Inhibition of fibrinogen-clotting activity of thrombin by TM is more rapid than inhibition by AT. In a previous study, excess AT without heparin gradually reduced fibrinogen-clotting activity of thrombin after >30 min [11], whereas excess rTM fully diminished it within a minute in this study (data not shown). The interaction of thrombin with TM is an enzyme–cofactor interaction that should be rapid and reversible so that the cofactor can convert many allosteric enzyme molecules simultaneously [12]. Most of the thrombin formed, at least in microcirculation, is believed to be bound to TM, representing the major anticoagulant mechanism at the intact endothelial surface; AT may irreversibly inhibit thrombin while bound to TM. Thus, it is assumed that TM plays a key role in the regulation of coagulation in damaged blood vessels, although AT is a pivotal molecule that can inhibit excessive clotting over the long term.

Thrombin is an allosteric enzyme controlled by sodium binding [13, 14]. Sodium-bound thrombin (known as the fast form) is optimized for procoagulant function because of increasing substrate specificity for fibrinogen, whereas sodium-free thrombin (known as the slow form) is an anticoagulant because of increasing specificity for cleaving PC [15]. The mutation in prothrombin Yukuhashi occurred at residue Arg596 (Arg221a in the chymotrypsinogen numbering system [16]) within the sodium-binding region of thrombin and can be expected to have an influence on the binding of sodium, resulting in a change in its protease activity and specificity. In this study, we demonstrated that rTM substantially enhanced APC generation activity of mutant thrombin, although the activity was still approximately half of that of the wild-type. In chromogenic assays, procoagulant activity of mutant thrombin was 57% of that of the wild-type and APC generation activity of the mutant in the presence of rTM was 54% of that of the wild-type. This suggests that APC generation activity can be associated with the protease activity of thrombin. It is appears that rTM can form a complex with both wild-type and mutant thrombins and enhance PC activation in equal proportion, suggesting that the prothrombin Yukuhashi mutation may not be TM-resistant in terms of APC generation enhancement.

Fukudome et al. demonstrated the endothelial cell–specific expression of endothelial cell protein C–APC receptor (EPCR) *in vivo*, particularly in the aorta and large arteries but not in the capillaries [17]. EPCR was found to greatly accelerate PC activation mediated by the thrombin–TM complex. Although the high-affinity binding of PC to EPCR on the endothelial cells was a critical step for activation, TM was an essential component for activation even in the presence of EPCR. In this study, we evaluated the effect of the prothrombin Yukuhashi mutation on the activation of PC in the presence of soluble rTM, but not in the presence of both TM and EPCR. Therefore, further experiments may be needed to evaluate the mutant effect in the presence of both TM and EPCR on the endothelial cell surface taking into account *in vivo*-situation.

In conclusion, we evaluated the effects of the prothrombin Yukuhashi (Arg596Leu) mutation on the TM–PC system and demonstrated that the mutation may attenuate immediate anticoagulant activity of TM. This possibly contributes to the susceptibility to thrombosis, although its enhancing effect on APC generation can be maintained.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authorship

Y.T. performed the experiments, analyzed the data, and drafted the manuscript. I.K., Y.A., Y.N., M.M., A.T., and T.M. interpreted the data and contributed to analytical methodology. T.K. designed the project, analyzed the data, and wrote the manuscript. All authors were involved in the critical review of the manuscript prior to submission.

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References

[1] Bauer KA. Management of thrombophilia. J Thromb Haemost 2003;1:1429–34.
[2] Bafunno V, Margaglione M. Genetic basis of thrombosis. Clin Chem Lab Med 2010; 48(Suppl. 1):S41–51.
[3] Miyawaki Y, Suzuki A, Fujita J, Maki A, Okuyama E, Murata M, et al. Thrombosis from a prothrombin mutation conveying antithrombin resistance. N Engl J Med 2012; 366:2390–6.
[4] Matsushita T, Saito H, Kojima T. Thrombosis from a prothrombin mutation conveying antithrombin resistance. The author reply. N Engl J Med 2012;367:1069–70.
[5] Djordjevic V, Kovac M, Miljic P, Murata M, Takagi A, Pruner I, et al. A novel prothrombin mutation in two families with prominent thrombophilia–the first cases of antithrombin resistance in a Caucasian population. J Thromb Haemost 2013;11: 1936–9.
[6] Esmon CT. The protein C pathway. Chest 2003;124(3 Suppl.):26S–32S.
[7] Esmon CT. Thrombomodulin as a model of molecular mechanisms that modulate protease specificity and function at the vessel surface. FASEB J 1995;9:946–55.
[8] Isermann B, Hendrickson SB, Zogg M, Wing M, Cummiskey M, Kisanuki YY, et al. Endothelium-specific loss of murine thrombomodulin disrupts the protein C anticoagulant pathway and causes juvenile-onset thrombosis. J Clin Invest 2001;108: 537–46.
[9] Mohri M, Suzuki M, Sugimoto E, Sata M, Yamamoto S, Maruyama I. Effects of recombinant human soluble thrombomodulin (rhs-TM) on clot-induced coagulation in human plasma. Thromb Haemost 1998;80:925–9.
[10] Mohri M, Sugimoto E, Sata M, Asano T. The inhibitory effect of recombinant human soluble thrombomodulin on initiation and extension of coagulation– a comparison with other anticoagulants. Thromb Haemost 1999;82:1687–93.
[11] Murata M, Takagi A, Suzuki A, Okuyama E, Takagi Y, Ando Y, et al. Development of a new laboratory test to evaluate antithrombin resistance in plasma. Thromb Res 2014;133:293–8.
[12] Baerga-Ortiz A, Rezaie AR, Komives EA. Electrostatic dependence of the thrombin-thrombomodulin interaction. J Mol Biol 2000;296:651–8.
[13] Dang OD, Vindigni A, Di Cera E. An allosteric switch controls the procoagulant and anticoagulant activities of thrombin. Proc Natl Acad Sci U S A 1995;92:5977–81.
[14] Pineda AO, Carrell CJ, Bush LA, Prasad S, Caccia S, Chen ZW, et al. Molecular dissection of Na + binding to thrombin. J Biol Chem 2004;279:31842–53.
[15] Di Cera E, Dang QD, Ayala YM. Molecular mechanisms of thrombin function. Cell Mol Life Sci 1997;53:701–30.
[16] Bode W, Turk D, Karshikov A. The refined 1.9-A X-ray crystal structure of D-Phe-Pro-Arg chloromethylketone-inhibited human alpha-thrombin: structure analysis, overall structure, electrostatic properties, detailed active-site geometry, and structure-function relationships. Protein Sci 1992;1:426–71.
[17] Fukudome K, Ye X, Tsuneyoshi N, Tokunaga O, Sugawara K, Mizokami H, et al. Activation mechanism of anticoagulant protein C in large blood vessels involving the endothelial cell protein C receptor. J Exp Med 1998;187:1029–35.

深部静脈血栓症に対する対策と治療

A 序論

深部静脈血栓症(deep vein thrombosis：DVT)は，本邦においても食生活の欧米化や高齢化に伴って近年増加傾向にある疾患である．発症危険因子としては，アンチトロンビン，プロテインC，プロテインSなど生理的抑制因子の欠乏症による血栓性素因，妊娠や加齢，さらには術後の長期臥床やロングフライト，災害時の車中泊などがあげられる（表1）．特にDVTに起因する肺血栓塞栓症（pulmonary embolism：PE）は重篤な症状を呈し，ときには致命的となるため迅速な対応が求められる．PEはDVTに合併することも多く，同じ病態で発症することが知られており，合わせて静脈血栓塞栓症（venous thromboembolism：VTE）と称する．

表1 DVTの付加的な危険因子の強度

危険因子の強度	危険因子
弱い	肥満
	エストロゲン治療
	下肢静脈瘤
中等度	高齢
	長期臥床
	うっ血性心不全
	呼吸不全
	悪性疾患
	中心静脈カテーテル
	がん化学療法
	重症感染症
強い	DVTの既往
	血栓性素因
	下肢麻痺
	ギプスによる下肢固定

2004年に肺血栓塞栓症/深部静脈血栓症（静脈血栓塞栓症)予防ガイドラインが策定された[1]．また，低分子量ヘパリンやXa阻害薬などの新規抗凝固薬の保険適用が開始されたことなどから，肺血栓塞栓症および深部静脈血栓症の診断，治療，予防に関するガイドライン（2009年改訂版）が発表された[2]．

B 指針

肺血栓塞栓症および深部静脈血栓症の診断，治療，予防に関するガイドライン（2009年改訂版）では検査法および治療法の適応に関する推奨基準を4段階に設定している（表2）．また，

表2 検査法および治療法の適応に関する推奨基準のクラス分類

Class Ⅰ		検査・治療が有効，有用であることについて証明されているか，あるいは見解が広く一致している．
Class Ⅱ	検査・治療の有効性，有用性に関するデータまたは見解が一致していない場合がある．	
	Class Ⅱa	データ・見解から有用・有効である可能性が高い．
	Class Ⅱb	データ・見解から有用性・有効性がそれほど確立されていない．
Class Ⅲ		検査・治療が有用でなく，ときに有害であるという可能性が証明されている．あるいは有害との見解が広く一致している．

