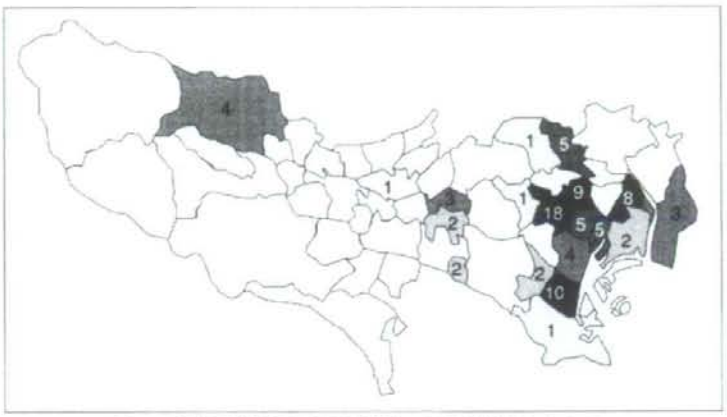

図3　血液悪性疾患の虎の門病院への紹介地域（東京都内）

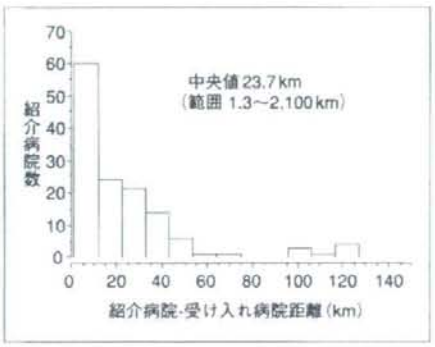

図4　紹介施設と受け入れ施設の間の距離（km）

ある地域・医療圏全体を病院とみなせ，各中核病院を中心に，各開業医は受け持つ各地域（病院でいえば病棟）のナースステーションに当たると考えることができる．当然，開業医でもそれぞれ専門分野をもっており，例えば開業医が循環器専門であれば，悪性疾患の終末期医療を任せられるか？ に関しては，“血液の病気は専門ではないので診ることができない”とよくいわれ，現状不可能である場合が多い．悪性疾患症例を十分に経験した医師が，今後開業し，死因第1位の悪性疾患の終末期医療を担えれば，あるいは開業医でも全身管理の十分な経験をもった医師（general physician）であれば，在宅での緩和・終末期ケアは可能になってくると考えられる．しかし残念ながら，医学生や若い医師は悪性疾患を専門にしない風潮があり，先細りになっていくことが予想される．今後の対策が必要である．

文　献

1) The international non-Hodgkin's lymphomaprognostic factors project：A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med **329**：987-994，1993.
2) Iwakiri R et al：Prognostic of elderly patients with acute myelogenous leukemia：analysis of 126 AML Cases. Int J Hematol **75**：45-50，2002.
3) 井形昭弘：高齢者におけるインフォームド・コンセント．Geriat Med **35**：1479-1483，1997.
4) 堤　　久ほか：化学療法後に重篤な骨髄抑制をきたした高齢者悪性リンパ腫症例の臨床的検討．日本老年医学会雑誌 **38**（Suppl）：103，2001.
5) 白杵憲祐，浦部晶夫：Palliative Chemotherapy―造血器腫瘍―．緩和医療学 **2**：329-336，2000.
6) Stalfelt AM et al：The final phase in acute myeloid leukaemia（AML）. A study on bleeding, infection and pain. Leuk Res **27**：481-488，2003.
7) Bauduer F et al：Characteristics of deaths in a

department of oncohaematology within a general hospital. A study of 81 cases. Support Care Cancer **8**：302-306, 2000.
8) 宮腰重三郎：新しい輸血の方向　血液疾患での濃厚血小板の適応基準　予防的血小板輸血をどこまで少なくできるか．臨床医　**24**：1224-1228, 1998.
9) 大田雅嗣：高齢者造血器悪性腫瘍—高齢者のターミナルケア．血液フロンティア　**13**：213-218, 2003.
10) 増子忠道：高齢者在宅医療における終末期医療．Geriat Med　**35**：1512-1516, 1997.

