1回点滴静注し，これを2～4回繰り返す方法である。20例に対して行われた成績では13例(65%)が血小板数15万/μL以上となり，完全寛解の13例中8例は7カ月から7年間(平均2.5年)完全寛解を維持しており，有用な治療法である。このほか高用量のシクロホスファミドを用いた後に自家末梢血幹細胞移植（auto-PBSCT：autologous peripheral blood stem cell transplantation）を行う報告もあり，移植に伴うリスクはあるが50%以上に良好な血小板数の増加がみられている（表12)。

（6）多剤併用化学療法[40]

CMOPP（シクロホスファミド＋ビンクリスチン＋プロカルバジン＋プレドニゾロン)，CMV（シクロホスファミド＋ビンクリスチン＋プレドニゾロン)，CEP(シクロホスファミド＋エトポシド＋プレドニゾロン）療法など，悪性リンパ腫に準じた化学療法が行われ，長期間の寛解が得られた報告がある。しかし骨髄抑制，消化器症状など副作用が強く支持療法や補充療法を必要とする症例もあり，これら治療による白血病など二次発がんが危惧され，適応を十分に吟味して選択すべきである。

（7）ダプソン療法[41]

本剤はハンセン病や乾癬などの治療薬であるが，難治性ITPに対し本剤100 mg/日経口服用後40%の症例に血小板の増加効果が認められ，持続効果もあると報告されている。作用機序は明らかではないが多くの症例に溶血所見が認められることから，赤血球破壊による網内系細胞のブロックが推測されている。

（8）リツキシマブ療法[42,43]

Bリンパ球に発現しているCD20抗原を標的としたキメラモノクローナル抗体（リツキシマブ）で，CD20陽性悪性リンパ腫の治療薬として化学療法との併用で良好な治療成績をおさめている。最近，抗体産生にかかわる非悪性Bリンパ球もその標的となることから，種々の自己免疫疾患への応用も試みられてきた。ITPにおいては1998年の症例報告以来，各国で散発的に成人の治療抵抗性ITPに使用されてきたが，今回その効果と副作用が評価可能な19報告，313例についてレビューされた(表13，図12，表14)[43]。それによると，リツキシマブ375 mg/m^{2}を週1回4週間点滴静注する方法で完全寛解は46.3%に認められ，血小板数5万/μL以上の増加反応を示した症例は62.5%と良好な反応を示した。平均血小板増加反応期間は10.5カ月で，10.5%が再発している。一般にCR(complete remission：完全寛

表12　ITPに対するauto-PBSCTの成績

対象		
14例，17～52歳，治療抵抗性ITP，血小板数＜2万/μL		
治療スケジュール		
PBSC（末梢血幹細胞）動員：G-CSF（顆粒球コロニー刺激因子）10 μL/kg/日，静注		
白血球除去 day 5 および/もしくは day 6		
CD34陽性細胞選択（免疫磁気ビーズ法）		
前処置		
シクロホスファミド 50 mg/kg/日，4日間		
結果		
CR（完全寛解）	血小板数＞10万/μL	6例（43%）－死亡1例
PR（部分寛解）	血小板数＞ 5万/μL	2例（14%）－死亡1例
NR（無反応）		6例

現時点では自家末梢血幹細胞移植（auto-PBSCT）しか行われていない。死亡例はいずれも移植に伴うものである。

（文献39より引用）

解）症例は再発が少ないのに対し，PR（partial remission：部分寛解）例は再発が多く，ITP としての罹病期間の長い症例（15 年以上）では反応が悪い傾向にある。

副作用として軽微なものが 306 例中 66 例（21.6 %），grade 3 ～ 4 の生命にかかわる重篤例が 10 例（3.7%），grade 5 の 9 例（2.9%）が死亡している。死因の多くは本療法に起因した肺感染症による呼吸不全や出血であるが，治癒に近い症例も多く，副作用に対する対策を考慮した上で今後使用する価値があると考えられる。

（9）血小板増殖因子療法[44~47]

最近 TPO レセプターアゴニストとしてペプチド や非ペプチド性の小分子，さらには TPO アゴニスト作用を有する抗体などが開発されている（表 15）。いずれも TPO レセプターに結合し，巨核球コロニーを増加させ，巨核球の成熟を促進し血小板産生が亢進することが確認されている。難治性 ITP を中心に 2 つの薬剤（AMG531〔romiplostim〕，eltrombopag）について臨床治験の結果が報告された[44, 45]。いずれも投与量依存性に 5 ～ 7 日目から血小板数が増加し始め，12 ～ 16 日目くらいに最大の血小板増加を認め，継続使用により血小板数の増加効果を維持することができる。TPO に対する抗体は出現せず，1 年以上の長期投与においても重篤な副作用は認められていない[46]。難治症例の 80%以上に血小板

表 13　難治性 ITP 症例に対するリツキシマブ療法の効果

報告者	報告年	症例数	反応症例	CR	PR	NR	長期反応例（期間）
Stasi R	2001	25	13（52%）	5（20%）	5（20%）	3（12%）	7（28%）（6 カ月<）
Giagoumidis A	2002	12	9（75%）	5（41%）	2（17%）	2（17%）	4（33%）（320 日<）
Zaja F	2003	20	13（65%）	9（45%）	4（20%）	−	6（30%）（180 日<）
Cooper N	2004	57	31（54%）	18（32%）	13（23%）	−	16CR（28%）（72.5 週<）
Braedndstrup P	2005	39 クール	17（44%）	7（18%）	6（15%）	4（10%）	13CR，PR（33%）（47 週）
Penalver F.J.	2006	89	49（55%）	41（46%）	8（9 %）	−	31（35%）（9 カ月）
Bennett C（小児）	2006	36	11（31%）	−	−	−	−
Parodi E（小児）	2006	19	13（63%）	13（63%）	−	−	7（37%）（33 カ月）

CR：完全寛解，PR：部分寛解，NR：無反応　　（文献 43 より引用）

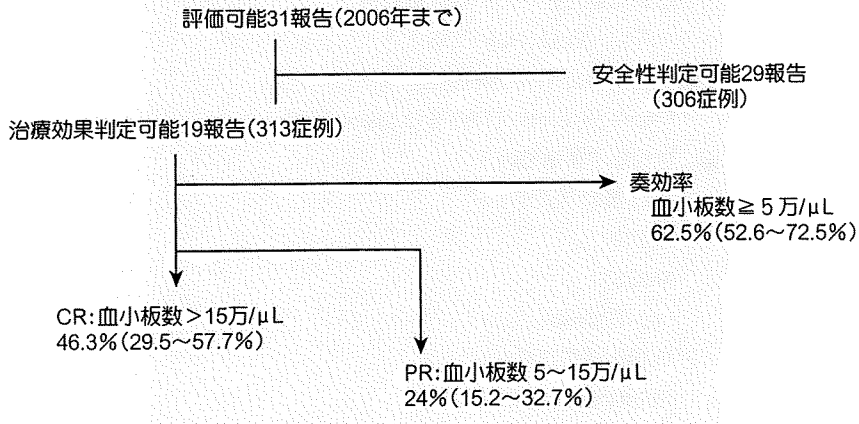


図 12　ITP に対するリツキシマブ療法の系統的 review

2006 年までに報告された評価可能な摘脾成績の論文は 31 であった。この論文の中で治療効果判定可能な 19 報告 313 症例についての治療成績を示したものである。全般的な奏効率は 62.5%となった。

CR：完全寛解，PR：部分寛解　　（文献 43 より引用）

増加効果が認められ，出血が回避されることから有用性が高い。しかし根本治療でないために使用し続ける必要のあること，使用中止後に血小板数は治療前値に戻り，その際出血傾向が増悪する例があること，骨髄で細網線維が増加する症例があること（投与を中止すれば回復するらしい）など，長期投与が余儀なくされる難治性ITPに関しては，長期使用による副作用の再確認が必要である（図13，14）。

(10)多剤併用療法による出血傾向に対する治療[48]