表3 リスクの階層化とDVTの発生率，および推奨される予防法

リスクレベル	下腿DVT (%)	中枢型DVT (%)	症候性PE (%)	致死性PE (%)	推奨される予防法
低リスク	2	0.4	0.2	0.002	早期離床および積極的な運動
中リスク	10～20	2～4	1～2	0.1～0.4	弾性ストッキングあるいは間歇的空気圧迫法
高リスク	20～40	4～8	2～4	0.4～1.0	間歇的空気圧迫法あるいは抗凝固療法
最高リスク	40～80	10～20	4～10	0.2～5	抗凝固療法と間歇的空気圧迫法の併用あるいは抗凝固療法と弾性ストッキングの併用

入院患者を対象としたDVTの一時予防を目的として，第6回ACCP Consensus Conference on Antithrombotic Therapyの予防ガイドライン（ACCPガイドライン）に準拠し，リスクレベルを4段階に分類している（表3）．血栓症の効果的な治療を目指す上で考慮すべき危険因子として，妊娠・分娩，外科手術などの静脈の機械的圧迫，がんなどの組織因子による凝固系機構のみでなく，プロテインC/S，アンチトロンビンなどの制御系タンパク質の異常や欠乏状態を把握することが重要である（Class Ⅰ）．予防法としては，理学的予防法と薬物的予防法がある．DVT予防の基本である下肢の運動は，血流うっ滞を減少させる．できる限りの早期離床（低リスク群でClass Ⅰ）を目指し，自力での運動が困難な場合は，弾性ストッキングの着用（中リスク群でClass Ⅱa），間歇的空気圧迫法（IPC：高リスク群でClass Ⅱa）などを行う．IPCは2004年に弾性ストッキングとともに診療報酬として認可された．スリーブの形状や圧力，圧迫回数などは装置により様々で，最近では日本人の体型に合ったものも出てきている．その効果は低分子量ヘパリンに匹敵するという報告もある[3]．薬物的予防法は，従来一般的に抗凝固療法（高リスク群でClass Ⅱa）に用いられてきたワルファリンの内服，未分画ヘパリンの投与に加えて，最近では低分子量ヘパリン（LMWH）やXa阻害薬の使用が認可された．DVT治療においては，PEの合併発症を防ぎ，速やかに血栓を除去もしくは溶解し，再発を防ぐ必要がある．血栓溶解療法においてプラスミノゲンアクチベータのウロキナーゼを用いる場合，初回6万～24万単位/日を点滴静注，7日目まで漸減し投与する（Class Ⅱa）．

C エビデンス

1 Wells PS, et al (JAMA. 2014; 311: 717-28)[4]

目的 VTEの病因，ならびに3つのVTE治療期間：急性（5～10日），長期（急性期治療の終了から3～6カ月），長期以降の延長（3～6カ月以上），について検索．

方法 ヒトでのVTE治療に関する文献検索（コクランレビュー，メタアナリシス，ランダム化比較試験，その他の臨床試験など）．

結果 LMWHと引き続きビタミンK拮抗薬(VKA)を用いることで，ほとんどの急性期DVT

患者の外来診療を可能にした．新規経口抗凝固薬の開発はさらに急性期治療を簡素化し，2つの経口薬はLMWHの必要性を回避する，単剤療法として使用することができる．PE患者であってもまた，予後およびPEの重症度に応じた判断のもと，外来患者として急性期治療をすることもできる血栓溶解療法は重症VTE患者に確保される最高のもので，下大静脈フィルター（理想的には回収可能なもの）は抗凝固療法が禁忌である場合に使用されるべきである．一般にVTE患者は，LMWHとVKA，あるいは直接Xa因子阻害薬や直接IIa因子阻害薬などによる薬物的抗凝固療法を3カ月必要とする．その後，VTEの病因（一過性リスク要因，孤発性，悪性腫瘍随伴），再発リスク，大出血リスクのバランスを取った治療法を選択する．大出血に対してより良い予測ツールが必要とされている．急性期，長期，および長期以降の延長された治療での選択肢として，新しい経口抗凝固薬の使用経験はまだ限定されているが，これらすべての時期での治療において，安全かつ有効であることが示されつつある．

結論 VTE治療の主力は抗凝固療法であるが，血栓溶解療法や下大静脈フィルターなどの介入は特殊状況下の選択肢として確保されている．抗凝固薬は出血リスクを増大させる可能性があり，病因，リスク，利益，コスト，および患者の好みを組み込んだ個人に合わせた治療戦略を必要とする．

2］ Nakamura M, et al（Circ J. 2014; 78: 708–17）[5]

目的 急性期VTEの疫学と臨床管理は日本での十分な情報がないため，これらを明らかにする．

方法 日本VTE治療全国疫学調査（JAVA）は，症候性急性PE，症候性急性DVT，または無症候性急性近位DVT患者の多施設コホート研究である．

結果 急性VTEで登録1,076人の患者のうち，68.7%が孤発性DVT，17.0%はPEのみを発症しており，14.4%は両者を合併していた．VTE管理では，高頻度の下大静脈フィルター挿入（40.6%），頻回の血栓溶解（21.1%），ワルファリン（INR＝1.5～2.5）に引き継がれる低用量の未分画ヘパリンによる抗凝固療法を特徴とした．平均252.5日間の観察期間中，VTEの再発は29例（発生率：3.9/100患者・年）であった．試験期間中での死亡は123例（16.6/100患者・年）であった．大出血の発生率は2例の致命的な出血と7例の頭蓋内出血を含み，3.2%/患者・年であった．

結論 日本のVTE管理は，低用量の抗凝固療法を使用するプロトコルとは対照的に，急性期での積極的な治療を特徴とする．日本や欧米でのVTE再発率は同様であるが，死亡率は日本で高く，患者とその管理法の特性に応じてかなりのばらつきがある．

D 根拠となった臨床研究の問題点と限界

DVTの予防や治療に関するデータの多くは欧米人を対象としたものであり，その結果をそのまま日本人に適応することには問題がある．その点，後者の日本人でのデータは貴重なものといえる．

E (本邦の) 患者に適応する際の注意点

ワルファリンのコントロールには一般にPT-INRを用いるが，欧米人は2.0～3.0でコントロールするのに対し，出血の副作用を起こしやすい日本人では2.0（1.5～2.5：Class IIb）が推奨されている．

F コメント

本邦におけるDVTの予防と治療に関するエビデンスは欧米に比較して乏しい．そのためガイドラインでは，出血合併症の頻度が明らかでない抗凝固療法による薬物的予防法より理学的予防法に比重を高めた推奨がなされている．今後，わが国でも新規抗凝固薬のVTE予防・治療への適応拡大が進むことが期待されるが，日本人に関するデータの蓄積と十分なエビデンスに基づく治療方針の決定が望まれる．

文献

1) 肺血栓塞栓症/深部静脈血栓症（静脈血栓塞栓症）予防ガイドライン作成委員会．肺血栓塞栓症/深部静脈血栓症（静脈血栓塞栓症）予防ガイドライン．東京：Medical Front International Limited；2004.
2) 安藤太三，伊藤正明，應儀成二．肺血栓塞栓症および深部静脈血栓症の診断，治療，予防に関するガイドライン（2009年改訂版）．2009：http://www.j-circ.or.jp/guideline/pdf/JCS2009_andoh_h.pdf.
3) Ginzburg E, Cohn SM, Lopez J, et al. Randomized clinical trial of intermittent pneumatic compression and low molecular weight heparin in trauma. Br J Surg. 2003；90：1338-44.
4) Wells PS, Forgie MA, Rodger MA. Treatment of venous thromboembolism. JAMA. 2014；311：717-28.
5) Nakamura M, Miyata T, Ozeki Y, et al. Current venous thromboembolism management and outcomes in Japan. Circ J. 2014；78：708-17.

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トピックス臨床シリーズ

新たな血栓性素因：
アンチトロンビンレジスタンス

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要　旨

静脈血栓塞栓症（VTE）は様々な先天的/後天的リスクにより発症する多因性疾患である．VTEは従来から欧米人に比べ日本人には少ないとされてきたが，診断技術の向上や食生活の欧米化などにより日本人にも決して少なくないことが判明してきている．日本人での血栓症リスクとなる先天性血栓性素因には，欧米人と同じくアンチトロンビン（AT），プロテインC（PC），プロテインS（PS）など生理的凝固抑制因子の遺伝子異常による欠乏症/異常症があり，特にPS Tokushima変異（p.K196E）は日本人特有の血栓性素因として知られている．一方，いまだに原因不明な遺伝性血栓症もあり，我々は長らく原因が不明であった静脈血栓症が多発する家系において，通常は出血症状が問題となるプロトロンビン遺伝子異常で逆に遺伝性血栓症の原因となる変異を発見し，新たな血栓性素因・アンチトロンビンレジスタンス（ATR）として報告した．本稿では，この血栓性素因・ATRについて最近の知見も踏まえて概説する．

はじめに

静脈血栓塞栓症（venous thromboembolism：VTE）は，深部静脈血栓症（Deep vein thrombosis：DVT）と肺血栓塞栓症（Pulmonary embolism：PE）の2つの概念を合わせた疾患群で，その発症要因には遺伝的リスクと環境的リスクが知られており，これらが複数重なることで発症する多因性疾患である[1]．VTEは，欧米人に多く見られ日本人には少ない疾患とされてきたが，食生活の欧米化や診断技術の向上により日本人における患者数も決して少なくはないことが明らかになってきた．VTE発症の誘因となる環境的リスクとしては，加齢，妊娠，長期臥床，ロングフライト（エコノミークラス症候群）などが挙げられる．また，その遺伝的リスクとして，生理的血液凝固抑制因子であるアンチトロンビン（antithrombin：AT），プロテインC（protein C：PC），プロテインS（protein S：PS）の欠乏症/異常症が広く知られており，日本人においてもそれぞれ数多くの遺伝子異常が報告されている．特に，PS Tokushima変異（p.K196E）は日本人特有の血栓性素因として知られ，55人に1人の頻度でヘテロが存在している[2]．一方，いまだに原因が不明な遺伝性血栓症もあり，我々は長らく原因が不明であった静脈血栓症が多発する家系において，通常は出血傾向となるプロトロンビン遺伝子異常で，逆に遺伝性血栓症の原因となることを発見し，新しい血栓性素因・アン

