★ 生物統計学セミナー第2回目

期間: 2012/2/14

参加: 中村秀文、佐古まゆみ、中島啓介、他院内多数

詳細: 生存時間解析　講師:スタットコム株式会社統計解析部　松尾富士男

場所: 国立成育医療研究センター研究所　2階セミナールーム

★ 生物統計学セミナー第3回目

期間: 2012/2/28

参加: 中村秀文、佐古まゆみ、中島啓介、他院内多数

詳細: 多変量解析(予後予測因子)　講師:スタットコム株式会社統計解析部　松尾富士男

場所: 国立成育医療研究センター研究所　2階セミナールーム

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
瀧本 哲也	臨床試験におけるデータセンターの役割	五十嵐　隆,菊地　陽	小児科臨床ピクシス10　小児白血病診療	中山書店	東京	2009	172-175

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kamei K, Ito S, Nozu K, Fujinaga S, Nakayama M, Sako M, Saito M, Yoneko M, Iijima K	Single dose of rituximab for refractory steroid-dependent nephrotic syndrome in children	Pediatr Nephrol	24(7)	1321-1328	2009
Isojima T, Yokoya S, Ito J, Horikawa R, Tanaka T	New reference growth charts for Japanese girls with Turner syndrome	Pediatrics International	51(5)	709-714	2009
中川雅生	適正な小児薬物治療の確立を目指して	日本小児科学会雑誌	114	7－14	2010
中川雅生	小児に使用する医薬品の現状と問題点	京都医学会雑誌	56	15-18	2009
栗山　猛	子どもで治験が必要な理由と小児治験の実施体制整備の現状	小児科臨床	62	1623-1629	2009
土田　尚	小児領域の臨床試験と医薬品開発を促進するための海外の取り組み	日本小児アレルギー学会雑誌	23	83-90	2009
土田　尚	小児領域の医薬品開発や標準的治療確立のための臨床試験	小児科診療	51	651-658	2009
土田　尚	医師主導治験によるフェンタニルクエン酸塩注射液の新生児・小児への適応拡大の経緯と臨床使用の注意点	Neonatal Care	22	793-798	2009
Isojima T, Yokoya S, Ito J, Naiki Y, Horikawa R, Tanaka T	Proposal of New Auxological Standards for Japanese Girls with Turner Syndrome	Clinical Pediatric Endocrinology	19	69-82	2010

研究成果の刊行物・別刷

小児白血病と臨床試験

臨床試験におけるデータセンターの役割

瀧本哲也

小児白血病の治療成績は，より高い長期生存率と治療毒性の軽減を目指した臨床試験によって向上してきた．

未承認薬などに対する治験をはじめとする企業が主導する臨床試験では，GCP[*1]の規制を受けるが，医師（研究者）主導型臨床試験[*2]においても，GCPに準拠して手続きの標準化や倫理性の担保を行うことが求められている．したがって，臨床試験の実施には，得られた結果の信頼性や倫理性が保証されるためのシステムが必要である．

白血病に限らず小児がん領域には成人領域とは異なる特性があり，臨床試験の実施にあたって考慮されなければならない（❶）．

❶ 小児白血病の臨床試験において考慮すべき特性

- 希少な疾患（最も多いALLでさえ日本の年間症例数は約400例，次いで多いAMLで約150例程度）である．
- 抗腫瘍薬（市販後）を組み合わせた多剤併用化学療法が治療の中心となる．
- 臨床試験の結果が確定するまでに長期の観察が必要である．
- 現在の化学療法で一定の成績が達成されており，劇的な向上は困難である．
- 日本では治療施設がセンター化されていないため，1施設あたりの症例数が少ない．
- 体格，体内での薬物動態，各種の正常値などが年齢によって異なり，個人差も大きいため，投与量設定，有害事象[*3]の評価などが複雑になる．
- 発症後の容態変化の早い例がまれでなく，臨床試験の結果にバイアス[*4]がかかる可能性がある．
- 治癒後の生存期間が長く，晩期障害やQOLの意義が高いため，長期のフォローアップを要する．これは，真のエンドポイント[*5]を容易には決められないということでもある．
- 小児特有の倫理的問題（インフォームドコンセントの取得が困難など）がある．

臨床試験組織とデータセンター

標準治療[*6]確立のための臨床試験は，後期治療開発[*7]と位置づけられる．しかし小児白血病の特性からも明らかなように，企業主導で行うことは難しく，必然的に医師（研究者）主導型の多施設共同臨床試験によらざるをえない．

多施設共同臨床試験は，症例集積や得られた結果の一般化の点で有利である半面，管理が複雑で責任の所在が不明確になりやすく，また多くの点でみられる施設間差をコントロールしなければならない．

多施設共同臨床試験のための研究組織は，おおむね臨床研究者グループ，研究支援部門，第三者的監視機構から成り，データセンターはこのうち研究支援部門に属している．

臨床試験のデザインから最終的なデータ解析までには多くのプロセスがあり，エラーはおのおののプロセスで生じうる（❷）．データセンターは臨床試験データを一元的に管理し，エラーを防止して臨床試験の信頼性を確保するために中心的な役割を果たしている．これは臨床試験の品質管理[*8]に直結する作業である．

*1 GCP (good clinical practice)
結果の科学性・信頼性や，被験者の権利・秘密が守られていることを客観的に保証するための，臨床試験の計画・実施・報告の基準．

*2 研究者主導型臨床試験
ヒトを対象として介入を行う臨床試験のうち，企業が主導して行う治験や製造販売後臨床試験を除いたもの．現状では多くは公的研究費によっている．

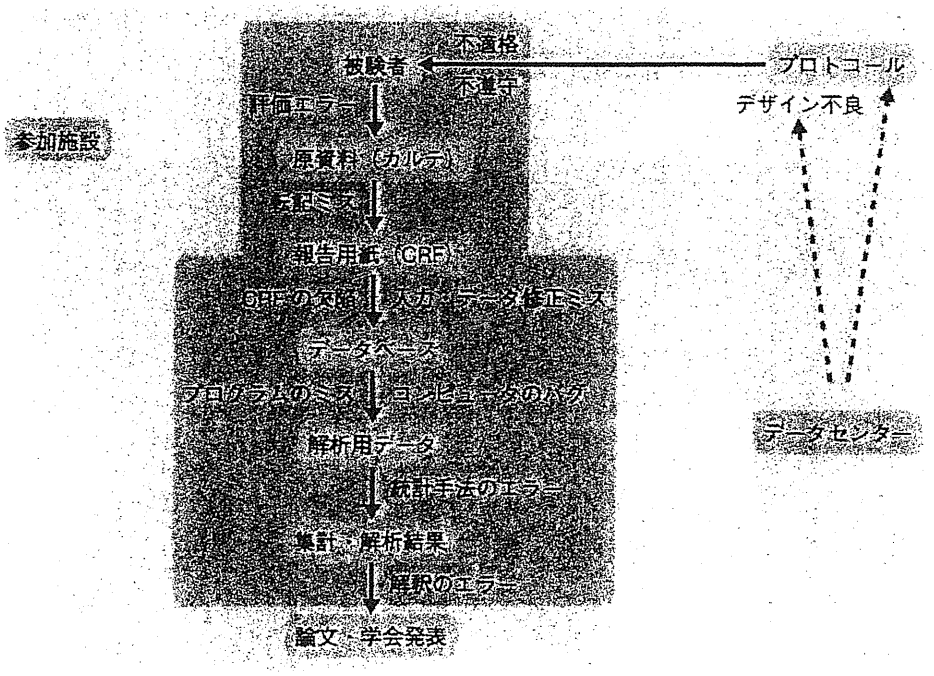
*3 有害事象
試験治療との因果関係の有無にかかわらず，試験期間中に被験者に生じたあらゆる好ましくない現象．薬剤などの“副作用”とは意味が異なる．

*4 バイアス
臨床試験の計画，実施，解析および結果の評価などに関連するさまざまな因子の影響によって，臨床試験で得られた結果と真の値とのあいだに生じた系統的な誤差．

*5 エンドポイント
臨床試験において試験仮説に直結する評価項目．患者の臨床的有用性（有効性ではない）を直接評価する項目は真のエンドポイント（true endpoint），また真のエンドポイントを用いることがなんらかの理由（測定が難しい，判明するのに時間がかかるなど）で困難な場合に代理として用いられる項目は代替エンドポイント（surrogate endpoint）とよばれる．

*6 標準治療
エビデンスに基づいた，その時点での最良の治療法．“一般に広く行われている治療”という意味ではない．

❷ 臨床試験のプロセスと生じうるエラー



臨床試験が計画され，最終的に論文などの形で公表されるまでには多くのプロセスがあり，どこかでエラーが生じれば，臨床試験の結果の信頼性は損なわれる．

- データセンターは，施設間格差の防止のみならず，プロトコールに記載しきれない予測不能の事象に対する対策の検討や検討結果の迅速な周知にも役立つ．小児の年齢差，個人差や速い容態変化に起因する複雑なデータの管理という点でも，データセンターの果たす役割は大きい．
- 有害事象の迅速な把握は臨床試験の安全性確保に必須である．参加施設からの有害事象報告はデータセンターで集められ，直ちに臨床試験の研究代表者に報告される．これによって，他の参加施設への迅速な周知や，必要に応じて治療プロトコールの変更などを行うことも可能となる．
- データセンターによる試験データの中央管理の利点は大きいが，欠点もある．たとえば，データセンターに集められた症例報告書（CRF）などに記載された情報が，本当に医療現場で起きた事実と一致しているかどうかはデータセンターでは確認できない．これを行うには，参加施設を訪問してSDV[*9]を行う必要があるが，実際には困難である．
- 臨床試験のプロトコールやCRFの作成から登録，データ収集，解析に至るプロセスを一つのデータセンターで標準化しておくことは効率的であり，また品質保証[*10]にも寄与するところが大きい．
- 小児がん領域では，その特性からみても明らかなように，臨床試験で小さな治療効果の検出は難しく，ポジティブな結果は期待しにくいため，臨床試験デザインは容易ではない．全国規模で症例を集めるだけではなく，臨床研究者とデータセンターの統計部門との共同作業で治療計画を立案することが今後いっそう重要になっていくと思われる．

