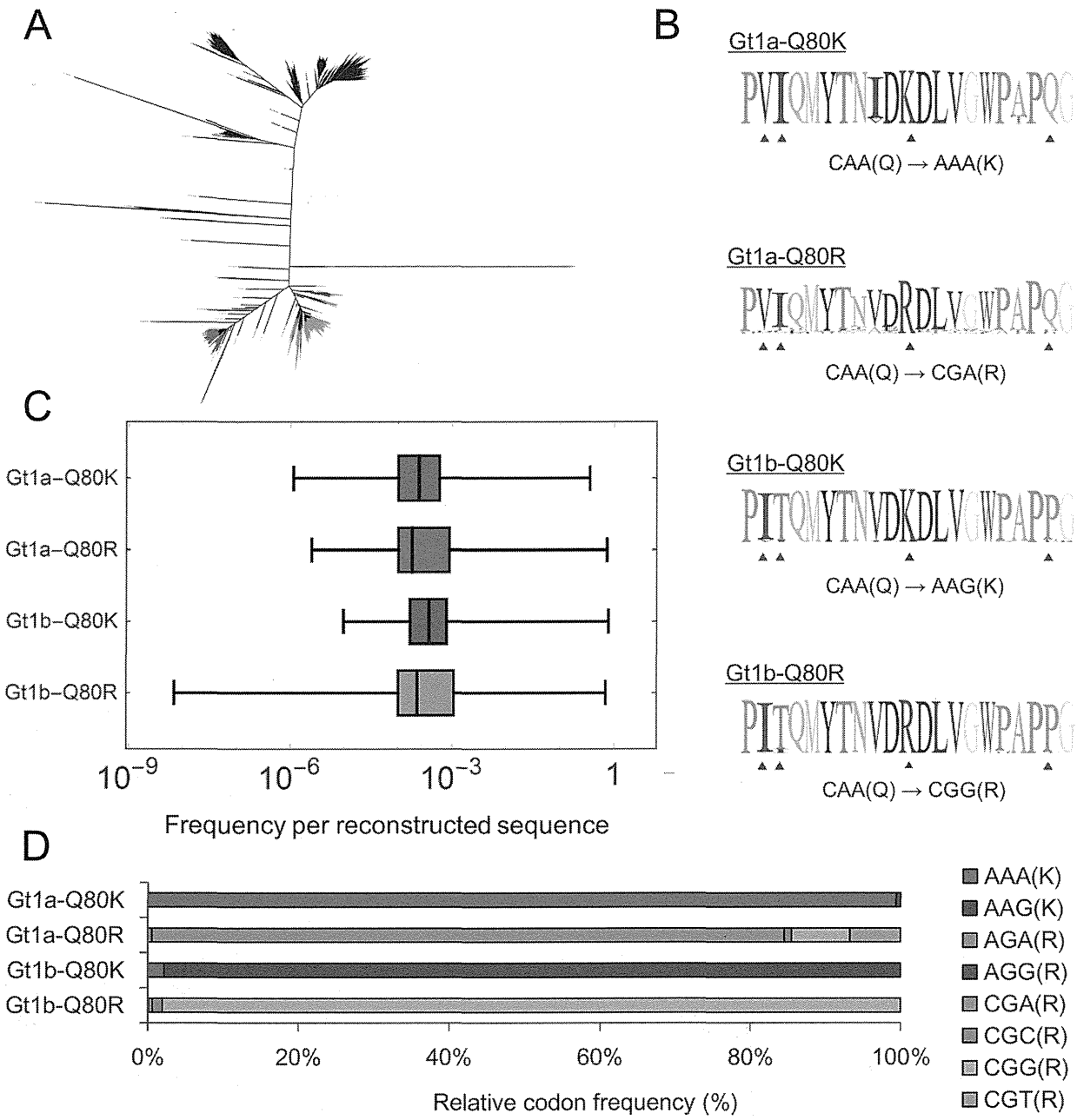

Fig 8. Integrated interdependence analysis of Gt and RAV enables high-throughput identification of distinct subpopulations with characteristic combinations of Gt and amino acid haplotype. (A) Phylogenetic analysis of all reconstructed sequences of NS3 protease region. Taxa are colored on the basis of their assigned Gt and Q80 RAV as follows: Gt1a-Q80K = blue; Gt1a-Q80R = purple; Gt1b-Q80K = red; Gt1b-Q80R = orange. A phylogenetic tree was generated using FigTree software. (B) Sequence logos from all sequences assigned to each pair of Gt and Q80 amino acid variant depicted in (A). Blue triangles denote NS3 Q80. Red triangles denote geno/subtype-specific amino acid polymorphisms at positions 71, 72, and 89. The codon change from reference to the most dominant variant at position 80 was denoted. (C) Distributions of estimated frequency per reconstructed sequence. (D) Relative codon frequencies for each Gt-RAV. The frequency was defined as the ratio of the number of reconstructed sequences possessing each codon and the total number of reconstructed sequences.

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core region and NS3 protease region. Furthermore, we achieved highly accurate estimations of Gts and RAVs by combining two QSR programs, QuRe and QuasiRecomb, both of which were based on different algorithm principles. Initially, we had a concern about artificial

recombination attributed to PCR amplification step and/or QSR calculation step. However, the simulation experiments demonstrated the accuracy of our QSR-based genotyping (Fig. 3 and Table 2) and RAV screening (Fig. 4 and Table 3) without *in silico* RAV recombination proven (Fig. 4A). A high PPV, rather than a high Sn, would be preferable for future investigation, because a high PPV would allow effective selection of patients having "true-positive" low-frequency RAVs, without the annoying false-positive RAVs. This is particularly important for research focusing on the impact of pre-existing minor RAVs, because a considerable number of false-positive RAVs at the preliminary screening stage might lead to a false conclusion that minor RAVs showed no correlation with the treatment outcome. In addition, note that sensitivity is in principle restricted by the coverage depth attainable with the sequencers currently available; therefore, so methodological improvement would be difficult. Lastly, by desterilizing the reconstructed haplotype information, we combined genotyping and RAV screening so as to find a novel relationship between them. The limitations of conventional SNV-based mutation screening are summarized into the following points: (1) it was difficult to gain genotype information; (2) it was difficult to link detected SNVs to correctly infer relevant RAVs, especially when multi-geno/subtype clones co-existed; and (3) it was impossible to gain insight on the basis of epistatic interactions between mutations, which has recently been predicted in HIV protease by a systems approach [45]. Our approach can overcome these limitations, which can reveal how the impact of one mutation depends on the presence or absence of other mutations in the context of clinical trials and post-trial surveys.

Recently, Jabara et al. have reported a novel solution to eliminating errors introduced during PCR amplification and pyrosequencing by using a single-molecular identifier [46,47]. The principle of this strategy is the use of a RT primer tagged by an 8 degenerate ID sequence. The resultant pyrosequencing reads having the same ID tag sequence are clustered, and the consensus sequence is generated on the majority basis, thus enabling the effective removal of artificial errors introduced during PCR, library preparation, and NGS. Polymerase error rate has been vigorously studied because of its potential impact on the inference of viral quasispecies diversity [48]. Although a promising technique, however, the analysis of this diversity could not yet be considered error-free owing to the error-prone nature of reverse transcriptase. The error rate of the commonly used, MMLV RTase was reported to be around 10^{-5}–10^{-4} per nucleotide [49], which might still be sufficient to artificially generate low-frequency false positive variants. Moreover, the read length of the NGS sequencer, a maximum of $\sim$1000 bp achieved using the Roche GS FLX+ system, would be an inevitable limitation of this methodology. Another solution that has recently been described by Acevedo et al. is circular sequencing (CirSeq), wherein circularized genomic RNA fragments are used to generate tandem repeats [50,51]. These repeated reverse transcriptions principally eliminate even the errors introduced by the reverse transcriptase use. The CirSeq approach in principle would provide completely error-free sequencing, but the target RNA must be fragmented into small pieces before amplification, which would be unfavorable for linkage analysis. In contrast to these emerging techniques, our analysis pipeline is much more practical. Moreover, our framework can be applicable even to previous NGS data obtained from ordinary RT-PCR experiments, as long as the read lengths are sufficiently large. NGS sequence meta-analysis is an emerging but promising strategy to integrate our knowledge leading to deeper insights on viral quasispecies dynamics.