難治性症例のなかにはIVIG(intravenous immunoglobulin：免疫グロブリン大量静注）療法に対しても抵抗性を示す症例が存在し，出血の回避に難渋することが多い。このような症例に対してIVIG(1 g/kg)に加え副腎皮質ステロイド療法(30 mg/kg)，ビンカアルカロイド点滴静注療法（VCR 0.03 mg/kg）あるいは抗D血清療法の3剤を併用したり，これら4剤を併用する治療が行われている。その結果，71%の症例に3万/μL以上の血小板増加反応が認められている。さらには引き続いてダナゾール10～15 mg/kg)，アザチオプリン（2 mg/kg）の併用維持療法が行われた17例中13例は血小板数が5万/μL以上となり日常の出血が回避され，予定

表14　治療抵抗性ITPの治療成績

	症例数	CR	PR	NR	長期効果
シクロスポリンA		5（41.7%）			
2002 Emilina G	12	4（33.3%）*	1（8.3%）	2	60%（26.6カ月）
リツキシマブ					
2003 Shanafeld	12	5（42%）			
2004 Cooper N	57	18（32%）	13（23%）**	26	16/18（72.5週）

*維持療法継続

**11例再発

CR：完全寛解，PR：部分寛解，NR：無反応　　（筆者作成）

表15　血小板増殖因子

第一世代血小板増殖因子
- 遺伝子組換えヒト血小板増殖因子
  - rhTPO，PEG-rHuMGDF
- 遺伝子組換えTPO融合蛋白
  - Promegapoietin（TPO/IL-3 fusion protein）

第二世代血小板増殖因子
- TPO類似ペプタイド
  - Fab59
  - AMG531
  - Peg-TPOmp
- TPO類似非ペプタイド
  - eltrombopag（SB-497115）
  - AKR-501（YM477）
- TPOアゴニスト抗体
  - Minibodies：c-Mplに結合するモノクローナル抗体
  - MA01G4G344：Mplに対するモノクローナル抗体

（筆者作成）

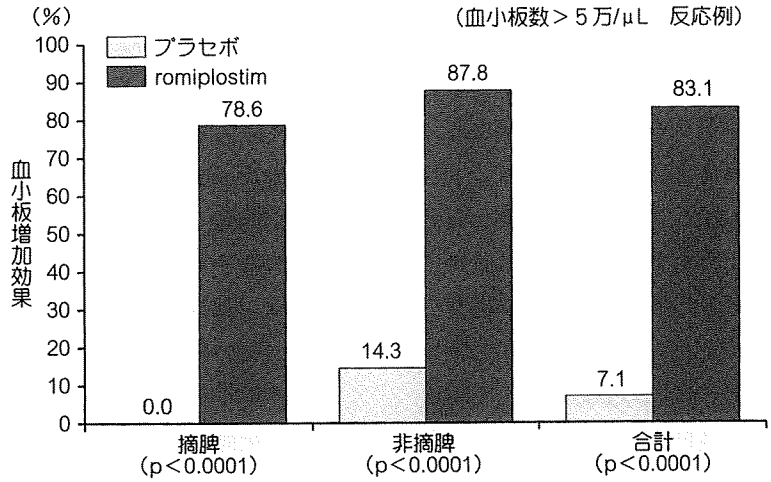


図 13　TPO アゴニストである AMG531 (romiplostim) の ITP に対する血小板増加効果

摘脾の有無にかかわらず 80%以上の症例で血小板増加効果が認められた。
本剤は皮下注射薬で，1 週間に 1 回の皮下注射で血小板数の維持が可能である。

(文献 44，46 より引用)

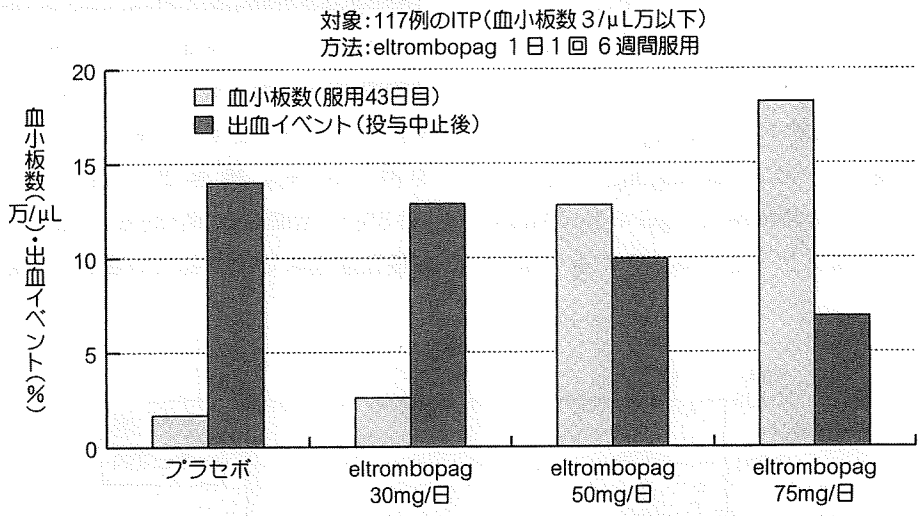


図 14　TPO アゴニスト eltrombopag による ITP に対する血小板数増加効果

本剤は経口薬で，連日服用の結果，服薬量依存性に出血のイベントが減少し，血小板数は増加することが示されている。

(文献 45 より引用)

手術が可能になったと報告されている。

(11) その他

難治性 ITP の治療には十分満足すべき方法がなく種々の治療法が研究的に行われている。抗 RhD 血清を用いて自己赤血球を感作し，この感作赤血球による網内系のブロック作用を利用する方法もあるが，わが国での基礎的検討はなされていない。また免疫抑制薬としてはシクロスポリン（シクロスポリン A）が有効であった症例の報告もみられている(表 14)。さらに，分子標的薬の 1 つとして Syk inhibitor が Fcγ レセプターを介するマクロファージの貪食機能を抑える点に着目し，難治性 ITP に対して良

好な成績が報告され，今後の発展が期待されている[49]。

c）緊急時あるいは外科的処置，分娩時などにおける治療

重篤な出血傾向を示す急性ITPや慢性ITPで経過中，主要臓器内への出血（脳，肺，消化管，腹腔内など）や血小板数5万/μL以下の手術，分娩時には一時的にでも血小板数を増加させることが必要である。これらの状態では血小板数は可能であれば10万/μL以上が望ましいが，8万/μL以上でも脳出血など頭部手術を除いて良好な経過をたどることが多い。特に分娩時には血小板数5万/μL前後でも自然分娩が可能で，子宮収縮がよければ出血量は必ずしも多くならない（図15）[50]。

（1）血小板輸血

緊急時に血小板を増加させるには有効であるが，血小板抗体が存在するので輸注血小板の寿命は短い。したがって時間的余裕があれば，後述するγグロブリン大量療法を併用すると血小板増加効果が上がる。

（2）γグロブリン大量療法（HIVG）[51]

完全分子型免疫グロブリン400 mg/kg/日を5日間連続点滴静注する。作用機序は，IgGのFc部分が網内系細胞のFcレセプターをブロックするため，感作血小板の貪食作用が阻止されて血小板が増加すると考えられている。本療法開始5～10日後血小板は増加し，平均7日後に最大値に達するが，多くは一過性に留まり，持続日数は数日である。64%の症例は血小板数が10万/μL以上となり，5万/μL以上の増加を示す例は83%で，有用性が高い。本療法が無効な例でも血小板輸血を併用すると血小板数は効率よく増加する例が多い。

（3）メチルプレドニゾロンパルス療法[51]

メチルプレドニゾロン1 g/日点滴静注を3日間行い，以後漸減する。急性ITPに使用され効果が上がっているが，最近慢性ITPにも用いられ，約80%の症例で10万/μL以上の血小板数の増加が認められている。反応は3日目くらいより現れるが一過性である。副作用は副腎皮質ステロイドに準じる。