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チトロンビンレジスタンス（AT resistance：ATR）として報告した[3)4)]．本稿では，この新しい先天性血栓性素因・ATRとそのスクリーニング検査法の開発について，最近の知見も踏まえて概説する．

既存の先天性血栓性素因

血管損傷時には，血液が血管外に漏れ出ないよう損傷部局所ですみやかな血栓形成による止血機構が働く．逆に，健常血管内では誤って血栓が生じないように，また血管損傷部での止血血栓形成部位においては過剰に血液凝固反応が進行しないように，ともに生理的凝固抑制機構が働いている．生体防御反応の一つでもあるこれらの止血血栓形成は，血管内皮，血小板，凝固線溶因子ならびにそれらの抑制因子などの巧妙な連携制御のもとに営まれており，この制御機構の異常によりそのバランスが崩れると病的な出血症状や血栓症が発生する．これらのうち病的血栓症の原因となる遺伝的リスク要因としての先天性血栓性素因には，凝固反応の抑制制御に問題が生ずる「凝固抑制因子の遺伝子異常」と過剰な凝固反応をもたらす「凝固因子の遺伝子異常」が知られている．

1．凝固抑制因子遺伝子異常による先天性血栓性素因

生理的な凝固抑制因子であるAT，PC，PSの遺伝子異常には，それぞれの欠乏症/異常症に伴う遺伝性血栓性素因となる変異が報告されており[5)~7)]，日本人の静脈血栓症患者からも数多くの遺伝子異常が同定されている[8)]．特に，PS Tokushima（p.K196E）変異は日本人特有の先天性血栓性素因として知られ，日本人の55人に1人がヘテロ接合体としてp.K196E変異を保有するとされる[2)]．

2．凝固因子の遺伝子異常による先天性血栓性素因

欧米人に多い遺伝的リスクとして，活性化PCレジスタンス（APC resistance）を示すFactor V Leiden（R506Q）変異[9)]や，プロトロンビン遺伝子の3'非翻訳領域の変異により血漿中プロトロンビン濃度が上昇するプロトロンビンG20210A変異[10)]が有名である．しかし，これらは日本人を含めてアジア人には報告がなく，欧米人に血栓症の頻度が多い要因の一つとされている．また，ごく希な報告としては，凝固第IX因子（FIX）遺伝子異常が先天性出血性素因（血友病B）ではなく，逆に活性が異常高値（700%）を示すため遺伝性血栓症の原因となるFIX Padua遺伝子変異がある[11)]．

新たな先天性血栓性素因・アンチトロンビンレジスタンス（ATR）

遺伝性血栓症の中にはいまだに原因不明なものがある．我々は長らく原因が不明で静脈血栓症が多発する家系において，通常は出血傾向となるプロトロンビンの異常で逆に静脈血栓症の原因となる遺伝子異常を発見し，新しい血栓性素因・ATRとして報告した[3)4)]．これは，FV Leidenが同じく凝固因子のミスセンス変異でありながら，凝固活性は保たれ，かつ活性化PC（APC）による不活化に抵抗性を示すため，結果として血栓傾向となる病態に似ている[9)]．

1．プロトロンビンYukuhashi変異の発見

発端者の日本人女性は11歳時に深部静脈血栓症を発症していた．この家系では3世代に渡って8名の静脈血栓塞栓症患者（3名は既に死亡）がみられ，静脈血栓症は代を経るにつれ若年性に発症する傾向が見られた．2001年に既知の先天性血栓性素因について調査がされていたが，本家系には異常は検出されなかった[12)]．こうした中，2009年にBostonで開催された国際血栓止血学会（ISTH）において，ある遺伝性血栓症家系のゲノムワイド連鎖解析から，その病態は不明なもののプロトロンビン遺伝子異常の存在が報告された[13)]．この報告を受け我々も本家系発端者の解析を行ったところ，プロトロンビン遺伝子にミスセンス変異・プロトロンビンYukuhashi変異（c.1787G>A，p.R596L）を同定した．この変異は，トロンビン（プロトロンビンの活性型）がATと複合体（TAT）形成して不活化される際でのAT分子との結合部に位置するものであった（図1）．

2．本家系での血栓症発症機序解析

2009年ISTH Bostonにて報告されたプロトロンビン遺伝子異常については変異の存在の報告のみで，血栓症発症に至る病態解析の報告は未だな

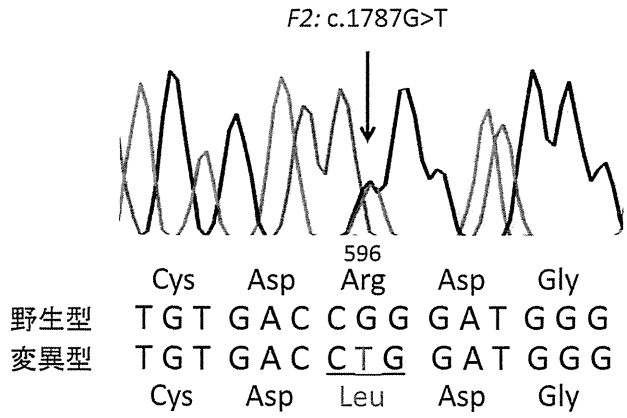


図1．アンチトロンビンレジスタンス（ATR）を示したプロトロンビン遺伝子変異
（Miyawaki, et al.：N Engl J Med, 2012から改変）
発端者のプロトロンビン遺伝子（*F2*）に，活性化後のトロンビンとATとの結合部に位置するアルギニン（596R）がロイシン（596L）に置換するミスセンス変異（c.1787G>A，p.R596L）を同定した．

されていない[13]．そこで我々は日本人家系におけるこのプロトロンビンYukuhashi変異での血栓症発症機序についての詳細な解析を行った．変異の見られたプロトロンビンのアルギニン（R596）は，活性化されたトロンビンがATと複合体（TAT）を形成して不活化される際に，ATのアスパラギン（N265）と水素結合を形成することが報告されている[14]．したがって，R596L置換はプロトロンビン活性化後の異常トロンビンの不活化不全を起こすことが予想され，また，このR596L置換変異は本家系の健常配偶者にはみられず，患者と家系内での他の血栓症患者にも検出されたことから，本家系での遺伝性血栓症の原因であることが強く疑われた．異常プロトロンビンの機能解析において，すでに血栓症治療のためにワルファリンを投与された患者血漿検体では解析困難であったため，我々は遺伝子工学的技法を用いて野生型/変異型プロトロンビンをリコンビナント蛋白としてHEK29細胞内で合成させ，その無血清培地での培養上清から濃縮し，そのトロンビンへの活性化動態，活性化後のATによる不活化動態を比較検討した．

3．トロンビンへの活性化動態

リン脂質とCaイオン存在下において，ウシ由来FXa/Fvaによるプロトロンビンからトロンビンへの転換を経時的にウェスタンブロット解析で観察したところ，変異型プロトロンビンは野生型とほぼ同様な開裂パターンを示したことから，それぞれトロンビンへの活性化はほぼ同等におこることが判明した（図2）．

次に，プロトロンビン欠乏血漿にそれぞれ野生型あるいは変異型リコンビナントプロトロンビンを添加して疑似血漿を作製し，3つの方法でプロトロンビン活性を解析した．すなわち，プロトロンビンからトロンビンへの活性化とフィブリノゲンを基質とする凝固活性を反映する凝固一段法，十分に活性化したトロンビンのフィブリノゲンに対する凝固活性のみを反映する凝固二段法，さらに，トロンビンに特異的な発色合成基質S-2238に対する活性を反映する合成基質法の，3種類の方法を用いて野生型/変異型プロトロンビンの活性を測定した（表1）．その結果，野生型疑似血漿はいずれの測定法でも正常血漿と同当な数値を示したが，変異型疑似血漿では3つ全ての方法で野生型を下回り，凝固一段法で最も低く（15%），次いで凝固二段法（32%），蛍光基質法（66%）の順で数値が大きくなった．すなわち，変異型プロトロンビンはトロンビンへの変換がやや遅延し，フィブリノゲンを基質とした活性も低下すること，フィブリノゲンと比較して分子量が小さい発色合成基質S-2238を用いて測定した活性はあまり低下しないことが観察された．ウェスタンブロット解析結果がほぼ同等の開裂パターンを示した結果と凝固一段法，二段法での明らかな差異とは一見矛盾しているようにみえるが，ウェスタンブロット法でのトロンビンへの開裂に要した最小時間は20秒と長く，数秒単位の差を反映する凝固法による際の検出限界には及ばなかったものと思われた．

4．変異トロンビンの不活化動態

変異型プロトロンビンをトロンビンに活性化した後，ATとの結合能（トロンビン・AT（TAT）複合体形成能）について野生型のそれと比較検討したところ，ヘパリン非存在下で変異型トロンビンはATにほとんど結合しないことが判明し，ほとんどATにより不活化されないことが予想さ