Among hemophiliacs frequently receiving coagulation factor concentrates, the prevalence of HCV infection was high (60–90%) [52,53]. Before 1984, preheating was not yet routinely performed during the preparation of coagulation factor concentrates to inactivate contaminating HIV [54,55]. Moreover, blood products were frequently imported from countries overseas including the United States, as there were insufficient blood donors in Japan. Thus, patients using blood products at that time were exposed to the risk of infection with not only HCV but

also HIV, which had not yet been prevalent in Japan. Considering this specific circumstance, we hypothesized that there were HCV quasispecies of different genetic and geographic origins among HCV monoinfected non-hemophiliacs and HCV/HIV coinfected hemophiliacs in Japan. As expected, our analyses demonstrated that the compositions of genotypes and RAVs were quite different between HCV/HIV coinfected hemophiliacs, HCV monoinfected patients with previous exposure to whole-blood transfusion (BLx), and HCV monoinfected patients without a history of exposure to BLx. Gt1b was dominant (10 out of 11 = 91%) among cases without HIV coinfection, whereas Gt1a was dominant (6 out of 11 = 54%) among HCV/HIV coinfected patients. The predominant infection with Gt2a and Gt2b was determined in 3 cases. No other genotypes such as Gt3 and Gt4 were detected in this study. Moreover, multi-geno/subtype overlapping infection was significantly more prevalent among hemophiliacs and patients with BLx. This high prevalence of overlapping infection might explain the changes in genotype frequently observed among hemophiliacs [56,57] and other at-risk populations [58]. Furthermore, investigation on the interrelationships between Gts and RAVs suggests that Q80K was more prevalent in HCV/HIV coinfected hemophiliacs, whereas Q80R was less prevalent in HCV monoinfected non-hemophiliacs (Tables 4 and 5). A notable finding is that Q80K was significantly associated with Gt1b quasispecies among the hemophiliacs in this study (Tables 4 and 5, and Fig. 8). The Q80K variant is observed in 5.7–38% and 0.0–0.8% of patients with Gt1a and Gt1b HCV infections, respectively [42]. Q80K confers a 9.3-fold resistance against simeprevir in the Gt1a replicon system [42], and one clinical Phase 2 trial of simeprevir showed reduced SVR 24 rates with patient with Q80K mutation compared to those without Q80K (70.6–85.5% vs 55.0–66.7%) [38,59]. Currently, however, there is still limited information available regarding the impact of Q80K on Gt1b HCV infection, despite the fact that the effect of Q80K has been well characterized for Gt1a. In the first place, the epidemiology and characteristics of Gt1 sequences having the Q80K/R variant should be further studied, as searches of the Los Alamos database yielded a very unsatisfying number of previously identified sequences (S3 and S4 Tables). Detailed examination of the linkage between genotype and several RAVs may provide additional insights the clinical relevance of low-frequency genotype, drug-resistant quasispecies and their impact on the DAA therapy outcome.

Similarly to all studies, this study has some limitations. Firstly, the number of cases studied was very small, thus, the statistical power was insufficient to certainly detect low-prevalence mutations (e.g., R155, A156, and D168) if present. Secondly, although randomly selected, there might be a certain bias in enrolling HCV mono-infecting samples with a history of blood transfusion available. In this study, sample information including age, sex, associated illness, and source of infection, was not taken into consideration in the analysis; thus, the possibility of confounding and selection biases still remains. Thirdly, this study does not include hemophiliacs with HCV infection without HIV coinfection, because of sample unavailability. Finally, since this is a single-time point observational study, no information on the dynamic evolution of viral quasispecies is available. We are currently in process of another study targeting NS3 and NS5A using paired serum samples of pre-therapy, post-therapy, and post-relapse for hemophiliacs previously treated with peg-IFN plus ribavirin. We will also conduct post-trial surveillance of DAAs including simeprevir and sofosbuvir, wherein the NS3 and NS5B would be the target regions.

In conclusion, we developed and validated novel genotyping and RAV screening pipelines for HCV using the emerging technologies of NGS and QSR, reinforcing their potentials for the deconvolution of low-frequency genotypes, RAVs, and their interrelationships. Our study clearly demonstrated how the compositions of pre-existing minor genotypes and RAVs are considerably different between hemophiliacs and nonhemophiliacs, and HCV monoinfected patients with or without a history of whole-blood transfusion. These results strongly warrant

further studies investigating the epidemiology and impacts of low-frequency variants on the clinical outcome of DAA therapies among hemophiliacs and other high-risk populations.

Supporting Information

S1 Fig. Pairwise SNV-to-SNV distance distributions of HCV core and NS3 protease region estimated from Los Alamos HCV reference sequences. (A, B) SNV-to-SNV distance distributions of all possible Gt pairs of (A) core and (B) NS3 protease region were estimated from aligned reference sequences obtained from Los Alamos HCV sequence database. (C-F) Intragenotype (C, D) and intrasubtype (E, F) SNV-to-SNV distance distributions of (C, E) core and (D, F) NS3, respectively. A white notch represents median, and a red bar represents mean in each box-whisker chart.
(TIF)

S2 Fig. Phylogenetic positions of reconstructed sequences assigned to false-positive genotypes. Normalized patristic distances from reference sequences of each Gt were averaged, and distances from Gt1b and Gt2a were plotted. Sequences assigned to Gt1b were depicted in blue; Gt2a in cyan; Gt1a in red; Gt2b in orange; Gt2k in purple.
(TIF)

S3 Fig. Quantitative accuracy of different QSR methods for detecting minor genotypes. Reconstructed abundances under different simulation conditions were paired with corresponding preset abundance values. The conditions tested were as follows: (A, C) QuRe-Low and QuRe-High, and (B, D) QuasiRecomb-Low and QuasiRecomb-High, wherein Low represents the total read count of 30,000, and High represents 100,000 for (A, B) core and (C, D) NS3 protease region (NS3). Note that the abundance threshold was set to 0.001, and values below 0.001 were replaced with 0.001 for descriptive purposes.
(TIF)

S4 Fig. NS3 PI RAVs reproducibly detected by QuRe. QSR was performed using QuRe, and relative abundances of resistance-associated variants (RAVs) in the NS3 protease region were estimated in each subject. The x-axis labels are sample IDs colored on the basis of their history of exposure to blood (see Table 1 for details). The y-axis RAV labels were colored on the basis of the effects of RAVs on simeprevir susceptibility: susceptible (FC < 2) substitutions are in cyan, moderately resistant substitutions ($2 <$ FC < 50) in magenta. No highly resistant substitutions (FC > 50) were detected. The threshold was set at a frequency of 0.0001.
(TIF)

S5 Fig. NS3 PI RAVs reproducibly detected by QuasiRecomb. QSR was performed using QuasiRecomb, and relative abundances of resistance-associated variants (RAV) in the NS3 protease region were estimated in each subject. The x-axis labels are sample IDs colored on the basis of their status of exposure to blood (see Table 1 for details). The y-axis RAV labels are colored on the basis of the effects of RAVs on simeprevir susceptibility: susceptible (FC < 2) substitutions are in cyan, moderately resistant substitutions ($2 <$ FC < 50) in magenta; highly resistant substitutions (FC > 50) are in brown. The threshold was set at a frequency of 0.0001.
(TIF)

S1 Table. Primers used for RT-PCR.
(XLSX)

S2 Table. Parameter settings for QSR simulations.
(XLSX)

S3 Table. Counts of NS3 amino acid at position 80 binned by genotype in reported sequences retrieved from the Los Alamos HCV sequence database.
(XLSX)

S4 Table. Counts of NS3 amino acid at position 80 binned by sampled country in reported sequences retrieved from the Los Alamos HCV sequence database.
(XLSX)

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Author Contributions

Conceived and designed the experiments: MO HY. Performed the experiments: MO HY TT. Analyzed the data: MO HY TT HO WS. Contributed reagents/materials/analysis tools: HG SO. Wrote the paper: MO HY HO WS KM SK KK.

