In the present study, DynabeadsCD4 obtained by using the original protocol also showed a good result (see Table 1). During the operation, we found 2 problems with the original protocol, however. One was the transfer of 200 μL of blood from 500 μL of blood to a new tube after monocyte depletion. This step might lead to inaccurate results because we could not mix the blood well while the tube was on the Dynal MPC-S. The other was that too many free Dynabeads (which did not attach to CD4 cells) and red blood cells were identified when the cells were counted under a light microscope. This might be the reason for recommending lysis of the cells, staining the nuclei, and using a fluorescent microscope in the last step of the original protocol. In our modified protocol, the entire sample was transferred to a new tube after monocyte depletion. The number of free Dynabeads decreased after the volume of CD4 Dynabeads was reduced. Furthermore, we washed the sample 4 times after CD4 cell separation in spite of the original protocol recommending washing only twice. The red blood cells could be almost completely removed by 4 washes, especially when the washing buffer had been discarded completely at each wash. These modifications made a direct count under a light microscope possible.

After reduction of the volume of Dynabeads used in the assay, the cost of reagents used for analysis of 1 sample decreased to less than $1.00. Thus, the total cost of 1 CD4 count, including other disposable materials such as syringes, tubes, and tips, could be less than $3.00.

In conclusion, the present study demonstrated that our final modified protocol of Dynabeads assay could be used as a good alternative to flow cytometry with sufficient accuracy, reliability, and simplicity at a reasonable cost. Therefore, the assay could be suitable for monitoring ART in resource-limited settings.

ACKNOWLEDGMENTS

REFERENCES

1. World Health Organization. Scaling up antiretroviral therapy in resource limited settings: guidelines for a public health approach. Available at: http://www.who.int/hiv/. Accessed April 2002.
2. Department of Health and Human Services. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Available at: http://AIDsinfo.nih.gov. Accessed July 14, 2003.
3. Brando B, Barnett D, Janossy G, et al. Cytofluorimetric methods for assessing absolute numbers of cell subsets in blood. *Cytometry.* 2000; 42:327–346.
4. Crowe S, Turnbull S, Oelrichs R, et al. Monitoring of human immunodeficiency virus infection in resource-constrained countries. *Clin Infect Dis.* 2003;37(Suppl 1):S25–S35.
5. Badri M, Wood R. Usefulness of total lymphocyte count in monitoring highly active antiretroviral therapy in resource-limited settings. *AIDS.* 2003;17:541–545.
6. Jacobson AM, Liu L, Khayam-Bashi H, et al. Absolute or total lymphocyte count as a marker for the CD4 T lymphocyte criterion for initiating antiretroviral therapy. *AIDS.* 2003;17:917–919.
7. Spacek AL, Griswold M, Quinn CT, et al. Total lymphocyte count and hemoglobin combined in an algorithm to initiate the use of highly active antiretroviral therapy in resource-limited settings. *AIDS.* 2003;17:1311–1317.
8. Diagbouga S, Chazallon C, Kazatchkine MD, et al. Successful implementation of a low-cost method for enumerating CD4+ T lymphocytes in resource-limited settings: the ANRS 12-26 study. *AIDS.* 2003;17:2201–2208.
9. Brinchmann JE, Leivestad T, Vartdal F. Quantification of lymphocyte subsets based on cells directly from blood. *J Immunogenet.* 1989;16:177–183.
10. Didier J, Kazatchkine M, Demouchy C, et al. Comparative assessment of five alternative methods for CD4 T-lymphocyte enumeration for implementation in developing countries. *J Acquir Immune Defic Syndr.* 2001; 26:193–195.
11. Lyamuya EF, Kagoma C, Mbena EC, et al. Evaluation of the FACScount, TRAx CD4 and Dynabeads methods for CD4 lymphocyte determination. *J Immunol Methods.* 1996;195:103–112.
12. Nicholson JK, Velleca W, Jubert S, et al. Evaluation of alternative CD4 technologies for the enumeration of CD4 lymphocyte. *J Immunol Methods.* 1994;177:43–54.
13. Lyamuya EF, Schechter M, Echeverria de Perez G, et al. Multicentre evaluation of alternative methods for CD4 lymphocyte determination [abstract Mo.A.513]. Presented at the International AIDS Conference, Vancouver, July 1996.
14. Carella AV, Moss MW, Provost V, et al. A manual bead assay for the determination of absolute CD4+ and CD8+ lymphocyte counts in human immunodeficiency virus-infected individuals. *Clin Diagn Lab Immunol.* 1995;2:623–625.
15. Gaudernack G, Lundin KE. Rapid immunomagnetic phenotyping of cells. *J Immunogenet.* 1989;16:169–175.

四病協合同感染症対策委員会より

四病協感染症対策委員会では，問題になっている重要な感染症に焦点を当て，その分野を専門とする委員から最新の情報の提供を受け，検討等を行っています。今回は平成16年度第3回四病協合同感染症対策委員会(16.12.3) より，その一部を報告させていただきます。

<div align="right">

感染症対策委員会委員長　佐藤　眞杉

</div>

I HIV感染症の現況と 予防啓発事業等について

国立国際医療センターエイズ治療・研究開発センター長　　木村　哲

HIV感染症の最近の情報についてお話しいたします。

1. 日本でもHIV感染者／患者が急増

図1に示しますように，現在，世界でHIVに感染している患者／感染者数は3,940万人と推定されており，また，毎年500万人が新たに感染し，毎年300万人がエイズで亡くなっていると推定されています。途上国を中心として非常な勢いで増えています。途上国が感染者の95％くらいを占めているというのが現状です。

日本では図2にありますように献血者のなかで10万人当たりの陽性率が87年からどんどん上がっていて，2004年9月末までのものでは10万人当たり1.78人という数字にまでなってきています。この数字は欧米の献血血液の陽性率よりも高いという状況にあります。

検査の代わりに献血をして確かめるという人が若干はあるようですけれども，このグラフは実際に感染者が増えているということを示しているものであろうと思います。

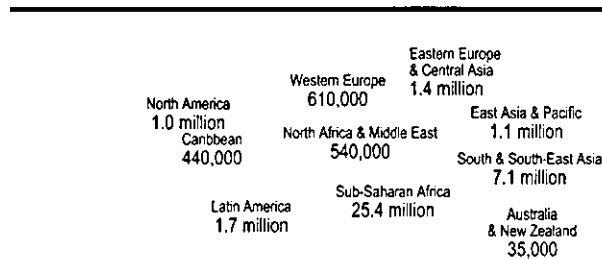図 1　Adults and children estimated to be living with HIV/AIDS as of end 2004

North America 1.0 million

Caribbean 440,000

Western Europe 610,000

North Africa & Middle East 540,000

Eastern Europe & Central Asia 1.4 million

East Asia & Pacific 1.1 million

South & South-East Asia 7.1 million

Sub-Saharan Africa 25.4 million

Latin America 1.7 million

Australia & New Zealand 35,000
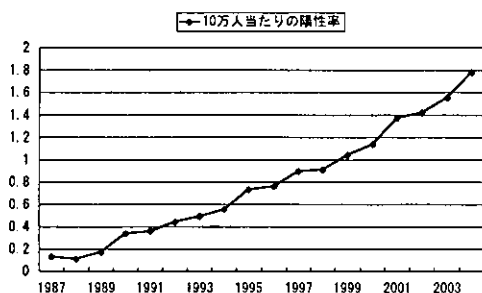
UNAIDS

Total: 39.4 million

図2 日本の献血血液の抗体陽性率

図3 HIV感染者の国籍別年次推移

国籍別・男女別の感染者状況をエイズ動向委員会のほうに報告されていた数字をもとにグラフで示したのが図3です。いちばん伸びているのが日本人男性です。これは毎年の，新規に報告されている該当者数で，累積ではないのですが，このように感染者は右肩上がりで伸びています。女性のほうは横ばい傾向です。

図4は私の所属しているエイズ治療・研究開発センター（ACC）の患者数です。折れ線グラフが累積で，これまでに1,500名を超えております。棒グラフのほうは，毎年の新規の受診者数です。ACCは1997年に新設されましたので，その年とその後2～3年は他施設からの感染者／患者が集まってきたため，新患数が多いのですが，それが落ち着いた2000年以後，新規の患者がだんだん増えてきており，今年はもうすでに去年の204名という患者を超えています。

2. 治療法の進歩

治療ガイドラインが，最近，大きく変わりました。治療薬の新しいものがどんどんできています。日本でも去年の末から今年の初めにかけて，新しい治療薬アタザナビル（ATV）とテノホビル（TDF）が承認され（表1），また新たに臨床試験の結果が次々と出て，それに伴って，治療にどういう組み合わせがいちばん良いのかという推奨が変わりました。

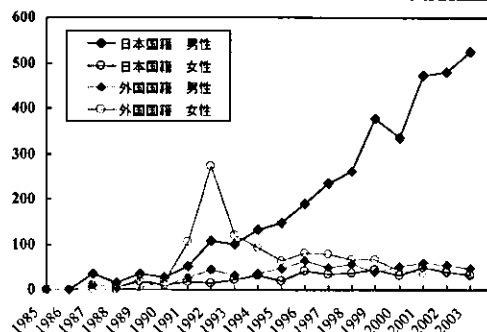図5が2004年3月に出たアメリカのガイドラインに沿った組み合わせ方ですが，同年10月29日

図4 ACC患者数の推移
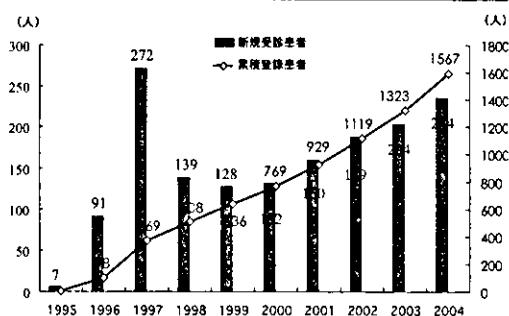
表1 抗HIV薬の開発状況

逆転写酵素阻害薬		プロテアーゼ阻害薬
核酸系	非核酸系	
ZDV	Nevirapine	Saquinavir
ddI	Delavirdine	Ritonavir
ddC	Efavirenz	Indinavir
d4T		Nelfinavir
3TC		Amprenavir
Abacavir		Lopinavir/RTV
TDF		Atazanavir