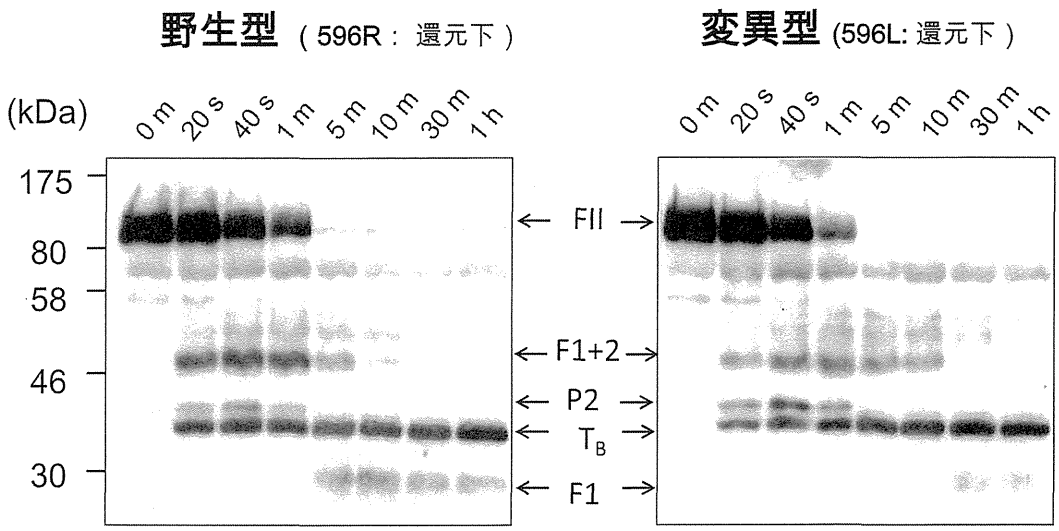


図 2. リコンビナントプロトロンビンの FXa/FVa による活性時開裂パターン
(Miyawaki, et al. : N Engl J Med, 2012 から改変)
野生型 (596R : 左), 変異型 (596L : 右) リコンビナントプロトロンビンのウシ由来 FXa/FVa によるトロンビンへの活性化開列パターンをウェスタンブロッティング解析した.
FII : prothrombin, F1, 2 : fragment 1+2, P2 : prethrombin-2, T_B : B chain of thrombin, F1 : fragment-1

表 1. プロトロンビン活性測定

	抗原量	活性値		
		凝固一段法	凝固二段法	合成基質法
正常型 (596R)	112%	91%	109%	88%
変異型 (596L)	118%	15%	32%	66%

プロトロンビン欠乏血漿にそれぞれ野生型／変異型リコンビナントプロトロンビンを添加して疑似血漿とし, 凝固一段法, 凝固二段法, 合成基質法の 3 種の方法を用いて野生型 (596R)／変異型 (596L) プロトロンビンの活性を測定した.
(Miyawaki, et al. : N Engl J Med, 2012 から改変)

れた (図 3). ヘパリン存在下では変異型でも野生型に似た継時的な TAT 複合体上昇を示したが, 1 分以内に形成された TAT 複合体は野生型の約半分程度にとどまっていた. これらの結果から, 変異型トロンビンでは AT によるトロンビン不活化反応が強く障害されていることが予想された.

一方, プロトロンビン欠乏血漿に変異型/野生型プロトロンビンを 50% ずつ添加した疑似患者血漿におけるトロンビン生成試験 (Thrombin generation assay : TGA) では, 野生型プロトロンビンを加えた疑似正常血漿や正常プール血漿と比較して, 最高トロンビン活性がやや低いものの不活化が著しく遅延しており, 結果として総トロンビン活性量に相当する ETP (Endogenous thrombin potential : 活性値と持続時間の積分値) の著しい増大を認めた (図 4). すなわち, 患者血漿中の異常プロトロンビンは, 血液凝固活性は低いものの一旦活性化されると ATR を示して凝固活性 (フィブリン生成能) を保ち続けることが予想され, これが本家系の遺伝性血栓症の原因になるこ

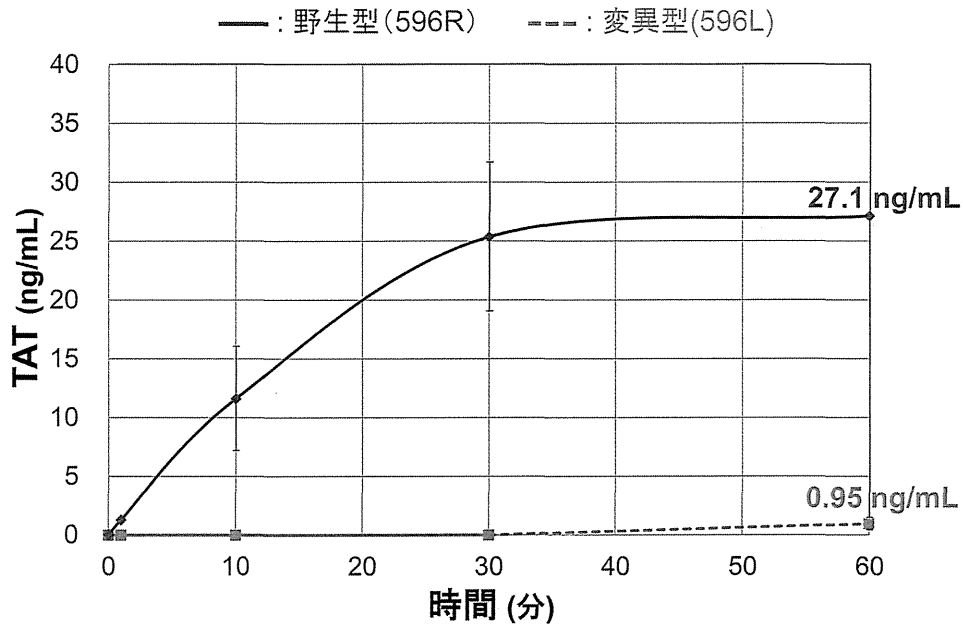
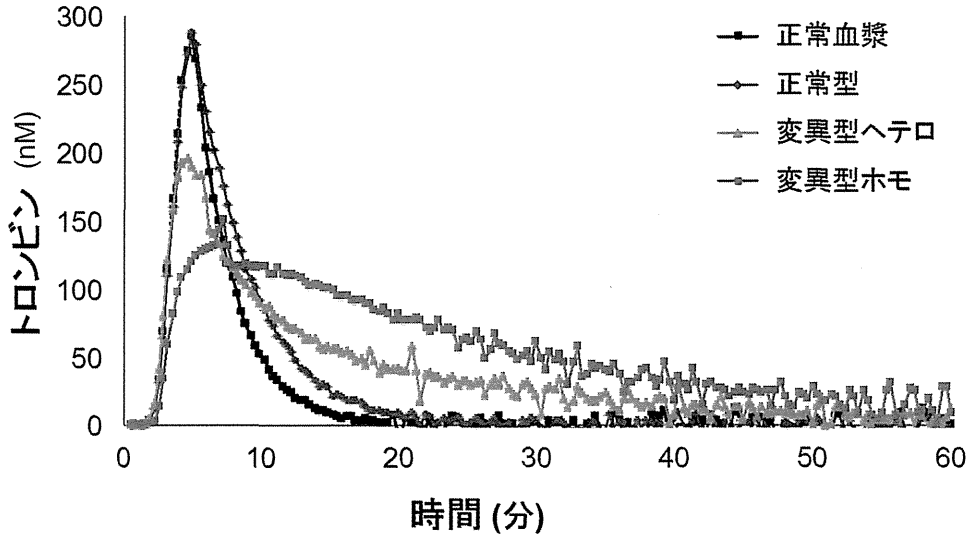


図3.　トロンビン・アンチトロンビン（TAT）複合体形成
（Miyawaki, et al. ：N Engl J Med, 2012から改変）
リコンビナントプロトロンビン（正常型/596Rおよび変異型/596L）をトロンビンに変換し，それぞれアンチトロンビンとの複合体（TAT）をELISA法にて測定．正常型でのTAT形成は経時的増加が見られたが，変異型では30分まで検出限界未満，60分後でもごくわずかにしか見られなかった．

とが示唆された．

5. ATRスクリーニング臨床検査法の開発

我々は，ヘパリン存在下，非存在下でのATによるトロンビンの不活化動態を合成基質法で解析観察することでATRを検出する臨床検査法を開発し，報告した[15]．これは，血漿検体を用いてプロトロンビナーゼ様活性をもつTypan蛇毒（*Oxyuranus scutellatus*（Ox））によるプロトロンビンを十分に活性化し，ATによるトロンビン不活化動態を各反応時間での残存トロンビン活性を測定して観察するものである．本法の開発にあたっては，反応各相での試薬，反応溶液組成，反応時間等の至適条件を検討し，ATRを検出する臨床検査法を考案した．本検査法において，プロトロンビン欠乏血漿に野生型ATを添加し作製した野生型再構成血漿検体で，ヘパリン非存在下で血中濃度5倍量のAT添加から30分で約10%にまでトロンビン活性が阻害されるのに対し，同様に作製した変異型ホモ再構成血漿検体では30分後でも90%以上残存していた（図4A）．また，静脈血栓症患者検体を想定し，ワルファリン服用検体を用いてワルファリンが本測定法に及ぼす影響についても検討した結果，考案した検査法は相対的なATによるトロンビン活性の不活化動態を観察することでワルファリン服用時にも解析可能であった．すなわち，ヘパリン非存在下でR596L変異非保有者検体は30分後にはいずれも20%程度までトロンビン活性が阻害されるのに対し，2名のR596L変異保有患者血漿ではともに30分後に約50%のトロンビン活性の残存が観察されたことから，ATR検体であると判定できる（図4B）．本検査法を用いて，原因不明であった静脈血栓塞栓症症例を解析することにより，静脈血栓塞栓症における新規血栓性素因・ATRの関与の実態が明らかとなることが期待される．ごく最近，我々はN Engl J Med誌に報告した家系とは異なる日本人静脈血栓症家系において，上述の血漿検体スクリーニング法での解析により新たなATR日本人症例を同定している[16]．