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原　著

ICF（国際生活機能分類）コアセット7項目版尺度の信頼性と因子妥当性の検証
—血液凝固因子製剤によるHIV感染被害者を対象とした分析—

久地井寿哉[1)]，柿沼　章子[1)]，岩野　友里[2)]，藤谷　順子[3)]，大金　美和[4)]，大平　勝美[1)]，木村　哲[2)]

[1)] 社会福祉法人はばたき福祉事業団，[2)] 公益財団法人エイズ予防財団，
[3)] 独立行政法人国立国際医療研究センター病院リハビリテーション科，
[4)] 独立行政法人国立国際医療研究センター病院エイズ治療・研究開発センター

背景：近年，全国の血液凝固因子製剤によるHIV感染被害者は，高死亡率，HIVに関する慢性炎症や全身性の代謝異常，HIV/HCV重複感染など病態の多様性の影響により，生活機能・社会的機能の低下が顕著である。適切な支援戦略・構想を実現するため，信頼性・妥当性の高い生活機能に関する指標の開発・導入が必要である。

目的：全国の血液凝固因子製剤によるHIV感染被害者を対象に，ICF（国際生活機能分類，WHO）に基づく生活機能尺度の開発を行い，信頼性・因子妥当性の検証を行う。

方法：研究同意の得られた全国の血液凝固因子製剤によるHIV感染被害者93名を対象に，対象者に対する支援歴10年以上の支援者および面接調査を行った研究者等の合議により，ICFコアセット7項目について評定を行い，合計スコアを算出し，尺度化を行った。因子分析ならびに共分散構造分析を行い，信頼性・因子妥当性の検証を行った。

結果：7項目合計スコア（range 0〜28）Cronbach $\alpha=0.821$。下位尺度は「b130活力と欲動の機能」「b152情動機能」「d230日課の遂行」「d850職業」4項目合計スコア（range 0〜16），Cronbach $\alpha=0.865$ および，「b280痛みの感覚」「d450歩行」「d455移動」3項目合計スコア（range 0〜12），Cronbach $\alpha=0.887$ からなる2因子性であった。

考察：尺度，下位尺度（2因子）ともに十分な信頼性が認められた。また因子構造は，ICF（国際生活機能分類）の構成概念である「活動」と「参加」を反映しており，各下位尺度単独での利用も可である。

結論：本尺度は，血液凝固因子製剤によるHIV感染被害者の生活機能に関する社会保障の基準策定や支援具体化に関する有望な尺度であると考えられる。

キーワード：血友病，HIV/AIDS，ICF，長期療養，信頼性・妥当性

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目　的

血液製剤によるHIV感染では感染後約30年が経過し，近年ではHIVによる慢性炎症や全身性の代謝異常[1)]や，HCVとの重複感染による肝機能の低下，抗HIV療法の血友病も含む長期副作用，原疾患である血友病性の関節障害などや，長期療養と高齢化に伴う多くの課題などが深刻化してきている[2,3)]との報告がある。

これらの問題を抱えた全国の血液凝固因子製剤によるHIV感染被害者（以下，被害者と記す）が全国に散在しているため，医療機関同士の情報共有・医療の連携が上手く行われておらず，被害者が孤立している状況があり[4)]，医療と社会福祉が連結して最良の医療やケアを提供できる仕組みを早急に確立することが求められている[5〜7)]。現状では既知の脅威および新たな脅威を前にして，生活機能を確保し，維持・向上するという課題もある[5)]。こうしたことから，生活機能に影響する諸因子を解析し，適切な支援戦略・構想を実現することや公平な社会保障の実現や実践的な指針を提示する必要があり，生活機能に関する信頼性の高い包括的指標の迅速な開発・導入が不可欠である。また，被害者救済戦略・施策を科学的に評価し，生活支援のための社会資源の開発・導入を円滑にすることなどが期待される。そのため，今後の被害者の治療・長期療養支援に必要となる科学的・論理的・実践的な枠組みが必要であり，その基幹となる生活機能評価法の確立を目指したい。そこで，本研究では，ICF（国際生活機能分類）に基づき，一般的な生活機能の核となる要因として先行研究[8)]で提案された7項目を用いて，「活動性」と「参加性」の両面からの評価をひとつの指標として評価が可能となるようICFコアセット7項目尺度（generic set）として尺度化し，そ

著者連絡先：久地井寿哉（〒162-0814　東京都新宿区新小川町9-20 新小川町ビル5階　社会福祉法人はばたき福祉事業団）

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の信頼性について検証し，さらに用いた項目の妥当性と想定した因子の妥当性を検討することを目的とする。ICF（生活機能分類）コアセット7項目（generic set）について本邦のような目的で尺度化し検討した先行研究は見当たらず，その目的下での検証は本例が初となる。

方　　法

研究同意の得られた全国の血液凝固因子製剤によるHIV感染被害者（以下「被害者」と記す）93名を対象に，ICFに基づく生活困難度を評価し，これらを基に尺度化を行った。これらは尺度開発法[9]に基づいて行われ，ひとつの総合的な尺度と，ICFの構成概念である「活動」と「参加」を反映するよう二つの下位尺度を開発した。総合的な指標として用いた項目は先行研究[8,10]で提案されている以下の項目を採用した。「b130 活力と欲動の機能（Energy and drive functions）」「b152 情動機能（Emotional functions）」「b280 痛みの感覚（Sensation of pain）」「d230 日課の遂行（Carrying out daily routine）」「d450 歩行（Walking）」「d455 移動（Moving around）」「d850 職業（Remunerative employment）」。それぞれの項目について，困難度に応じて0点（困難なし），1点（軽度の困難），2点（中等度の困難），3点（重度の困難），4点（完全な困難）の素点を与えた。これらの評定は，調査対象者となる薬害HIV被害者に対して10年以上の支援経験のある複数の支援者ならびに，2011年から2012年にかけて同じく調査対象者に対し半構造化面接調査を行った経験のある調査者・研究者等の合議により評価した。また，二つの下位尺度で用いる項目選択のために，共分散構造に基づく確証的因子分析を行った。この分析の目的は，「活動」と「参加」というICFの構成概念間の関係について検討し，またこれら二つの構成概念を規定する要因を検討することができる。これにより因子分析により選択された項目が，想定した因子として妥当性があるかどうかを確認した。あわせて，その項目を尺度として利用した場合の各項目の合計点の内的整合性について信頼性係数を求め信頼性を検討した。

1. ICF（国際生活機能分類）[11]について

定義：国際的な体系化された生活機能の分類・評価にはICF（International Classification of Functioning, Disability and Health：国際生活機能分類）がある。人間の生活機能と障害の分類法として，2001年5月，世界保健機関（WHO）総会において採択された。この特徴は，これまでのWHO国際障害分類（ICIDH）がマイナス面を分類するという考え方が中心であったのに対し，ICFは，生活機能というプラス面からみるように視点を転換し，さらに環境因子等の観点を加えたことである。障害に関する国際的な分類としては，これまで，世界保健機関（以下「WHO」）が1980年に「国際疾病分類（ICD）」の補助として発表した「WHO国際障害分類（ICIDH）が用いられてきたが，WHOでは，2001年5月の第54回総会において，その改訂版として「ICF（International Classification of Functioning, Disability and Health）」を採択した。

ICFは，人間の生活機能と障害に関して，アルファベットと数字を組み合わせた方式で分類するものであり，人間の生活機能と障害について「心身機能・身体構造」「活動」「参加」の3つの次元および「環境因子」等の影響を及ぼす因子で構成されており，約1,500項目に分類されている。