Ⅷ 予後

わが国における本症の長期予後は，急性型62例中治癒53例（85.4%）である。慢性型186例中非摘脾例127例の予後は，治癒45例（35.4%），治療中70例（55.1%），摘脾群59例中では治癒35例（59.3%），治療中23例（39%）であった[52]。したがって慢性型全体では約43%が治癒し，残りは何らかの

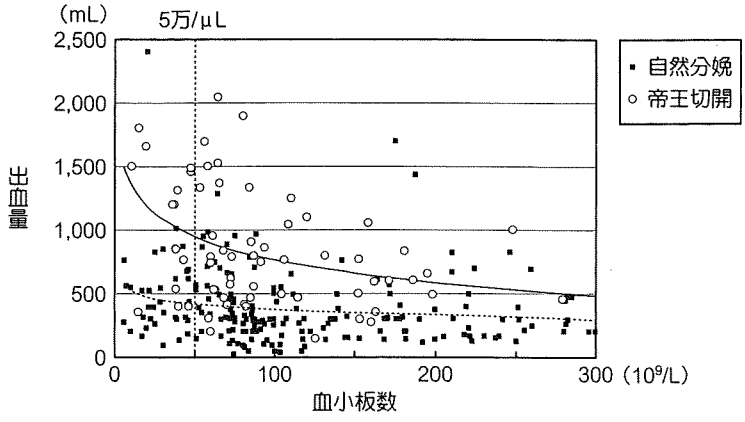


図15　分娩時の血小板数と出血量

血小板数5万/μL以下でも1例を除き自然分娩では出血量が1,000 mL以下であるのに対し，帝王切開では半数以上が1,000 mLを超える。帝王切開時には血小板数は少なくとも5万/μL以上であることが望ましい。

（文献50より引用）

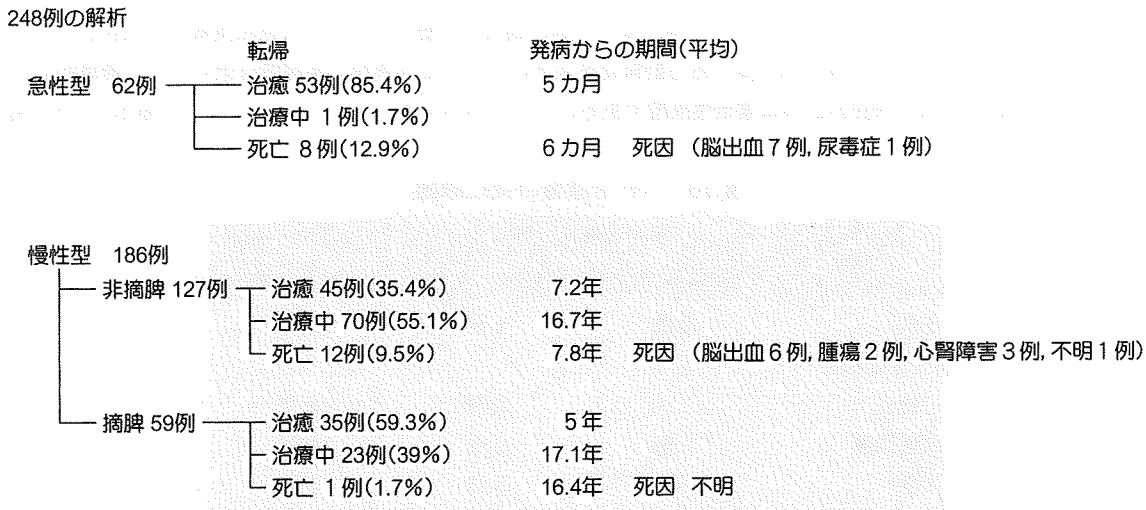
かたちで治療を継続する必要があり，本症が慢性に出血傾向を繰り返して経過する病気であることを裏づけている（図 16）。外国における単一施設での長期予後の報告を図 17 に示す[53]。

わが国における全体の死亡例は 21 例（8.5%）で急性型の 12.9%，慢性型非摘脾群の 9.4%，摘脾群の 1.7%であった。死因の多くは脳出血（13 例）で，本症の病態をあらわしている（図 16）。しかし 1983 年から始めたプロスペクティブ研究では観察対象症例 250 例中 16 例（6.4%）が死亡し，死亡率の減少が認められている。直接死因となった疾病は悪性腫瘍が 5 例，感染症（炎症）5 例，出血が原因となった例は脳出血の 1 例のみで，死亡直前の血小板数は 4 例が 5 万 / μL 以上，6 例が 10 万 / μL 以上に保たれていた（表 16）。

このことより，本症の予後は治療法の進歩によって向上し，特に出血に基づく死因は極端に減少したのに対し，治療薬の副作用が関係している感染症死や，罹患年齢の高齢化に伴う悪性腫瘍死が増加傾向にある。

外国における長期予後の検討では，Portielje らによると，2 年以上経過観察した 134 例の予後は，治療後血小板数 3 万 / μL 以上となり，以後無治療で経過している症例の長期死亡の危険率は一般人と変わりがない。しかし 3 万 / μL 以下の症例では死亡の危険率が一般人に比し 4.2 と高く，出血や感染が主たる死亡原因であると報告している（表 17）[54]。2 年以内に 4 例が ITP 関連死し，それ以降に 17 名（15%）が死亡し，2 例は ITP に関連した出血死や感染症死で，15 例は ITP とは関連のないがん，心・脳血管障害などであった。また長期間の追跡調査中 ITP に関連した入院の頻度は，治療抵抗群や維持療法必要群において高いと報告している（表 16）。

また血小板数 3 万 / μL 以下の症例 1,817 例のメタ解析の結果，49 例の致死的出血症例が集積された。致死的出血や重篤な出血は年齢に比例して頻度が増



※当初ITPとされ，その後非ITPとなった症例11例（SLE 7例，再生不良性貧血1例，その他3例）は含まず。

図 16　わが国における ITP の長期予後（1974 年以前 10 年間に集積した症例）における アンケート調査による 10 年後（1983 ～ 1984 年）の予後調査

アンケートによる予後調査で慢性型の約 43%の症例が治癒し，50%が長期にわたって何らかの治療を継続している。死亡は約 7 %で ITP 関連した出血死は約半数である。

急性型は多くは治癒するが，死亡例の多くは出血が原因であり急性型は出血傾向が強いことを示している。

SLE：全身性エリテマトーデス

（文献 52 および山中　學ほか：臨床血液 27：1737-1741, 1986 より引用）

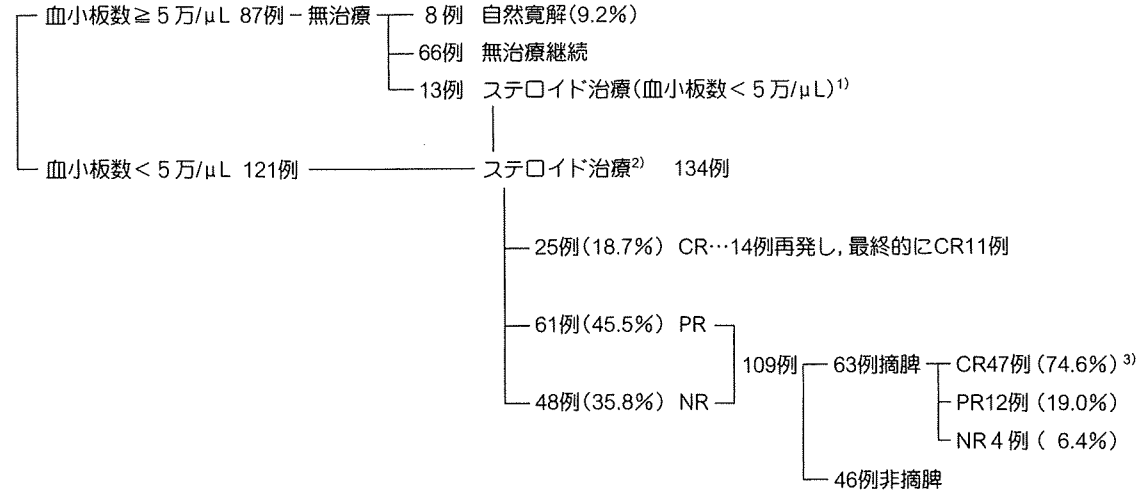


図 17　ITP の治療予後

1) 経過観察中に血小板数 5 万 /μL 未満となった症例

2) プレドニン　1 mg/kg/ 日

3) 長期間の CR は 23 例となった

CR (完全寛解)：血小板数 12 万 /μL 以上　PR (部分寛解)：血小板数 5 ～ 12 万未満　NR (無反応)：血小板増加なし，5 万 /μL を超えない