	正常血漿	正常型	変異型ヘテロ	変異型ホモ
総トロンビン活性量 (nM.min)	1276	1658	2374	3620
最高トロンビン活性 (nM)	284	283	194	144
トロンビン活性消失時間 (min)	23.5	26.5	78.0	105.0

図 4. トロンビン生成試験 (TGA)
(Miyawaki, et al. : N Engl J Med, 2012 から改変)
プロトロンビン欠乏血漿に各リコンビナントプロトロンビンを加えた疑似患者血漿 (変異型ヘテロ) では, 疑似正常血漿 (正常型) に比べ最高トロンビン活性がやや低いが消失時間が著しく長く, 結果として総トロンビン活性量 (ETP : Endogenous thrombin poteitial) の著しい増大を認めた.

6. ATR を示す新たな変異症例

2013 年 Djordjevic らは, 遺伝性血栓症をもつセルビア人 2 家系でのプロトロンビン遺伝子変異 (c.1787G>A, p.Arg596Gln) が報告したが, 我々が開発した検出法によりいずれも ATR を示すことが明らかとなった[17]. また, 同年にインド人血栓症患者においても同じ変異が同定され報告されている[18]. ごく最近, 上述のごとく我々は日本人 2 家系目の ATR 家系を血漿検体スクリーニング法により同定したが, 本家系の遺伝子解析の結果セルビア人変異と同じ c.1787G>A 変異を検出し[16], さらに別家系の日本人血栓症症例でも ATR を検出し, やはり c.1787G>A 変異を同定している (日本人 3 家系目). したがって, 血栓性素因・ATR は日本人だけでなく欧米人をはじめ他の人種にも遺伝性血栓症の原因として存在していることが明らかとなった. 血漿検体スクリーニング法の普及は, 厳密な倫理的配慮が必要な遺伝子解析に比べて利便性が高く, さらなる ATR 症例の検出にきわめて有用であると考えられる.

おわりに

遺伝性血栓症の原因として, 現在までに様々な凝固関連因子の遺伝子異常が同定されているが, いまだに原因不明な遺伝性血栓症も多くある. 我々は, 通常では出血傾向が予想される凝固因子・プロトロンビンの遺伝子変異が, 逆に静脈血栓症の原因となる詳細な分子病態を解明し, 新規血栓性素因・ATR を世界で初めて報告した. この新しい血栓性素因の発見は, 日本人だけでなく欧米人をはじめ他の人種での遺伝性血栓症においてもその病態解明による血栓症発症予防につながる

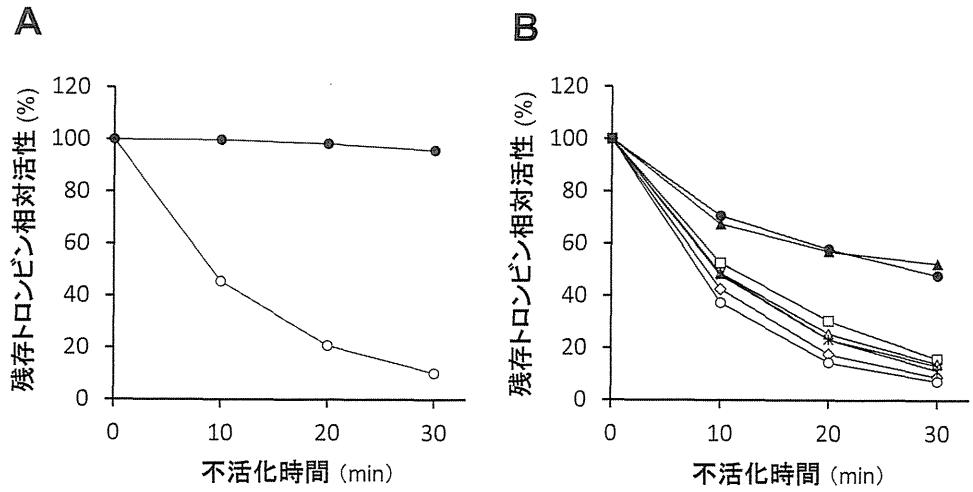


図5.　ATRスクリーニング検査法による血漿検体評価
(Murata, et al. : Thromb Res, 2014から改変)
A：ヘパリン非存在下において血中濃度5倍量ATの混和後30分でのトロンビン活性は，野生型（596R）再構成血漿では10%程度にまで阻害されるのに対して，変異型（596L）ホモ再構成血漿は90%以上の残存がみられた．
B：ヘパリン非存在下でのトロンビン活性は，プロトロンビン遺伝子に異常のないワルファリン服用患者血漿（□，△，◇，＊）では健常人血漿（○）と同様の不活化動態を示し，プロトロンビンR596L変異をヘテロにもつ2名の患者のワルファリン服用時血漿（●，▲）では明らかにATによる阻害を受けにくい結果を示した．

ことが期待でき，今後更なるATR病態についての研究成果の蓄積が望まれる．

文　献

1) Rosendaal F: Venous thrombosis: a multicausal disease. Lancet 353: 1167—1173, 1999.
2) Kimura R, et al: Protein S K196E mutation as a genetic risk for deep vein thrombosis in Japanese patients. Blood 107: 1737—1738, 2006.
3) Miyawaki Y, et al: Thrombosis from a prothrombin mutation conveying antithrombin resistance. N Engl J Med 366: 2390—2396, 2012.
4) Matsushita T, et al: Thrombosis from a prothrombin mutation conveying antithrombin resistance. The authors reply. N Engl J Med 367: 1069—1070, 2012.
5) Egeberg O: Inherited antithrombin deficiency causing thrombophilia. Thromb Diath Haemorrh 13: 516, 1965 (Abstract).
6) Griffin JH, et al: Deficiency of protein C in congenital thrombotic disease. J Clin Invest 68: 1370—1373, 1981.
7) Comp P, Esmon C: Recurrent venous thromboembolism in patients with a partial deficiency of protein S. N Engl J Med 311: 1525—1528, 1984.
8) Miyata T, et al: Prevalence of genetic mutations in protein S, protein C and antithrombin genes in Japanese patients with deep vein thrombosis. Thromb Res 124: 14—18, 2009.
9) Bertina RM, et al: Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369: 64—67, 1994.
10) Poort SR, et al: A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 88: 3698—3703, 1996.
11) Simioni P, et al: X-Linked Thrombophilia with a Mutant Factor IX (Factor IX Padua). N Engl J Med 361: 1671—1675, 2009.
12) 酒井道生，他：乳児発症例を含む血栓症多発の1家系. J UOEH(産業医科大学雑誌)　23：297—305, 2001.
13) ten Kate M, et al: A genome wide linkage scan for thrombosis susceptibility genes identifies a novel prothrombin mutation. XXII Congress of ISTH, Boston, July 11—16, 2009 (Abstract).
14) Li W, et al: Structure of the antithrombin-thrombin-

heparin ternary complex reveals the antithrombotic mechanism of heparin. Nat Struct Mol Biol 11: 857—862, 2004.
15) Murata M, et al: Development of a new laboratory test to evaluate antithrombin resistance in plasma. Thromb Res 133: 293—298, 2014.
16) 村田　萌，他：原因不明であった静脈血栓塞栓症にみられたアンチトロンビン抵抗性を示す本邦2家系目のプロトロンビン異常症．第33回日本臨床検査医学会東海・北陸支部例会・抄録集　2014.
17) Djordjevic V, et al: A novel prothrombin mutation in two families with prominent thrombophilia—the first cases of antithrombin resistance in a Caucasian population. J Thromb Haemost 11: 1936—1939, 2013.
18) Sivasundar S, et al: Molecular defect of 'Prothrombin Amrita': substitution of arginine by glutamine (Arg 553 to Gln) near the Na (+) binding loop of prothrombin. Blood Cells Mol Dis 50: 182—183, 2013.

Abstract

A new thrombophilia: Antithrombin resistance

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Venous thromboembolism (VTE) is a multifactorial disease that develops due to a variety of congenital and/or acquired factors. Previously, it was thought that the incidence of VTE is lower in Japanese than in Caucasians, but it has now been recognized that it occurs quite frequently in Japanese based on improved diagnosis technology and the westernization of eating habits. Congenital thrombophilia due to a genetic deficiency of natural anticoagulant factors such as antithrombin (AT), protein C (PC) and protein S (PS), is known to exist in Japanese, as in Caucasians, and many of those gene defects have been identified. In particular, PS Tokushima mutation (p.K196E) is known as a thrombophilia peculiar to Japanese. On the other hand, there is still idiopathic hereditary thrombosis. In a Japanese family in whom venous thrombosis occurred frequently and the cause was unknown for a long time, we found that hereditary thrombosis was caused by a mutation in the prothrombin gene that is usually reversely involved in bleeding symptoms. We thus reported a case of antithrombin resistance (ATR) as a new thrombophilia. In this review, I will discuss an outline of this thrombophilia, ATR, based on recent findings.