2. ICFコアセットについて

近年の研究により，慢性期の臨床実践や，生活支援の基幹となる項目選定が行われ，長期療養にかかわるICF項目のコアセットの実践利用が提言されている[12]。コアセットは，一般項目群（Generic Set），短縮項目群（Brief ICF Set），拡大短縮項目群（Enlarged Brief Set），包括項目群（Comprehensive）がある。本研究で被害者の生活機能の評価に採用した一般項目群（Generic Set）は，7項目のICFカテゴリからなり[10]，長期療養における活用，公衆レベルの健康統計にも利用が想定され選定されている[8]

3. 倫理的配慮

血友病HIV感染被害者の聞き取り調査対象者，個別の症例評価，についてエイズ予防財団の倫理委員会に提出し，承認を受けた（公益財団法人エイズ予防財団倫理審査委員会，「疫学研究に関する倫理指針」および「臨床研究に関する倫理指針」承認番号：公エ予240821号，承認日：平成24年8月1日）。調査対象者にはインフォームドコンセントによる同意を書面で得た。個人情報については，担当者以外には連結できない形とし，情報データベースは外部と接続されていないパソコンに保管し管理した。

結　　果

1. 対象者の属性・特性

対象者の年齢内訳は30代（30名），40代（31名），50代（17名），60代（8名），不明（7名）であった。またその他の対象者の属性・特性については表1に，血友病の型，血友病重症度，ならびにCD4値を対象者の健康状態として表2に示した。今回総合的な指標を作成する際に採用した各項目の合計点をICF generic set 7項目の合計スコアとしてそれぞれの対象者に与えた。その結果，HIV薬害被害者のICF generic set 7項目の合計スコアの平均±標準偏差は，11.2±6.1であった。

2. ICF（国際生活機能分類）コアセット7項目尺度の信頼性

ICF generic setの7項目版の特性は，7項目合計スコアのrange 0～28，Cronbach $\alpha = 0.809$ となり，尺度として用い

表 1　対象者の属性・特性　(N=93)

	N	%
性別　男性	93	100.0
年齢		
30～34	9	9.7
35～39	21	22.6
40～44	17	18.3
45～49	14	15.1
50～54	9	9.7
55～59	8	8.6
60～64	8	8.6
不明	7	7.5
居住地域		
北海道	14	15.1
東北	9	9.7
東京	10	10.8
関東（東京除く）	14	15.1
甲信越	5	5.4
東海	7	7.5
北陸	0	0.0
近畿	5	5.4
中・四国	7	7.5
九州・沖縄	22	23.7
最終学歴		
中学	5	5.4
高校	32	34.4
専門学校・短大	13	14.0
大学	29	31.2
大学院	6	6.5
不明	8	8.6
婚姻状況		
未婚	50	53.8
既婚	36	38.7
離別	2	2.2
死別	0	0.0
不明	5	5.4

注）端数処理のため％の合計は 100％とならない。

表 2　対象者の健康状態　(N=93)

	N	%
血友病の型		
A	51	54.8
B	8	8.6
不明・その他	34	36.6
血友病重症度		
重症	34	36.6
中等度	7	7.5
軽症	6	6.5
不明・その他	46	49.5
CD4		
＜200	9	9.7
201～350	24	25.8
351～500	23	24.7
501～600	13	14.0
＞601	14	15.1
不明・その他	10	10.8

注）端数処理のため％の合計は 100％とならない。

る場合の十分な信頼性が得られた（Nunnally（1978）の基準によれば，0.72 より大きい値である必要がある[13]。また，7 項目のうち，第一因子負荷量（以下，因子負荷量）の高い上位 3 項目は「痛みの感覚」「活力と欲動の機能」「歩行」であった。因子負荷量ならびに因子ごとの相関係数については表 3 に示す。項目「職業」を除いたすべての項目で，第一因子負荷量は 0.7 以上と十分に高く，また「職業」の項目を加えた 7 項目尺度として用いた場合でも十分な信頼性が確保された。

3. ICF（国際生活機能分類）コアセット 7 項目版下位尺度の信頼性

7 項目に対し，因子分析を行い，プロマックス回転を行ったところ，2 因子性を示した（表 4）。そこで，「活力と欲動の機能」「情動機能」「日課の遂行」「職業」の 4 項目の合計得点（range 0～16），ならびに，「痛みの感覚」「歩行」「移動」の 3 項目の合計得点（range 0～12）を算出し，それぞれ「参加」「活動」を表す下位尺度の得点として対象者に与えた。下位尺度「参加」「活動」の信頼性係数はそれぞれ，Cronbach α=0.865，0.887 となり十分な信頼性が得られた。下位尺度「活動」と「参加」の間に r=0.248（p<0.05）の有意な相関がみられ，完全に独立であるという帰無仮説（この帰無仮説は r=0）は棄却されるが，相関係数の値は 0.248 であるので，二つの因子（下位尺度）は，それぞれ異なる尺度を表すものと解釈できる（表 5）。

そこで，下位尺度がそれぞれ異なる尺度を表し，実際のデータに対してどの程度の当てはまりの良さがあるかについて確認するため，確証的因子分析を行った。モデル全体の当てはまりの良さを以下の 3 指標を用いて検定した。GFI（Goodness of Fit Index）：適合度指標，AGFI（Adjusted GFI）：調整済み適合度指標，RMSEA（The Root Mean Square Error of Approximation）。GFI と AGFI は，モデルの説明力（GFI は回帰分析での R^2，AGFI は自由度調整済み R^2 に対応）を表し，1.00 に近い値をとるほど望ましく，0.90 以上

表 3 ICF コアセット 7 項目版尺度各項目の因子負荷量および相関行列 ($N=87$)

	因子負荷量[1]	相関係数[2]						
		1	2	3	4	5	6	7
1. 痛みの感覚	0.875	1						
2. 活力と欲動の機能	0.859	0.185	1					
3. 歩行	0.813	0.801**	0.215*	1				
4. 日課の遂行	0.808	0.269*	0.757**	0.259*	1			
5. 情動機能	0.803	0.193	0.858**	0.215*	0.747**	1		
6. 移動	0.762	0.725**	0.175	0.640**	0.137	0.206	1	
7. 職業	0.477	0.194	0.517**	0.184	0.546**	0.410**	0.043	1

欠損値は除外した。[1] 因子抽出法：主成分分析，分散の 77.1％を説明。[2] ピアソンの積率相関係数。
*$p<0.05$, **$p<0.01$, ***$p<0.001$

表 4 ICF コアセット 7 項目版尺度 探索的因子分析（主因子法，プロマックス回転）
($N=87$)

参加（$\alpha=0.866$）	因子 1	因子 2
活力と欲動の機能	**0.922**	0.097
情動機能	**0.888**	0.119
日課の遂行	**0.888**	0.140
職業	**0.688**	0.067
活動（$\alpha=0.880$）		
痛みの感覚	0.132	**0.926**
歩行	0.150	**0.889**
移動	0.054	**0.871**
回転後の負荷量平方和	2.94	2.46
累積寄与率（％）	42.04	77.11

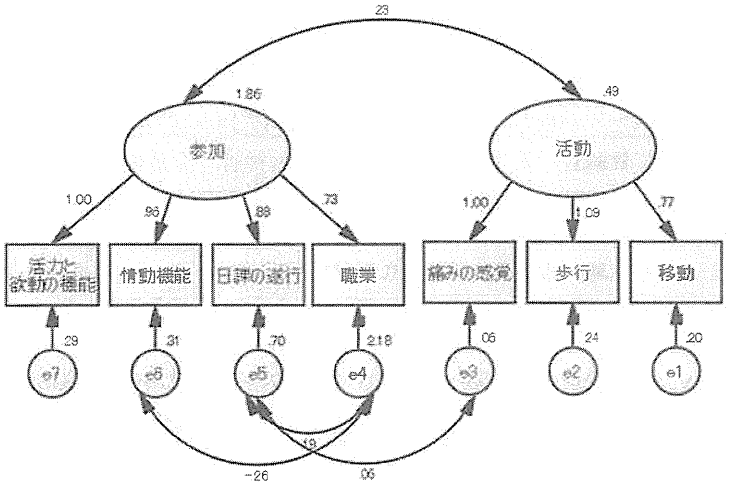
欠損値は除外した。α：クロンバックの α 係数。ICF コアセット 7 項目版尺度全合計値：$\alpha=0.809$。
太字は，（因子負荷量）＞0.6 の項目。

