the American Society of Hematology (ASH), the diagnosis of ITP should be made in patients with thrombocytopenia who lack findings that are atypical or suggest another diagnosis in their history, physical examination, complete blood count, and peripheral blood film, and no further laboratory tests are considered necessary [3]. A similar guideline has been proposed in the UK [4]. However, it is difficult for physicians who are not experts in ITP to exploit the guidelines' recommendations, because these guidelines are principally aimed at detecting alternative causes of thrombocytopenia and require comprehensive expertise and experience in platelet disorders. One potential solution to this problem is development of consistent and reproducible criteria, which do not rely on the skill of the clinicians. For this purpose, it is potentially useful to

incorporate reliable laboratory tests in the existing guidelines for the diagnosis of ITP. In this regard, the presence of antiplatelet antibodies is a hallmark of this autoimmune disease, and the detection of antibody responses to platelet surface glycoproteins, especially to GPIIb/IIIa, is useful to identify ITP [5–7]. In addition, the percentage of reticulated platelets and the circulating thrombopoietin (TPO) level are useful in discriminating accelerated platelet destruction from decreased platelet production [8,9].

Recently, two of us (M.K. and Y.I.) conducted a prospective study to identify initial laboratory findings that were useful for predicting a diagnosis of ITP and identified six: normal erythrocyte count, normal leukocyte count, increased anti-GPIIb/IIIa antibody producing B cell frequency, increased platelet-associated anti-GPIIb/IIIa antibody level, increased proportion of reticulated platelets, and normal or slightly increased plasma TPO [10]. These results led us to propose diagnostic criteria for ITP that depend solely on non-invasive laboratory tests using peripheral blood samples (Table 1). To diagnose ITP using these criteria, thrombocytopenia without other morphologic abnormalities in the peripheral blood film and the presence of three or more of the six ITP-associated laboratory findings listed above, including at least one of three positive findings, for example, increased anti-GPIIb/IIIa antibody producing B cell frequency, increased platelet-associated anti-GPIIb/IIIa antibody level, and increased proportion of reticulated platelets, are required. As ITP also occurs secondary to an underlying disorder, such as SLE [11], antiphospholipid syndrome [12], lymphoproliferative disorders, infection with HIV [13], and liver cirrhosis [14], a detailed clinical evaluation is still necessary to discriminate idiopathic from secondary forms. Invasive procedures, such as bone marrow examination are not necessary, although these tests are recommended for patients over 60 years or who show findings that are atypical for ITP, as previously mentioned [3].

Here, we report the results of a multi-center prospective study performed to verify the usefulness and accuracy of our preliminary laboratory based criteria in routine hematology clinics.

Table 1 Preliminary laboratory based criteria for the diagnosis of immune thrombocytopenic purpura (ITP) at presentation

1. Thrombocytopenia ($< 100 \times 10^9$ L^{-1}) without morphologic evidence for dysplasia in the peripheral blood film
2. The presence of any three or more, including at least one of (iii), (vi), and (iv), of the following laboratory findings:
   (i) absence of anemia,
   (ii) normal leukocyte count,
   (iii) increased anti-GPIIb/IIIa antibody producing B cell frequency,
   (vi) increased platelet-associated anti-GPIIb/IIIa antibody level,
   (v) elevated percentage of reticulated platelets, and
   (iv) normal or slightly increased plasma TPO level (< 300 pg mL^{-1})

For the diagnosis of ITP, both criteria must be met. Idiopathic or primary ITP can be diagnosed in the absence of conditions that potentially cause secondary ITP, for example SLE, antiphospholipid syndrome, lymphoproliferative disorders, infection with HIV, and liver cirrhosis. Bone marrow examination is recommended in patients over 60 years or with findings that are atypical for ITP. TPO, thrombopoietin; SLE, systemic lupus erythematosus; HIV, human immunodeficiency virus.

Materials and methods

Study design

This multi-center prospective study was conducted at nine medical centers in Japan from November 2002 to April 2004, and involved 127 consecutive patients who had been referred to tertiary care medical centers because of low platelet count or a question of ITP. At the first visit, detailed assessments of complete medical histories, physical examination, and routine laboratory tests, including complete blood count, a peripheral blood film, chemistry profiles, serologic evaluations such as tests for antinuclear antibody, antidouble stranded antibody, anti-β_2-glycoprotein I antibody, were performed for all patients. Serologic tests for HIV, and hepatitis B and C virus were performed only in patients with risk of these pathogens. Additional evaluations, including imaging studies and/or biopsies, were carried out in selected patients who were suspected of having underlying diseases that can cause secondary ITP, such as SLE, antiphospholipid syndrome, lymphoproliferative disorders, infection with HIV, or liver cirrhosis. The inclusion criteria were: (i) *de novo* patients; (ii) thrombocytopenia (platelet count $< 100 \times 10^9$ L^{-1}); (iii) the absence of any morphologic evidence for dysplasia in the peripheral blood film; and (iv) no previous treatment with corticosteroids or immunosuppressants. Patients who were diagnosed as having definitive diseases that can cause secondary ITP were excluded at entry. Diagnoses of SLE, antiphospholipid syndrome, and liver cirrhosis were made according to the published criteria [15–17] and lymphoproliferative disorders were diagnosed based on cytomorphologic analysis and immunophenotyping, while infection with HIV was diagnosed solely based on serologic analysis. The patients who had some features of diseases that can cause secondary ITP, but did not have definitive diagnosis were included in this study. These patients were carefully followed, and were excluded if they meet the above criteria during the observation period.