CRF：case report form

*7 **治療開発**
臨床試験を行ってよりよい治療をつくっていくこと．おおむね医薬品や医療機器としての規制当局による承認を目的とするものが早期治療開発，市販後の多種の薬剤や集学的治療で治療法を開発するものが後期治療開発と位置づけられる．

*8 **品質管理**
臨床試験実施中に，すべてのプロセスに介入してエラーの発生を抑制することによって，全体としてのエラーを最小（臨床試験の結論に影響を及ぼさない程度）にする行為．

*9 **SDV(source document verification)**
カルテなどの原資料とCRFなどの報告書の内容の整合性を直接照合して確認すること．

*10 **品質保証**
臨床試験の終了後に，それが定められた手順どおりに実施され，求められる基準を満たすことを確認することによって，得られた結果が信頼できることを証明する行為．

JPLSG：Japanese Pediatric Leukemia/Lymphoma Study Group

＊11
JPLSGは，既存の4つの白血病研究グループが共同して設立されたもので，日本の白血病症例のほとんどをカバーすることができる．

＊12
モニタリング
品質管理のための具体的活動．臨床試験の進行中に，試験がプロトコールどおり適切に実施されているかをすべての登録症例の全データを対象として調査し，発見されたエラーを修正し，必要に応じてエラーの予防策を講じていく作業．データセンターのスタッフが，プロトコールに定められた各種の遵守事項が実施されていることを確認したり，提出されたCRFの記載の誤りや記載もれについて当該施設に問い合わせて修正するものは中央モニタリングとよばれる．

＊13
監査
臨床試験の終了後に，原資料を報告書などと照合することにより，データが正しく処理・報告されたことを検証すること．エラーの頻度や内容が，臨床試験の結論に影響を及ぼしていないことを確認する目的で行われる一種の抜き取り調査である．本来は部外者によって行われるが，研究者主導の臨床試験では一般的ではない．

＊14
IRB（institutional review board）
機関（施設内）審査委員会．医学・科学の専門家と非専門家から構成される．通常は臨床試験の参加施設内に設けられ，その臨床試験をその施設で行うかどうかを審査し，客観的な立場から臨床試験の再保証を与える機関．臨床試験に参加する被験者の安全性と人権保障に重点をおいた審査を行う場合は倫理委員会とよばれるが，明確な区別がなされていないのが現状である．

＊15
臨床試験コーディネーター（clinical research coordinator：CRC）
医師の代行として臨床試験のスケジュール管理，CRFへのデータ転記，その他各種の原資料の整理と保管，さらには試験参加患者の支援などの臨床試験業務支援を行う．企業主導で行われる治験と異なり，医師主導の臨床試験においては現在のところ一般的ではない．

日本小児白血病・リンパ腫研究グループ（JPLSG）での実例

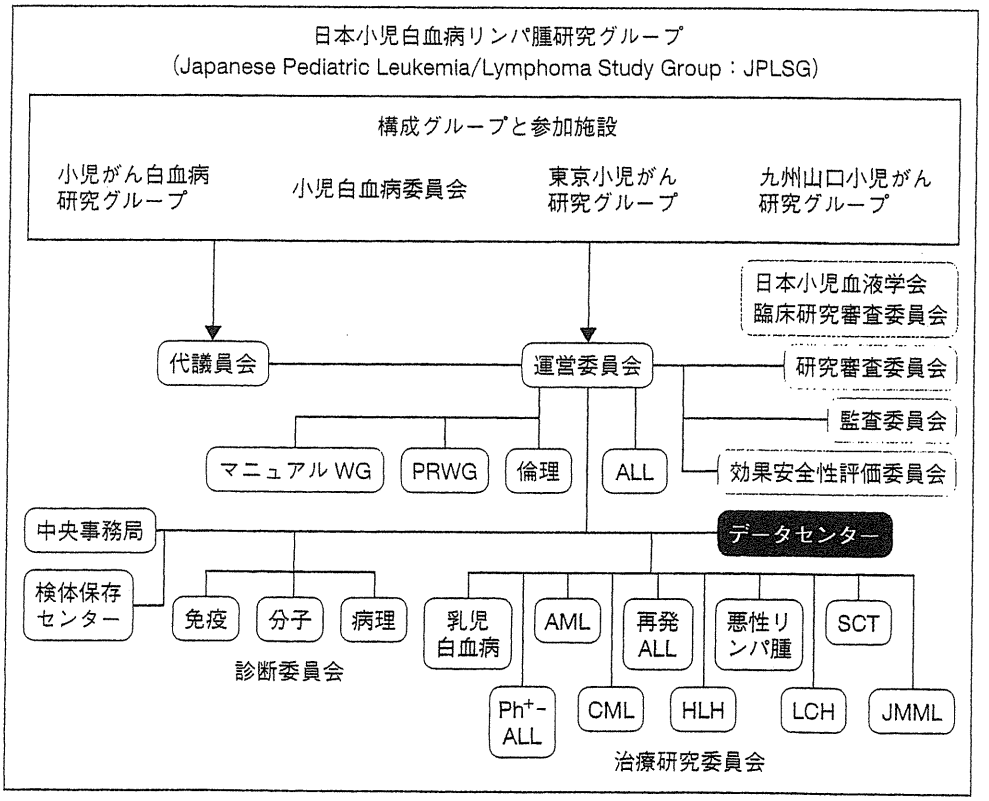
現在，小児造血器腫瘍領域で日本の全国規模の臨床試験実施の中核となっているJPLSG＊11の組織図を❸に示す．

JPLSGのデータセンターで実際に行われている具体的な作業を❹に示す．中心となるのは患者登録，CRFに基づくデータマネージメントとモニタリング＊12および統計解析であるが，これらを効率よく行えるようにするため，CRF設計などのプロトコールの作成段階から関与を行っている．

監査＊13は品質保証のための具体的な行為である．JPLSGでは施設訪問モニタリングの代用として，臨床試験の研究期間中にJPLSG内部の監査委員会が実施している．

監査の主な対象は臨床試験参加施設およびデータセンターである．施設ではSDVと同様の内容（ただし監査では仮にエラーが発見されても，それを修正することはしない）やプロトコール（修正版を含む）などの文書の

❸ JPLSGの組織図（2008年3月現在）



各種委員会の役割
治療研究委員会：臨床試験を立案し，臨床試験遂行の主体となる．
診断委員会：分野ごとに診断法や診断基準の標準化を行う．
PRWG：プロトコールレビューワーキンググループ．臨床試験の実施計画書の作成段階でレビューを行って質の向上を図る．ここで承認されなければJPLSGプロトコールと認知されない．
倫理委員会：小児特有の倫理的問題や，中央診断後の余剰検体利用の手続きなどについて検討し，統一的な見解を示す．
臨床研究審査委員会：臨床試験の実施計画書と最終的な試験結果について医学的，科学的，倫理的な面から審査し承認する．客観性担保のため外部機関（日本小児血液学会）内の組織に委託している．
研究審査委員会：余剰検体を利用する基礎研究の審査を行う．
効果安全性評価委員会：臨床試験の実施中にモニタリングレポート，有害事象報告，中間解析結果などに基づいて進捗状況，安全性，有効性を評価し，試験の継続，変更，中止を勧告する．
監査委員会：臨床試験のすべての過程を検証して品質保証を行う．

保存や，施設 IRB[*14]/倫理委員会のプロトコール承認文書・同意文書の確認などが，またデータセンターではデータの修正や解析プロセスなどについて履歴や記録に基づいた確認作業が行われる．

今後の課題

CRF の記入などの臨床試験への協力が，施設での臨床医の負担となっている．このような負担軽減のためには臨床試験コーディネーター（CRC）[*15] の配置を推進していく必要がある．

各施設の IRB や倫理委員会の見解には，施設間で差がみられることが珍しくない．小児白血病の臨床試験をどの程度科学的・倫理的に審査できているかについての検証手段もなく，今後の課題と考えられる．

入院直後に容態の変化する症例は，臨床試験に登録されにくく，また予後もよくないことが多い．このような症例が除かれることは，臨床試験の結果の解釈に大きな影響を及ぼす．したがって臨床試験参加症例のみでなく不参加症例の転帰を把握することは，臨床試験結果の一般化可能性を検証するために重要である．

治癒すれば長期の生存が期待される小児では，内分泌学的合併症や二次がんなど多くの長期的合併症が生じる可能性がある．これらの実態を把握し，予防や治療を系統的に行っていくために，臨床試験の研究期間終了後も，フォローアップを継続する体制を構築していく必要がある．

今後は，データセンターを，多施設共同臨床試験を管理するだけでなく，小児がん登録などと提携し，かつできるだけ臨床試験不参加症例も把握するような機能をもたせる形で拡充していくことが必要と考えられる．