表 5 ICF コアセット 7 項目版尺度の記述統計と相関係数

	n	平均（SD）	相関係数[2]		
			1	2	3
1. 参加［0～16］[1] 4 項目	87	6.1（5.2）	1		
2. 活動［0～12］[1] 3 項目	87	5.1（2.1）	0.248*	1	
3. 全合計点［0～28］[1] 7 項目	87	11.2（6.1）	0.941***	0.531***	1

欠損値は除外した。[1]［数字］は range：「0 点（困難なし）～4 点（完全な困難）」の 5 件法。[2] ピアソンの積率相関係数。*$p<0.05$, **$p<0.01$, ***$p<0.001$。

が求められる。また，RMSEA は，モデルの分布と真の分布との乖離を 1 自由度あたりの量として表現した指標であり，RMSEA＜0.05 なら適合が良いモデルとされる。職業と日課の遂行，情動機能と職業，職業と情動機能，痛みの感覚と職業の誤差分散の相関を設定した最終モデルでは，AGFI＝0.930，GFI＝0.975，RMSEA＜0.001 のそれぞれ高い適合度が得られた。その結果を図 1 に示す。最終モデルに示されるように，確証的因子分析において想定した二つの因子は，それぞれ参加と活動に対応するものと考えられる。この意味において，因子分析の結果と確証的因子分析の結果とは相補的な解釈が可能であり，因子の妥当性が認められた。すなわち，二つの因子（下位尺度）は，それぞ



CMIN=7.811 p=.647
AGFI=.930 GFI=.975 NFI=.981
RMSEA=.000 AIC=43.811

図 1 ICF コアセット 7 項目版尺度確証的因子分析（$N=87$）
長方形：それぞれ観測変数「b130 活力と欲動の機能（Energy and drive functions）」「b152 情動機能（Emotional functions）」「b280 痛みの感覚（Sensation of pain）」「d230 日課の遂行（Carrying out daily routine）」「d450 歩行（Walking）」「d455 移動（Moving around）」「d850 職業（Remunerative employment）」を表す。楕円：潜在変数，直接測定できない構成概念「活動」「参加」を表す。丸：誤差変数：構成概念だけでは説明できない誤差。楕円から長方形へひかれた矢印に付された係数：構造方程式における標準化されたパラメータ推定値。楕円間に引かれた矢印に付された係数：因果係数，標準化された推定値。矢印：因果の方向を示す。双方向矢印：共分散関係を示す。モデルの適合度：共分散構造モデルの適合度指標として CMIN, p, AGFI, GFI, NFI, RMSEA, AIC を用いた。

れ異なる尺度を表すものと解釈できる。

考　　察

ICF（国際生活機能分類）コアセット 7 項目尺度，ならびに二つの下位尺度について十分な信頼性が確保された。

その後の因子分析により，当初想定していた ICF の構成概念「活動」「参加」の 2 つの下位尺度とその項目に分かれたため，内容的にも解釈可能な構造であった。ただし，項目別には，項目「職業」については，7 項目からなる指標として用いた場合は第一因子負荷量が 0.477 と相対的には低かったが，内容的に尺度を構成する重要な項目と考え今回は削除せず，今後は，あわせて社会経済的地位を示す尺度と合わせて評価する必要があると考えられた。

二つの下位尺度「活動」と「参加」については，相関分析では完全に独立であるという仮説は棄却されたものの $r=0.248$ と低かった。加えて，共分散構造分析を用いた確証的因子分析を行ったところ，二つの因子を想定したモデルを想定し，実際のデータとの適合度を検討したところ，十分に当てはまりの良いモデルが得られた。これらのことより，二つの下位尺度「活動」「参加」について，それぞれ単独での使用が可能であると考えられる。

すなわち，本研究の ICF（国際生活機能分類）コアセット 7 項目尺度は，構成概念として従来の研究でも見られたいわゆる「生活困難度」としての指標であることを示唆するとともに，下位尺度として「活動」「参加」に関する生活機能を測定する目的での，それぞれ単独での利用が可能であることが示唆された。これらの利用により疾病予防・生活改善の手がかりがより明らかになる可能性がある。本尺度は，まず，生活機能における能力や状況といった面からの改善可能性について，問題の把握，理解，治療や支援が可能になる点で意義がある。特に長期療養においては，臨床実践に加え，生活実践や地域連携の具体的なガイドライン化の際の指標として有望であり，十分に利用可能な因子的な信頼性・妥当性を示した。さらに，本研究においては，HIV 薬害被害者に対する支援経験豊富な複数の支援者ならびに研究者等による合議による評点を行った。関連領域の専門家の合議の基に評価を行ったことで一定の信頼性・妥当性が認められたと考えられる。また，迅速な生活

機能評価に十分活用できると考えられる。今後，より正確な生活機能の評価と活用のために，対象者の拡大，再検査法による検討（自己評価，専門家による改善可能性の評価，支援者による評価，評価妥当性の向上）が課題である。制度運用に関して本尺度は全体として信頼性・妥当性が確認され，迅速な生活機能評価尺度としての使用可能性が示された。

一方で，本研究には限界と課題もある。本研究の対象者は，全国を対象にアウトリーチ可能な被害者であることもあり，比較的健康状態の良い集団である可能性がある。そのため，この尺度を用いた生活機能実態については，接近困難層，接近不可層は分析対象に含まれないことから，全体的な生活機能水準に関しては下方修正が必要な可能性がある。また，今後は，アウトリーチによる対象者の拡大，また，一般化のために他疾患との比較，疾患状況を考慮した検討が必要である。

次に，信頼性・妥当性の検討についての本研究の限界もある。合議による評価により十分な信頼性・妥当性が得られたが，今後は再検査法による信頼性の検証や，評価者によるバイアスの調整，健康の自己評価ツールとしての活用，外的妥当性の検証など，より信頼性・妥当性を高めるため，さらなる検討が必要である。また，被害者の長期療養施策においては，縦断調査や介入研究により検証することで，長期療養の効果についてもより戦略的に改善する可能性がある。また本尺度の改良を視野に入れながら，簡便な評価指標として迅速に医療・保健・介護・障害・地域等の生活支援の現場で利用されることが期待される。喫緊の課題として，調査・研究目的にとどまらず，被害者の生活の原状回復を目的とした，社会保障水準の適正化に貢献が期待できる。そのため，迅速な運用導入と，尺度改善の取組みを，同時かつ速やかに進めることが望ましい。

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利益相反：本研究では利益相反に相当する事項はない。

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Reliability and Factor Validity of ICF Core Sets (7 Item Generic Version) for Hemophilia Patients with HIV in Japan

Toshiya KUCHII[1)], Akiko KAKINUMA[1)], Tomosato IWANO[2)], Jyunko FUJITANI[3)], Miwa OGANE[4)], Katsumi OHIRA[1)] and Tetsu KIMURA[2)]

[1)] Social Welfare Corporation HABATAKI Welfare Project, Tokyo, Japan,
[2)] Japan Foundation for AIDS Prevention,
[3)] Physical Medicine and Rehabilitation, National Center for Global Health and Medicine,
[4)] AIDS Clinical Center, National Center for Global Health and Medicine

Objective : This study was conducted to evaluate reliability and factor validity of ICF Core Sets (7 item generic version) for hemophilia patients with HIV in Japan.

Methods : Semi-structured interviews were conducted among HIV patients with hemophilia (HIV victims, $N=93$, male, age 30–64, in Japan). The variable of ICF Core Sets (7 item generic version) was "b130 Energy and drive functions" "b152 Emotional functions" "b280 Sensation of pain" "b230 Carrying out daily routine" "d450 Walking" "d455 Moving around" "d850 Remunerative employment". Reliability and Factor analysis were conducted structural equation modeling (SEM).