最終的に CR43 例 (20.6%)，死亡 11 例 (5.3%)。このうち ITP 関連死 5 例，非関連死 6 例

慢性 ITP では血小板数が 5 万 /μL 以上あれば無治療で長期の観察が可能で，なかには自然寛解する症例が 9.2%存在することが明らかとなった。第一選択薬である副腎皮質ステロイドによる長期の寛解率は低く，また最終的な CR 率も低く，長期間の治療，観察が必要な難治性疾患であることがうかがえる。　(文献 53 より引用)

表 16　ITP 治療後の死亡原因

死亡原因	厚生省研究班報告		Portielje, et al
	1973 ～ 1984	1983 ～ 1991	1974 ～ 1996
出血 (脳出血など)	6 例	1 例	2 例
血栓症	1 例	2 例	4 例
悪性腫瘍	2 例	5 例	4 例
炎症	0	5 例	4 例
腎，心不全	2 例	1 例	
その他			7 例
不明	4 例	2 例	3 例
合計	15 例	16 例	24 例

ITP 治療指針が定まる以前 1984 年までは出血死が多いが，治療指針が定まってからは悪性腫瘍，治療関連死 (炎症) が増えてきている。この傾向は外国でも同様である。その他は ITP と関連しない死因である。

(文献 52，54 より引用)

表 17　一般人口に対する血小板減少症の死亡危険率

治療に対する反応	症例数	死亡危険率 (95%CI)
完全寛解	90	0.7 (0.4 ～ 1.3)
不完全寛解	24	1.8 (0.6 ～ 5.5)
維持療法	8	1.8 (0.6 ～ 5.5)
反応なし	12	4.2 (1.7 ～ 10.0)

ITP の死亡危険率の一般人口との比較。死亡危険率は二次性免疫性血小板減少症で高く，2 年間の治療後血小板数が 3 万 / μL 未満で推移する症例は死亡危険率が一般人口に比し 4.2 倍高い。3 万 / μL 以上維持している症例では一般人口に比しやや高くなる程度である。

完全寛解：15 万 / μL 以上

不完全寛解：5 万～ 15 万 / μL 未満

維持療法：3 万～ 5 万 / μL 未満

反応なし：3 万 / μL 未満

（文献 54 より引用）

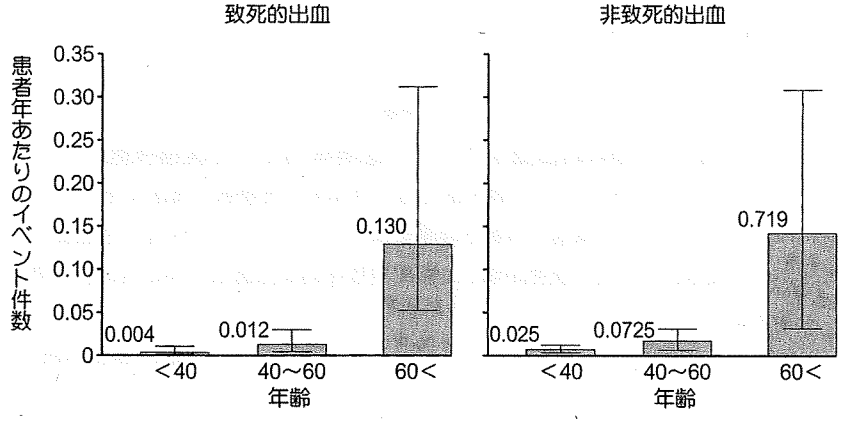


図 18　年齢による致死的，非致死的出血の年間発生数の見積もり

既報告論文を集積しメタ解析を行ったもので，患者年あたりの致死的，非致死的イベント数を各年齢群別に見積もったものである。

40 歳未満の症例では 60 歳を超える症例に比し致死的イベント発生件数が 30 倍以上高く，非致死的イベント発生件数も 28 倍高い。すなわち，加齢が出血イベント発生を増加させる要因である。

（文献 55 より引用）

し，40 歳未満と 60 歳を超える症例の比較では約 30 倍違う（図 18）。5 年間の予測死亡率は 40 歳以下では患者年あたり 2.2%，60 歳以上では 47.8% と計算されている。また年齢が高くなるに従い経年的に出血のイベントが増加し，予後は年齢と密接に関係している（図 19）[55]。

以上のように ITP は難病として扱われているが，その生命予後は多くの例において決して悪い疾病ではない。しかし，いかなる治療法によっても血小板数のコントロールが困難な難治症例（5 ～ 30%）においては，出血傾向にさらされ生命予後は 60 歳以上で悪い（表 18）[56]。

一方，重症 ITP130 症例に対し血小板数 1 万 / μL 以上を維持することを目標に治療し，10 年間経過観

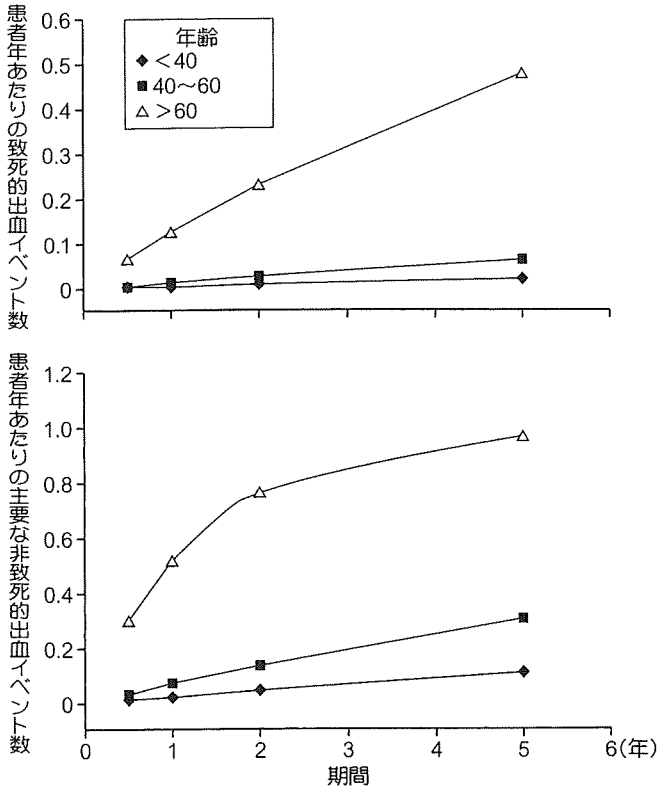


図 19　経年的出血イベントの患者年あたりの累積件数

いずれのイベント数も 60 歳を超えると経年的に累積数が増加するのに対し，それ以下では累積増加件数が非常にゆっくりしている。すなわち加齢に伴って経年的に出血の頻度が増し，特に非致死的な出血は 2 年以内に多く発生する。

（文献 55 より引用）

表 18　生命予後，出血症状を規定する因子

血小板数 3 万 /μL 以下が持続すると

（文献 54）

死亡危険率が 4.2 と一般人に比し高い

（文献 56）

60 歳以上では 40 歳以下に比し出血の危険率（オッズ比）が 28.9 と高い

（文献 55）

5 年間の予測死亡率　60 歳＜　47.8%

40 歳＞　2.2%

生命予後が一般人に比し各年代で短い

血小板数が 3 万 /μL 以下が持続すると死亡危険率が高くなり，特に 60 歳以上では出血の危険率が高まる。したがって血小板数 3 万 /μL 以上を，治療目標にする根拠となっている。