❹ データセンターの主な業務

1. プロトコール作成の支援
   - 登録手順（付随研究も含む）作成
   - 各種検体提出手順（中央診断・付随研究）作成
   - データ収集内容の検討と収集時期の決定
   - 標準的構成に則ったプロトコール原案作成（治療研究委員会と共同）
   - 統計学的事項の検討
   - 各種 CRF 作成（登録票，検体提出用紙，初診時レポート，フローシート，有害事象報告書など）
   - 登録システム，データベース統計（システムエンジニアと共同）
2. 登録
   - 参加施設管理
   - プロトコールの IRB（倫理委員会）承認の確認
   - 適格性の確認，不適格例の記録（目視トリプルチェック）
   - 登録確認票送付
3. データマネージメント
   - 各種 CRF の受領と記録（未回収の場合には督促）
   - 各種 CRF 記載内容の目視トリプルチェック（空欄・不整合など）と問い合わせ・修正
   - データベースへの入力（コンピュータによるロジカルチェック）と入力内容の目視ダブルチェック
   - 定期追跡調査と記載内容チェック（未回収の場合には督促）
   - 解析用データファイルの作成とデータの最終クリーニング
4. モニタリング
   - 臨床試験の進捗状況管理（IRB/倫理委員会承認状況，症例登録ペース，各種 CRF 提出状況など）
   - プロトコール逸脱・中止例のチェック
   - 有害事象報告の記載内容チェック，治療研究委員会への報告
   - 定期モニタリングレポートの作成
   - 研究代表者による CRF レビューへの協力
   - 施設訪問モニタリング（あるいは監査）の支援
5. 統計解析
   - 中間解析
   - 最終解析
6. その他
   - データセンターニュースによる参加施設への情報発信（注意事項など）

Pediatr Nephrol
DOI 10.1007/s00467-009-1191-0

ORIGINAL ARTICLE

Single dose of rituximab for refractory steroid-dependent nephrotic syndrome in children

Koichi Kamei · Shuichi Ito · Kandai Nozu · Shuichiro Fujinaga · Makiko Nakayama · Mayumi Sako · Mari Saito · Maki Yoneko · Kazumoto Iijima

Received: 12 February 2009 / Revised: 25 March 2009 / Accepted: 26 March 2009

Abstract We conducted a multicenter prospective trial to evaluate the efficacy, safety and pharmacokinetics of a single dose of rituximab (375 mg/m^2 body surface area) for the treatment of children with refractory steroid-dependent nephrotic syndrome (SDNS). All patients (n=12) were able to discontinue steroids at a median of 74 days after treatment. The frequency of relapses per 6 months was significantly reduced and the steroid-free period per 6 months was significantly increased after treatment compared with those before treatment. The condition in nine of the patients (75%) relapsed at a median of 129 days after treatment, and seven patients were given additional rituximab due to steroid dependency. Most of the relapses developed simultaneously with recovery of B-cells. However, three patients (25%) did not have a relapse with B-cell recovery and the disease was kept in remission for more than 1 year. None of the patients developed life-threatening adverse events. This is the first report of a prospective study of a single dose of rituximab for refractory SDNS. Treatment with a single dose of rituximab may be effective for refractory SDNS, but its efficacy to prevent relapses was transient in most of the patients.

Keywords Refractory steroid-dependent nephrotic syndrome · Children · Clinical trial · Rituximab · Pharmacokinetics

Introduction

Idiopathic nephrotic syndrome is the most frequent glomerular disease of childhood. Most cases respond to steroid treatment, but approximately 40% of the children develop

K. Kamei · S. Ito · M. Nakayama · M. Sako · K. Iijima
Department of Nephrology,
National Center for Child Health and Development,
Tokyo, Japan

K. Kamei
Department of Pediatrics and Developmental Biology,
Tokyo Medical and Dental University,
Tokyo, Japan

S. Ito
Department of Pediatrics,
Yokohama City University Medial Center,
Yokohama, Japan

K. Nozu
Department of Pediatrics,
Kobe University Graduate School of Medicine,
Kobe, Japan

S. Fujinaga
Department of Nephrology,
Saitama Children's Medical Center,
Saitama, Japan

M. Saito
Biostatistics/Epidemiology and Preventive Health Sciences,
School of Health Science and Nursing,
University of Tokyo, Tokyo, Japan

M. Yoneko
Division of Clinical Research,
National Center for Child Health and Development,
Tokyo, Japan

K. Iijima (✉)
Division of Child Health and Development,
Department of Pediatrics,
Kobe University Graduate School of Medicine,
5-1 Kusunoki-cho 7 chome, Chuo-ku,
Kobe 650-0017, Japan
e-mail: iijima@med.kobe-u.ac.jp

Published online: 07 May 2009

frequent-relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome (SDNS). In Japan these patients are usually treated with immunosuppressants such as cyclosporine (CyA), cyclophosphamide (CPA), mizoribine (MZR) and mycophenolate mofetil (MMF). However, some patients continue to have relapses, and the condition remains steroid-dependent despite these treatments. Such patients often suffer from severe steroid toxicity, such as cataracts, obesity, osteonecrosis and growth failure. Moreover, these immunosuppressants also have significant adverse effects. Chronic nephrotoxicity is a well-known side effect of CyA, and long-term use of this drug is known to carry a high risk for chronic CyA nephrotoxicity [1], although Kranz et al. reported that long-term use of cyclosporine for SDNS was relatively safe [2]. Because of the possibility of gonadotoxicity (azoospermia), it is recommended that CPA be used within limited cumulative doses. MZR and MMF are still off label for SDNS in Japan. Therefore, even if these immunosuppressants are effective, it is difficult for these drugs to be used for a long period.

It has recently been reported that rituximab (anti-CD20 monoclonal antibody) was successfully used to treat patients with refractory nephrotic syndrome [3–14]. To date, there have been eight reports of a total of 34 patients with SDNS who received rituximab treatment [3–10]. In those reports, rituximab treatment for SDNS that was resistant to immunosuppressants prevented relapses and resulted in the patients' discontinuation of steroids. Thus, it is likely that rituximab is very effective for SDNS. In most of the previous studies, rituximab was given at a dose of 375 mg/m^2 body surface area (BSA) once weekly for 4 weeks, because this dosage is recommended for patients with B-cell lymphoma. However, Smith reported successful treatment of a SDNS patient with a single dose of rituximab [6]. We also successfully treated two patients with refractory steroid-resistant nephrotic syndrome with a single dose of rituximab [12]. Thus, the optimal dose of rituximab for childhood refractory SDNS has not been established.

Therefore, we conducted a multicenter prospective study to examine the efficacy and safety of a single dose of rituximab for refractory SDNS in children. Since there have been no previous reports of rituximab pharmacokinetics in children, we also examined the pharmacokinetics of rituximab and time courses of peripheral B-cell counts after rituximab administration in children with refractory SDNS.

Materials and methods

Patient population

Patients included in this trial met the following criteria: (1) they were younger than 20 years old, (2) they had idiopathic nephrotic syndrome, (3) the disease was steroid-dependent, and conventional immunosuppressants such as CyA, CPA, MZR and MMF were being taken, and (4) they had no history of rituximab treatment. Approval of the off-label use of rituximab and of the study protocol was obtained from the institutional review boards of the National Center for Child Health and Development, Kobe University Graduate School of Medicine, Yokohama City University Medical Center and Saitama Children's Medical Center. All patients' parents gave their written informed consent.

Treatment

Before rituximab infusions, complete blood counts, biochemical parameters (total protein, albumin, blood urea nitrogen, creatinine, uric acid, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, electrolytes, etc.), peripheral B-cell counts, and titers of hepatitis B virus and hepatitis C virus were examined. Chest radiographs and electrocardiograms were obtained, and echocardiography was also carried out. We administered rituximab intravenously, in a single dose of 375 mg/m^2 BSA (maximum 500 mg) after obtaining remission with prednisolone (PSL). In order to minimize infusion reactions, we gave the patients acetaminophen and cyproheptadine hydrochloride 30 min prior to rituximab infusions. Steroids were not infused prior to rituximab infusions. Patients were observed in the hospital for at least 24 h after rituximab infusion.

Follow-up

For all patients, clinical and laboratory parameters, including complete blood counts, biochemical parameters, serum immunoglobulin levels and CD19+ B-cell counts by flow cytometry were monitored once a month for 6 months. Steroid dosages were tapered and discontinued by 6 months after the rituximab infusions, although the detailed protocol for tapering steroid dosage was not restricted. The protocols for discontinuation of immunosuppressants and PSL for relapses were not restricted in this trial. Patients were followed-up for at least 1 year after they had been given the rituximab infusion. Frequency of relapses, steroid-free periods, and dosages of steroids were evaluated before and after rituximab treatment. B-cell depletion was defined as a CD19+ countof fewer than five cells per cubic millimeter at any time, and B-cell recovery was defined as a CD19+ cell count of more than15 cells per cubic millimeter.

Measurement of serum rituximab levels

Serum rituximab levels were quantified by a proprietary enzyme-linked immunosorbent assay (ELISA) at Covance

Laboratories, Inc. (Chantilly, VA, USA). The ELISA-0145 method is a sandwich ELISA format for the quantitative determination of rituximab in human serum. The 1/100 diluted calibrators, controls, and samples were pipetted on to a microtiter plate precoated with polyclonal goat anti-rituximab, incubated, and then washed. A goat anti-mouse IgG $F(ab')^2$ horseradish peroxidase (HRP) conjugate was then incubated, followed by another plate washing. Bound HRP conjugate was detected with a tetramethyl benzidine (TMB) substrate, which was read colorimetrically on a plate reader.

Rituximab pharmacokinetic analysis

We obtained serum samples to examine serum rituximab levels before and just after infusion, after 24 hours, and after 1 week, and then once a month until 6 months after treatment. The maximum serum concentration (Cmax) of rituximab was an actual measured value. The area under the serum concentration–time curve (AUC), total clearance (CL), elimination half-life (T1/2) and volume of distribution (Vdss) were calculated by non-compartmental methods using the computer program MOMENT [15].

Statistical analysis

We used the Wilcoxon signed-rank test to compare the number of relapses and steroid-free periods before and after rituximab infusion, and Student's *t*-test and analysis of variance (ANOVA) to analyze PSL dosages, B-cell counts, and serum immunoglobulin levels. The Kaplan–Meier method was used for the analysis of the probabilities of relapse-free survival and B-cell depletion. Statistical significance was established at $P<0.05$.