Result : We found that the ICF scale showed high internal consistency reliability (Cronbach's $\alpha=0.821$) and high factor validity (two latent variable model, AGFI = 0.930, GFI = 0.975, RMSEA < 0.001).

Discussion and Conclusion : This scale analysis showed the good reliability and factor validity of ICF core sets (7 item generic version). These results suggested that ICF core sets (7 item version) is a useful and hopeful for assessment index for social security and provide social support for any patients includes hemophilia patients with HIV in Japan.

Key words : hemophilia, HIV/AIDS, ICF, long-term care, reliability/validity

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Brief communication

CD4 T lymphocyte counts in patients undergoing splenectomy during living donor liver transplantation☆

Koji Natsuda [1], Susumu Eguchi *,[1], Mistuhisa Takatsuki [1], Akihiko Soyama [1], Masaaki Hidaka [1], Takanobu Hara [1], Tota Kugiyama [1], Zhassulan Baimakhanov [1], Shinichiro Ono [1], Amane Kitasato [1], Fumihiko Fujita [1], Kengo Kanetaka [1], Tamotsu Kuroki [1]

Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

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ABSTRACT

The role of splenectomy in increasing the CD4-positive T lymphocyte counts (hereafter: CD4 counts) and the CD4 to CD8 ratio have not yet been fully investigated, especially in the case of HIV-positive patients undergoing liver transplantation (LT).

Methods: The change in the total lymphocyte counts of 32 patients who underwent one-stage splenectomy with living donor (LD) LT with (n = 13) or without rituximab (RTX, n = 19) therapy were examined to validate our cohort of ABO-incompatible LDLT with RTX. Subsequently, perioperative changes in CD4 counts and the CD 4 to CD8 ratio were measured in 13 patients who underwent ABO-incompatible LDLT/RTX with splenectomy.

Results: (1) The administration of RTX did not significantly affect the total lymphocyte counts of patients after LDLT/splenectomy in any of the observation periods. (2) The CD4 counts were significantly higher at 2 years after LDLT in comparison to the perioperative CD4 counts but not within the 3-month period ($p = 0.039$). The CD4/CD8 ratio gradually decreased after LDLT/splenectomy under RTX treatment.

Conclusions: An immediate increase in the CD4 counts therefore cannot be expected after LDLT with splenectomy. The total lymphocyte and CD4 counts were rather stable in the peritransplant period even in ABO incompatible LDLT with RTX.

1. Introduction

In general, liver transplantation (LT) is indicated in HIV-positive end-stage liver failure patients with CD4-positive T lymphocyte counts (hereafter: CD4 counts) of at least 200 or 100/μL in order to prevent opportunistic infection [1,2]. However, patients with hepatic cirrhosis whose HIV is well controlled are sometimes not indicated for liver transplantation if they have a CD4 count that is below baseline due to pancytopenia, which decreases total lymphocyte counts due to portal hypertension. If combined splenectomy improves CD4 counts, subsequent liver transplantation may enable those patients to survive [3]. However, no report is available whether splenectomy can increase CD4 counts when performed during living donor (LD) LT.

In order to clarify the answer to the clinical question, our cohort of ABO incompatible LDLT was assessed and validated, because we measured the CD4 counts only in this cohort to evaluate changes in the T cell and B cell percentages. In fact, in Asian countries, ABO-incompatible LDLT is performed for patients with end-stage liver cirrhosis with the aid of rituximab (RTX) [4,6]. After RTX treatment 1–2 weeks before LT, B cells (CD19/20) are eliminated to almost zero percent. However, there are few reports in the literature regarding the changes of the total lymphocyte counts and CD4 counts in patients who receive RTX treatment before LDLT. If RTX does not affect the CD4 count, our cohort of ABO incompatible LDLT could be valid to investigate the changes in the CD4 counts combined with splenectomy.

2. Objective

We investigated the role of splenectomy in increasing CD4 counts and the CD4 to CD8 ratio performed during LDLT. Analysis 1 was performed to validate our cohort of ABO-incompatible LDLT with RTX. In analysis 2, the changes in the CD4 counts in patients who received RTX treatment before LDLT were clarified.

Abbreviations: CD4, CD4-positive T lymphocyte; LDLT, living donor liver transplantation; LT, liver transplantation; RTX, rituximab.

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* Corresponding author at: Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
E-mail address: sueguchi@nagasaki-u.ac.jp (S. Eguchi).

[1] All authors have no disclosures.

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3. Patients and methods

3.1. Subjects

Analysis 1: Thirty-two patients who underwent LDLT for various diseases combined with splenectomy in Nagasaki University Hospital between November 2006 and June 2013, and who were observed for at least 1 year, were included. Splenectomy was indicated for thrombocytopenia less than 50,000/μL and hepatitis C liver cirrhosis. Of those 32 patients, 13 patients received RTX 1 week before ABO incompatible LDLT while 19 did not because of ABO matching.

Analysis 2: The above-mentioned 13 patients who underwent ABO incompatible LDLT combined with splenectomy were included in the analysis. All patients received RTX therapy before LDLT. Our method of LDLT was previously reported elsewhere [5].

3.2. Analysis

Analysis 1: The total lymphocyte counts were measured before and after LDLT at various time points until 5 years after LDLT.

Analysis 2: To specifically clarify the effect of splenectomy on CD4 T cell counts and CD4/CD8 ratio, 13 patients were analyzed. The net CD4 counts and the CD4 to CD8 T cell ratio were analyzed at various time points.

3.3. Statistics

All of the data are expressed as the mean and standard deviation or as median values with ranges. The statistical analyses were performed using the Mann–Whitney U test for continuous values and the chi-square test for categorical values. A p-value of <0.05 was considered to be statistically significant. The GraphPad PRISM version 5.0 software program (GraphPad Software, San Diego, CA) was used for all of the statistical analyses.

The study was conducted in accordance with the Declaration of Helsinki of 2013.

4. Results

As shown in Fig. 1, the total lymphocyte counts after LDLT combined with splenectomy did not differ significantly between the patients who received RTX and those who did not in any of the observation periods.

The median CD4 counts (/μL) of the LDLT recipients who underwent splenectomy before the administration of RTX and at 1 month, 3 months, 1 year, and 2 years after treatment were 298, 287, 247, 359, and 441, respectively (Fig. 2). The CD4 counts increased slowly after LDLT, and were significant higher at 2 years and after in comparison to the perioperative count ($p = 0.039$). Furthermore, there was no significant difference in the CD4 counts regardless of splenectomy (Fig. 3).

In addition, the administration of RTX did not influence the CD4/8 ratio after LDLT and splenectomy (Fig. 4). It signifies that CD8 was more enhanced than CD4.

5. Discussion

In the present study we demonstrated that the administration of RTX did not affect the total lymphocyte counts after LDLT combined with splenectomy. Therefore, using the cohort of ABO incompatible LDLT, we found that splenectomy in order to increase the CD4 count before and after LDLT had no therapeutic effect. The present study revealed that in ABO incompatible LDLT with RTX, the total lymphocyte and CD4 counts were rather stable in the peritransplant period and an immediate rise of CD4 count cannot be expected after LDLT with splenectomy.