察した成績では出血による死亡はなく，2例が感染，7例はITPと関連のない死亡であった。このことからITPは比較的予後の良い良性の疾患で，アグレッシブな治療は必要なく，血小板数を1万/μL以上に保っていれば出血が防げるとの報告もある[57]。

ITPの合併症

合併症としては，出血傾向の結果として，出血性（鉄欠乏性）貧血や臓器出血による臓器障害（たとえば脳出血後遺症）などが認められるが，特に後者の頻度は少ない。一方，各種治療による副作用としての合併症は多くの症例で認められ，特に治療対象年齢が50歳を超えると治療薬による種々の副作用の出現頻度が増加する。副腎皮質ステロイドによる糖尿病，高血圧症，不眠症，内分泌障害，感染症，骨粗鬆症，大腿骨頭壊死などに加えて，免疫抑制薬の併用による感染症の増悪などが日常認められる。またダナゾールによる男性化作用や肝機能障害，ビンカアルカロイドによる末梢神経障害，脱毛などはよく知られている。

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BRIEF COMMUNICATION

Single nucleotide polymorphism of interleukin-1β associated with *Helicobacter pylori* infection in immune thrombocytopenic purpura

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Key words
Helicobacter pylori; immune thrombocytopenic purpura; infection; interleukin-1

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Abstract

To examine the role of genetic factors in development of immune thrombocytopenic purpura (ITP) in association with *Helicobacter pylori* infection, gene polymorphisms within the loci for human leukocyte antigen class II, interleukin (IL)-1β (−511), tumor necrosis factor-β (+252), immunoglobulin (Ig)G1 heavy chain (+643), and Igκ light chain (+573) were determined in 164 adults with ITP and 75 healthy controls. Of these gene polymorphisms, the IL-1β (−511) T allele was less frequently detected in *H. pylori*-infected than in *H. pylori*-uninfected (58% *vs* 81%, $P = 0.01$, odds ratio = 0.31) ITP patients diagnosed before age 50. These findings suggest that a single nucleotide polymorphism within the IL-1β (−511) may affect susceptibility to early-onset ITP associated with *H. pylori* infection.

Immune thrombocytopenic purpura (ITP) is an autoimmune disease characterized by the presence of autoantibodies against platelet membrane glycoproteins, such as GPIIb/IIIa (1). Its pathogenic process primarily involves an accelerated clearance of opsonized platelets by phagocytes in the reticuloendothelial system (1). The etiology of ITP remains unclear, but both genetic and environmental factors are thought to play a role in the development of the disease. Several genes involved in immune system regulation, including human leukocyte antigen (HLA)-DPB1 and tumor necrosis factor (TNF)-β, are associated with susceptibility to ITP (2, 3). On the other hand, the potential association of ITP with infectious agents, such as HIV and hepatitis C virus, has also attracted many investigators (4). In addition, several recent lines of evidence have indicated that platelet recovery occurs in a subset of ITP patients infected with *Helicobacter pylori*, a gram-negative bacterium that establishes chronic infection in the gastric mucosa, after the successful eradication of *H. pylori* (5). Several potential mechanisms for the role of *H. pylori* infection in the ITP pathogenesis include molecular mimicry between *H. pylori* components and platelet antigens and modulation of host's immune system by *H. pylori* infection (6), but we have recently proposed a mechanism in which *H. pylori* modulates the Fcγ receptor balance of monocytes/macrophages toward inhibitory FcγRIIB, thereby enhancing phagocytosis and antigen presentation (7). In addition, chemokine production in response to *H. pylori* infection may contribute to the pathogenesis of ITP because serum levels of monocyte chemoattractant protein-1, regulated on

activation normally T-cell expressed and secreted, and epithelial cell-derived neutrophil attractant-78 were elevated in *H. pylori*-infected than in *H. pylori*-uninfected ITP patients (8). Interestingly, platelet recovery after *H. pylori* eradication was observed in nearly half of the patients in cohorts from Japan (9) and Italy (10), but in <15% of the patients in cohorts from Spain and the United States (11, 12). This apparent ethnic difference suggests a potential role for host genetic factors in the development of *H. pylori*-associated ITP. To investigate this issue, in this study, we examined polymorphisms of genes for selected immune system-regulating molecules, including TNF-β, interleukin (IL)-1β, immunoglobulin (Ig), and HLA class II in Japanese patients with ITP, with and without an associated *H. pylori* infection.

We studied 164 unrelated Japanese adult patients with chronic ITP, defined as thrombocytopenia (platelet count $<100 \times 10^9$/l) for at least 6 months, normal or increased bone marrow megakaryocytes, and no secondary diseases that could account for the thrombocytopenia (1). Refractory ITP was defined as a platelet count of $<50 \times 10^9$/l despite treatment with high-dose corticosteroids and splenectomy (1). The patients were enrolled consecutively and were followed at Keio University Hospital, Tokyo, Japan. None of the patients had ever been treated for *H. pylori* eradication. They included 54 men and 110 women with a mean age of 45.4 ± 17.0 years (range 17–80). Controls included 75 race-matched healthy volunteers living in the Tokyo metropolitan area. Written informed consent was obtained from all the participants in accordance with the Keio University Institutional Review Board guidelines.

Helicobacter pylori infection was defined by the presence of IgG anti-*H. pylori* antibody, which was measured in plasma samples using an enzyme-linked immunosorbent assay kit, HEL-pTEST (Amrad, Kew, Australia). The results showed that 85 patients (52%) were infected with *H. pylori*. Comparisons of the demographic and clinical characteristics were made between *H. pylori*-infected and *H. pylori*-uninfected ITP patients. Differences between two groups were evaluated for statistical significance using the Mann–Whitney *U*-test or 2 × 2 chi-square test as appropriate. The mean age at diagnosis was significantly higher for the *H. pylori*-infected than the *H. pylori*-uninfected patients (50.8 ± 16.0 *vs* 39.5 ± 16.3 years, $P = 0.002$). The female dominance was more prominent in the *H. pylori*-uninfected compared with *H. pylori*-infected patients (79% *vs* 57%, $P = 0.003$), but there was no difference in the frequency of refractory ITP, which was defined to be poor response to corticosteroids and splenectomy. The number of circulating B cells producing IgG autoantibodies to platelet GPIIb/IIIa measured using an enzyme-linked immunospot assay (13) were not different between the two groups.

We first compared the distribution of single nucleotide polymorphisms (SNPs) within TNF-β (+252 G/A), IL-1β (−511 C/T), IgG1 heavy chain (+643 G/A), and Igκ chain (+573 C/G) among *H. pylori*-infected and *H. pylori*-uninfected ITP patients and healthy controls. These SNPs were determined by the specific amplification of genomic DNA that contained individual SNPs by polymerase chain reaction (PCR), combined with separation on an agarose gel to detect restriction fragment length polymorphisms (RFLPs) or hybridization with sequence-specific oligonucleotide probes (3). The distribution of individual SNP phenotypes in each group conformed to the Hardy–Weinberg principle. Phenotypic frequencies were tested for statistical significance using the chi-square test. There was no significant difference in the distribution of these SNPs between *H. pylori*-infected and *H. pylori*-uninfected ITP patients, *H. pylori*-infected patients and healthy controls, or *H. pylori*-uninfected patients and healthy controls (Table 1). Our control samples are likely to be a representative for the Japanese population based on the similar allele and diplotype frequencies to the published data in terms of IL-1β (−511) SNP (14). The HLA-DRB1, -DQB1, and -DPB1 alleles were also determined using the PCR-RFLP method (15), but again there was no significant difference in the relative frequencies of individual alleles among *H. pylori*-infected and *H. pylori*-uninfected ITP patients and healthy controls (data not shown). In contrast, Veneri et al. reported that ITP patients with *H. pylori* infection show significantly higher frequencies of HLA-DRB1*11 and *14 and DQB1*03 and a significantly lower frequency of DRB1*03 than those without the infection (16). The reason for this inconsistent result is unknown, but it might be because of the difference in HLA class II allele distribution between the Italians and the Japanese: DRB1*03 is lacking in the Japanese population.