Results

Patients' characteristics

Between February and September 2007, 12 patients (eight boys and four girls) with SDNS were enrolled in our trial at four institutions. The clinical characteristics of the patients are shown in Table 1. Renal biopsies revealed minor glomerular abnormalities (minimal change disease) in 11 patients and focal segmental glomerulosclerosis in one patient. All patients had a history of CyA treatment. All patients suffered from severe steroid-dependent disease and could not discontinue steroid usage under one or two immunosuppressants at the time of rituximab infusion. Mean duration of steroid therapy before rituximab treatment was 6.1±3.2 years. The effects of severe steroid toxicity, such as short stature, obesity, osteoporosis, hypertension and cataract, were observed in almost all patients.

Clinical courses after rituximab treatment

Clinical courses after rituximab infusion are shown in Table 2. All patients were able to discontinue PSL usage at a median of 74 days after the infusion. For eight patients, immunosuppressants were also able to be discontinued. Complete B-cell depletion was achieved in ten of 12 patients (88%). In

Table 1 Patients' characteristics (*IS* immunosuppressant, *Rtx* rituximab, *M* male, *F* female, *FSGS* focal segmental glomerulosclerosis, *CyA* cyclosporine A, *CPA* cyclophosphamide, *MZR* mizoribine, *PSL* prednisolone, *MGA* minor glomerular abnormalities, *CHL* chlorambucil, *FK* tacrolimus, *MMF* mycophenolate mofetil, *SD* standard deviation)

Patient no.	Gender	Age at onset (years)	Duration of steroid treatment (years)	Age at Rtx treatment (years)	Renal histology	History of previous IS	Treatments at the time of Rtx therapy
1	M	5	9.3	14	FSGS	CyA, CPA, MZR	PSL, CyA, MZR
2	M	8	7.7	16	MGA	CyA, MZR	PSL, MZR
3	M	15	1.5	18	MGA	CyA	PSL, CyA
4	M	4	8.3	12	MGA	CyA, CPA, CHL, MZR	PSL, CyA, MZR
5	M	2	2.7	5	MGA	CyA, CPA, MZR	PSL, CyA
6	M	10	10.6	19	MGA	CyA, MZR	PSL, MZR
7	F	8	1.9	10	MGA	CyA, CPA, MZR	PSL, CyA
8	M	2	7.9	10	MGA	CyA, FK, CPA, MZR	PSL, FK, MZR
9	M	2	9.0	15	MGA	CyA, CPA, MMF	PSL, CyA, MMF
10	F	9	2.5	12	MGA	CyA, CPA, MZR, MMF	PSL, CyA, MMF
11	F	1	4.5	11	MGA	CyA, CPA	PSL, CyA
12	F	3	6.7	10	MGA	CyA, MMF	PSL, MMF
Mean		5.8	6.1	12.7			
SD		4.3	3.2	3.9			

Table 2 Clinical courses after rituximab infusion (*IS* immunosuppressant, *Rtx* rituximab, *D/C* discontinued, *MZR* mizoribine, *CyA* cyclosporine A, *FK* tacrolimus, *MMF* mycophenolate mofetil)

Patient no.	Cessation of PSL after Rtx infusions (days)	IS treatment after Rtx infusions	B-cell recovery after Rtx infusions (days)	First relapse after Rtx infusions (days)	Summary of the clinical courses
1	63	D/C MZR on day 0 D/C CyA on day 175	119	129	Relapses on days 129 and 190, additional Rtx on day 190
2	83	D/C MZR on day 116	146	161	Relapse on day 161, additional Rtx on day 175, relapse on day 316, four doses of additional Rtx from day 328
3	68	D/C CyA on day 89	245	Not relapsed	Maintaining remission on day 365
4	73	D/C MZR on day 0 D/C CyA on day 122	118	104	Relapses on days 104 and 128, additional Rtx on day 141, relapses on day 253, four doses of additional Rtx from day 273
5	57	D/C CyA on day 112	Not depleted	199	Relapses on days 199 and 221, restarted CyA on day 233
6	75	D/C MZR on day 0	152	124	Relapses on days 124 and 194, four doses of additional Rtx from day 203, relapse on day 217
7	58	D/C CyA on day 140	147	128	Relapses on days 128 and 156, additional Rtx on day 167, relapse on day 213, restarted CyA on day 272
8	76	D/C MZR on day 0 FK→CyA on day 8	104	8	Relapses on days 8, 139 and 173, additional Rtx on day 196, Relapses on days 197, 253 and 302, two doses of additional Rtx from day 343
9	123	D/C CyA on day 74 Continued MMF	151	353	Relapse on day 353
10	120	D/C CyA on day 78 Continued MMF	Not depleted	Not relapsed	Maintaining remission on day 365
11	55	D/C CyA on day 112	84	125	Relapses on days 125 and 146, additional Rtx on day 153
12	172	Continued MMF	171	Not relapsed	Maintaining remission on day 365
Median	74.0		146.5	129.0	
Range	55–172		84–245	8–353	

two patients (patients 5 and 10), B-cells decreased but were not depleted (fewer than five per cubic millimeter). Minimum CD19+ B-cell counts were 61 per cubic millimeter and 44 per cubic millimeter, and the reduction in CD19+ B-cells was 87% and 91%, respectively.

Nine patients had relapses of nephrotic syndrome (NS) at a median of 129 days after the rituximab infusion. Most of the relapses developed simultaneously with the recovery of B-cells. Seven of these patients (patients 1, 2, 4, 6, 7, 8, and 11) were given additional rituximab infusions at an average of 175 days after the first rituximab infusion, due to steroid dependency. Patient 8 suffered relapse 8 days after the first rituximab infusion, although complete B-cell depletion was achieved, but his steroids were discontinued on day 76. After two steroid-free months, he suffered from two more relapses. Although he was given an additional single-dose rituximab infusion on day 196, the condition relapsed again, which led to two additional doses of rituximab infusions from day 343. Two patients (patients 5 and 7) were restarted on CyA. The disease in three patients (patients 3, 10, and 12) remained in remission for 12 months after infusions.

Comparison of clinical parameters before and after rituximab treatment

The frequency of relapses per 6 months was significantly reduced, and the steroid-free period per 6 months was significantly increased after treatment, compared with those before treatment (Table 3). Mean PSL dosages

Table 3 Frequency of relapses and steroid-free periods before and after rituximab infusion (*Rtx* rituximab)

Patient no.	Number of relapses		Steroid-free period (days)	
	During the 6 months before Rtx infusions	During the 6 months after Rtx infusions	During the 6 months before Rtx infusions	During the 6 months before Rtx infusions
1	3	2	12	64
2	1	1	0	77
3	3	0	47	114
4	2	2	0	34
5	4	0	5	126
6	2	1	0	64
7	4	2	13	72
8	2	3	0	63
9	4	0	0	60
10	5	0	7	64
11	2	2	0	69
12	2	0	0	9
Mean	2.83*	1.08*	7.0**	68.0**
S.D.	1.19	1.08	13.5	30.7

*P=0.016; **P=0.0005

(before infusion 0.82±0.36 mg/kg per day) were significantly reduced between 2 months and 5 months after rituximab infusion (2 months 0.32±0.17 mg/kg per day; 3 months 0.08±0.09 mg/kg per day; 4 months 0.07±0.15 mg/kg per day; 5 months 0.39±0.44 mg/kg per day), but they increased again at 6 months (0.45±0.63 mg/kg per day) (Fig. 1a).

B-cell counts and serum immunoglobulin levels

B-cells were depleted in ten of 12 patients. Peripheral B-cell counts (before infusion 238.7±314.7 per cubic millimeter) rapidly decreased immediately after rituximab infusion (24 hours 28.3±29.2 per cubic millimeter). B-cells gradually increased at 4 months (105.2±120.6 per cubic millimeter) and returned to baseline levels at 6 months (218.0±166.2 per cubic millimeter) (Fig. 1b). Serum immunoglobulin (Ig)G and IgA levels did not change during the clinical course, but serum IgM levels gradually decreased (before infusion 135.7±58.4 mg/dl; 1 month 94.1±58.3 mg/dl; 6 months 61.2±25.4 mg/dl) after rituximab treatment (Fig. 1c–e).

Pharmacokinetics

Figure 1f shows serum rituximab levels after the drug infusion. Cmax obtained just after the drug infusion was 220.0±78.9 μg/ml. Serum rituximab levels at 24 hours,