2004.12.04 現在

にまた新しいガイドラインが出ました（図6）。FTCという新しい薬が出てきたということにも関係しますけれども，組み合わせ方がこういうふうに変わってきますので，キャッチアップしていくことが大切です。FTCは日本でも近々承認される見込みです。

1987年にAZTが最初に出まして，単剤で治療しました。そのうち1剤ではだめだということで

図5 抗HIV薬の組み合わせ HAART

```
AZT  +  3TC ┐   ┌─────────┐
            ├──→│ LPV / RTV │
d4T  +  3TC ┤   └─────────┘
            │   ┌─────────┐
TDF  +  3TC ┘   │   EFV   │
                └─────────┘
```

DHHS Guideline 2004.03.23

図6 HAARTにおける抗HIV薬の組み合わせ法 最新の国際的ガイドライン

```
AZT or TDF ── 3TC or FTC ── EFV

AZT ── 3TC or FTC ── LPV/RTV
```

DHHS Guideline 2004.10.29
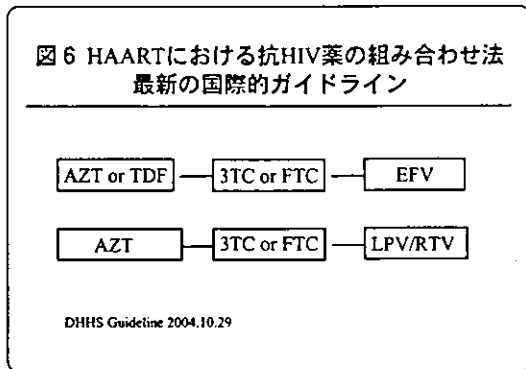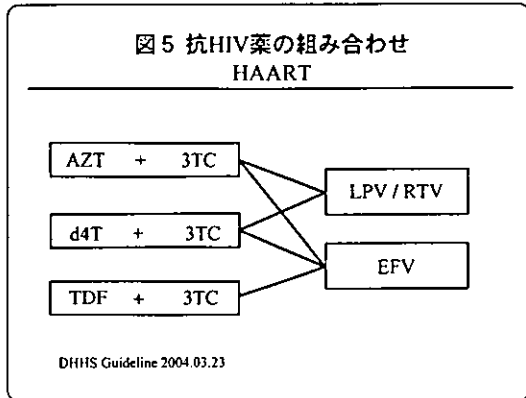
図7 HAARTの課題

- 服薬アドヒアランスの保持・耐性化の防止
  - より単純な服薬法へ
  - 服薬個数をより少なく
- 副作用の回避
  - Efavirenzによる中枢神経系副作用
  - D drugによるミトコンドリア障害
  - Abacavirによる過敏性症候群
- 免疫再構築症候群の回避

2剤併用になったり，交互に投与したりという試みがありましたが，いずれも駄目でした。日本でいえば97年ごろからやっと3剤併用，いわゆるhighly active antiretroviral therapy（HAART）が導入されて患者さんの予後が非常に良くなってきました。

長期治療が必要なので，ときどき中断する方法はどうか。経済性も高いのではないかということ

で，structured treatment interruption（STI）が一時注目されましたけれども，これもあまり効果がないことが分かり，最近はやはりHAARTで継続的にやるのがいいという考え方に変わってきています。

治療の開始時期についても，HAARTが可能となった1995年頃は，なるべく早い時期，感染が見つかってから直ちに治療を開始するコンセプトだったのですが，間もなくCD4陽性リンパ球※が500以下になってからでもよく，500以上は経過観察ということになりました。その後さらに，もうちょっと下がってからでも治療をすればCD4陽性リンパ球が回復するということが分かり，副作用もあり，あるいはあまり早くから開始すると結局は耐性ウイルスが出てしまうということもあって，200～350くらいの間で治療開始するという動きになっております。HAARTにはまだ多くの課題も残っています（図7）。

※CD4陽性リンパ球：別名ヘルパーT細胞，HIV感染，結核等で低値となる。

3. 患者の受け入れ体制：拠点病院の二極化が問題

日本でもHIV感染者／患者がどんどん増えてきているので，それを受け入れる医療体制を整備する必要があります。ACCのみならず，主要な拠点病院では，どこも患者数が増え続けています。首都圏，特に東京都で患者が最も多いわけですが，回答のあった都内の32拠点病院で患者さん400人以上を診ている施設が3施設です。300人台，200人台が全くなく，199人から100人までというのが1施設，99人から50人が6施設，49人から10人が10施設，9人以下が12施設となっていて，二極化しています（表2）。非常にたくさん患者さんが集中しているところと，数十人以下の患者さんしか診ていないところと二極化していて，患者さんの多いところでは，患者さんがどんどん増え，医療体制，受け入れ体制が追いついていない状況が出てきています。あと2年くらいで，今のままの体制だと限界に達するだろう，それ以上患者が

増えると，1人ひとりの診療時間が減って，医療の質を低下させざるを得ないような状態になってしまうだろうということが分かりました。

患者さんが，もう少しいろいろな拠点病院に行けるように，患者さんの選択肢を増やせるように拠点病院の充実が急務となってきました。

4. 抗体検査の遅れ

もう1つの問題は感染しておりながら，感染を知らずにいるという人が感染者の8割に達すると考えられる点です。感染を知って治療を受けている人は免疫力が回復し，エイズを発症しなくなります。しかも，血中のウイルスが低くなるので他人へ伝播する確率も減ります。HAARTを始めていなくても通院している人は診療のたびに医療機関からの啓発活動，情報を得るため，行動に配慮するようになり，HIVの伝播を防止できるようになっていきます。しかし，なにぶんにも8割の人がそれを知らずに，自分の行動も従来どおり続けているために，感染が広がっているのです。そのポピュレーションにメッセージがなかなか伝わりません。

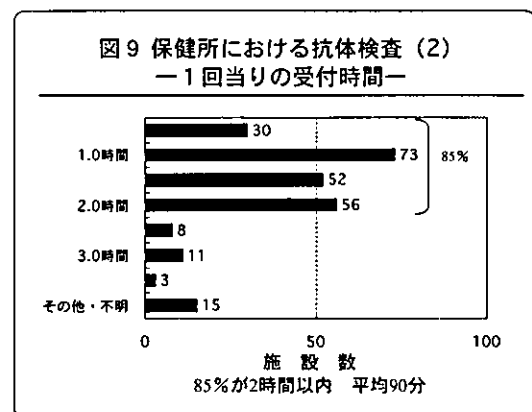1つには検査をきちんと受けることが重要で，そのためにはもっと簡単に検査が受けられるような体制をつくらないといけません。

現在保健所でやっている抗体検査の実態を見て

みますと，1週間に1回とか2回くらいしか，検査を受け入れていません（図8）。その検査も1回あたりの受付時間が90分と短いのが現状です（図9）。多くの感染者の方々は，仕事を持っているわけですけれども，電話して検査予約をとり，指定された時間に保健所に行って，その1週間後か2週間後に結果を聞きにいってという非常にハードルの高いことをクリアしないと検査を受けられないのです。仕事に追われている現代人が月2ないし4回の限られた90分間に保健所に行くのは難しいし，そこで同じ目的で来た知人に会ってしまうかも知れません。このような状況を変えていかないと，迷っている感染者を把握できません。

感染が分かれば，適切な時期に適切な治療を受けられるようになりますので，感染者自身にまずメリットになります。検査を受けやすいような体制をつくる必要があります。この体制をつくることが，日本における感染の拡大をくい止めることにつながるのではないかと思われます。

表2 患者数別の施設数（2003年）

患者数	≧400	399-300	299-200	199-100	99-50	49-10	9-1	0
東京都	3	0	0	1	6	10	11	1

図8 保健所における抗体検査（1）
ー検査回数ー

施 設 数
約90%が週1回以下しか行っていない

図9 保健所における抗体検査（2）
ー1回当りの受付時間ー

施 設 数
85%が2時間以内　平均90分
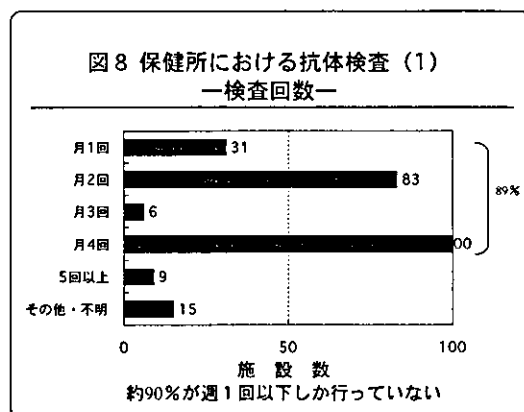
Viral Load of Human Herpesvirus 8 (HHV-8) in the Circulatory Blood Cells Correlates with Clinical Progression in a Patient with HHV-8-associated Solid Lymphoma with AIDS-associated Kaposi's Sarcoma

JIAN SONG[a], ATSUSHI YOSHIDA[b], YOSHIHIKO YAMAMOTO[c], HARUTAKA KATANO[d], KEISUKE HAGIHARA[a], SHINICHI OKA[c], SATOSHI KIMURA[b,c] and KAZUYUKI YOSHIZAKI[a,*]

[a]*Department of Medical Science I, School of Health and Sport Sciences, Osaka University, Osaka, Japan;* [b]*Department of Infection Control and Prevention, Graduate School of Medicine, University of Tokyo, Tokyo, Japan;* [c]*AIDS Clinical Center, International Medical Center of Japan, Tokyo, Japan and* [d]*Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan*

We encountered a case of a rapidly progressive HHV-8-associated solid lymphoma with AIDS-associated Kaposi's sarcoma (KS). HHV-8 DNA load in whole blood cells was analyzed quantitatively by real-time PCR using amplification of the HHV-8-encoded ORF26 gene. Ours is the first observation that the rapid increase in the HHV-8 viral load (from 1.9×10^4 copies/μg to 1.6×10^6 copies/μg in 40 days) in conjunction with low CD4 + cell counts was accompanied by an accelerated clinical disease progression. The results indicate that the quantity of circulating HHV-8 is measurable with real-time PCR and can provide clinically useful information.