Because *H. pylori* infection is established during infancy and childhood and sustained throughout life (17), a long incubation period must be required for *H. pylori*-associated ITP to develop. Therefore, it is possible that the pathophysiologic process of ITP differs among *H. pylori*-infected patients according to the length of the incubation period. Based on this assumption, we divided the ITP patients into two groups according to their age at diagnosis: early-onset ITP (diagnosis before age 50) and late-onset ITP (diagnosis at or after age 50). When demographic and clinical characteristics were compared between these two groups (Table 2), females were significantly more common among the patients with early-onset ITP than those with late-onset ITP. *H. pylori* infection was significantly less frequent in the early-onset ITP *vs* late-onset ITP patients, indicating that the majority of the patients with *H. pylori* infection developed ITP more than 50 years after the original infection. As shown in Table 3, among patients with early-onset ITP, the distribution of IL-1β (−511) differed

Table 1 Phenotypic frequencies of single nucleotide polymorphisms within the genes for tumor necrosis factor (TNF)-β, interleukin (IL)-1β, immunoglobulin (Ig)G1 heavy chain, and Igκ chain in *Helicobacter pylori*-infected and *H. pylori*-uninfected immune thrombocytopenic purpura (ITP) patients and healthy controls

		ITP patients		
Gene location	Phenotype	*H. pylori* infected ($n = 85$), n (%)	*H. pylori* uninfected ($n = 79$), n (%)	Healthy controls ($n = 75$), n (%)
TNF-β (+252)	G/G	10 (12)	13 (16)	18 (24)
	G/A	42 (49)	36 (46)	27 (36)
	A/A	33 (39)	30 (38)	30 (40)
IL-1β (−511)	C/C	33 (39)	20 (25)	23 (31)
	C/T	30 (35)	39 (50)	35 (47)
	T/T	22 (26)	20 (25)	17 (22)
IgG1 heavy chain (+643)	G/G	1 (1)	1 (1)	1 (2)
	G/A	14 (17)	14 (18)	18 (28)
	A/A	68 (82)	64 (81)	45 (70)
Igκ chain (+573)	C/C	7 (8)	6 (8)	4 (7)
	C/G	36 (44)	31 (41)	30 (51)
	G/G	40 (48)	38 (51)	25 (42)

significantly between the *H. pylori*-infected and the *H. pylori*-uninfected individuals (corrected $P = 0.04$). The pairwise comparisons showed that IL-1β (−511) C/C was increased and C/T was decreased in patients with *H. pylori* infection compared with those without [$P = 0.01$, odds ratio (OR) = 3.2, 95% confidence interval (CI): 1.3–8.0 and $P = 0.02$, OR = 0.34, 95% CI 0.14–0.83, respectively]. Finally, the T allele at IL-1β (−511) was less common in the *H. pylori*-infected than in the *H. pylori*-uninfected patients (58% *vs* 81%, $P = 0.01$, OR = 0.31, 95% CI 0.13–0.77). There was no significant association between *H. pylori* infection and gene polymorphisms within other loci. In addition, in patients with late-onset ITP, there was no difference in distribution of any gene polymorphisms, including IL-1β (−511), between the presence and the absence of *H. pylori* infection.

This is the first report representing a potential association between SNPs within the IL-1β locus and the prevalence of *H. pylori* infection in patients with ITP. In particular, we found that a lack of the IL-1β (−511) T allele was associated with *H. pylori* infection in patients with early-onset ITP but not in those with late-onset ITP. IL-1β, a proinflammatory cytokine, is known to be upregulated in the *H. pylori*-infected gastric mucosa (18), suggesting that it is important in the pathogenic processes of *H. pylori*-associated gastric diseases, such as chronic gastritis and gastric cancer. IL-1β is not only involved in the inflammatory response at the gastric mucosa but also influences the level of gastric acid secretion. Namely, IL-1β inhibits the secretion of gastric acid by parietal cells, resulting in *H. pylori*'s distribution to the corpus (19). Although there are some conflicting data regarding the functional effect of the allelic dipolymorphism of IL-1β (−511) on the production of IL-1β, the T allele is associated with increased IL-1β production in patients with gastric cancer and in healthy controls (20, 21). The IL-1β (−511) T allele has nearly complete linkage disequilibrium with the IL-1β (−31) C allele, which amplifies the interaction between genomic DNA and transcriptional factors at the TATA-box and is associated with increased transcription efficiency (20). In fact, in *H. pylori*-infected patients with gastritis, the gastric juice pH is significantly higher in those with the IL-1β (−511) T allele than in those without (21). In addition, subjects

Table 2 Demographic and clinical characteristics of patients with early-onset and late-onset ITP[a]

Demographic and clinical findings	Early-onset ITP ($n = 97$)	Late-onset ITP ($n = 67$)	P value
Age at diagnosis (years)[b]	33.1 ± 9.0 (17–49)	63.1 ± 7.6 (50–80)	N/A
Female[c]	73%	58%	0.045
Refractory ITP[c]	8%	7%	0.8
Anti-GPIIb/IIIa antibody-producing B cells (per 10^5 PBMC)[b,d]	6.4 ± 4.5 (0.2–17.7)	6.6 ± 4.2 (1.4–18.9)	0.7
Helicobacter pylori infection[c]	39%	70%	<0.001

ITP, immune thrombocytopenic purpura; N/A, not applicable; PBMC, peripheral blood mononuclear cells.

[a] ITP patients were divided into two groups according to their age at diagnosis (<50 years as early onset and ≥50 years as late onset).

[b] Continuous variables are shown as the mean ± SD (range) and were statistically evaluated using the Mann–Whitney *U*-test.

[c] Categorized variables were evaluated for statistical significance using the 2 × 2 chi-square test.

[d] This assay was performed in 57 patients with early-onset ITP and 44 patients with late-onset ITP.

Table 3 Phenotypic frequencies of single nucleotide polymorphisms within the genes for tumor necrosis factor (TNF)-β, interleukin (IL)-1β, immunoglobulin (Ig)G1 heavy chain, and Igκ chain in early-onset immune thrombocytopenic purpura patients with and without *Helicobacter pylori* infection

Gene location	Phenotype	*H. pylori* infected ($n = 38$), *n* (%)	*H. pylori*-uninfected ($n = 59$), *n* (%)	Overall corrected *P* value[a]
TNF-β (+252)	G/G	4 (11)	11 (19)	0.7
	G/A	19 (50)	28 (47)	
	A/A	15 (39)	20 (34)	
IL-1β (−511)	C/C	16 (42)	11 (19)	0.04
	C/T	10 (26)	30 (51)	
	T/T	12 (32)	18 (30)	
IgG1 heavy chain (+643)	G/G	1 (3)	1 (2)	0.7
	G/A	4 (11)	11 (19)	
	A/A	32 (86)	47 (79)	
Igκ chain (+573)	C/C	4 (11)	6 (10)	1
	C/G	18 (47)	26 (46)	
	G/G	16 (42)	25 (44)	

[a] Phenotypic frequencies were tested for statistical significance using the 2 × 3 chi-square test. Corrected *P* value was obtained by multiplying the observed *P* values by the number of comparisons made.

carrying the T allele are reported to have an increased risk of developing gastric atrophy and cancer (20–22). This association is thought to be because of both the more extensive gastritis and the lower acid production resulting from the high IL-1β production in subjects carrying the T allele, who would increase the exposure of gastric epithelium to exogenous and endogenous stimulants.