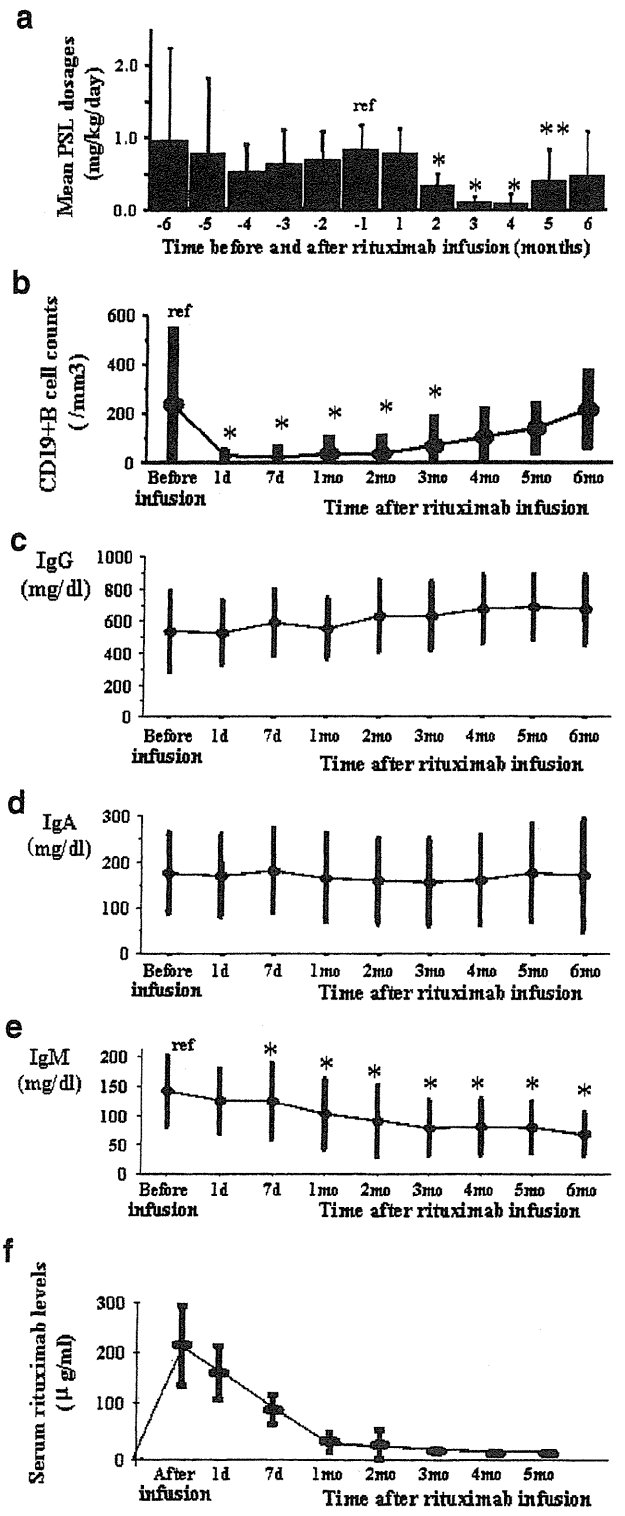


Fig. 1 Clinical parameters after rituximab treatment. Values are means±standard deviations (SDs). **a** Mean PSL dosages before and after rituximab treatment. *P<0.01, **P<0.05 compared with before infusion (reference). **b** CD19+B-cell counts after rituximab treatment. *P<0.01 compared with before infusion (reference). **c–e** Serum immunoglobulin (Ig)G, IgA and IgM levels after rituximab treatment. *P<0.01 compared with before infusion (reference). **f** Serum rituximab levels after infusion. *d* days, *mo* months

1 week, 1 month, 2 months and 3 months after the infusion were 166.2±56.2 μg/ml, 92.5±29.8 μg/ml, 27.4±18.4 μg/ml, 18.5±29.8 μg/ml and 3.0±4.4 μg/ml, respectively. They were undetectable (<0.5 μg/ml) in all patients at 5 months. A detailed pharmacokinetics study was carried out in five patients. T1/2 was 14.6±5.2 days, AUC was 83.2± 53.1 mg·h/ml, Vdss was 2.2±0.37 l/m^2 and CL was 5.83± 2.97 ml/h per square millimeter.

Probability of relapse-free survival and B-cell depletion

Figure 2a shows the probability of relapse-free survival by the Kaplan–Meier method. The median time to first relapse (50% survival time) in our patients was 129 days. Figure 2b shows the probability of CD19+ B-cell depletion by the Kaplan–Meier method. The median time to B-cell recovery in our patients was 119 days (50% survival time).

Adverse events

Mild reactions to the infusions were observed in five of 12 patients (42%). These reactions included fever and hypotension (one patient), tachycardia (one patient), hypertension (one patient), facial flushing (one patient), and mild respiratory distress (one patient). None of the patients developed serious adverse events that required discontinuation of the trial. One patient (patient 5) developed a fever of unknown etiology between days 45 and 98 but recovered spontaneously.

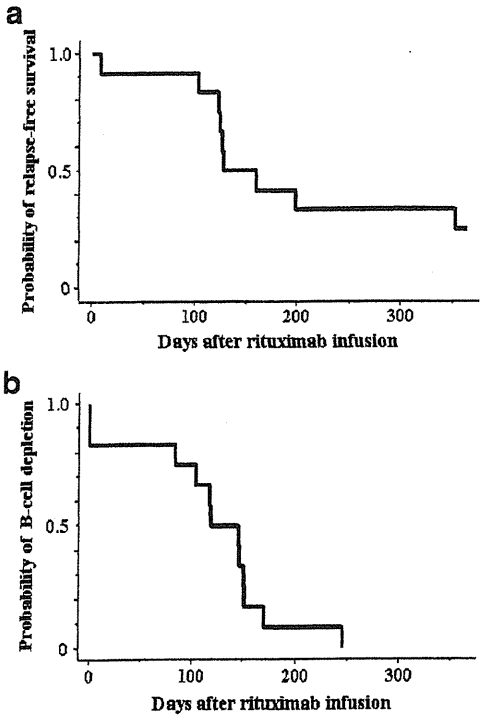


Fig. 2 Probability of relapse-free survival (**a**) and of B-cell depletion (**b**)

Discussion

This is the first report of a prospective study of single-dose therapy with rituximab for refractory SDNS. All patients were able to discontinue steroids after rituximab treatment. We found that the frequency of relapses was significantly reduced, the steroid-free period was significantly increased and mean steroid dosages were significantly reduced after rituximab treatment, suggesting that rituximab is effective for patients with SDNS. However, the efficacy of the treatment was transient in most patients. Nine of 12 patients (75%) had relapses within 1 year, and most of the relapses developed simultaneously with the recovery of B-cells. Seven of the patients needed additional rituximab treatment due to steroid dependency. It is of note that two out of the three patients who did not have a relapse and one of the patients who did not suffer relapse until day 353 did not stop their PSL until day 120 or later. Therefore, it is possible that the speed of PSL withdrawal was related to relapses, although further studies are required.

There have been no previous reports on rituximab pharmacokinetics in children. Table 4 shows previous reports of rituximab pharmacokinetics [16–22]. Most of the previous pharmacokinetic studies were carried out on adult patients with B-cell lymphoma, and most of them were given four doses of rituximab. The only large rituximab pharmacokinetics study of patients that did not have lymphoma was reported by Breedveld et al. [22]; however, they treated their patients with two doses of 1,000 mg rituximab. Vieira et al. reported pharmacokinetics of single-dose therapy with rituximab in kidney transplanted adults [16]. In that study, nine patients were treated with a single dose of rituximab (n= 3 per group) at 50 mg/m^2, 150 mg/m^2 or 375 mg/m^2 and the data for Cmax per dose (micrograms per milliliter per milligram) were almost the same among the three groups (0.30, 0.35, 0.30 each). Our data (Cmax 220.0 μg/ml; T1/2 14.6 days; AUC 83.2 mg·h/ml) were comparable with those of the patients who were treated with a dose of 375 mg/m^2 in the study by Vieira and colleagues, although the disease was different, suggesting that the rituximab pharmacokinetics profile in children is similar to that in adults.

Cmax of rituximab in our study (220.0 μg/ml) were comparable to serum levels after initial infusion of rituximab in previous reports on B-cell lymphoma (range 188.5–245.3 μg/ml) (Table 4). The peak level after four doses of rituximab was almost twice as high as that of a single dose in previous reports. Apart from in this study, we treated children with refractory SDNS with four doses of rituximab (n=4). In those patients treated with four doses of rituximab, the maximum serum levels of rituximab (403.4±122.9 μg/ml; range 318.7–584.8 μg/ml) were similar to those in the previous studies (Table 4) and were almost twice as high as those in this study. Berinstein et al. showed that the mean T1/2 was longer and the mean CL was smaller after four doses of

Table 4 Previous reports on rituximab pharmacokinetics data (*T1/2* elimination half-life, *AUC* area under the concentration–time curve, *Vss* volume of distribution at steady state, *CL* clearance)

Author	Disease	Number of patients	Age (years)	Dose of rituximab	Serum levels after the initial infusion (μg/ml)	Serum levels after the last infusion (μg/ml)	T1/2 (days)	AUC (mg·h/ml)	Vss	CL
Bernstein et al. [17]	B-cell lymphoma	166 (14)[a]	22–79	375 mg/m^2 ×4	239.1	460.7	8.6[d,e]	–	–	9.2 ml/h[e]
Davis et al. [18]	B-cell lymphoma	38 (10)[b]	31–80	375 mg/m^2 ×4	245.3	426.4	12.7[e]	–	–	–
Tobinai et al. [19]	B-cell lymphoma	4	31–66	250 mg/m^2 ×4	–	64.3	23.4[d,e]	91.3[e]	10.76 l [d,e]	168 ml/h[d,e]
	B-cell lymphoma	8	36–75	375 mg/m^2 ×4	–	92.1	16.2[d,e]	118.2[e]	11.16 l [d,e]	44 ml/h[d,e]
Iacona et al. [20]	B-cell lymphoma	7	Adults	375 mg/m^2 ×4	188.5	347.2	19.9[e,f]	–	–	5.4 ml/h per square meter[e]
Regazzi et al. [21]	B-cell lymphoma	22	Adults	375 mg/m^2 ×4	203.3	377.7	21.4[e,f]	–	3.52 l/m^2 [e]	5.1 ml/h per square meter[e]
	Autoimmune disease	14	Adults	375 mg/m^2 ×4	192.9	323.6	20.2[e,f]	–	4.45 l/m^2 [e]	6.6 ml/h per square meter[e]
	AL amyloidosis	4	Adults	375 mg/m^2 ×8	203.7	493.5	–	–	–	–
	Refractory lymphoma	8	Adults	375 mg/m^2 ×6	188.5	248.0	22.4[e,f]	–	4.03 l/m^2 [e]	5.2 ml/h per square meter[e]
Breedveld et al. [22]	Rheumatoid arthritis	111	Adults	1,000 mg ×2	–	453	20.7[e]	233.0[e]	4.56 l [e]	9.6 ml/h[e]
Vieira et al. [16]	Transplanted kidney	3	30–44	50 mg/m^2 ×1	25.90	–	9.88[e]	5.19[e]	–	–
	Transplanted kidney	3	34–55	100 mg/m^2 ×1	88.57	–	14.14[e]	21.3[e]	–	–
	Transplanted kidney	3	46–52	375 mg/m^2 ×1	232.67	–	12.36[e]	47.8[e]	–	–