Keywords: HHV-8-associated solid lymphoma; HHV-8; Viral load; ORF26; Real-time PCR

INTRODUCTION

Human herpesvirus-8 (HHV-8) is etiologically linked to Kaposi's sarcoma (KS), the most common malignancy in patients with AIDS [1,2]. HHV-8 DNA is consistently found in KS tissues, and is detected in peripheral blood mononuclear cells (PBMC) of human immunodeficiency virus (HIV)-infected individuals [3,4]. Detection of HHV-8 DNA in PBMC from HIV type 1-infected persons is associated with an increased risk of subsequent development of KS [4,5] and with the clinical stage of KS [5,6]. AIDS-associated primary effusion lymphoma (PEL, body-cavity based lymphoma), a distinct subtype of non-Hodgkin's lymphoma (NHL), is another HHV-8-associated neoplasm, which is typically present as a malignant effusion without solid tumor masses in the body cavity of AIDS patients [7]. Recently, solid organ involvement of HHV-8-associated lymphomas has also been reported in some AIDS patients [8–11]. HHV-8-associated solid lymphomas were characterized by expressing CD30, exhibiting anaplastic large cell morphology and carrying clonal immunoglubulin gene rearrangement that indicates B-cell origin despite the usual presence of a non-specific immunophenotype. HHV-8 DNA and latency-associated nuclear antigen (LANA) have been detected in lymphoma cells from HHV-8-associated solid lymphoma patients. Epstein-barr virus (EBV) co-infection was also found in most of these patients. There have been no reports, however, of an elevated HHV-8 DNA load in serum nor in whole blood cells with HHV-8-associated solid lymphoma in AIDS patients. Yamamoto [12] reported a case of rapidly progressive HHV-8-associated solid lymphoma with anaplastic large cell morphology followed by systemic KS, which is complemented by our kinetic study of the HHV-8 DNA load and CD4 + cell counts in blood cells in the same patient. The quantitative real-time PCR (Taqman PCR) technique provides an accurate and reproducible measurement of the level of HHV-8 in the circulatory blood cells. We used this technique to quantify the HHV-8 DNA load in whole blood cells. Ours is the first report of the correlation between a rapid

*Corresponding author. Address: Department of Medical Science I, School of Health and Sport Sciences, Osaka University, 2-1 Yamadaoka, Suita, Osaka, 565-0871, Japan. Tel.: + 81-6-6879-8960. Fax: + 81-6-6879-8971. E-mail: kyoshizaki@hpc.cmc.osaka-u.ac.jp

increase of HHV-8 viral load in conjunction with low CD4 + cell counts and disease progression.

CASE REPORT

Patient History

The clinical history, as well as the physical and laboratory findings including treatment modalities of this patient were described previously [12]. The patient was a 30-year-old Caucasian male with homosexual behavior and intravenous drug use history for 10 years. He had a 6-year HIV-1 infection history but did not contract any opportunistic infections until red spots were found on the skin of the arms, chest and abdomen and later on diagnosed as KS. Upon diagnosis with KS, the CD4 + cell count was 75/μl and HIV-1 plasma viral load was 431,992 copies/ml. He was transferred to the AIDS Clinical Center of the International Medical Center of Japan in Tokyo due to loss of consciousness, where Highly Active Antiretroviral Therapy (HAART) was stopped because he was unconscious. KS lesions were observed on his whole body. Liposomal doxorubicin (20 ng/m^2) was administered twice intravenously to treat KS. Whole blood cells were obtained before and after chemotherapy and the purified DNA was used for HHV-8 DNA quantification by means of real-time PCR. Biopsy results from the enlarged axillary and cervical lymph nodes showed large cell lymphoma morphology. HHV-8 and Epstein-Barr virus (EBV) producing proteins were detected in the lymphoma cells. Enzyme immunoassay (EIA) and immunofluorescence assay (IFA) were negative for the serum anti-HHV-8 antibody. The patient died 6 days after the induction of a CHOP regimen [12]. In autopsy, lymphoma cells were found not only in the cervical, mediastinal and inguinal lymph nodes, but also in the spleen, tonsils, gastrointestinal mucosa, lungs, adrenal glands and bone marrow. The lymphoma cells displayed anaplastic large blastic cell morphology and had an undeterminant phenotype. In immunohistochemistry, CD30-positive, CD43-positive, CD45RO-positive, CD45-positive, CD4-negative, CD5-negative, CD8-negative, CD15-negative and CD20-negative were detected in the lymphoma cells. Also, HHV-8-encoded LANA was expressed in most lymphoma cells. EBV-encoded small RNA-1 (EBER-1) was expressed in some lymphoma cells by in situ hybridization. KS was found in skin and perilymph node soft tissue in the inguinal region.

Establishment of Quantitative Real-time PCR Assay for HHV-8-Encoded ORF26 Gene

The amount of HHV-8 DNA purified from whole blood cells was determined by real-time PCR using amplification of the conserved region of the open reading frame (ORF) 26 gene. The primers and probe of ORF26 were conducted by the method described by White [13]. The amount of human genomic DNA present in the same sample was also determined by real-time PCR using amplification of the human GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene. The quantification of human GAPDH was used to normalize the target DNA. The primers and probes used to quantify ORF26 and GAPDH are shown in Table I. Quantitative real-time PCR was performed in duplicate with the aid of the Taqman Universal PCR Master Mix kit and the PE Biosystem 5700 sequence detector (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's protocol. Briefly, the reaction volume of 50 μl contained 25 μl of 2 × master buffer, 15 pmoles of 1 of primers, 10 pmoles of the dual-labeled probe, and 0.1 μg DNA of 1 of samples to be tested. All assays showed a linear relationship between the value of threshold cycle (Ct) for standards and the logarithm of the amount of ORF26 DNA added to the reaction. The PCR products were further examined by acrylamide gel electrophoresis for confirmation of specific HHV-8 DNA amplification.

Relationship Analysis of HHV-8 DNA Load, CD4 + cell Counts and Clinical Course

Real-time quantitative PCR of the HHV-8 ORF26 gene showed that the HHV-8 DNA load in whole blood cell was constitutively high (1.9 × 10^4 copies/μg) at the beginning of anti-KS therapy (Liposomal doxorubicin, 20 mg/m^2) (Fig. 1), while the CD4 + cell count was very low (32/μl) even though the patient had been treated with HAART. Systemic KS treatment with liposomal doxorubicin (20 ng/m^2) was administered twice intravenously

TABLE 1   Oligonucleotide primers used for real-time PCR assay

Name	Position[a]		Polarity	Sequence[b]
Taq26F	379	399	Sense	5'-CTCGAATCCAACGGATTTGAC-3'
Taq26R	434	452	Antisense	5'-TGCTGCAGAATAGCGTGCC-3'
Probe26	410	429	Sense	5'-F-CCATGGTCGTGCCGCAGCA-T-3'
GapdhF	6	24	Sense	5'-GAAGGTGAAGGTCGGAGTC-3'
GapdhR	212	231	Antisense	5'-GAAGATGGTGATGGGATTTC-3'
GapdhP	183	202	Antisense	5'-J-CAAGCTTCCCGTTCTCAGCC-T-3'

[a]Numbers indicate nucleotide position of the ORF26 and GAPDH sequence.
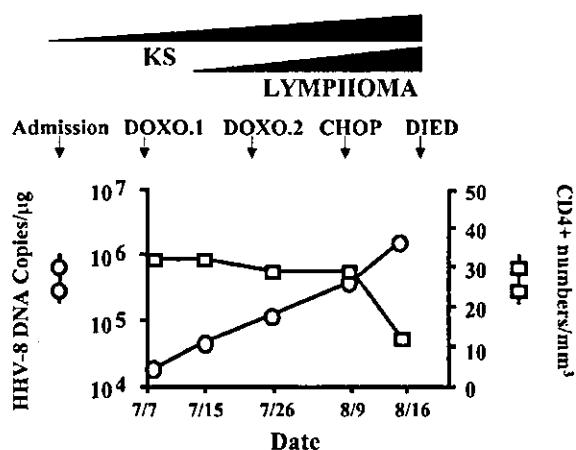[b]F, FAM reporter dye; T, TAMRA quencher dye; J, Joe reporter dye.

FIGURE 1 The relationship between HHV-8 viral load in whole blood cells and CD4 + cell counts on the one hand, and clinical course on the other in an HHV-8-associated solid lymphoma patient with AIDS-KS. DOXO.1: first administration of Liposomal Doxorubicin. DOXO.2: second administration of Liposomal Doxorubicin.
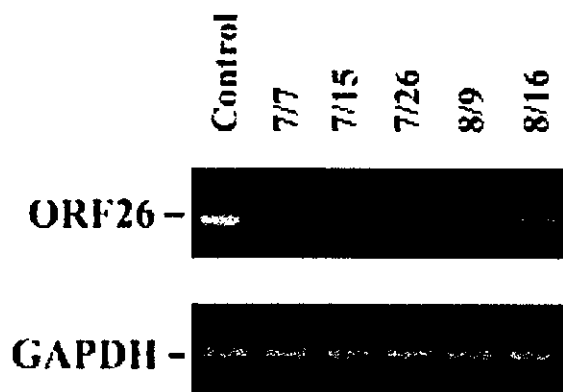

FIGURE 2 Specific amplification of real-time PCR products corresponding to HHV-8-encoded ORF26 gene and GAPDH internal control was confirmed by gel electrophoresis. The control was BCBL-1 (a B cell line derived from body-cavity-based lymphoma, which is latently infected with HHV-8) derived DNA. 7,7, 7/15, 7/26, 8,9 and 8/16 represent dates when whole blood cells were obtained from the patient.

with a 12-day interval. During treatment, right axillary and left cervical lymph nodes grew to 2 and 3–5 cm in diameter, respectively. The HHV-8 viral load was increased to 4.7×10^4 copies/μg shortly after the first course of treatment. Examination of biopsy material from these lymph nodes showed large cell lymphoma morphology, and the lymphoma spread rapidly to the eyelids, neck and arms, which appeared as growing lymphadenopathy. The HHV-8 viral load increased to 1.3×10^5 copies/μg during the first week after the second treatment course. Since the rapidly progressive lymphoma did not respond to liposomal doxorubicin, a CHOP regimen consisting of prednisolone (120 mg), doxorubicin (30 mg; reduced by 30% due to the co-existing thrombocytopenia), vincristine (2 mg, reduced by 7.4%) and cyclophosphamide (750 mg, reduced by 50%) was administered. However, the level of HHV-8 continued to increase to 3.9×10^5 copies/μg in a blood sample obtained a few days after CHOP administration, while the CD4 + cell counts remained low (29/μl). The patient died 6 days after the induction of the CHOP regimen. In the final sample obtained 2 days before the patient died, the HHV-8 viral load was 1.6×10^6 copies/μg, and the CD4 + cell counts were reduced to 12/μl. In addition, neither effusion lymphoma nor lymphocytic leukemia was detected in this patient during the whole clinical course. Overall, the HHV-8 DNA load in the whole blood cells increased by a factor of about 100 despite the therapy for KS, while the CD4 + cell counts stayed low during the progression of the lymphoma (Fig. 1). All of the real-time PCR products were confirmed as specific and the amplified product was verified as correct by acrylamide gel electrophoresis (Fig. 2).