According to Nomura et al., there is a decrease in the number of CD4 cells in the peripheral blood of patients with liver cirrhosis after splenectomy, which leads to a significant decrease in the CD4/CD8 ratio [7]. As a consequence, splenectomy may not significantly increase the CD4 count before LT. However, Hashimoto et al. reported that the function of CD4 cells in the production of interferon-gamma and CD4 proliferation were increased after splenectomy [8]. Accordingly, it remains controversial whether or not splenectomy should be performed

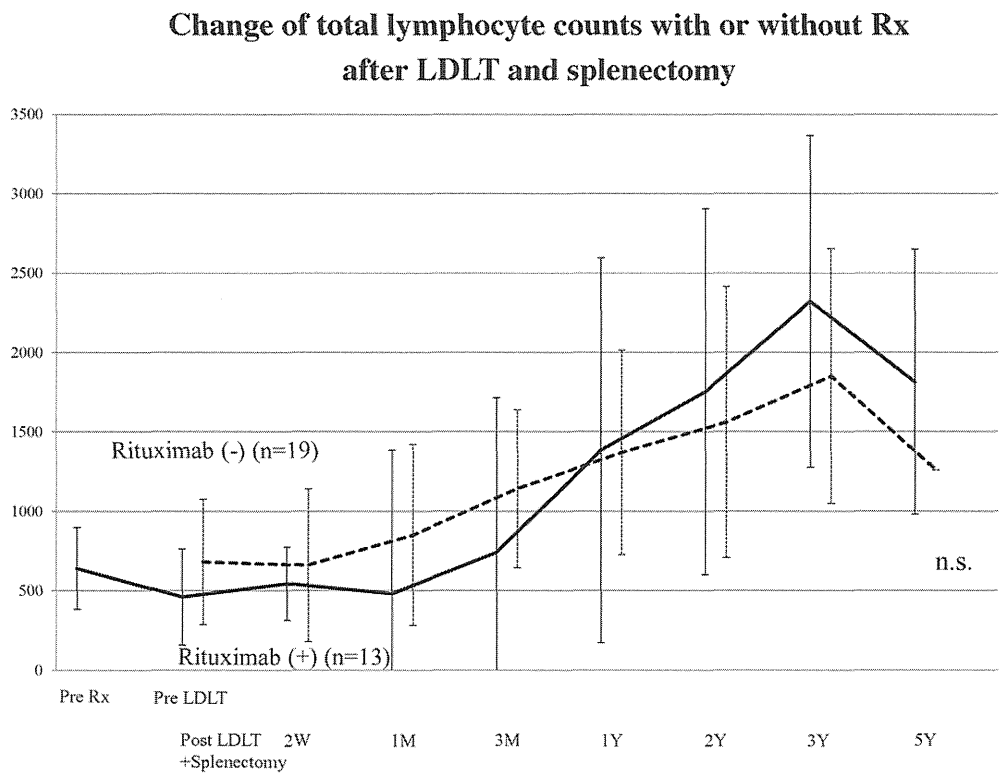

Fig. 1. Change of total lymphocyte counts after LDLT and splenectomy according to the rituximab administration.

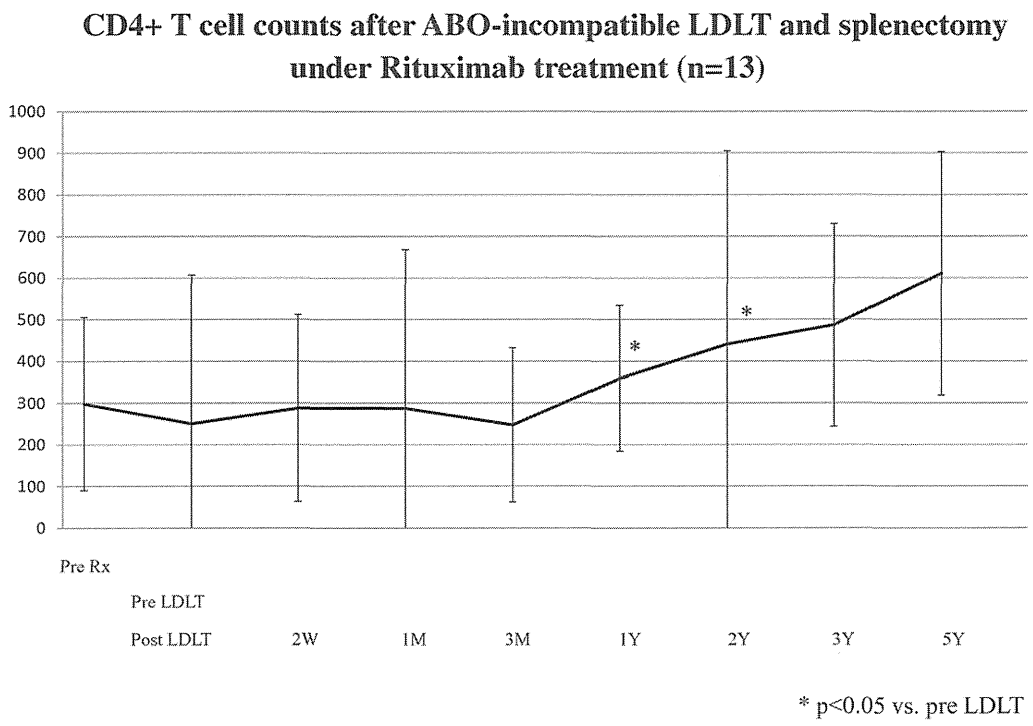

Fig. 2. Changes of CD4 + T cell counts after ABO-incompatible LDLT and splenectomy under rituximab treatment,

before LT in order to increase the number of CD4 cells or the function of the CD4 cells, especially in patients with HIV [9].

In the present study, there was a gradual, long-term increase in the CD4 counts of patients who underwent splenectomy at the time of LT. The short-term decrease in the CD4 count was probably due to surgical stress and the effects of other drugs (e.g., mycophenolate mofetil and interferon). Although our investigation showed that splenectomy did not affect the CD4 count, this may be due to the small number of patients under the specific conditions of the present study (including RTX administration), and should be the subject of a prospective analysis in the future.

Our indication for LT for HIV infected patients was a CD4 count of above 100, since hypersplenism existed due to severe portal hypertension [9]. Immediately after the diseased donor (DD) LT, although the CD4 count dropped below 100, it recovered spontaneously, probably with the aid of splenectomy, which had been planned before DDLT [10]. We believe that if the HIV titer is controlled by antiretroviral therapy, the absolute CD4 count for the indication of LT could be

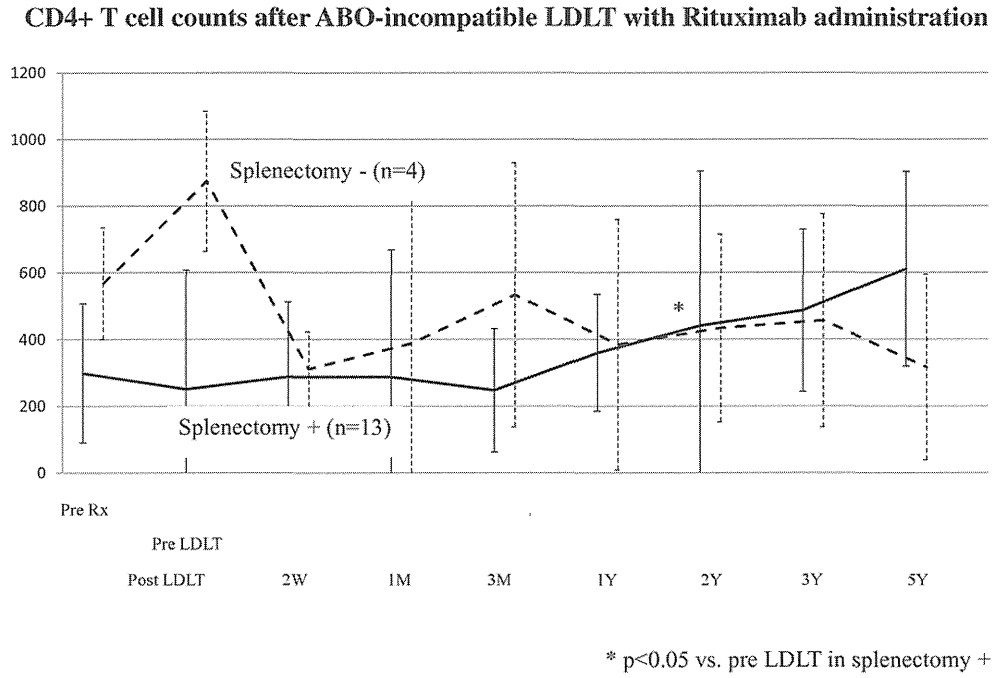

Fig. 3. CD4 + T cell counts after ABO-incompatible LDLT with rituximab administration with or without splenectomy.