[a] Pharmacokinetics data were analyzed in 14 patients

[b] Pharmacokinetics data were analyzed in ten patients

[c] Non-compartment model

[d] One-compartment model

[e] Two-compartment model

[f] T1/2β

rituximab than those of the initial dose, and that this phenomenon was probably due to circulating B-cells, which played the role as antibodies of rituximab at the time of the initial infusion [17]. Collectively, four doses of rituximab may provide higher maximum serum levels, a longer T1/2 and smaller CL, even in childhood refractory SDNS. In addition, only two of six patients had a first relapse within 1 year after the treatment (261 days and 270 days after the treatment, respectively) in previously reported studies using a dose of 375 mg/m^2 BSA once weekly for 4 weeks [3–5, 7, 9], whereas the median time to first relapse was 129 days in our patients. Also, the time to B-cell recovery was 270 days and 360 days after the treatment, respectively, in a previously reported two patients on a dose of 375 mg/m^2 BSA once weekly for 4 weeks [4, 5], while the median time to B-cell recovery was 119 days in our patients. Therefore, a multicenter, randomized, double-blind, placebo-controlled trial for patients with childhood refractory SDNS is in progress in Japan to evaluate the efficacy and safety of four doses of rituximab. Alternatively, a single dose of rituximab every several months could be effective for childhood refractory SDNS, for which further studies are needed to examine the optimal protocol of this drug.

Mechanisms by which rituximab can prevent relapses in patients with refractory SDNS remain unclear. For more than 30 years, nephrotic syndrome was thought to be primarily a disorder of T-cell function. However, recently, a number of clinical observations provided evidence of an important role for B-cells in the development of nephrotic syndrome. Kemper et al. found increased levels of both sCD23 (a marker of B-cell activation) and sCD25 (a marker of T-cell activation) during relapses of SDNS [23]. Cho et al. also observed a significantly higher expression of CD23 in fresh B-cells from patients with active minimal change nephrotic syndrome [24]. Several studies have shown increased production of interleukin (IL)-13 and elevated expression of IL-13 mRNA in patients with minimal change nephrotic syndrome [24, 25]. IL-13 is one of the cytokines associated with type 2 T helper (Th2) cells, leading to antibody production and allergic reactions, which are caused by immune responses involving B-cells.

As well as being the source of plasma cells, B-cells are also involved in the presentation of antigens to T-lymphocytes, and they secrete co-stimulatory signals required for CD4 T-cell activation [26]. Therefore, B-cell depletion by rituximab may block T-cell activation induced by B-cells or B-cell-derived factors. Tokunaga et al. showed that rituximab decreases CD40- and CD80-expressing cells among activated B-cells in patients with systemic lupus erythematosus (SLE), and it also down-regulates CD40L and CD69 on CD4-positive cells [27]. Those results imply that rituximab can inhibit the interaction between these B-cells and activated T-cells. However, the roles of CD40L in patients with active SDNS are not yet known and will need to be investigated in the future.

In our study adverse events associated with rituximab were acceptable. Mild reactions to the infusion were observed in five of 12 patients (42%). Steroids were not infused before rituximab treatment in our protocol. There might have been fewer infusion reactions if steroids had been given immediately before the rituximab treatment. There were no serious adverse events during the patients' clinical courses.

In conclusion, a single dose of rituximab may be effective for patients with childhood refractory SDNS. However, its efficacy to prevent relapses was transient in most of the patients.

Acknowledgments This study was supported by the Health and Labor Sciences Research Grant for the Large Scale Clinical Trial Network Project (to K.I.). The authors are grateful to Professor Shuki Mizutani, Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University, and the Zenyaku Corporation, for their helpful advice.

References

1. Iijima K, Hamahira K, Tanaka R, Kobayashi A, Nozu K, Nakamura H, Yoshikawa N (2002) Risk factors for cyclosporine-induced tubulointerstitial lesions in children with minimal change nephrotic syndrome. Kidney Int 61:1801–1805
2. Kranz B, Vester U, Büscher R, Wingen AM, Hoyer PF (2008) Cyclosporine-A-induced nephrotoxicity in children with minimal-change nephrotic syndrome: long-term treatment up to 10 years. Pediatr Nephrol 23:581–586
3. Benz K, Dötsch J, Rascher W, Stachel D (2004) Change of the course of steroid-dependent nephrotic syndrome after rituximab therapy. Pediatr Nephrol 19:794–797
4. Gilbert RD, Hulse E, Rigden S (2006) Rituximab therapy for steroid-dependent minimal change nephrotic syndrome. Pediatr Nephrol 21:1698–1700
5. François H, Daugas E, Bensman A, Ronco P (2007) Unexpected efficacy of rituximab in multirelapsing minimal change nephrotic syndrome in the adult: first case report and pathophysiological considerations. Am J Kidney Dis 49:158–161
6. Smith GC (2007) Is there a role for rituximab in the treatment of idiopathic childhood nephrotic syndrome? Pediatr Nephrol 22:893–898
7. Kemper MJ, Möller K, Ludwig K, Rink N, Muller-Wiefel DE (2006) Rituximab in the treatment of refractory steroid-sensitive nephrotic syndrome (abstract). Pediatr Nephrol 21:1528
8. Dallachio IA, Trimoreau F, Feuillard J, Guigoinis V (2006) A case of steroid-dependent nephrotic syndrome treated with rituximab (abstract). Pediatr Nephrol 21:1615
9. Hofstra JM, Deegens JK, Wetzels JF (2007) Rituximab: effective treatment for severe steroid-dependent minimal change nephrotic syndrome? Nephrol Dial Transplant 22:2100–2102
10. Guigonis V, Dallocchio A, Baudouin V, Dehennault M, Hachon-Le Camus C, Afanetti M, Groothoff J, Llanas B, Niaudet P, Nivet H, Raynaud N, Taque S, Ronco P, Bouissou F (2008) Rituximab treatment for severe steroid- or cyclosporine-dependent nephrotic syndrome: a multicentric series of 22 cases. Pediatr Nephrol 23:269–279
11. Bagga A, Sinha A, Moudgil A (2007) Rituximab in patients with the steroid-resistant nephrotic syndrome. N Engl J Med 356:2751–2752
12. Nakayama M, Kamei K, Nozu K, Matsuoka K, Nakagawa A, Sako M, Iijima K (2008) Rituximab for refractory focal segmental glomerulosclerosis. Pediatr Nephrol 23:481–485
13. Yang T, Nast CC, Vo A, Jordan SC (2008) Rapid remission of steroid and mycophenolate mofetil (MMF)-resistant minimal change nephrotic syndrome after rituximab therapy. Nephrol Dial Transplant 23:377–380
14. Suri M, Tran K, Sharma AP, Filler G, Grimmer J (2008) Remission of steroid-resistant nephrotic syndrome due to focal and segmental glomerulosclerosis using rituximab. Int Urol Nephrol 40:807–810
15. Tabata K, Yamaoka K, Kaibara A, Suzuki S, Terakawa M, Hata T (1999) Moment analysis program available on Microsoft Excel. Xenobiot Metab Dispos 14:286–293
16. Vieira CA, Agarwal A, Book BK, Sidner RA, Bearden CM, Gebel HM, Roggero AL, Fineberg NS, Taber T, Kraus MA, Pescovitz MD (2004) Rituximab for reduction of anti-HLA antibodies in patients awaiting renal transplantation: 1. Safety, pharmacodynamics, and pharmacokinetics. Transplantation 77:542–548
17. Berinstein NL, Grillo-López AJ, White CA, Bence-Bruckler I, Maloney D, Czuczman M, Green D, Rosenberg J, McLaughlin P, Shen D (1998) Association of serum rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. Ann Oncol 9:995–1001
18. Davis TA, Maloney DG, Grillo-López AJ, White CA, Williams ME, Weiner GJ, Dowden S, Levy R (2000) Combination immunotherapy of relapsed or refractory low-grade or follicular non-Hodgkin's lymphoma with rituximab and interferon-alpha-2a. Clin Cancer Res 6:2644–2652
19. Tobinai K, Kobayashi Y, Narabayashi M, Ogura M, Kagami Y, Morishima Y, Ohtsu T, Igarashi T, Sasaki Y, Kinoshita T, Murate T (1998) Feasibility and pharmacokinetic study of a chimeric anti-CD20 monoclonal antibody (IDEC-C2B8, rituximab) in relapsed B-cell lymphoma. The IDEC-C2B8 Study Group. Ann Oncol 9:527–534
20. Iacona I, Lazzarino M, Avanzini MA, Rupolo M, Arcaini L, Astori C, Lunghi F, Orlandi E, Morra E, Zagonel V, Regazzi MB (2000) Rituximab (IDEC-C2B8): validation of a sensitive enzyme-linked immunoassay applied to a clinical pharmacokinetic study. Ther Drug Monit 22:295–301
21. Regazzi MB, Iacona I, Avanzini MA, Rupolo M, Arcaini L, Astori C, Lunghi F, Orlandi E, Morra E, Zagonel V, Regazzi MB (2005) Pharmacokinetic behavior of rituximab: a study of different schedules of administration for heterogeneous clinical settings. Ther Drug Monit 27:785–792
22. Breedveld F, Agarwal S, Yin M, Ren S, Li NF, Shaw TM, Davies BE (2007) Rituximab pharmacokinetics in patients with rheumatoid arthritis: B-cell levels do not correlate with clinical response. J Clin Pharmacol 47:1119–1128
23. Kemper MJ, Meyer-Jark T, Lilova M, Müller-Wiefel DE (2003) Combined B- and T-cell activation in childhood steroid-sensitive nephrotic syndrome. Clin Nephrol 60:242–247
24. Cho BS, Yoon SR, Jang JY, Pyun KH, Lee CE (1999) Up-regulation of interleukin-4 and CD23/FcepsilonRII in minimal change nephrotic syndrome. Pediatr Nephrol 13:199–204
25. Kimata H, Fujimoto M, Furusho K (1995) Involvement of interleukin (IL)-13, but not IL-4, in spontaneous IgE and IgG4 production in nephrotic syndrome. Eur J Immunol 25:1497–1450
26. Martin F, Chan AC (2004) Pathogenic roles of B cells in human autoimmunity; insights from the clinic. Immunity 20:517–527
27. Tokunaga M, Saito K, Kawabata D, Imura Y, Fujii T, Nakayamada S, Tsujimura S, Nawata M, Iwata S, Azuma T, Mimori T, Tanaka Y (2007) Efficacy of rituximab (anti-CD20) for refractory systemic lupus erythematosus involving the central nervous system. Ann Rheum Dis 66:470–475