Immunohistochemical Studies

Immunohistochemistry was performed as described previously [14]. After endogenous peroxidase was blocked

with methanol-0.6% H_2O_2 for 30 min at room temperature, the anti-ORF50 or anti-ORF59 polyclonal antibodies were allowed to react at 4^{c}C. Immunohistochemical staining showed that lymphoma cells expressing HHV-8 encoded ORF50 and ORF59 (Fig. 3a,b) antigens in the cells' nuclei. This finding suggests that HHV-8 replication did occur in the tumor mass of this patient.

DISCUSSION

Our observation of a nearly 100-fold increase of the HHV-8 DNA load in whole blood cells within 40 days and the detection of HHV-8 lytic protein in the lymphoma cells indicate that HHV-8 replicates in HHV-8-associated solid lymphoma with AIDS-associated KS. HHV-8 is etiologically associated with KS, primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD). The HHV-8-associated solid lymphoma has recently been proposed as a new type of lymphoma. It is a solid lymphoma and is often complicated with other HHV-8-associated diseases such as KS [8]. HHV-8 usually establishes latent infection in the natural host cells. Activation of HHV-8 replication in the latently infected cells, reflecting an increase in HHV-8 DNA load, is responsible for viral spread and presumed to contribute to the development of HHV-8-associated diseases [15]. It has been reported that detection of HHV-8 DNA in PBMC from HIV type 1-infected persons is associated with an increased risk of subsequent development of KS [4,5] and with KS disease progression [5,6]. Some studies have suggested quantification of the HHV-8 viral load might be useful for monitoring the therapeutic response of patients with HHV-8-associated diseases [16]. Poor prognosis is common among cases of HHV-8-associated lymphoma. Liposomal doxorubicin and the CHOP regimen are first-line agents for the treatment of KS
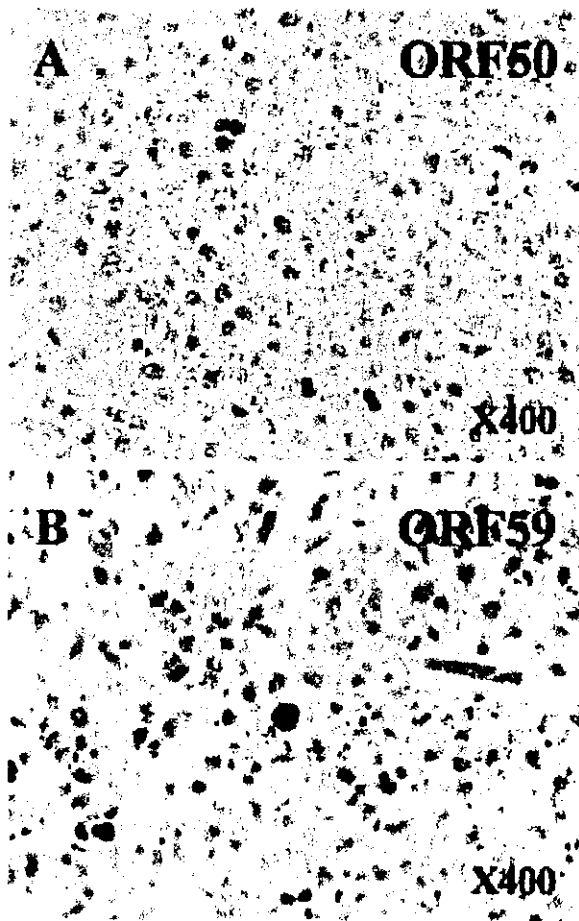
FIGURE 3   Immunohistochemical staining showed that the lymphoma cells of this patient expressed HHV-8 encoded ORF50 (A) and ORF59 (B) antigens in the cells' nuclei. Specimens were obtained at autopsy.

and lymphoma, but were not able to suppress the progression of the disease in our patient, as evidenced by the nearly 100-fold increase in the HHV-8 viral load during the 40 days of chemotherapy, and by the rapid lymphoma progression. The clinical deterioration seen in our patient accompanied by the increase in the HHV-8 viral load suggests that HHV-8 replication had occurred in this patient, and that HHV-8 had been involved in lymphoma progression. Our finding that detection of high load HHV-8 DNA is associated with lower CD4 + cell counts is consistent with data reported by others [4], providing further evidence that advanced immunosuppression is responsible for HHV-8 replication and development of lymphoma as well as KS. Co-infection with HIV may also affect HHV-8 replication and HHV-8-associated disorders through cytokine production and HIV-1 Tat protein secretion from HIV-infected cells [17–19]. Interferon γ (IFNγ) and oncostatin M (OSM) reportedly induce HHV-8 replication [20–21]. We have found that IL-6 activates HHV-8 replication in PEL-derived BCBL-1 cells [15]. In addition, EBV coinfection reported in most cases of HHV-8-associated solid

lymphoma suggests HHV-8 may act in conjunction with EBV in the progression of lymphoma. However, our previous publication of this case suggested that HHV-8, rather than EBV, is the main viral agent associated with the pathogenesis of lymphoma in the patient [12]. EBV co-infection found in our patient probably contributed to the progression of HIV infection and the loss of functional immune responses.

In summary, our results suggest that the HHV-8 viral load in whole blood cells measured by real-time quantitative PCR may be useful for monitoring the response to therapies used to treat HHV-8-associated diseases. Further investigation is needed to identify the precise role of HHV-8 as well as inflammatory cytokines in HHV-8-associated solid lymphoma.

References

[1] Chang, Y., Cesarman, E., Pessin, M.S., Lee, F., Culpepper, J., Knowles, D.M., *et al.* (1994) "Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma", *Science*, 266, 1865–1869.

[2] Said, J.W., Tasaka, T., Takeuchi, S., Asou, H., de Vos, S., Cesarman, E., *et al.* (1996) "Primary effusion lymphoma in woman: report of two cases of Kaposi's sarcoma herpesvirus-associated effusion-based lymphoma in human immunodeficiency virus-negative woman", *Blood*, 88, 3124–3128.

[3] Kikuta, H., Itakura, O., Ariga, T. and Kobayashi, K. (1997) "Detection of human herpesvirus 8 DNA sequences in peripheral blood mononuclear cells of children", *Journal of Medical Virology*, 53, 81–84.

[4] Whitby, D., Howard, M.R., Tenant-Flowers, M., Brink, N.S., Copas, A., Boshoff, C., *et al.* (1995) "Detection of Kaposi's sarcoma associated herpes virus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma", *Lancet*, 346, 799–802.

[5] Campbell, T.B., Borok, M., Gwanzura, L., MaWhinney, S., White, I.E., Ndemera, B., *et al.* (2000) "Relationship of human herpesvirus 8 peripheral blood virus load and kaposi's sarcoma clinical stage", *AIDS*, 14, 2109–2116.

[6] Cattani, P., Capuano, M., Lesnoni La Parola, I., Guido, R., Santangelo, R., *et al.* (1998) "Human herpesvirus 8 in Italian HIV-seronegative patients with Kaposi sarcoma", *Archives of Dermatology*, 134, 695–699.

[7] Cesarman, E., Chang, Y., Moore, P.S., Said, J.W. and Knowles, D.M. (1995) "Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas", *New England Journal of Medicine*, 332, 1186–1191.

[8] Katano, H., Suda, T., Morishita, Y., Yamamoto, K., Hoshino, Y., Nakamura, K., *et al.* (2000) "Human herpesvirus8-associated solid lymphoma occurring in AIDS patients take anaplastic large cell morphology", *Modern Pathology*, 13, 77–85.

[9] Oksenhendler, E., Boulanger, E., Galicier, L., Du, M.Q., Dupin, N., Diss, T.C., *et al.* (2002) "High incidence of Kaposi sarcoma-associated herpesvirus-related non-Hodgkin lymphoma in patients with HIV infection and multicentric Castleman disease", *Blood*, 99, 2331–2336.

[10] DePond, W., Said, J.W., Tasaka, T., de Vos, S., Kahn, D., Cesarman, E., et al. (1997) "Kaposi's sarcoma-associated herpes-virus and human herpesvirus 8 (KSHV/HHV8)-associated lympho-ma of the bowel. Report of two cases in HIV-positive men with secondary effusion lymphomas", American Journal of Surgical Pathology, 21, 719 724.

[11] Buske, C., Hannig, H., Hiddemann, W. and Bodemer, W. (1997) "Human herpesvirus-8 (HHV-8) DNA associated with anaplastic large cell lymphoma of the B-cell type in an HIV-1-positive patient". International Journal of Cancer, 73, 303 304.

[12] Yamamoto, Y., Teruya, K., Katano, H., Niino, H., Yasuoka, A., Kimura, S., et al. (2003) "Rapidly progressive human herpesvirus 8-associated solid lymphoma in a patient with AIDS-associated Kaposi sarcoma", Leukemia & Lymphoma, 44, 1631 1633.

[13] White, I.E. and Campbell, T.B. (2000) "Quantitation of cell-free and cell-associated Kaposi's sarcoma-associated herpesvirus DNA by real-time PCR", Journal of Clinical Microbiology, 38, 1992 1995.

[14] Katano, H., Sato, Y., Kurata, T., Mori, S. and Sata, T. (2000) "Expression and localization of human herpesvirus 8-encoded proteins in primary effusion lymphoma, Kaposi's sarcoma, and multicentric Castleman's disease", Virology, 269, 335 344.

[15] Song, J., Ohkura, T., Sugimoto, M., Mori, Y., Inagi, R., Yamanishi, K., et al. (2002) "Human interleukin-6 induces human herpesvirus-8 replication in a body cavity-based lymphoma cell line", Journal of Medical Virology, 68, 404 411.