If this is the case, our results suggest that individuals lacking the T allele, who are infected with *H. pylori* and thus have less extensive gastritis and high acid production, have an increased risk for developing ITP before age 50. It is likely that the *H. pylori* strains that colonize the stomach are different between subjects with and without the T allele because the intragastric environment is apparently different. In this regard, a recent report showed the eradication rates for *H. pylori* by a standard triple regimen to be 77%, 90%, and 95% in subjects with the C/C, C/T, and T/T phenotypes of IL-1β (−511), respectively (23), suggesting that the IL-1β (−511) SNP could be a prognostic indicator of the success or failure of *H. pylori* eradication. Moreover, the frequency of *H. pylori* strains possessing the *cagA* gene is significantly higher in ITP patients than in healthy controls (24). These particular strains may induce ITP in infected individuals within a shorter incubation period compared with other *H. pylori* strains. Interestingly, IL-1β (−511) T carriers infected with *cagA*-positive strains were found to have an increased risk for developing gastric carcinoma (22). Taken together, a combination of host genetic factors and infectious agents may be important for determining an individual's susceptibility to ITP in the setting of *H. pylori* infection. Further studies investigating the interactive effects of host genetic factors and colonized *H. pylori* strains on the development of ITP are necessary to elucidate the role of *H. pylori* in the pathogenesis of ITP.

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Heterogeneous pathogenic processes of thrombotic microangiopathies in patients with connective tissue diseases

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Summary

To clarify the pathogenic processes of thrombotic microangiopathies (TMAs) in patients with connective tissue disease (CTD), we analysed clinical characteristics and plasma ADAMTS13 levels in 127 patients with CTD-TMAs, including patients with systemic lupus erythematosus (SLE), systemic sclerosis, polymyositis/dermatomyositis, and rheumatoid arthritis (RA), and 64 patients with acquired idiopathic thrombotic thrombocytopenic purpura (ai-TTP). Plasma levels of ADAMTS13 activity, antigen, and inhibitors were determined by enzyme immunoassays. IgG type anti-ADAMTS13 antibodies were also detected by immunoblots using purified ADAMTS13. ADAMTS13 activity was significantly decreased in CTD-TMAs, regardless of the underlying disease, but the frequency of severe deficiency (defined as <0.5% of normal) was lower in CTD-TMA patients than in ai-TTP patients (16.5% vs. 70.3%, $p<0.01$). Severe deficiency of ADAMTS13 activity was predominantly detected in patients with RA- and SLE-TMAs, and was closely associated with the presence of anti-ADAMTS13 IgG antibodies. CTD-TMA patients with severe deficiency of ADAMTS13 activity appeared to have lower platelet counts and better therapeutic outcomes. At least two phenotypic TMAs occur in patients with CTDs: a minor population with deficient ADAMTS13 activity caused by neutralising autoantibodies, and a major population with normal or moderately reduced activity. Classifying CTD-TMAs by ADAMTS13 activity may be useful in predicting the clinical course and therapeutic outcomes, as patients with moderately reduced activity are likely to have more prominent renal impairment and poor prognoses.

Keywords

Connective tissue disease, TMA, ADAMTS13, autoantibody

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Introduction

Thrombotic thrombocytopenic purpura (TTP) and haemolytic uraemic syndrome (HUS) are life-threatening diseases, characterised pathologically by thrombotic microangiopathies (TMAs), microangiopathic haemolytic anaemia, destructive thrombocytopenia, and organ dysfunction caused by platelet-thombi (1). The discovery of ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motif 13) (2–7), which specifically cleaves the Tyr1605-Met1606 bond in the von Willebrand factor (VWF)-A2 domain, facilitated the recognition that enzymatic deficiency due to genetic mutation or acquired autoantibodies was a more specific feature of TTP (8, 9). In the absence of ADAMTS13, unusually large VWF multimers (UL-VWFMs) are not appropriately cleaved in circulation; as a result, platelets aggregate excessively under high shear stress (10, 11).

In 1939, Gitlow and Goldmark (12) first reported a close relationship between TTP and systemic lupus erythematosus (SLE). Since then, this concept has become well accepted (13–16), but investigations of the underlying pathogenic processes are still lacking. More recently, several investigators (17–21) have observed deficient ADAMSTS13 activity caused by inhibitory IgG-autoantibodies in some patients with SLE-related TTP; however, many other patients have slightly reduced or almost normal activity (18). Thus, the pathogenesis of SLE-related TTP is still controversial.

During 1997–2006, we identified a total of 783 patients with TMAs by analysing their clinical and laboratory findings, including the levels of ADAMTS13 activity (ADAMTS13:AC)

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and ADAMTS13 inhibitor (ADAMTS13:INH), at Nara Medical University (22). In this database, we found 33 patients with congenital ADAMTS13:AC deficiency (termed Upshaw-Schulman syndrome), a rare thrombo-haemorrhagic disease, and elucidated their ADAMTS13 genetic status (23, 24). Using the same database, we identified 127 patients who developed TMAs in association with connective tissue diseases (CTDs), including SLE, systemic sclerosis (SSc), polymyositis/dermatomyositis (PM/DM), and rheumatoid arthritis (RA).

In this study, to further characterise the clinical expression and underlying pathogenesis of CTD-TMAs, we have extensively evaluated ADAMTS13 profiles, antibodies to ADAMTS13, and clinical features in patients with CTD-TMAs in comparison with 64 patients with acquired idiopathic (ai)-TTP.

Patients, materials and methods

Study subjects

One hundred twenty-seven patients with CTD-TMAs in a Nara Medical University database (22) included 64 SLE patients, 42 SSc patients, 11 PM/DM patients, and 10 RA patients. All patients fulfilled the corresponding classification criteria (25–28). SSc patients with complicating classical hypertensive renal crisis were excluded from the analysis. Blood samples and detailed clinical information were provided by referring physicians in area hospitals across Japan, and only patients who were confirmed to have TMAs were included in the database.

Based on the previous reports (29–31), TMAs were defined as having all of the followings: (i) microangiopathic haemolytic anemia (haemoglobin ≤12 g/dl), Coombs test negative, undetectable serum haptoglobin (<10 mg/dl), more than two fragmented red cells (schistocytes) in a microscopic field with a magnification of 100, and concurrent increased serum lactate dehydrogenase (LDH) above institutional baseline; (ii) thrombocytopenia (platelet count ≤100 x 10^9/l); and (iii) a variety of organ dysfunction (renal or neurological involvement) devoid of the stigmata of disseminated intravascular coagulation.

Sixty-four ai-TTP patients in a Nara Medical University database generated during the past two years (March 2006-April 2008) were used in this study for comparison. A diagnosis of ai-TTP was made for patients (i) without apparent underlying disease, and (ii) with the aforementioned clinical and laboratory features of TMAs.

Normal plasma samples were prepared from 20 healthy individuals (10 male, 10 female) between 20 and 40 years of age for use as a control. The study protocol conformed to the ethical principles of the World Medical Association Declaration of Helsinki as reflected in a *priori* approval from the Ethics Committee of Nara Medical University, and written informed consent was obtained from all patients at each referral hospital.

Therapeutic regimens and outcomes

Steroid pulse therapy is administration of intravenous methylprednisolone 1 g/day for 3 days, followed by a moderate or high dose of steroid therapy (0.5–1.0 mg of oral prednisolone/kg body weight/day). Therapeutic plasma exchange was conducted according to the following regimen: daily plasma exchange (PE) was performed at 1.5-fold body plasma volume with fresh frozen plasma (FFP) for the first 3 days, and PE was then performed at one body plasma volume daily for up to 14 days, until normal platelet counts (>150 x 10^9/l) were achieved. Response to therapy for TMAs was evaluated in two ways: (i) remission, defined as normalisation of platelet count (>150 x 10^9/l) and clinical manifestations without requiring PE, or (ii) death due to TMA.

Blood sampling

Before therapeutic plasma exchange or plasma infusions, blood samples (4.5 ml) were taken from each patient into plastic tubes containing 0.5 ml of 3.8% sodium citrate. Plasma was isolated by centrifugation at 3,000 x g for 15 minutes (min) at 4°C. Plasma samples were kept in aliquots at –80°C until testing, and sent to our institution.