Pediatrics International (2009) **51**, 709–714 doi: 10.1111/j.1442-200X.2009.02838.x

Original Article

New reference growth charts for Japanese girls with Turner syndrome

Tsuyoshi Isojima,[1] Susumu Yokoya,[1,2] Junko Ito,[2,3] Reiko Horikawa[1,2] and Toshiaki Tanaka[2,4]

[1]*Clinical Research Center, National Center for Child Health and Development, Ohkura,* [4]*Tanaka Growth Clinic, Taishidoh, Setagaya-ku,* [2]*The Foundation for Growth Science, Hongo, Bunkyo-ku and* [3]*Toranomon Hospital, Toranomon, Minato-ku, Tokyo, Japan*

Abstract ***Background***: Currently used growth charts for Japanese girls with Turner syndrome (TS) were constructed with auxological data obtained before the secular trend in growth reached a plateau. These charts were published in 1992 and may no longer be valid for the evaluation of stature and growth in girls with TS in clinical settings. Thus, we need to establish new clinical growth charts.

Methods: The samples for analysis were obtained by a retrospective cohort study. A total of 1867 Japanese girls with TS were registered between 1991 and 2004 for growth hormone (GH) treatment and their pretreatment anthropometric measurements were obtained. Reference growth charts were newly constructed using the LMS method from 1447 girls' cross-sectional data after exclusion of measurements derived from those with the presence of puberty, with previous growth-promoting treatment, or without cytogenetic evidence of TS.

Results: The new clinical reference growth charts differ from the old charts. Secular trends can be detected in both height and weight. Mean adult height on the new chart is 141.2 cm, 3.0 cm taller than the old data. This result seems attributable to the secular trend observed during the same period in Japanese women.

Conclusions: The newly constructed clinical reference growth charts for Japanese girls with TS seem to be better for the evaluation of growth in girls with TS born after approximately 1970, although selection bias and some other limitations in the present study should be kept in mind.

Key words growth chart, LMS method, secular trend, Turner syndrome.

Background

Turner syndrome (TS) is the most common chromosomal disorder in girls and affects about one in 1500 to 2500 live-born female infants.[1] One of the most significant features of the syndrome is short stature. Untreated girls are reported to be approximately 20 cm shorter than normal girls within their respective populations.[2] Growth hormone (GH) has been used to accelerate growth, and it is known to increase adult height.[3]

Growth patterns of girls with TS are different from those in normal populations mainly because of the short stature homeobox-containing gene on the X chromosome (SHOX) haploinsufficiency and their ovarian insufficiency. TS-specific growth curves have been published in various countries[4–11] including Japan,[12] and they have been clinically used for the evaluation of stature and growth. Those of the Japanese were constructed with data from subjects whose body measurements were obtained by sending questionnaires to their follow-up hospitals. The data consisted of 6255 measurements from 705 girls born between 1955 and 1989.

Correspondence: Tsuyoshi Isojima, MD, Clinical Research Center, National Center for Child Health and Development, 2-10-1 Ohkura, Setagaya-ku, Tokyo 157-8535, Japan. Email: isojima-t@ncchd.go.jp

Received 15 July 2008; revised 24 December 2008; accepted 14 January 2009.

Japan has experienced extremely rapid changes in eating habits together with vast socioeconomic changes since the end of the Second World War, and these changes have affected Japanese children's growth. The physical size of Japanese children has increased along with these environmental changes, and nutrition is thought to be the most important contributing factor. In Japan the food supply has been sufficient or even excessive since 1970. The acceleration of growth is reported to have been most prominent between 1955 and 1970,[13] but it has reached a plateau since around 1990, as discussed later. Thus the subjects analyzed in the currently used charts were born before the secular trend approached the recent plateau. Therefore, use of the presently available growth charts may be inadequate for the evaluation of recent cases of TS. In this context, construction of new reference charts and their validation have become necessary.

Methods

Population

The samples were obtained from a database compiled by the Foundation for Growth Science, Japan. The Foundation has been controlling the use of GH through its registration system in Japan, which judges candidates' eligibility for supplemental GH treatment according to the diagnostic criteria for GH deficiency established by the Ministry of Health, Labor and Welfare's Study

Table 1 Age distribution

Age (years)	Number
0	1
1	9
2	14
3	41
4	74
5	104
6	105
7	104
8	113
9	152
10	160
11	168
12	131
13	75
14	68
15	52
16	38
17	22
18	11
19	2
20	3
Total	1447

Group for Hypothalamo-Pituitary Disorders.[14] Medical doctors are encouraged to have each candidate registered for GH treatment at the Foundation using an application form that includes his/her pre-treatment anthropometric measurements, karyotype (in the case of TS), presence or absence of puberty, and evidence of informed consent from each subject regarding the use of the data for scientific purposes.

Between 1991 and 2004, 1867 girls were registered as TS subjects in this cohort. The diagnosis of TS was confirmed by reviewing all the reported karyotypes of cultured peripheral blood lymphocytes. In this study TS was defined as a karyotype that contains a cell line of monosomy lacking at least a distal major part in the short arm of the X chromosome. Subjects having no evidence of such karyotypic features, missing a description regarding puberty status, with secondary pubertal signs, with a history of previous growth-promoting therapy, or whose age was over 20 were excluded.

Statistical analysis

Data were cleaned in several stages. Bivariate plots of height and weight were used to identify gross disproportions. Data points were scrutinized, going back to the source data if necessary, and transcription errors were corrected. If a value was deemed highly unlikely (more than 5 standard deviation scores [SDS] from the mean), such a point was deleted, even in the absence of any evidence of a transcription error.

Reference growth charts were obtained using the LMS method,[15] which assumes that the data can be transformed to normality by a suitable power transformation (L); the distribution is then summarized by the median (M) and the coefficient of variation (S). The values of L, M, and S are constrained to change smoothly with age, and fitted values can be used to construct any required centile curves. The karyotypes of 45,X and non-45,X were compared for body height using analysis of covariance (ANCOVA) with covariates of age and age-karyotype interaction. This analysis was performed using JMP 6.0.3 (SAS Institute Inc., Cary, NC, USA.) and *P*-values less than 0.05 were considered statistically significant.

Results

In total, 420 subjects were excluded because of insufficient or inadequate cytogenetic evidence for the diagnosis (31 subjects), secondary pubertal signs (107 subjects), lack of records about puberty (14 subjects), previous growth-promoting treatment (264 subjects), age over 20 (one subject) and highly unlikely measurements (three subjects). The remaining 1447 subjects were analyzed. Table 1 lists the number of subjects according to age. Their birth years range from 1970 to 2002 (median: 1985). Perinatal information and their parents' anthropometric measurements were collected whenever possible. Gestational age is 39.6+/−1.6 weeks (n = 1268), birth length 46.8+/−2.7 cm (n = 633), birthweight 2.68+/−0.44 kg (n = 1322), and target height 157.6+/−7.2 cm (n = 1289). Target height was calculated by the formula adjusted for the Japanese before the secular trend reached a

Table 2 Karyotypes of 1447 subjects

	Non-Mosaic	Number of subjects	Mosaic	Number of subjects
Aneuploidy	45,X	432	45,X/46,XX	87
			45,X/47,XXX	91
			45,X/46,XY	16
			45,X/46,XX/47,XXX	6
		432		200
Structural abnormality	46,X,i(Xq)	128	45,X/46,X,i(Xq)	309
	46,X,del(Xp)	55	45,X/46,X,del(Xp)	22
	46,X,r(X)	3	45,X/46,X,r(X)	106
	others	10	45,X/46,X,+mar	109
			others	73
		196		619
Total		628		819

Table 3 LMS values of height and weight for the Japanese girls with Turner syndrome