At the study entry, two tubes of peripheral blood [one anticoagulated with heparin and the other with ethylenediaminetetraacetic acid (EDTA)] were obtained for the evaluation of the anti-GPIIb/IIIa antibody response and platelet turnover. Heparinized blood was sent to Keio University School of Medicine for the measurement of anti-GPIIb/IIIa antibody-producing B cell frequency, platelet-associated anti-GPIIb/IIIa antibody, and plasma TPO, and EDTA-anticoagulated blood was sent to Osaka University Hospital for the measurement of the percentage of reticulated platelets. Investigators at the individual participating centers recorded the gender, age at first visit, erythrocyte count, leukocyte count, and platelet count for each patient at study entry. An erythrocyte count of < 4.3 (male) or < 3.7 (female) $\times 10^{12}$ L^{-1} was regarded as anemia. Investigators at the participating centers were blinded to the

results of the four specialized tests for evaluating the anti-GPIIb/IIIa antibody response and platelet turnover, so the clinical diagnoses were not influenced by these laboratory findings. In addition, all other clinical data were kept at the participating centers, and the investigators at the laboratories were blinded to them. All blood samples were obtained after the patients gave their written informed consent, as approved by the corresponding Institutional Review Boards.

Clinical diagnosis

At study entry, the patients were first evaluated for tentative clinical diagnosis (ITP or non-ITP) by at least one of the authors (skilled hematologists) on the basis of clinical history, physical examination, complete blood test, and bone marrow findings if available, according to the guidelines proposed by the ASH [3], as usually performed in regular hematology clinics. Patients who might have ITP, but had atypical features, such as unexplained leukopenia and reduced megakaryocytes in the bone marrow without morphologic evidence for dysplasia, were included in the non-ITP group. Final clinical diagnosis was re-evaluated > 6 months after the first visit, taking account of clinical course over at least 6 months, especially therapeutic responses to corticosteroids, splenectomy, and *Helicobacter pylori* eradication. It is important that tentative and final clinical diagnoses were made independent of the specialized studies performed as part of this study. Patients whose follow-up period was < 6 months were excluded from the study.

Sample preparation

Platelet-rich plasma (PRP) was prepared from heparinized venous blood by centrifugation, followed by separation into platelets and platelet-poor plasma. The remaining cell components were subjected to centrifugation through a Lymphoprep (Nycomed Pharma AS, Oslo, Norway) density gradient to isolate the peripheral blood mononuclear cells. PRP was also prepared by centrifugation of blood that was anticoagulated with EDTA.

Evaluation of anti-GPIIb/IIIa antibody responses

B cells producing IgG anti-GPIIb/IIIa antibodies were detected using the enzyme-linked immunospot assay as described [7]. Each experiment was conducted in five independent wells, and the results represent the mean of the five values. The frequency of circulating anti-GPIIb/IIIa antibody-producing B cells was calculated as the number per 10^5 peripheral blood mononuclear cells, and the cut-off value was defined as 2.0 [7]. IgG anti-GPIIb/IIIa antibodies in platelet eluates (from 5×10^7 platelets) were measured by enzyme-linked immunosorbent assay using purified human GPIIb/IIIa as the antigen [18,19]. Antibody units were calculated from the OD_{450} results, based on a standard curve obtained from serial concentrations of pooled plasma with a high titer of IgG anti-GPIIb/IIIa antibodies. All samples were examined in duplicate, and the results were calculated as the mean of the two values. The cut-off value for platelet-associated anti-GPIIb/IIIa antibodies was 3.3 U [19].

Evaluation of platelet turnover

Reticulated platelets were detected by staining paraformaldehyde-fixed platelets with thiazole orange (Retic-COUNT; Becton Dickinson, San Jose, CA, USA) followed by flow-cytometric analysis, as described previously [9]. The cut-off for the percentage of reticulated platelets was 9.3%. The plasma TPO level was measured using a commercially available enzyme-linked immunosorbent assay kit (Quantikine; R&D Systems®, Minneapolis, MN, USA) according to the manufacturer's protocol.

Statistical analysis

All continuous variables were expressed as the mean ± SD, and compared using the Mann–Whitney *U*-test. Differences in the frequency between two groups were compared using the chi-squared test or Fisher's exact test when applicable. A stepwise multiple regression analysis was conducted to identify independent variables associated with the diagnosis of ITP, and the odds ratio (OR) and its 95% confidence intervals (95% CI) were calculated for all statistically significant differences. All statistical procedures were performed using the STATVIEW software (SAS Institute, Cary, NC, USA).

Results

Clinical diagnoses

A total of 127 consecutive patients were enrolled in this study. After the final clinical diagnosis, which was made > 6 months after the first visit, the clinical information recorded at study entry by the investigators at the individual participating centers and the results for the four specialized laboratory tests for the anti-GPIIb/IIIa antibody response and platelet turnover were combined and analyzed. Fifteen patients were excluded from the analysis because of a platelet count > 100×10^9 L^{-1} at entry ($n = 2$), the subsequent diagnosis of a lymphoproliferative disorder or liver cirrhosis ($n = 3$), spontaneous resolution of thrombocytopenia ($n = 1$), previous treatment with corticosteroids ($n = 6$), and a follow-up period of < 6 months ($n = 3$). In the end, we analyzed data for 112 patients between 1 and 80 years of age (45.7 ± 22.6), including 18 children. The final clinical diagnosis after > 6 months of follow-up included ITP in 88 patients (79%), aplastic anemia in 11 (10%), myelodysplastic syndrome (MDS) in 10 (9%), Fanconi anemia in one (1%), May-Hegglin anomaly in one (1%), and myelofibrosis in one (1%).

Application of the preliminary diagnostic criteria

As all 112 patients were selected because they had thrombocytopenia without any other morphologic evidence for dyspla-