Plasma ADAMTS13:AC, ADAMTS13:INH, ADAMTS13:AG, and VWF:Ag

Plasma levels of ADAMTS13:AC were determined using a commercially available chromogenic act-ELISA (Kainos Inc., Tokyo, Japan) (32). The detection limit of ADAMTS13:AC by this method was 0.5% of the control. A good correlation in plasma levels of ADAMTS13:AC between the classic VWFM assay and the chromogenic ADAMTS13-act-ELISA has previously been shown in normal individuals (R^2=0.72, $p<0.01$) (32, 33).

In terms of plasma levels of ADAMTS13:AC, since SSC-ISTH (Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis) has not defined "severe" deficiency of ADAMTS13:AC, we have here tentatively categorized into three types of plasma ADAMTS13:AC: less than 0.5%, 0.5% to less than 25%, and 25% or higher of the normal control as severe deficiency, moderate-to-mild deficiency, and subnormal-to-normal, respectively.

ADAMTS13:INH titers were evaluated by act-ELISA using plasma that was heat-inactivated at 56°C for 30 min. Inhibitor titers are expressed as Bethesda units (BU) (34). One BU is defined as the amount necessary to reduce ADAMTS13:AC to 50% of control levels. Titers greater than 0.5 Bethesda U/ml were classified as inhibitor-positive.

Plasma ADAMTS13 antigen (ADAMTS13:AG) levels were analysed by sandwich antigen (ag)-ELISA, using two murine anti-ADAMTS13 mAbs, A10 and C7. The A10 antibody recognizes an epitope in the disintegrin-like domain, completely inhibiting enzyme activity at a final concentration of 10 μg/ml (35). The C7 antibody recognizes the 7th and 8th thrombospondin-1 domains without affecting activity. The detection limit of the ag-ELISA for plasma ADAMTS13:AG was 0.1% of the normal control (35).

Plasma levels of VWF:Ag were assayed by sandwich ELISA using rabbit anti-human VWF polyclonal antibodies (DAKO, Denmark). The detection limit of this assay was 0.3% of the normal control.

Detection of IgG autoantibodies specific to ADAMTS13

Plasma-derived ADAMTS13 was purified using A10-agarose immunoaffinity chromatography followed by size-exclusion chromatography. Purified ADAMTS13 had a specific activity of 302 units/mg. SDS-5% polyacrylamide gel electrophoresis (PAGE) analysis revealed a 170kD-band before and a 190 kD-band after reduction (36).

To detect IgG-type autoantibodies specific for ADAMTS13, 0.15 μg purified ADAMTS13 per lane was separated by SDS-5% PAGE under non-reducing conditions, and electrophoretically transferred to polyvinylidine fluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). After blocking nonspecific binding with 5% skim milk, PVDF membranes were cut longitudinally into small pieces (3 x 800 mm). Each strip was incubated overnight at 4°C with 3 ml 5% skim milk containing 50 μl heat-treated plasma from each CTD-TMA patient. The heat-treated plasma was prepared by incubation at 56°C for 30 min. After centrifugation, the supernatant was used in assays. Human IgG bound to the purified ADAMTS13 on PVDF membranes was detected using a horseradish peroxidase (HRP)-conjugated anti-human IgG polyclonal antibody (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA). Binding was visualised using the Western Lightning Chemiluminescence reagent (Perkin-Elmer Life Science Inc., Boston, MA, USA) and imaged by X-ray autoradiography (Eastman Kodak, Rochester, NY, USA) (37, 38). Heated plasma from a patient with acquired idiopathic TTP with IgG inhibitors against ADAMTS13 was used as a positive control, while that from a normal individual without ADAMTS13:INH was used as a negative control.

Statistical analysis

All continuous values are shown as median values (25, 75 percentile). All comparisons among three (severe deficiency, moderate-to-mild deficiency, and subnormal-to-normal ADAMTS13 activity in both CTD-TMAs and ai-TTP) or five (SLE, SSc, PM/DM, RA, and ai-TTP) patient groups were tested for statistical significance using the Kruskal-Wallis H test or chi-square tests with Yates' correction for 2 x3 or 2 x 5 tables. Significant differences between three or five groups (overall $p < 0.05$) were further analysed by the Mann-Whiteney U-test or chi-square test. A two-tailed p-value less than 0.05 was considered to be significant.

Results

Clinical and laboratory features of CTD-TMAs

The clinical features and therapeutic outcomes of 127 patients with CTD-TMAs in comparison to 64 patients with ai-TTP are summarised in Table 1. SLE patients were younger at age at onset than patients with PM/DM or RA. There was a gender disparity, with female predominance, for patients with CTD-TMAs relative to ai-TTP patients. Platelet counts in SSc-TMA patients appeared to be higher than in patients with other CTD-TMAs or

Table 1: Clinical features and therapeutic outcomes of patients with CTD-TMAs and ai-TTP.

	CTD-TMAs (n=127)				ai-TTP (n=64)	Overall *P* *
	SLE (n=64)	SSc (n=42)	PM/DM (n=11)	RA (n=10)		
Clinical features						
Median age at onset of TMAs, years (25, 75 percentile)	44 (30, 54)	59 (54, 70)	57 (49, 63)	62 (56, 73)	54 (40, 69)	<0.01[a]
Female (%)	84	95	82	90	64	<0.01[b]
Renal involvement (%)	91	95	100	100	83	NS
CNS involvement (%)	69	48	64	80	70	NS
Laboratory findings at TMA diagnosis						
Median platelet count, 10^9/l (25, 75 percentile)	29 (9, 40)	50 (31, 74)	32 (9, 46)	23 (14, 28)	9 (9, 20)	NS
Median haemoglobin, g/dl (25, 75 percentile)	7.5 (6.1, 8.8)	8.3 (7.3, 9.3)	7.4 (6.6, 9.0)	7.2 (6.9, 8.1)	8.1 (6.4, 9.2)	NS
Median serum creatinine, mg/dl (25, 75 percentile)	1.6 (0.7, 2.6)	2.8 (1.9, 3.3)	1.5 (1.2, 2.3)	3.1 (1.1, 4.4)	2.1 (0.7, 2.1)	<0.01[c]
Median VWF:Ag , % (25, 75 percentile)	207 (147, 325)	256 (191, 370)	339 (225, 461)	302 (245, 454)	147 (114, 202)	<0.01[d]
Therapies						
Plasma exchange (%)	70	79	81	60	77	NS
Plasma infusion without plasma exchange (%)	27	21	18	40	25	NS
Steroid therapy without pulse therapy (%)	53	40	82	60	39	NS
Steroid pulse therapy (%)	38	26	0	20	30	NS
Rituximab (%)	0	0	0	0	9	NS
Immunosuppressants (%)	31	14	9	20	10	NS
Therapeutic response	(n=50)	(n=26)	(n=7)	(n=9)	(n=61)	
Remission of TMAs (%)	74	42	57	33	79	<0.01[e]
Death due to TMAs (%)	26	58	43	67	21	<0.01[f]

NS: not significant differences ($P \geq 0.05$). Overall *P* values were calculated using the Kruskal-Wallis H test or chi-square tests with Yates' correction for 2 x 5 tables. Significant differences between 5 groups (overall $P < 0.5$) were further analyzed by Mann-Whiteney U-test or chi-square test. [a] $P<0.01$ between SLE and PM/DM, RA. [b] $P<0.01$ between SLE, SSc. PM/DM, RA and ai-TTP. [c] $P<0.05$ between SLE and RA. [d] $P<0.01$ between SLE, and PM/DM. $P<0.01$ between PM/DM, RA and ai-TTP. [e] $P<0.01$ between SLE and SSc. $P<0.01$ between SSc, RA and ai-TTP. $P<0.05$ between SLE and RA. [f] $P<0.01$ between SLE and SSc. $P<0.01$ between SSc, RA and ai-TTP. $P<0.05$ between SLE and RA.