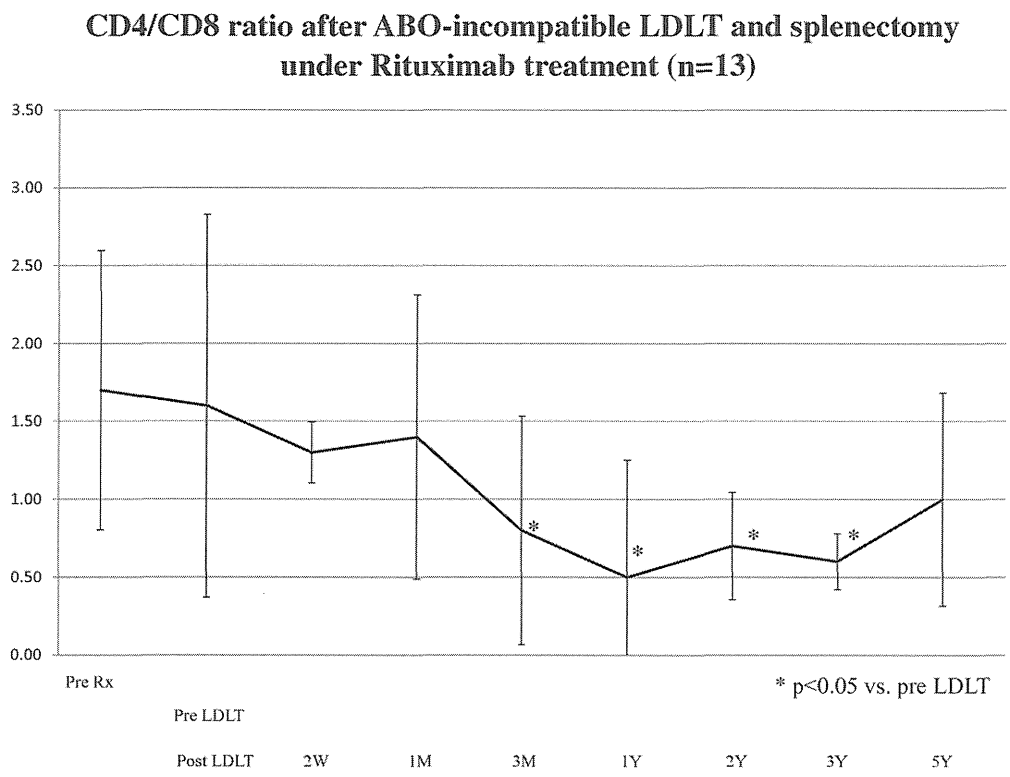

Fig. 4. Changes of CD4/CD8 ratio after ABO-incompatible LDLT and splenectomy under rituximab treatment.

lowered to 100 not 200, since hypersplenism can mask the real immunological function of patients with HIV/HCV coinfection. Furthermore, splenectomy may increase the CD4 count and strengthen the immune function before or during LT. Our results indicate that because the number of CD19/20 B lymphocytes decreased to almost zero after the administration of RTX, the CD8 count should be expected to increase in the early period after LDLT/splenectomy [11]. Since CD4 and CD8 T cells cooperate together, we need to await further investigation on the rate of infection or tumor recurrence after LDLT with RTX and splenectomy in larger studies [12,13].

In conclusion, this is the first report of the effect of splenectomy on the number of CD4 T cells after LDLT. An immediate increase in the CD4 counts therefore cannot be expected after LDLT with splenectomy. The total lymphocyte and CD4 counts were rather stable in the peritransplant period even in ABO incompatible LDLT with RTX.

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Short Communication

The First Case of Deceased Donor Liver Transplantation for a Patient with End-Stage Liver Cirrhosis Due to Human Immunodeficiency Virus and Hepatitis C Virus Coinfection in Japan

Susumu Eguchi*, Mitsuhisa Takatsuki, Akihiko Soyama, Masaaki Hidaka, Tota Kugiyama, Koji Natsuda, Tomohiko Adachi, Amane Kitasato, Fumihiko Fujita, and Tamotsu Kuroki

Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8501, Japan

SUMMARY: We previously reported that progression of liver cirrhosis is quicker and survival is dismal in patients with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) coinfection, especially when acquired in childhood through contaminated blood products. Recently, we performed the first deceased donor liver transplantation (DDLT) for an HIV/HCV-coinfected hemophilic patient in Japan. A 40-year-old man was referred to our hospital for liver transplantation. Regular DDLT was performed using the piggyback technique with a full-sized liver graft. Cold ischemia time was 465 min, and the graft liver weighed 1,590 g. The antiretroviral therapy (ART) was switched from darunavir/ritonavir to raltegravir before the transplant for flexible usage of calcineurin inhibitors postoperatively; tenofovir was used as the baseline treatment. The postoperative course was uneventful, and the patient was discharged home on day 43. He started receiving anti-HCV treatment on day 110 with pegylated interferon, ribavirin, and simeprevir after the DDLT. Herein, we report the first case of DDLT in Japan. Meticulous management of ART and clotting factors could lead to the success of DDLT.

Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infection after the use of HIV/HCV-contaminated imported blood products for hemophilia patients in the 1980s has led to increased mortality rates due to end-stage liver disease resulting from chronic hepatitis C infection (1,2). In the meantime, the development of antiretroviral agents made it possible to nearly eliminate HIV-related morbidity and mortality (3). Therefore, an urgent need has developed to establish a system to salvage those patients with HIV/HCV coinfection. It is important to note that these patients usually develop end-stage liver cirrhosis at a young age, such as in their 30s and 40s. They may also develop hepatocellular carcinoma (4,5).

In Japan, the Tokyo University group has made an intense effort to salvage those patients undergoing living donor liver transplantation (LDLT) and to yield a good survival rate after LDLT (6). However, liver transplantation from a deceased donor has not been performed thus far. In the world literature, there have been some case series of deceased donor liver transplantation (DDLT) in patients with HIV infection (7); however, an optimal antiretroviral therapy (ART) regimen and anti-HCV treatment has not been clarified yet. Herein, we report the first case of DDLT for an HIV/HCV coinfected hemophilic patient, with special consideration for antiretroviral conversion and immunosuppressive agent selection.

The patient was a 40-year-old man who was infected with HIV and HCV through imported contaminated blood products used for treating hemophilia when he was an infant. He received treatment with antiretroviral agents, and the HIV RNA levels remained under the detectable range. However, chronic hepatitis with HCV infection persisted, and he recently developed cirrhosis. Pegylated-IFN therapy combined with ribavirin was discontinued owing to mental depression, which was induced by the pegylated-IFN. He was also treated for esophageal varices with endoscopic variceal ligation. A computer tomography scan showed a relatively hypertrophic left lobe of the cirrhotic liver with ascites.

The inferior vena cava was completely surrounded by the enlarged caudate lobe of the liver. No tumor formation was noted inside or outside of the liver. The patient's Child-Pugh status was class C with 10 points and the Model for End-Stage Liver Disease score was 19 points. To HIV RNA level was below detection limits and his absolute CD4 number was around 150. However, the patient had a high HCV RNA titer. A clotting profile indicated that he had hemophilia A with a low factor VIII level, which necessitated administration of factor VIII 3 times per week. Finally, he was indicated for liver transplantation (LT) and waited 3 years with low points. However, over the 3 years his liver function progressively deteriorated. He obtained extra points on the waiting list because the mortality of HIV/HCV coinfected patients without LT is higher than that of HCV-mono-infected patients. Before LT, his ART was changed from darunavir/ritonavir to raltegravir in order to exercise flexible control of the calcineurin inhibitor. Tenofovir was used as the basic ART.

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*Corresponding author: Mailing address: Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. Tel: +81-95-819-7316, Fax: +81-95-819-7319, E-mail: sueguchi@nagasaki-u.ac.jp