[16] Tedeschi, R., Enbom, M., Bidoli, E., Linde, A., De Paoli, P. and Dillner, J. (2001) "Viral load of Human herpesvirus in peripheral blood of Huam immunodeficiency virus-infected patients with Kaposi's sarcoma", Journal of Clinical Microbiology, 39, 4269 4273.

[17] Ensoli, B., Barillari, G. and Gallo, R.C. (1992) "Cytokines and growth factors in the pathogenesis of AIDS-associated Kaposi's sarcoma (Review)", Immunological Reviews, 127, 147 155.

[18] Harrington, W.J., Sieczkowski, L., Sosa, C., Chan-a-Sue, S., Cai, J.P., Cabral, L., et al. (1997) "Activation of HHV-8 by HIV-1 tat", Lancet, 349, 774 775.

[19] Varthakavi, V., Browning, P.J. and Spearman, P. (1999) "Human immunodeficiency virus replication in a primary effusion lymphoma cell line stimulates lytic-phase replication of Kaposi's sarcoma-associated Herpesvirus", Journal of Virology, 73, 10329 10338.

[20] Blackbourn, D.J., Fujimura, S., Kutzkey, T. and Levy, J.A. (2000) "Induction of human herpesvirus-8 gene expression by recombinant interferon gamma", AIDS, 14, 98 99.

[21] Mercader, M., Taddeo, B., Panella, J.R., Chandran, B., Nickoloff, B.J. and Foreman, K.E. (2000) "Induction of HHV-8 lytic cycle replication by inflammatory cytokines produced by HIV-1-infected T cells", American Journal of Pathology, 156, 1961 1971.

ORIGINAL ARTICLE

Hirohisa Endo · Yoshimi Higurashi · Katsuko Okuzumi
Shigemi Hitomi · Satoshi Kimura

Changes in drug susceptibility and toxin genes in *Staphylococcus aureus* isolated from blood cultures at a university hospital

Abstract We studied changes in toxin-producing genes and drug susceptibility in *Staphylococcus aureus* isolated from blood cultures at the University of Tokyo Hospital between 1980–1984 (six *mecA* gene-positive methicillin resistant *S. aureus* [MRSA] strains and 20 *mecA* gene-negative methicillin-susceptible *S. aureus* [MSSA] strains) and 1999 (11 MRSA and 20 MSSA strains). The prevalence of strains with toxin-producing genes increased from 66.7% to 90.9% in MRSA, and from 30.0% to 55.0% in MSSA during the interval. Among toxin-producing gene-positive *S. aureus*, the dominant strains shifted from those with the entero-toxin (ET) – A gene in 1980–1984 to those with both the toxic shock syndrome toxin-1 and the ET-C genes in 1999. All strains were susceptible to vancomycin and teicoplanin. Mupirocin and arbekacin inhibited all strains at concentrations of less than or equal to 0.5 μg/ml and 4 μg/ml, respectively. More than half of the MRSA strains in 1999 were considered to be nonsusceptible to flomoxef. Because almost all MRSA and more than half of MSSA among recently isolated strains possessed the toxin-producing genes, we should pay attention to whether toxin-related diseases caused by MRSA and MSSA are increasing.

Key words *Staphylococcus aureus* · *mecA* · Drug susceptibility · Toxin genes

H. Endo (✉) · Y. Higurashi · K. Okuzumi · S. Kimura
Department of Infection Control and Prevention, University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
Tel. +81-3-3815-5411; Fax +81-3-5800-8796
e-mail: hiroendo-tky@umin.ac.jp

S. Hitomi
Department of Infectious Diseases, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan

S. Kimura
AIDS Clinical Center, International Medical Center of Japan, Tokyo, Japan

Introduction

Staphylococcus aureus is an important human pathogen causing infections involving the skin, soft tissues, lung, bone, and endocardium.[1,2] The drug susceptibility of and toxin production by the organism provide important clues for the treatment of these infections. These characteristics of *S. aureus* isolated at the University of Tokyo Hospital in 1982 and 1992 have been reported previously.[3] However, the strains reported in that study were collected from various specimens that contained both organisms from infected patients and colonized organisms. In the present study, therefore, we focused on organisms isolated from blood cultures at the hospital and examined changes in their characteristics.

Materials and methods

We examined 26 *S. aureus* strains isolated from the blood in 1980–1984, which were the oldest strains available for the present study, and 31 strains isolated in 1999. The strains were reconfirmed with a gram stain test, a catalase test, a coagulase test,[1] and VITEC Gram Positive Identification Cards (Japan bioMérieux, Tokyo, Japan). Suspected *S. aureus* strains with an ambiguous result on these tests were confirmed with a Slidex Staph Kit (Japan bioMérieux). When an organism was isolated repeatedly from the same patient within a year, the oldest isolate was used. In the present study, we defined methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) as *S. aureus* with and without the *mecA* gene, respectively. The *mecA* gene was detected with a polymerase chain reaction method described by Hiramatsu et al.[4] Genes for toxic shock syndrome toxin-1 (TSST-1) and enterotoxin A, B, C, D, and E (ET-A, ET-B, ET-C, ET-D, and ET-E) were amplified with the Toxic Shock Syndrome Toxin Gene Detection Primer Set TST-1 and 2 and the *S. aureus* ET Gene Detection Primer Set SEA-1 and 2, SEB-1 and 2, SEC-1

and 2, SED-1 and 2, and SEE-1 and 2 (Takara Shuzo, Tokyo, Japan), respectively, and detected according to the manufacturer's instructions. The minimum inhibitory concentrations (MICs) of oxacillin, vancomycin, teicoplanin, mupirocin, arbekacin, and flomoxef were examined with the microbroth dilution method recommended by the Japanese Society of Chemotherapy,[5] and interpreted according to the criteria of the National Committee for Clinical Laboratory Standards,[6] or other descriptions (mupirocin:[7] ≤2μg/ml, susceptible; ≥4μg/ml, resistant; arbekacin:[8] ≤2μg/ml, susceptible; ≥4μg/ml, resistant; flomoxef:[9] ≤8μg/ml, susceptible; 16μg/ml, intermediately-resistant; ≥32μg/ml, resistant).

Results and discussion

The mecA gene was detected in 6 of the 26 strains (23.1%) in 1980–1984 and in 11 of the 31 strains (35.5%) in 1999. There was one mecA gene-positive but oxacillin-susceptible strain in 1980–1984. All oxacillin-resistant S. aureus strains were mecA gene-positive. Toxin genes were detected in 4 of the 6 MRSA (66.7%) and 6 of the 20 MSSA strains (30.0%) in 1980–1984 and in 10 of the 11 MRSA strains (90.9%) and 11 of the 20 MSSA strains (55.0%) in 1999 (Table 1). The total number of S. aureus strains with toxin-producing genes increased significantly during the interval (P = 0.026; χ^2 test). In the period 1980–1984, the ET-A gene was the most frequent gene, found in 3 of the 4 toxin-gene-positive MRSA strains and 4 of the 6 toxin-gene-positive MSSA strains. Only 1 MRSA strain and no MSSA strain in the period had both TSST-1 and ET-C genes. In 1999, on the contrary, all 10 toxin-gene-positive MRSA strains and 5 of the 11 toxin gene-positive MSSA strains possessed both of these toxin genes (Table 1). Previous studies in Japan reported that the dominant MRSA strains shifted from those producing ET-A to those producing both TSST-1 and ET-C in about 1990,[3,10] that MRSA strains producing the two toxin proteins prevailed in the 1990s,[11,12] and that around half of MSSA strains in the 1990s were toxin-producing

strains.[3,11] Our results indicate that, in the late 1990s, MRSA strains producing both TSST-1 and ET-C were strongly dominant and the prevalence of toxin-producing MSSA strains, although lower than that of toxin-producing MRSA strains, had also increased, to more than 50%.

All of the examined S. aureus strains were susceptible to vancomycin, teicoplanin, and mupirocin. Two strains, one MRSA and one MSSA in 1999, were resistant to arbekacin, inhibited at a concentration of 4μg/ml. However, because we applied the breakpoint for respiratory tract infection recommended by the Japan Society of Chemotherapy[8] for interpretation of the MIC, it is unclear whether the two strains isolated from blood culture could be definitely interpreted as resistant to the drug. Although MRSA strains in 1980–1984 and all MSSA strains were susceptible to flomoxef, only 2 of the 11 MRSA strains in 1999 were susceptible to the drug. Therefore, now, flomoxef can hardly be used for the treatment of MRSA infection. The ranges and 50%, and 90% values for the MICs of the antibiotics for MRSA and MSSA in each period are listed in Table 2.

Table 1. Occurrence of toxin-producing genes

Toxin types[a]	Numbers of strains (%)			
	1980–1984[b]		1999	
	MRSA	MSSA	MRSA	MSSA
TSST-1	–	–	–	1 (5.0)
TSST-1 and ET-C	1 (16.7)	–	10 (90.9)	5 (25.0)
ET-A	3 (50.0)	3 (15.0)	–	1 (5.0)
ET-B	–	–	–	3 (15.0)
ET-C	–	1 (5.0)	–	–
ET-D	–	1 (5.0)	–	–
ET-E	–	–	–	–
ET-A and ET-B	–	1 (5.0)	–	1 (5.0)
ND	2 (33.3)	14 (70.0)	1 (9.1)	9 (45.0)
Total	6 (100)	20 (100)	11 (100)	20 (100)

MRSA, methicillin-resistant *Staphylococcus aureus* (with *mecA* gene);
MSSA, methicillin-susceptible *S. aureus* (without *mecA* gene)
[a] TSST-1, toxic shock syndrome toxin-1; ET-A, enterotoxin A; ET-B, enterotoxin B; ET-C, enterotoxin C; ET-D, enterotoxin D; ET-E, enterotoxin E; ND, not detected
[b] Year(s) of isolation

Table 2. Ranges and 50% and 90% MIC values in MRSA and MSSA

Organism	Antibiotic	MIC					
		1980–1984[a]			1999		
		Range	50%	90%	Range	50%	90%
MRSA	Vancomycin	0.5–1	1	1	0.5–1	1	1
	Teicoplanin	0.25–2	0.5	2	0.25–2	1	2
	Mupirocin	0.25–0.5	0.25	0.5	0.13–0.25	0.13	0.25
	Arbekacin	≤0.06–1	0.5	1	0.25–4	1	2
	Flomoxef	0.5–8	2	8	8–128	16	64
MSSA	Vancomycin	0.5–1	1	1	1–2	1	2
	Teicoplanin	0.13–2	0.5	1	0.5–2	1	2
	Mupirocin	≤0.06–0.5	0.25	0.5	≤0.06–0.5	0.13	0.25
	Arbekacin	≤0.06–2	0.25	0.5	0.25–4	0.5	1
	Flomoxef	0.13–1	0.5	0.5	0.25–1	0.5	0.5

MIC, minimum inhibitory concentration
[a] Year(s) of isolation

10

Today, only limited numbers of antibiotics are effective for overcoming MRSA infection. In addition, almost all of the recently isolated MRSA strains possessed toxin-producing genes. The prevalence of strains with toxin-producing genes is also increasing among MSSA strains. Therefore, we should pay attention to whether toxin-related diseases caused by MRSA and MSSA strains may increase in the future, and we should encourage the promotion of infection control practices to prevent expansion of the strains.