Key words: Thrombophilia, Antithrombin resistance, Prothrombin, Gene mutation

ELSEVIER

Analysis of the Hepatic Functional Reserve, Portal Hypertension, and Prognosis of Patients With Human Immunodeficiency Virus/Hepatitis C Virus Coinfection Through Contaminated Blood Products in Japan

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ABSTRACT

Background. As the survival of human immunodeficiency virus (HIV)-infected individuals has improved due to the widespread use of antiretroviral therapy, the mortality rate due to hepatitis C virus (HCV)-related liver disease has increased in HIV/HCV-coinfected patients.

Aim. The aims of this study were to establish the appropriate therapeutic strategy for HIV/HCV-coinfected patients by evaluating the liver function, including the hepatic functional reserve and portal hypertension, and to investigate the prognosis of HIV/HCV-coinfected patients in Japan.

Patients and Methods. In addition to regular liver function tests, the hepatic functional reserve of 41 patients with HIV/HCV coinfection was evaluated using the indocyanine green retention rate and liver galactosyl serum albumin-scintigraphy. The data for 146 patients with HIV/HCV coinfection through blood products were extracted from 4 major HIV centers in Japan. In addition to liver function tests, the platelet counts (PLT) were evaluated as a marker of portal hypertension.

Results. In spite of the relatively preserved general liver function test results, approximately 40% of the HIV/HCV-coinfected patients had an impaired hepatic functional reserve. In addition, while the albumin and bilirubin levels were normal, the PLT was <150,000/μL in 17 patients. Compared with HCV mono-infected patients with a PLT <150,000/μL, the survival of HIV/HCV-coinfected patients was shorter (HCV, 5 years, 97%; 10 years, 86% and HIV/HCV, 5 years, 87%; 10 years, 73%; $P < .05$).

Conclusion. These results must be taken into account to establish an optimal therapeutic strategy, including the appropriate timing of liver transplantation in HIV/HCV-coinfected patients in Japan.

FROM 1970 until the early 1980s, blood products were imported to Japan, and contaminated blood products were unknowingly used to treat patients with hemophilia. It was later revealed that these patients were sometimes infected with both human immunodeficiency virus (HIV) and hepatitis C virus (HCV; HIV/HCV coinfection) [1].

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However, as the survival of HIV-infected people has improved due to the widespread use of antiretroviral therapy, the mortality due to HCV-related liver disease has increased in HIV/HCV-coinfected patients [2,3].

The main aims of this investigation were to investigate the status of portal hypertension and the prognosis in HIV/HCV-coinfected patients, and to establish an appropriate therapeutic strategy for HIV/HCV-coinfected patients, including the timing of liver transplantation, in Japan.

PATIENTS AND METHODS

Routine hematology and blood chemistry tests (general liver function), abdominal ultrasonography, and contrast-enhanced computed tomography (CT) were performed for 30 patients with HIV/HCV coinfection at Nagasaki University Hospital. To investigate the hepatic functional reserve, liver GSA-scintigraphy and the indocyamine green retention test at 15 minutes were performed. In addition, upper gastrointestinal tract endoscopy to diagnose gastroesophageal varices was performed.

The data of the 146 patients who had acquired HIV/HCV coinfection through blood products were extracted from 4 major HIV centers in Japan, including the AIDS Clinical Center, Osaka National Hospital, Yokohama Municipal Hospital, and Kyushu Medical Center. In addition to liver function tests, platelet counts (PLT) were evaluated as a marker of portal hypertension. As a control, HCV mono-infected patients from Nagasaki Medical Center were used for comparison.

RESULTS

In spite of the relatively well-maintained general liver functions, approximately 40% of the HIV/HCV-coinfected patients had an impaired hepatic functional reserve (Table 1). In addition, in spite of maintained albumin and bilirubin levels, the PLT was <150,000/μL in 17 coinfected patients, indicating the presence of ongoing portal hypertension.

Even with Child-Pugh A liver function, the HIV/HCV-coinfected patients showed a worse prognosis than the HCV mono-infected patients. The prognosis was especially poor in those with lower PLT than in the patients with a normal PLT (Table 2). When compared with HCV mono-infected patients with a PLT <150,000 μL, the survival of HIV/HCV-coinfected patients was much shorter (HCV, 5 years, 97%; 10 years, 86% and HIV/HCV, 5 years, 87%; 10 years, 73%; $P < .05$).

Table 1. Patient Characteristics

Child-Pugh A/B/C	38 (93%)/1 (2%)/2 (5%)
ICG R15 (%)	
<10/10–20/20–30/30<	24 (59%)/8 (20%)/3 (7%)/6 (14%)
GSA schincigram LHL15	
>0.9/0.8–0.9/0.8>	28 (69%)/6 (15%)/7 (16%)
Liver configuration on CT	
Normal/CH/LC	10 (24%)/17 (42%)/14 (34%)
Splenomegaly	
Yes/no	26 (63%)/15 (37%)
Esophageal varices	
Yes/no	13 (32%)/28 (68%)

CH, chromic hepatitics; LC, liver cirrhosis.

Table 2. Patient Survival after Diagnosis

	5Y OS	10Y OS	
HCV mono-infection (Child-Pugh A)	97%	86%	
HIV/HCV coinfection (Child-Pugh A)			
PLT > 150,000	94%	85%	
PLT < 150,000	87%	73%	$P < .05$ vs HCV mono-infection

5Y OS, 5 year patient survival; 10Y OS, 10 year patient survival.

DISCUSSION

In HIV/HCV-coinfected patients, liver failure due to HCV hepatitis was previously reported to be enhanced by antiretroviral therapy ART-related hepatotoxicity, especially manifesting as noncirrhotic portal hypertension (NCPH) [4,5]. One of the ART drugs, Didanosin (DDI), has been suspected to be related to the serious morbidity observed in coinfected patients [6]. Thus, not only in patients with deteriorated liver function, such as in Child-Pugh B or C cases, but also even in Class A cases, the patients' liver function can easily deteriorate abruptly [7]. The natural course of pure NCPH is unknown because it can be modulated by HCV or other causes, and has only been reported as case series. An important study of "NCPH in HIV Mono-Infected Patients Without HCV" was published in 2012 [8]. All 5 patients had portal hypertensive symptoms, such as ascites or variceal bleeding, after receiving antiretroviral therapy.

Therefore, all HIV/HCV-coinfected patients should be carefully followed up so as not to miss an opportunity for liver transplantation (LT) [9]. The prognosis for HIV/HCV-coinfected patients was reported to be worse than that for HCV mono-infected patients [10]. In the present study, coinfected patients with a PTL <150,000 μL had an especially poor prognosis, with a shorter survival than mono-infected patients. Our results should be taken into account to establish a therapeutic strategy, while also considering the appropriate timing of LT in HIV/HCV-coinfected patients.

In 2013, based on the evidence of rapid progression of the liver cirrhosis and portal hypertension in patients with HIV/HCV coinfection, a rank-up system for the waiting list for deceased donor LT was set up in Japan. Even HIV/HCV-coinfected liver cirrhotic patients with Child-Pugh class A can be listed for LT as "point 3" because of the NCPH (noncirrhotic portal hypertension) nature. Coinfected patients with Child-Pugh class B and C disease can be listed as "point 6" and "point 8," respectively, based on the data collected by the HIV/acquired immunodeficiency syndrome (AIDS) project team of the Ministry of Health, Labor, and Welfare of Japan, and the published literature [11]. This primarily covers victims who received contaminated blood products for hemophilia.

Future perspectives on LT for HIV/HCV coinfection include the following: new anti-HCV agents should be

developed to improve the control against HCV; new ART drugs, such as Raltegravir, should facilitate post-transplantation immunosuppressive therapy; noninvasive tests for portal hypertension, such as the fibroscan, should be performed for hemophilic patients; and the development of guidelines for the management hemophilia in the peri-operative period should facilitate better outcomes.

In conclusion, the present results should be taken into account to establish an optimal therapeutic strategy, including the appropriate timing of LT in HIV/HCV-coinfected patients.

REFERENCES

[1] Eguchi S, Soyama A, Hidaka M, Takatsuki M, Muraoka I, Tomonaga T, et al. Liver transplantation for patients with human immunodeficiency virus and hepatitis C virus coinfection with special reference to hemophiliac recipients in Japan. Surg Today 2011;41:1325–31.

[2] Merchante N, Merino E, Lopez-Aldeguer J, Jover F, Delgado-Fernandez M, Galindo MJ, et al. Increasing incidence of hepatocellular carcinoma in HIV-infected patients in Spain. HIV/AIDS 2013;56:143–50.

[3] Cusinato CT, Koetz AP, Barcellos NT, Wolff FH. The prevalence of cirrhosis and hepatocellular carcinoma in patients with human immunodeficiency virus infection. Hepatology 2013;57:249–57.

[4] Vispo E, Moreno A, Maida I, Barreiro P, Cuevas A, Albertos S, et al. Noncirrhotic portal hypertension in HIV-infected patients: unique clinical and pathological findings. AIDS 2010;24:1171–6.

[5] Mendizabal M, Craviotto S, Chen T, Silva MO, Reddy KR. Noncirrhotic portal hypertension: another cause of liver disease in HIV patients. Ann Hepatol 2009;8:390–5.