Height				Weight			
Age (years)	L	M	S	Age (years)	L	M	S
1	1	66.75	0.024	1	1.63	6.92	0.094
1.5	1	71.25	0.025	1.5	1.37	8.02	0.094
2	1	75.44	0.026	2	1.11	9.10	0.094
2.5	1	79.1	0.026	2.5	0.86	10.06	0.095
3	1	82.39	0.027	3	0.64	10.90	0.096
3.5	1	85.46	0.028	3.5	0.44	11.65	0.097
4	1	88.38	0.028	4	0.24	12.37	0.099
4.5	1	91.11	0.029	4.5	0.06	13.04	0.102
5	1	93.68	0.029	5	−0.12	13.71	0.106
5.5	1	96.23	0.030	5.5	−0.32	14.44	0.111
6	1	98.75	0.030	6	−0.51	15.24	0.117
6.5	1	101.24	0.031	6.5	−0.69	16.10	0.124
7	1	103.81	0.031	7	−0.87	17.12	0.131
7.5	1	106.39	0.032	7.5	−1.03	18.32	0.139
8	1	108.79	0.032	8	−1.14	19.58	0.147
8.5	1	111.02	0.033	8.5	−1.19	20.83	0.154
9	1	113.18	0.033	9	−1.16	22.12	0.160
9.5	1	115.32	0.034	9.5	−1.04	23.52	0.165
10	1	117.53	0.034	10	−0.84	25.08	0.170
10.5	1	119.89	0.035	10.5	−0.60	26.76	0.176
11	1	122.35	0.035	11	−0.40	28.51	0.182
11.5	1	124.76	0.036	11.5	−0.28	30.26	0.187
12	1	127.03	0.036	12	−0.25	31.94	0.189
12.5	1	129.14	0.037	12.5	−0.26	33.50	0.190
13	1	131.03	0.037	13	−0.26	34.93	0.191
13.5	1	132.69	0.037	13.5	−0.23	36.23	0.191
14	1	134.14	0.038	14	−0.17	37.40	0.191
14.5	1	135.37	0.038	14.5	−0.10	38.43	0.191
15	1	136.38	0.038	15	−0.01	39.33	0.192
15.5	1	137.24	0.038	15.5	0.08	40.11	0.192
16	1	137.96	0.039	16	0.16	40.79	0.192
16.5	1	138.56	0.039	16.5	0.23	41.39	0.192
17	1	139.07	0.039	17	0.28	41.93	0.192
17.5	1	139.49	0.039	17.5	0.32	42.43	0.192
18	1	139.87	0.039	18	0.37	42.91	0.192
18.5	1	140.23	0.039	18.5	0.40	43.36	0.191
19	1	140.58	0.039	19	0.44	43.81	0.191
19.5	1	140.91	0.039	19.5	0.47	44.24	0.191
20	1	141.24	0.039	20	0.51	44.67	0.190

plateau.[16] Table 2 summarizes the number of subjects grouped by karyotype. There was no significant difference in height between 45,X and non-45,X subjects (regression coefficient: 0.19+/−0.14 cm, $P = 0.17$).

Centile curves were fitted to the data of all subjects together using the LMS method. For height, the distribution was assumed to be normal, while for weight there was appreciable skewness, to which the age-varying power transformation was adjusted. Table 3 provides values for L, M and S of height and weight by age. Clinical growth references for height and weight are shown in Figures 1 and 2, respectively. References for height and weight expressed as SDS are superimposed on those that are currently used in Figures 3 and 4.

Discussions

We produced new clinical reference growth charts for Japanese girls with TS who did not present with puberty. The charts were constructed using the LMS method, which we believe is one of the most widely applied approaches.[17] The LMS method is often used to construct age-related references not only of normal populations[18] but also of Down syndrome[19] and Williams syndrome[20] disease-specific populations. The number of subjects analyzed in this study was sufficient, being comparable to numbers analyzed in the construction of other TS-specific charts. All subjects were confirmed by chromosomal analyses to meet the definition of TS and were properly selected, excluding subjects who had undergone pubertal development or previous growth-promoting treatment or both. Although these charts were not derived from a totally unbiased TS population, they can be presumed to represent growth in girls with TS who are ordinarily seen in clinical practice, because the charts were constructed using adjacent data before GH treatment. We believe that these charts have been adequately and successfully produced taking these points into consideration.

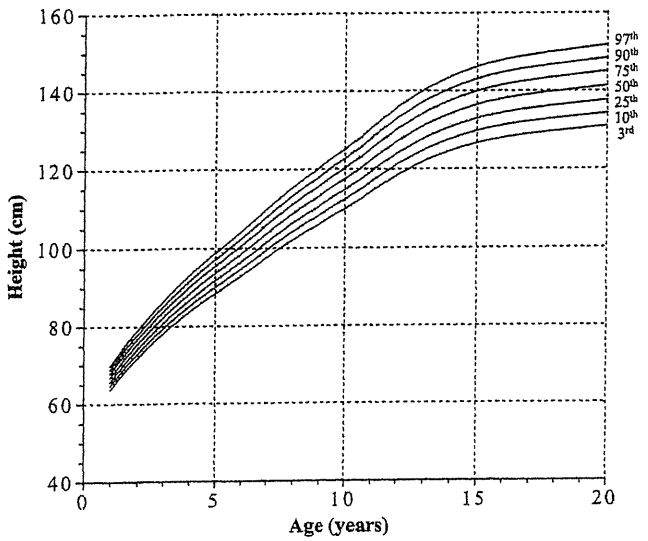


Fig. 1 Height chart for Japanese girls with Turner syndrome without puberty.

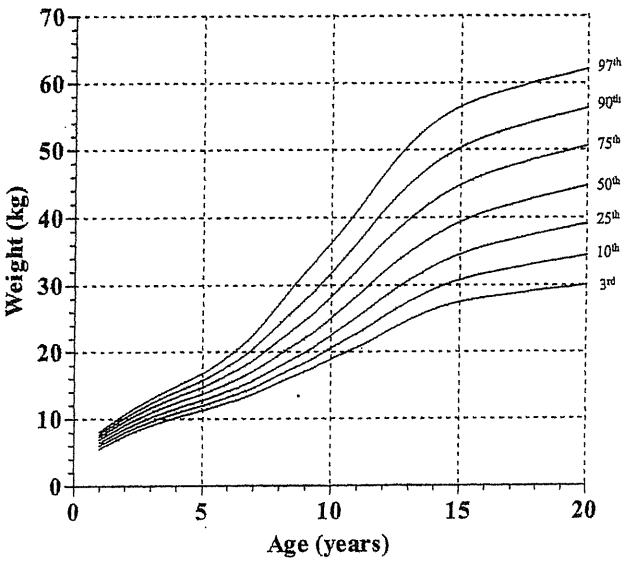


Fig. 2 Weight chart for Japanese girls with Turner syndrome without puberty.

Differences can be detected between the two charts in both height and weight growth (Figs 3 and 4). For example, the adult height from the new chart is 141.2 cm, which is 3.0 cm taller than the previous height when it is defined as the mean height at the age of 20 years. In the previous study, birth years ranged from 1955 to 1989 (median unknown). Given the year of publication, the adult height in the study had to be derived from subjects born before 1972. The standard adult heights of Japanese women in 1970, 1975, 1980, 1985, 1990, 1995, 2000 and 2005 were 155.6 cm, 156.3 cm, 157.0 cm, 157.6 cm, 157.9 cm, 158.0 cm, 158.1 cm and 158.0 cm, respectively.[21] This indicates that the secular trend in adult height has reached a plateau since approximately 1990 in Japan. Judging from the birth-year distribution, we know therefore that the old Japanese charts for TS were constructed with data from subjects the majority of whom were born before growth in height reached a plateau. On the other hand, the birth years in the present study ranged from 1970 to 2002 and 85.2% of the subjects were born after 1980. The new TS-specific growth charts therefore differ because they were constructed with data from a generation in which appreciable advances in secular height growth had disappeared. Secular growth trends in TS subjects have also been noted and studied in other countries.[9,22] In countries where secular trends

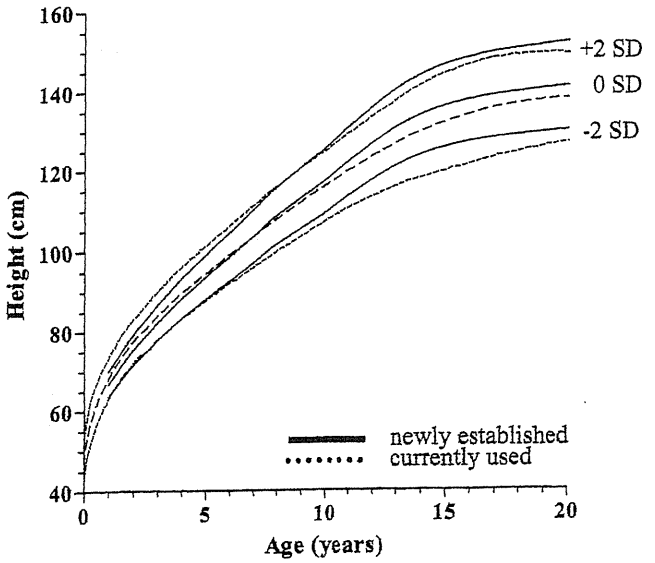


Fig. 3 Height chart for Japanese girls with Turner syndrome without puberty in comparison with the currently used one.[12] Solid line, newly established; dotted line, currently used.

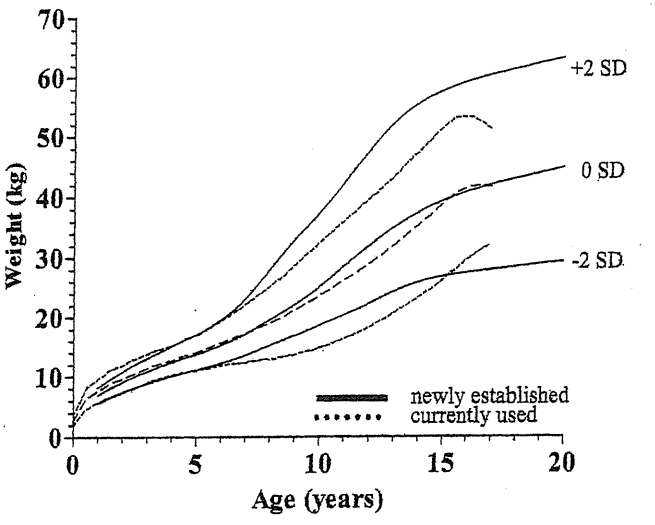


Fig. 4 Weight chart for Japanese girls with Turner syndrome without puberty in comparison with the currently used one.[12] Solid line, newly established; dotted line, currently used.