References

1. Kloos WE, Bannerman TL. *Staphylococcus* and *Micrococcus*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of clinical microbiology, 7th Ed. Washington, DC: American Society for Microbiology; 1999. p. 264–82.
2. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med 1998;339:520–32.
3. Tanaka T, Okuzumi K, Iwamoto A, Hiramatsu K. A retrospective study of methicillin-resistant *Staphylococcus aureus* clinical strains in Tokyo University Hospital. J Infect Chemother 1995;1:40–9.
4. Hiramatsu K, Kihara H, Yokota T. Analysis of borderline-resistant strains of methicillin-resistant *Staphylococcus aureus* using polymerase chain reaction. Microbiol Immunol 1992;36:445–53.
5. Goto S, Okada J, Oguri T, Kanno S, Yamaguchi K, Watanabe T. Report of the Committee for Japanese Standards for Antimicrobial Susceptibility Testing for Bacteria (in Japanese). Chemotherapy 1990;38:102–5.
6. The National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; twelfth informational supplement M100-S12. The National Committee for Clinical Laboratory Standards: Wayne; 2002.
7. Fuchs PC, Jones RN, Barry AL. Interpretive criteria for disk diffusion susceptibility testing of mupirocin, a topical antibiotic. J Clin Microbiol 1990;28:608–9.
8. Saito A. Clinical breakpoints for antimicrobial agents in pulmonary infections and sepsis: report of the Committee for Japanese Standards for Antimicrobial Susceptibility Testing for Bacteria. J Infect Chemother 1995;1:83–8.
9. Grimm H. Interpretive criteria of antimicrobial disk susceptibility tests with flomoxef. Infection 1991;19(Suppl 5):S258–63.
10. Nonoguchi R. Methicillin-resistant Staphylococci isolated from blood culture: epidemiologic characteristics and induction of penicillin-binding protein-2' by β-lactams (in Japanese). Chemotherapy 1990;38:90–101.
11. Nakazaki N, Uchida K, Hirata Y, Inoue M. Biological properties of blood-derived *Staphylococcus aureus* strains and their sensitivity to antimicrobial agents (in Japanese). Jpn J Chemother 1998;46:484–90.
12. Okada T, Furukawa S, Miwa K, Sakai R, Sugiyama J. A new exanthematous disease in newborn infants caused by exotoxins producing *Staphylococcus aureus*; exotoxin production of the isolates and serum levels of antitoxin antibody in the patients and umbilical cord blood (in Japanese). J Jpn Assoc Infect Dis 1999;73:893–900.

ORIGINAL RESEARCH ARTICLE

A discriminative study of health-related quality of life assessment in HIV-1- infected persons living in Japan using the Multidimensional Quality of Life Questionnaire for persons with HIV/AIDS

Megumi Watanabe RN[1], Koichi Nishimura MD[2], Tomoko Inoue RN[3], Satoshi Kimura MD[1], Shinichi Oka MD[1] and the QoL Research Group of the AIDS Clinical Centre and eight regional AIDS treatment hospitals in Japan*

[1]AIDS Clinical Centre, International Medical Centre of Japan, Tokyo; [2]Kyoto-Katsura Hospital, Kyoto; [3]Division of Comprehensive Health Nursing Sciences, Graduate School of Allied Health Sciences, Tokyo Medical Dental University, Tokyo, Japan.

Summary: The aim of this study is to evaluate the discriminative properties of the Multidimensional Quality of Life Questionnaire for HIV infection (MQoL-HIV) and to determine those factors contributing to the health-related quality of life (HRQoL) of HIV-1 infected persons living in Japan. The MQoL-HIV, the Nottingham Health Profile (NHP) as a generic instrument, and the Center for Epidemiologic Studies–Depression Scale (CES–D) as a psychological measure were administered in 375 patients as a multiple-centre study. The score distribution of the MQoL-HIV showed a unimodal distribution. The Cronbach's α coefficient scored more than 0.7 in seven out of 10 domains, but was low in both the physical functioning and sexual functioning domains. There was a strong correlation between the CES–D and MQoL-HIV index scores (R–0.73). Relatively high coefficient values were found between psychiatric and nervous symptoms and the index score (R = −0.60). In total, the MQoL-HIV may possess discriminative properties.

Keywords: health-related quality of life, Multidimensional Quality of Life Questionnaire for HIV/AIDS, Nottingham Health Profile

Introduction

HIV/AIDS is an infectious disease that is also considered to be a chronic disorder. The health-related quality of life (HRQoL) or health status has become an important consideration in the treatment of patients with chronic disorders. The purpose of medical intervention for chronic disorders is defined as improvement in both the quantity and quality of life. The former corresponds to an improvement in mortality, whereas the latter indicates improvement in the HRQoL. The importance of HRQoL as a health index, especially in the evaluation of health care services for treatment of chronic disorders, has long been emphasized. However, there have been few reports examining the HRQoL of AIDS patients.

This disease is thought to be significantly influenced by social and cultural background. When compared with Western countries, the prevalence of HIV infection is low in Japan[1]. A rare disease in a closed society like Japan may easily lead to prejudice by the general public against AIDS patients and asymptomatic carriers (AC), resulting in the patients experiencing social isolation. Several disease-specific instruments to measure HRQoL for AIDS patients have been developed and validated in the literature[2-7]. Smith and colleagues developed the Multidimensional Quality of Life Questionnaire for HIV/AIDS (MQoL-HIV), which is one of the most comprehensive disease-specific measures currently available for evaluating HIV/AIDS[5]. We first initiated our study to establish a Japanese version of the MQoL-HIV. Ultimately, the goal was to investigate the contributions of symptoms, laboratory findings, and psychosocial status to the HRQoL of AIDS patients to highlight the characteristics of the

Correspondence to: Shinichi Oka MD, Director, AIDS Clinical Centre International Medical Centre of Japan, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162-8655, Japan
E-mail: oka@imcj.hosp.go.jp

*Contributors to the QoL Research Group are listed in the Appendix

107

MQoL-HIV, and to identify its most influential factors.

Materials and methods

Translation of the MQoL-HIV

The MQoL-HIV was translated into Japanese in accordance with standardized methodology[8]. After obtaining from the author permission to translate the MQoL-HIV, the translation followed an established forward–backward translation procedure, with both independent and counter translations. Briefly, two researchers with clinical experience in treating AIDS patients independently translated the questionnaire into Japanese. These two translations were then proofread by a specialist (KN). A native English speaker who is fluent in Japanese but who has no medical experience translated the questionnaire back into English, and then this English translation was again translated into Japanese and carefully examined. Finally, 10 physicians who are routinely engaged in treating AIDS patients reviewed the questionnaire, and based on these reviews, the specialist finalized the Japanese translation.

The ethics committee of the International Medical Centre of Japan approved this study (IMCJ-H12-27).

Patients in a cross-sectional study

A total of 423 consecutive patients with HIV/AIDS from the AIDS Clinical Centre, International Medical Centre of Japan and eight other regional HIV treatment hospitals in Japan were recruited for this cross-sectional study from January to May 2000. All eligible patients completed all of the examinations described below, and an informed consent was obtained from each patient.

The patients were requested to complete a self-administered booklet. This booklet included the Japanese versions of the MQoL-HIV and the Nottingham Health Profile (NHP) for health status measurements[9], and the Japanese version of the Center for Epidemiologic Studies–Depression Scale (CES–D) for psychological evaluation[10]. In addition, the booklet included questions concerning personal issues such as disclosure of the HIV infection to others, employment status, and support from others, as an evaluation of their lifestyle and social background. Lastly, the booklet included questions concerning subjective symptoms.

For the subjective symptom analysis, 46 typical subjective symptoms of AIDS patients were selected from published reports. We used a five-point Lickert scale ranging from 'having no problem' to 'having serious problems' to obtain answers. We then classified the answers into two categories: '1, with symptoms' and '2, without symptoms'. Patients who answered 'having a problem' and 'having a serious problem' were grouped into the former category, whereas patients who chose any other answers were grouped into the latter. The results indicated that the patients with lower scores had more self-reported symptoms. In addition to the self-administered questionnaires, the following laboratory findings were recorded by coordinating nurses from individual medical records: age, gender, infectious route (blood product transfusion or sexual relationship), stage of disease (AC or AIDS), the presence or absence of treatment with anti-HIV drugs at the time of the examination, co-morbidity, CD4-positive lymphocyte count, and HIV-RNA levels. Furthermore, the attending nurses' opinions on the functional capacity of the patients were expressed by the Karnofsky Performance Status Scale (KPSS). The scale has a range of values from 0–100, where 0 means the patient is dead and 100 means the patient is healthy.

Assessment of HRQoL and psychological status

The HRQoL was measured by the MQoL-HIV[5], a disease-specific instrument, and the NHP[9], a generic instrument. The MQoL-HIV is a self-administered questionnaire consisting of the following 10 domains, totalling 40 items: mental health, physical health, physical functioning, social functioning, social support, cognitive functioning, financial status, partner intimacy, sexual functioning, and medical service (Table 1). Each domain consists of four items, and each item has seven response options. The score from each domain ranged from four to 28, and a lower score indicated a poorer HRQoL. In addition, we used a score range of 12 to 84 calculated from the equation, Mental health×2+Physical functioning, as the index score for the overall HRQoL.