[6] Kovari H, Ledergerber B, Peter U, Flepp M, Jost J, Schmid P, et al., Swiss HIV Cohort Study. Association of non-cirrhotic portal hypertension in HIV-infected persons and antiretroviral therapy with didanosine: a nested case-control study. Clin Infect Dis 2009;49:626–35.

[7] López-Diéguez M, Montes ML, Pascual-Pareja JF, Quereda C, Von Wichmann MA, Berenguer J, et al., GESIDA 37/03-FIPSE 36465/03-NEAT IG5 Study Group. The natural history of liver cirrhosis in HIV-hepatitis C virus-coinfected patients. AIDS 2011;25:899–904.

[8] Jackson BD, Doyle JS, Hoy JF, Roberts SK, Colman J, Hellard ME, et al. Non-cirrhotic portal hypertension in HIV mono-infected patients. J Gastroenterol Hepatol 2012;17:1512–9.

[9] Soyama A, Eguchi S, Takatsuki T, Hidaka M, Muraoka I, Kanematsu T. Analysis of hepatic functional reserve in HIV_HCV co-infected patients. Acta Hepatol Japonica (KANZO) 2012;53:403–8 (In Japanese).

[10] Takatsuki M, Eguchi S, Soyama A, Kanematsu T, Nakao K, Shirasaka T, et al. Evaluation of portal hypertension and prognosis of patients with HIV-HCV co-infection through comtaminated blood product. Acta Hepatol Japonica (KANZO) 2012;53:586–90 [in Japanese].

[11] Eguchi S, Takatsuki M, Kuroki T. Liver transplantation for patients with human immunodeficiency virus and hepatitis C virus co-infection: update in 2013. J Hepatobiliary Pancreat Sci [e-pub ahead of print]. Accessed September 11, 2013.

J Hepatobiliary Pancreat Sci (2014) 21:263–268
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REVIEW ARTICLE

Liver transplantation for patients with human immunodeficiency virus and hepatitis C virus co-infection: update in 2013

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Abstract Because of the progress of anti-retroviral therapy (ART) for human immunodeficiency virus (HIV), mortality due to opportunistic infection resulting in AIDS has been remarkably reduced. However, meanwhile, half of those patients have died of end-stage liver cirrhosis due to hepatitis C virus (HCV) with liver cirrhosis and early occurrence of hepatocellular carcinoma. Recently, in 2013, non-cirrhotic portal hypertension due to ART drugs or still unknown mechanisms have become problematic with early progression of the disease in this patient population. Liver transplantation (LT) could be one treatment of choice in such cases, but the indications for LT perioperative management, including both HIV and HCV treatments and immunosuppression, are still challenging. In this review, we update the literature on HIV/HCV co-infection and LT as well as recent effort for modifying allocation system for those patients.

Keywords Co-infection · Hepatitis C virus · HIV · Human immunodeficiency virus · Liver transplantation

Introduction

The causes of death of human immunodeficiency virus (HIV) infected patients have dramatically changed since 1995. A major background factor behind these trends is the improved HIV control achieved with anti-retroviral therapy (ART) [1]. Despite dramatic reduction of death due to acquired immunodeficiency syndrome (AIDS), co-infected hepatitis C virus (HCV)-related death due to liver failure or hepatocellular carcinoma (HCC) became a serious problem, not only in Japan but all over the world, including England [2]. In Japan, in the late 1980s, contaminated blood products for hemophilia caused co-infection by HIV and HCV. In such cases, liver transplantation (LT) is the only possible treatment option to achieve long-term survival, but several modifications of perioperative management are required recently for better outcome.

In this review, the outcome and the points of management of LT for HIV/HCV co-infected patients were reviewed to save relatively young patients with HIV/HCV co-infection bearing HCC [3, 4], non-cirrhotic portal hypertension (NCPH) [5–7], and decompensated liver cirrhosis [8, 9]. An updated critical review of the literature in 2013 was performed, and new information on problems and results for LT for HIV/HCV co-infection were included.

Upcoming topics regarding LT indications for HIV/HCV co-infection in 2013

Non-cirrhotic portal hypertension

In HIV/HCV coinfected patients, liver failure due to HCV hepatitis was enhanced by ART-related hepatotoxicity, especially manifesting as non-cirrhotic portal hypertension [5–7]. One of the ART drugs, Didanosin (DDI), has been suspected for serious morbidity. Thus, not only in cases with deteriorated liver function, such as in Child–Pugh B or C cases, but also even in Class A cases, patients' liver function can easily deteriorate abruptly [10, 11]. The actual natural course of pure NCPH is unknown, because it can be modulated with HCV or other causes and reported as only case series. However, an important study regarding "Non-cirrhotic portal hypertension in HIV mono-infected patients without HCV" was published in 2012 [12]. All five patients had portal hypertensive symptoms such as ascites or variceal bleeding after ART medication. We need to await their prognostic information, since it can be extrapolated into HIV/HCV co-infected patients after successful HCV eradication.

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Therefore, all HIV/HCV co-infected patients should be carefully followed up so as not to miss the opportunity for LT. Recently, in Japan, a scoring system was created for listing a deceased donor LT for those patients with HIV/HCV co-infection due to previous contaminated blood products.

Hepatocellular carcinoma

Recently it became evident that HCC in HIV/HCV co-infected patients develop HCC at a very early stage of life, such as in the 30s and 40s [3, 4]. The molecular mechanism of its development still remains unclear, but surveillance in those patients should be considered for HCC strictly. In Japan, HIV/HCV co-infected hemophilic patients have been undergoing periodic examination for liver-related disease on a research basis. Early detection could contribute to treatment choices such as liver resection or liver transplantation. Regardless of the infectious status of HIV, treatment strategy for HCC in HIV/HCV infected patients should be the same in HCV mono-infected patients. Namely, whether liver resection could be performed or not should be based on the liver functional reserve. Also radio frequency ablation and transarterial chemoembolization can be selected according to the location, size and number of HCC.

Current results of LT for HIV/HCV co-infected patients in 2013

Indications for LT

As HCV mono-infected patients, LT should be considered when patients develop deteriorated liver function as indicated by a Child–Pugh score of class B or C in co-infected patients. Recently, Murillas et al. reported that the Model for End-stage Liver Disease (MELD) score is the best prognostic factor in HIV-infected patients [13]. HIV/HCV co-infected patients might be considered for LT before their MELD score increases to achieve comparable results with HCV mono-infected patients. Several studies showed that aggressive fibrosis in HIV/HCV co-infected patients compared with HCV mono-infected patients [14, 15], but the mechanism of this aggressive fibrosis remains unclear. Recently, transient elastography or acoustic radiation force impulse (ARFI) imaging to check for liver stiffness has been introduced as an effective and noninvasive modality to determine patients' candidacy for LT [16, 17].

Regardless of the presence of hemophilia, the indications and methods for performing liver transplantation remains unchanged for patients with HIV/HCV co-infection. In fact, after a successful liver transplantation, hemophilia can normally be cured. Usually, the conditions for liver transplantation are as follows: (1) AIDS symptoms have not surfaced; (2) CD4+ T lymphocyte count is 150–200/μl or above; and (3) as a result of ART, the amount of HIV RNA in the blood by PCR method is below the level of sensitivity of the assay.

In HIV/HCV co-infected patients, current studies show that a count of more than 100/μl CD4+ T lymphocytes is acceptable [18, 19], because patients generally have portal hypertension, which can cause leukocytopenia. In such patients, the ratio of CD4/CD8 is reported to be a realistic marker to predict postoperative complications including opportunistic infections. When the ratio is less than 0.15, the incidence of infectious complications is significantly higher [20].

In 2013, based on the evidence of rapid progression of the liver cirrhosis and portal hypertension in patients with HIV/HCV co-infection, a ranking system for waiting list of deceased donor LT has been set up in Japan. Even HIV/HCV co-infected liver cirrhotic patients with Child–Pugh class A can be listed for LT as "point 3" because of NCPH nature. Also co-infected patients with Child–Pugh class B and C can be listed as "point 6" and "point 8" based on the data from our HIV/AIDS project team of the Ministry of Health, Labor, and Welfare of Japan, and world literatures [21–23]. It is basically considered for previous victims of contaminated blood products for hemophilia.

Results of LT for patients with HIV/HCV co-infection

In the United States and Europe, liver transplantation from deceased donors has been performed in HIV patients since the 1980s. At that time, the outcomes of LT were very poor [11]. Recent series of reports are listed in Table 1 [24–31]. The reality is that, in addition to those listed therein, there have been many sporadic reports, such as reviews, expectations for liver transplantation, and assessment of indications.

In general, most reports concluded that the results were 10% worse than in the cases with HCV mono-infection, with a 3-year survival of around 60–70%. Recently, a 5-year patient survival of around 50% was reported, and there is debate whether these results can be accepted for patients of a younger age and were co-infected through previous use of a contaminated blood product. In Japan, the Tokyo group reported six cases of living donor liver transplantation (LDLT) between 2001 and 2004 [32]. Terrault et al. reported that older donor age, combined kidney-liver transplantation, an anti-HCV positive donor, and a body mass index <21 kg/m^2 were independent predictors of graft loss [33]. After LT, several studies showed that acute cellular rejection was more frequent and more severe in HIV/HCV co-infected patients than in HCV mono-infected patients, possibly due to difficulties in achieving optimal immunosuppression because of interactions between antiretroviral agents and immunosuppression.