The NHP is a self-administered questionnaire composed of two sections containing 45 items. In the present study, only the first section with 38 items was used to assess the following: energy (three items), pain (eight items), emotional reaction (nine items), sleep (five items), social isolation (five items), and physical mobility (eight items). All items have a yes/no answer format. The dimension scores ranged from 0–100. The higher the NHP score, the greater the health problems for that patient.

To assess the psychological status, a previously validated Japanese version of the CES–D was used for evaluating the patients' depression status[10]. The scores ranged from 0–60 with, 16 being the cut-off point. A score of 15 or less was regarded as normal, and a score of 16 or more as depression. Depression was classified into three categories according to the scores: mild (16–20), medium (21–30), and severe (over 31).

Table 1. Patient characteristics in 375 persons with HIV/AIDS

	Mean ± SD	Range
Age (years)	36.5 ± 10.3	20–74
CD4+ lymphocytes (/mL)	409 ± 227	2–1224
HIV-RNA (copies/mL)	11334 ± 52968	<400–680000
CES-D	16 ± 12	0–60

	Number (%)
Female	31 (8)
Stage	
Asymptomatic carrier	299 (80)
AIDS	76 (20)
Infection route	
Blood products	122 (33)
MSM	175 (47)
Heterosexual	68 (18)
Disclosure of HIV infection	314 (84)
Getting support	340 (91)
Having a job	265 (71)
Complications	200 (53)
No ongoing HIV treatment	69 (18)

CES-D = Center for Epidemiologic Studies–Depression Scale; MSM = men who have sex with men

Statistical analysis

All results are presented as means ± SD. Pearson's correlation test was used to analyse the relationship between two sets of data, and a P value of <0.05 was considered to be statistically significant. In addition, calculating the Cronbach's α coefficient enabled us to assess the internal consistency[11].

Factor analysis techniques were used to reduce or rearrange large sets of symptoms into smaller sets of factors of related symptoms[12]. All 46 symptoms were included in the analysis, and a maximum likelihood iterative solution was used. In this analysis, a matrix of correlation values between the variables was created, and then the data were transformed into linear combinations of variables that shared common variance between the measures. The correlation between the original variables and the linear combinations or factors is called factor loading. The factors were interpreted and defined based on which variables were highly loaded on each factor. The relationships between these factor scores and the index scores and the scores from the 10 domains of the MQoL-HIV were analysed, accordingly.

To identify those variables which influenced the index score and the scores from the 10 domains of the MQoL-HIV, a backward stepwise multiple logistic regression analysis was conducted, as previously described elsewhere[13]. The dependent variables included scores from each domain and the index score of the MQoL-HIV. The independent variables were selected from those variables that were significantly correlated with the MQoL-HIV scores, but were not strongly correlated with each other. The independent variables were as follows: age (years), gender (1: male, 2: female), infectious route (1: blood product transfusion, 2: sexual transmission), stage of disease (1:AC, 2: AIDS), HIV treatment (1: currently under treatment, 2: currently not under treatment), complications (1: present, 2: absent), CES–D (0–60), CD4-positive lymphocyte count, HIV-RNA quantity in actual numbers, disclosure of HIV infection to others (1: disclosed, 2: not disclosed), employment status (1: working 2: not working), support from others (1: supported, 2: not supported), eight symptoms determined by the factor analysis (1: present, 2: absent), and the KPSS (0–100). The dependent variables for this statistical model were the index score and the scores from the 10 domains of the MQoL-HIV. P values of <0.05 were considered to be statistically significant. These analyses were performed by a Statistical Package for the Social Sciences (SPSS).

Results

The booklets from a total of 375 of 423 patients (88.7%) were valid, and were used for further analysis. The clinical backgrounds of all the patients are summarized in Table 1. The average

Table 2. Score distribution* and internal consistency of the MQoL-HIV in 375 persons with HIV/AIDS

	Score			No. of persons with		Cronbach's α coefficient
	Median	Mean	SD	Minimum score	Maximum score	
Mental health	19	17.9	5.2	5	5	0.76
Physical health	23	21.6	4.9	0	26	0.76
Physical functioning	20	19.0	5.5	5	27	0.61
Social functioning	20	19.5	5.7	1	29	0.74
Social support	16	16.7	7.4	19	45	0.85
Cognitive functioning	24	23.1	4.6	1	84	0.84
Financial status	23	22.5	4.9	4	57	0.73
Partner intimacy	19	18.4	7.2	6	44	0.82
Sexual functioning	21	18.5	5.1	6	5	0.47
Medical service	23	22.4	4.8	1	52	0.67

MQoL-HIV = Multidimensional Quality of Life Questionnaire for HIV/AIDS
* The score can range from 4 (the minimum score) to 28 (the maximum score)

Table 3. Results of factor analysis used to reduce or rearrange large sets of symptoms into smaller sets of factors of related symptoms

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
Factor 1: Psychiatric and nervous symptoms									
Depression	0.78	0.12	0.19	0.08	0.09	0.06	0.09	0.29	0.12
Anxiety	0.71	0.08	0.20	0.13	0.07	0.13	0.11	0.19	0.18
Fatigue	0.70	0.27	0.09	0.15	0.21	0.19	0.13	0.04	−0.00
Feeling sluggish	0.70	0.28	0.22	0.12	0.18	0.06	0.09	0.14	−0.03
Easily fatigued	0.69	0.22	0.17	0.13	0.21	0.17	0.15	0.00	0.01
Irritability	0.65	0.14	0.22	0.13	0.09	0.17	0.06	0.11	0.21
Insomania	0.52	0.28	0.12	0.20	0.02	0.09	0.10	0.26	0.13
Factor 2: Upper digestive symptoms									
Nausea	0.20	0.76	0.15	0.11	0.19	0.11	0.14	0.07	−0.05
Upset stomach	0.28	0.66	0.08	0.09	0.21	0.15	0.18	0.12	0.36
Vomiting	0.20	0.66	0.21	0.25	0.06	0.06	0.19	0.16	−0.04
Heartburn	0.23	0.63	0.13	0.13	0.19	0.19	0.19	0.07	0.33
Factor 3: Respiratory and circulatory symptoms									
Short of breath	0.31	0.19	0.76	0.14	0.19	0.17	0.11	0.09	0.10
Choking	0.31	0.20	0.71	0.18	0.20	0.13	0.13	0.14	0.08
Palpitation	0.26	0.21	0.71	0.20	0.19	0.10	0.10	0.19	0.10
Factor 4: Inflammatory and haemorrhagic symptoms									
Haematuria	0.06	0.11	0.08	0.60	0.17	0.09	−0.02	0.09	0.01
Blood in stool	0.12	0.13	0.11	0.59	0.01	0.14	0.11	0.13	0.09
Pain on urination	0.03	−0.01	0.30	0.51	0.28	0.04	0.13	0.21	0.14
Factor 5: Sensory symptoms									
Change in taste	0.15	0.29	0.15	0.20	0.63	0.21	0.09	0.08	−0.02
Change in sensation around mouth	0.18	0.37	0.16	0.22	0.58	0.19	0.08	0.16	−0.05
Factor 6: Dermatological symptoms									
Itching	0.15	0.15	0.15	0.18	0.14	0.82	0.15	0.08	0.07
Dry skin	0.17	0.16	0.10	0.13	0.21	0.66	0.05	0.10	0.13
Rash	0.26	0.09	0.13	0.37	0.08	0.55	0.17	0.16	−0.13
Factor 7: Lower digestive symptoms									
Soft stool	0.11	0.19	0.08	0.09	0.13	0.13	0.82	0.14	0.02
Diarrhoea	0.15	0.17	0.10	0.13	0.07	0.07	0.80	0.05	0.05
Factor 8: Appearance									
Change in weight	0.27	0.17	0.06	0.09	0.21	0.17	0.12	0.52	0.14
Change in body shape	0.29	0.14	0.20	0.10	0.32	0.15	0.15	0.50	0.03
Other factors									
Headache	0.35	0.38	0.18	0.21	0.12	0.10	0.03	0.20	0.08
Dizziness	0.33	0.36	0.36	0.11	0.11	0.03	0.08	0.22	0.15
Hallucination	0.16	0.24	0.20	0.33	0.01	0.00	0.08	0.37	−0.02
Nightmare	0.39	0.17	0.31	0.23	0.01	0.05	0.01	0.39	0.02
Feverish	0.35	0.23	0.25	0.42	0.02	0.15	0.23	0.05	−0.03
Feeling a chill	0.31	0.30	0.39	0.43	0.14	0.12	0.11	0.10	−0.05
Cough	0.25	0.22	0.30	0.29	0.18	0.16	0.10	0.10	0.19
Change in appetite	0.31	0.49	0.13	0.14	0.22	0.14	0.19	0.21	0.07
Abdominal pain	0.22	0.28	0.20	0.25	0.07	0.20	0.41	0.07	0.29
Constipation	0.28	0.13	0.20	0.22	0.03	0.01	0.04	0.15	0.38
Gas	0.19	0.20	0.18	0.10	0.10	0.13	0.30	0.28	0.33
Swelling	0.26	0.14	0.18	0.21	0.36	0.20	0.16	0.40	0.20
Changing urine volume/time	0.14	0.22	0.18	0.27	0.45	0.11	0.09	0.26	0.20
Numbness	0.23	0.18	0.26	0.17	0.35	0.09	0.07	0.19	0.22
Changing eyesight	0.27	−0.04	0.36	0.29	0.36	0.17	0.14	0.13	0.18
Oral thrush	0.25	0.16	0.14	0.32	0.11	0.19	0.15	0.13	0.07
Increase in sweating	0.35	0.10	0.31	0.29	0.11	0.28	0.24	0.04	0.11
Muscle pain	0.29	0.09	0.34	0.31	0.36	0.15	0.17	−0.00	0.35
Bleeding tendency	0.13	0.11	0.02	0.46	0.18	0.13	0.09	−0.06	0.12

Table 4. Pearson's correlation coefficient between the score of the MQoL-HIV and Center for Epidemiologic Studies–Depression Scale (CES–D), the Karnofsky Performance Status Scale (KPSS) and self-reported symptoms

MQoL-HIV appearance	CES–D	KPSS	Psychiatric and nervous symptoms	Upper and digestive symptoms	Respiratory and circulatory symptoms	Inflammatory and haemorrhagic symptoms	Sensory symptoms	Dermatological symptoms		Lower digestive symptoms
Index score	−0.73	0.33	−0.60	−0.38	−0.43	−0.33	−0.34	−0.31	−0.30	−0.34
Mental health	−0.73	0.20	−0.60	−0.34	−0.38	−0.28	−0.29	−0.29	−0.30	−0.31
Physical health	−0.57	0.33	−0.60	−0.58	−0.52	−0.46	−0.48	−0.32	−0.40	−0.38
Physical functioning	−0.34	0.41	−0.28	−0.24	−0.29	−0.22	−0.23	−0.17	−0.13	−0.20
Social functioning	−0.61	0.18	−0.49	−0.29	−0.33	−0.29	−0.31	−0.22	−0.23	−0.26
Social support	−0.30		−0.16	−0.06	−0.11	−0.11	−0.18	−0.12	−0.11	
Cognitive functioning	−0.50	0.11	−0.49	−0.35	−0.38	−0.30	−0.41	−0.17	−0.26	−0.31
Financial status	−0.44		−0.36	−0.21	−0.26	−0.19	−0.22	−0.24	−0.25	−0.27
Partner intimacy	−0.33		−0.14	−0.07	−0.06	−0.08				
Sexual functioning	−0.23		−0.23	−0.19	−0.14	−0.11	−0.15	−0.12	−0.18	−0.14
Medical care	−0.41		−0.43	−0.24	−0.34	−0.28	−0.29	−0.18	−0.29	−0.35

MQoL-HIV = Multidimensional Quality of Life Questionnaire for HIV/AIDS
All values listed represent statistically significant relationship ($P<0.05$); missing values indicate no significant relationship

age was 37 years, and males, haemophilia patients infected through blood products, and patients with jobs accounted for 92, 33, and 71% of the total, respectively.

Internal consistency and score distributions

The mean index score was 55.1 ± 12.7 in a score range of 12–84 with unimodal distribution. Table 2 shows the score distribution from each of the 10 domains and the values for the Cronbach's α coefficient. The score from some domains tended to be skewed towards the lower end of the quality of life scale. The best possible score (ceiling effect) for the cognitive functioning domain was noted in 84% of the patients. The values for Cronbach's α coefficient were more than 0.7 in 7 domains out of 10. Low values of 0.61, 0.47 and 0.67 were observed in the remaining three domains: physical functioning, sexual functioning and medical service, respectively.

Factor analysis of self-reported symptoms

The factor analysis yielded eight factors, which accounted for 76.9% of the total variance of the data. Table 3 provides a summary of the varimax rotation of the factor analysis. Depression, anxiety, fatigue, feeling sluggish, easily fatigued, irritability and insomnia loaded predominantly on factor one, which appeared to be the factor for psychiatric and nervous symptoms. Factor two appeared to be related to upper digestive symptoms. Variables with high loading on this factor included nausea, upset stomach, vomiting and heartburn. Factor three appeared to represent respiratory and circulatory symptoms, including shortness of breath, choking and palpitation. Factor four included haematuria, blood in the stool and pain during urination, and appeared to be related to

inflammatory and haaemorrhagic symptoms. Sensory symptoms were found in factor five. Changes in taste and in sensation around the mouth loaded predominantly on this factor. Factor six appeared to represent dermatological symptoms. Factor seven was related to lower digestive symptoms where soft stool and diarrhoea loaded predominantly. Factor eight appeared to represent changes in appearance, including changes in weight and in body shape. The other 19 symptoms could not be grouped with any of the above factors, and we decided to focus on these eight different symptoms to further investigate their contribution to the MQoL-HIV score.

Relationship between symptoms, psychological status, functional capacity and the NHP

Table 4 shows the correlation coefficients between the following: the CES–D score as a psychological measure, the eight symptoms determined by the factor analysis, the KPSS as an index of functional capacity, and the index scores and the scores from each of the 10 domains. A strong correlation was observed between the CES–D score and the index score, with a coefficient of −0.73. With respect to the CES–D score, the same strong coefficient of 0.73 was found with the mental health domain, which was the strongest among the 10 domains. The correlation coefficients between the psychiatric and nervous symptoms and the index score, and between mental health and physical health showed the same value of −0.60, whereas the coefficients between the upper digestive symptoms and physical health and between the respiratory and circulatory symptoms and physical health showed values of −0.58 and −0.52, respectively. These relatively high values indicate that these symptoms are related to physical health. On the other hand, there was no significant correlation between the

Table 5. Pearson's correlation coefficient between MQoL-HIV and subscale of the Nottingham Health Profile (NHP)

MQoL-HIV	Subscale of the NHP					
	Energy	Pain	Emotional reaction	Sleep	Social isolation	Physical mobility
Index score	−0.51	−0.34	−0.69	−0.37	−0.62	−0.39
Mental health	−0.45	−0.24	−0.70	−0.37	−0.60	−0.23
Physical health	−0.56	−0.52	−0.52	−0.39	−0.45	−0.42
Physical functioning	−0.32	−0.35	−0.31	−0.18	−0.35	−0.48
Social functioning	−0.45	−0.21	−0.60	−0.32	−0.65	−0.21
Social support	−0.13		−0.25		−0.36	
Cognitive functioning	−0.51	−0.35	−0.47	−0.42	−0.41	−0.26
Financial status	−0.30	−0.20	−0.45	−0.28	−0.41	−0.10
Partner intimacy	−0.15		−0.25		−0.29	
Sexual functioning	−0.23		−0.30	−0.16	−0.23	
Medical care	−0.38	−0.21	−0.43	−0.31	−0.42	−0.16

MQoL-HIV = Multidimensional Quality of Life Questionnaire for HIV/AIDS
All values listed represent statistically significant relationship ($P<0.05$); missing values indicate no significant relationship

KPSS and social support, financial status, partner intimacy, sexual functioning, or medical care. The KPSS was not closely correlated to indices measured by the MQoL-HIV.

Table 5 summarizes the correlation coefficients between the index scores and the scores from the six sub-scales of the NHP, a generic instrument. The index score correlated strongly with the emotional reaction, showing the highest coefficient of −0.69 whereas its correlation to pain was the weakest, showing a coefficient of −0.34.

Stepwise multiple regression analyses for the identification of the best variables to predict the MQoL-HIV

To identify the factors contributing to the index scores and the scores from each of the 10 domains, we conducted stepwise multiple regression analyses using the seven background parameters of the patients as shown in Table 1, the CES–D scores, and the eight different symptoms determined by the factor analysis (Table 6). The CED–S was identified as the contributing factor for all 10 domains. In the mental health domain, age, CES–D, and psychiatric and nervous symptoms accounted for 60% of the variance. On the other hand, in the sexual functioning domain, the presence of numerous unknown factors was suggested because only 14% of the variance could be explained by variables used in this analysis.

Discussion

The results from the stepwise multiple regression analyses of all 10 domains suggested the CES–D as the most significant contributing factor to determine the scores of the MQoL-HIV. This is a predictable conclusion based on the concept that the HRQoL should be comprehensively evaluated on the basis of symptoms, functional capacity, psychosocial status and social interactions. The

KPSS, an index of functional capacity, has been suggested to be a significant determinant of the MQoL-HIV index score in addition to the scores from two domains (physical health and physical functioning), although the correlation coefficient was extremely low. Employment status, which is thought to be related to the concept of social interaction, contributed as a significant factor only in the financial status and sexual functioning domains. Therefore, psychosocial status appears to be the most important factor contributing to the scores of the MQoL-HIV.

The statistical method of factor analysis can be considered to be a data reduction technique, and has been utilized previously to clarify the relationships between various parameters[12]. Grouping of symptoms by factor analysis facilitated the selection of individual symptoms that most closely represent the conceptual meaning of a composite variable. In the present study, it is reasonable to presume that depression, anxiety, fatigue, feeling sluggish, easy fatiguibility, irritability and insomnia, which comprised factor one, have a common conceptual meaning. Likewise, we were able to group 46 separated self-reported symptoms of AIDS patients into eight categories. As a consequence, we were able to determine that the psychiatric and nervous symptoms contributed significantly to the MQoL-HIV index score, as did the scores from five domains, and the former were the most important determinant of self-reported symptoms for the MQoL-HIV.

Age has been reported to be one independent determinant of HRQoL in the general population and in patients with chronic diseases[14,15]. When we examined each domain and the patient's age as contributing factors, we found that older age was associated with a favourable HRQoL in the mental health, financial status and partner intimacy domains. In contrast, younger age was correlated with a better HRQoL in the physical functioning, social functioning, and social support domains. We were therefore unable to confirm the widely

Table 6. Results of backward stepwise multiple logistic regression analysis to identify those variables which influenced the index score and the scores from the 10 domains of the MQoL-HIV

	Index score	Mental health	Physical health	Physical functioning	Social functioning	Social support	Cognitive functioning	Financial status	Partner intimacy	Sexual functioning	Medical care
R^2	0.59	0.60	0.52	0.27	0.45	0.27	0.36	0.29	0.21	0.14	0.25
Age		0.11		-0.17	-0.09	-0.16		0.13	0.11		
Gender						0.11					
Disease duration										0.12	
Infection route				-0.11	-0.11			-0.12			-0.13
With or without treatment				0.14	0.09						
No. of CD4-positive lymphocytes								-0.11			
With or without complications			0.09		0.11		0.12			-0.13	0.12
Disclosure of HIV infection						-0.24		0.10	-0.24		0.10
With or without support					-0.12	-0.20	0.11	-0.16	-0.13	0.15	
Employed or not	-0.58		-0.28	-0.20	-0.12		-0.33	-0.38		-0.21	-0.26
CES-D	0.16	-0.60	0.11	0.22	-0.48	-0.29			-0.34		
KPSS	-0.19	-0.32	-0.17				-0.25	0.15			
Psychiatric and nervous symptoms			-0.24		-0.18	0.13					-0.17
Upper digestive symptoms				-0.12							0.14
Respiratory and circulatory symptoms											
Inflammatory and haemorrhagic symptoms			-0.12								
Sensory symptoms							-0.26				
Dermatological symptoms							0.14				
Lower digestive symptoms							0.12			-0.12	
Appearance	0.93	1.15	1.01	0.96	1.30	1.13	1.33	1.15	0.81	0.73	0.92

MQoL-HIV Multidimensional Quality of Life Questionnaire for HIV/AIDS; CES-D: Center for Epidemiologic Studies–Depression Scale; KPSS the Karnofsky Performance Status Scale