Table 1 Updated outcome of liver transplantation for HIV positive recipients

Authors	Year	Country	*n*	Patient survival (%)			
				1 year	3 years	5 years	
Duclos-Vallee et al. [25]	2008	France	35	–	73	51	
Tsukada et al. [32]	2011	Japan	6	66	66	50	Only LDLT, only hemophilia
Terrault et al. [33]	2012	US	89	76	60	–	
Miro et al. [26]	2012	Spain	84	88	62	54	
Anadol et al. [27]	2012	Germany	32	90	65	60	
Harbell et al. [28]	2012	USA	125	91	67	–	
Baccarani et al. [31]	2012	Italy	32	–	79	69	
Di Benedetto et al. [46]	2012	Italy	30	75	65	50	with HCC
Ragni et al. [29]	2013	USA	15	71	38	–	only hemophilia

HCC hepatocellular carcinoma, *LDLT* living donor liver transplantation

Lowered outcome can be presumed from previous reports. Final mortality (graft loss) after LT was usually due to infection and multiorgan failure. As in Miro's report the causes due to the higher proportion of organs from donation after cardiac death (DCD) donors, higher rate of combined liver-kidney transplantation, increased rate of acute cellular rejection, HBV co-infection and infection. However, it was of note that there was no death due to infections related to HIV.

Preoperative management of HIV/HCV in liver transplantation

The number of HIV-RNA copies before LT is suggested as an independent risk factor of postoperative mortality, so that HIV should be controlled sufficiently before LT [30]. Accordingly, in patients who are under consideration to receive LT, ART can be safely stopped before LT, because HIV is generally well controlled for a long period by ART. Also ART can be toxic for the virgin graft, which underwent ischemia/reperfusion injury and liver resection in a donor. Once it is settled down after liver transplant, especially in LDLT cases, ART can be resumed with meticulous adjustment with calcineurin inhibitors.

Actually, after LT, ART should be restarted as soon as possible, because HIV-RNA appears at 3 to 30 days after ART is stopped [34], but the timing of restart of ART depends on the patient's condition, including liver function [35]. As long as the liver function has not fully recovered, or partial liver graft such as in LDLT has not yet sufficiently regenerated, ART cannot be started. Castells et al. reported in their case-control study that ART was started at a median of 8 days after LT (range 4–28 days) [36]. ART administered after LT should be the same as the preLT regimen, but the majority of ART drugs, including protease inhibitors and non-nucleoside reverse transcriptase inhibitors, have interactions with calcineurin inhibitors (CNI) or mammalian target-of-rapamycin (mTOR) [37], so that the monitoring of blood levels of immunosuppression is extremely important to avoid infectious complications or rejection. It can easily overshoot beyond the therapeutic level. Currently, a novel HIV-1 integrase inhibitor, raltegravir, is expected to be a feasible drug because it has no interactions with CNI, unlike other drugs [38, 39]. Therefore, the current recommended strategy in the light of LT could be to try raltegravir as ART before LT and see if HIV can be controlled with raltegravir. If it is the case, CNI could be used as usual after LT. However, if raltegravir cannot control HIV or cannot be applied due to other reasons, meticulous management of CNI (e.g. once a week administration with frequent trough monitoring) or Mycophenolate mofetil protocol should be considered. In fact, the novel protease inhibitor anti-HCV drug, telapreir, has the same character as ART drugs for HIV, and transplants team learn to overcome such drug interactions when post-LT HCV mono-infected patients are treated with telaprevir.

The treatment strategy for HCV in HIV/HCV co-infected patients is the same as in HCV mono-infected patients. Combination therapy of pegylated interferon (Peg-IFN) and ribavirin is the standard treatment both before and after LT in 2013. The treatment should be started as soon as possible, because in HIV/HCV co-infected patients, HCV recurrence may be accelerated in an immunocompromised state [40, 41]. As mentioned above, the novel protease inhibitor telaprevir is currently being introduced as an effective drug to achieve sustained viral response (SVR) of 70%, even in genotype 1b, with Peg-IFN/ribavirin in a non-transplant setting [42], but this drug is metabolized via cytochrome P450, as are CNI and various protease inhibitors of ART for HIV. Close monitoring of the CNI trough level should be performed, and although triple therapy with telaprevir/Peg-IFN/ribavirin or even without Peg-IFN is currently reported to be effective to prevent HCV recurrence after LT in HCV mono-infected cases, special attention should be paid when

this regimen is adapted for HIV/HCV co-infected patients. Additionally, mutational status of the IL28 B genotype should be investigated before interferon therapy for both donor and recipient.

Immunosuppression

Several reports have demonstrated both the in vitro and in vivo effectiveness of rapamycin in reducing HIV replication [43–45]. Di Benedetto et al. found that rapamycin monotherapy was significantly beneficial in long-term immunosuppression maintenance and HIV control after LT [46]. Mycophenolate mofetil is expected to be an effective immunosuppressive drug because of its efficacy in reducing HIV infection by both virological and immunological mechanisms. Mycophenolic acid, a selective inhibitor of the de novo synthesis of guanosine nucleotides in T and B lymphocytes, has been proposed to inhibit HIV replication in vitro by depleting the substrate (guanosine nucleotides) for reverse transcriptase. Using these drugs, a more effective regimen of immunosuppression with ART may be established. However, more information needs to be obtained to establish concrete immunosuppressive protocol.

As to steroids, several studies proposed that a steroid-free regimen can be safely applied and effective in LT for HCV cirrhosis. In HIV/HCV co-infected patients, a steroid-free protocol may play a beneficial role in preventing both HIV and HCV recurrence after LT [47, 48].

Hepatocellular carcinoma

Liver transplantation has been performed also for indication of HCC. The most updated study indicated that the existence of HCC did not change the outcome of LT provided that HCC was downstaged preoperatively for UCSF criteria [49]. Also for these cases sirolimus tended to be used as primary immunosuppressive agents. This encouraging result awaits further reports [50].

Conclusions

The above is an overview of liver transplantation performed to date in HIV/HCV- co-infected patients. Although, the results are 10% lower in patient survival after LT than those for HCV mono-infected patients, LT could be feasible in selected cases with HIV/HCV co-infection after careful evaluation within suitable stages of the disease. In light of the fact that most HIV/HCV co-infected patients in Japan are the victims of contaminated blood products, it is believed that the importance of liver transplantation will increase in the future in the context of medical relief as well. Our investigating team under the Ministry of Health, Labor, and Welfare of Japan has made all possible efforts to clarify the appropriate timing to put HIV/HCV co-infected patients on a waiting list for LT.

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Conflict of interest None declared.

References

1. Eguchi S, Soyama A, Hidaka M, Takatsuki M, Muraoka I, Tomonaga T, et al. Liver transplantation for patients with human immunodeficiency virus and hepatitis C virus coinfection with special reference to hemophiliac recipients in Japan. Surg Today. 2011;41:1325–31.
2. Darby SC, Ewart DW, Giangrande PL, Spooner RG, Rizza CR, Dusheiko GM, et al. Mortality from liver cancer and liver disease in haemophilic men and boys in UK given blood products contaminated with hepatitis C. UK Haemophilia Centre Directors' Organisation. Lancet. 1997;350:1425–31.
3. Merchante N, Merino E, Lopez-Aldeguer J, Jover F, Delgado-Fernandez M, Galindo MJ, et al. Increasing incidence of hepatocellular carcinoma in HIV-infected patients in Spain. HIV/AIDS. 2013;56:143–50.
4. Cusinato CT, Koetz AP, Barcellos NT, Wolff FH. The prevalence of cirrhosis and hepatocellular carcinoma in patients with human immunodeficiency virus infection. Hepatology. 2013;57:249–57.
5. Vispo E, Moreno A, Maida I, Barreiro P, Cuevas A, Albertos S, et al. Noncirrhotic portal hypertension in HIV-infected patients: unique clinical and pathological findings. AIDS. 2010;24:1171–6.
6. Mendizabal M, Craviotto S, Chen T, Silva MO, Reddy KR. Noncirrhotic portal hypertension: another cause of liver disease in HIV patients. Ann Hepatol. 2009;8:390–5.
7. Kovari H, Ledergerber B, Peter U, Flepp M, Jost J, Schmid P, et al. Swiss HIV Cohort Study. Association of noncirrhotic portal hypertension in HIV-infected persons and antiretroviral therapy with didanosine: a nested case-control study. Clin Infect Dis. 2009;49:626–35.
8. Merchante N, Girón-González JA, González-Serrano M, Torre-Cisneros J, García-García JA, Arizcorreta A, et al. Survival and prognostic factors of HIV-infected patients with HCV-related end-stage liver disease. AIDS. 2006;20:49–57.
9. Weber R, Sabin CA, Friis-Moller N, Reiss P, El-Sadr WM, Kirk O, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. Arch Intern Med. 2006;166:1632–41.
10. Ragni MV, Eghtesad B, Schlesinger KW, Dvorchik I, Fung JJ. Pretransplant survival is shorter in HIV-positive than HIV-negative subjects with end-stage liver disease. Liver Transpl. 2005;11:1425–30.
11. de Vera ME, Dvorchik I, Tom K, Eghtesad B, Thai N, Shakil O, et al. Survival of liver transplant patients coinfected with HIV and HCV is adversely impacted by recurrent hepatitis C. Am J Transplant. 2006;6:2983–93.
12. Jackson BD, Doyle JS, Hoy JF, Roberts SK, Colman J, Hellard ME, et al. Non-cirrhotic portal hypertension in HIV mono-infected patients. J Gastroenterol Hepatol. 2012;17:1512–19.