〔筆者連絡先〕　宮腰重三郎　〒173-0015　東京都板橋区栄町35-2　東京都老人医療センター血液科

第7回 雄田校医のゆっくり懇談会

在宅医療の特集を受けて、今回は雄田先生たっての希望で、訪問診療専門の「あおぞら診療所」の医師、川越正平先生と、訪問看護のパイオニアとして活躍中の村松静子さんをお招きし、在宅医療の現実について話し合っていただきました。

撮影／小川拓洋
イラスト／石森愛彦

「命」と「生活」を支える 在宅医療で 自分らしい生き方を

ゲスト 川越正平さん
（医療法人財団千寧健愛会あおぞら診療所所長）

ゲスト 村松静子さん
（開業ナース・在宅看護研究センター代表）

救急救命・高度医療の現場から在宅へ

雄田 お二人とも経歴を見ると、村松さんはICU（集中治療室）で救命医療に、川越先生は虎の門病院で高度医療に携わっていたのに、なぜ在宅医療に路線変更したのですか？

村松 救急救命は常に生と死が背中合わせですが、呼吸器につながれて話せない人が、生命維持装置の外を指さして「家に帰りたい」と願っていることがありました。私は祖父が家で亡くなっていますし、看護の原点は在宅にある、その人らしく生きていくためには家がいいと常に考えていました。

川越 私は骨髄移植など最先端の高度医療の現場に携わっていましたが、難病になると助けられる人は少なく、助からない人が「家に帰りたい」と願っても、地域には家まで来てくれる医師がいないという現実がありました。そこで自分がそちらの側に回って、在宅で緩和ケアなどができたらと思いました。

雄田 村松さんはまだ「訪問看護」という言葉がなかった頃から、「開業ナース」として、在宅医療で看護を提供してきたパイオニアですね。

村松 在宅で看護が必要な方には、いつでも誰にでも必要な時に必要なだけ、24時間365日お手伝いしますというのが理念です。サービスの提供は東京23区内が基本ですが、医療行為を必要とする人の旅行への同行や、IVH（中心静脈栄養法）でチューブを入れている方の結婚式の出席への付き添いなどでは全国どこへでも行きます。

雄田 川越先生の「あおぞら診療所」は松戸市にもう一カ所あるんですね。

川越 はい。現在、常勤の医師4人に研修医、非常勤医師に加えて看護師、ソーシャルワーカー、事務員と協働で、それぞれ200人、計約400人の在宅の患者さんを訪問しています。

雄田 訪問エリアは松戸市だけ？

川越 一応松戸市内なんですが、うちがお断りすると行き場のないがんの方などは、無理をしてでも

お引き受けしています。医師の一人は小児在宅医療が専門で、かなり遠方へも出かけています。

在宅での医師・看護師・ヘルパーそれぞれの役割

雄田 在宅医療は医師、看護師の、双方が補完し合わないと実現しないと思うのですが、それぞれの役割の違いを教えてください。

川越 在宅医療の本質は、命だけでなく生活を支えることだと思っています。

医師は限られた時間で診断をし、「これは病院に行ったほうがいい」「今回は看取りになるかもしれない」など、タイミングを逃さず決断し、患者さんとご家族に説明をする。看護師などチームには「こういう方針でがんばりましょう」と伝達しと全体の責任をもちますが、患者さんのことを一番わかっているのは接している時間の長い看護師ですから、協力が得られなければ続けられません。

村松 在宅においては、医師と看護師は車の両輪で、互いにキャッチボールのできる仲間だと思っています。

私たち看護師は、医師の指示に従うだけでなく、たとえば一つのチューブが体に入ることの意味を説明し、本人やご家族の不安感を解消したり、容態を観察しながら患部をきずったりマッサージをするなど、五感を駆使した看護ができないことですよね。

雄田 では、介護と看護はどう違うのかな。

村松 あえて分けるとすれば、介護のほうはまさに生活に密着していて、生活をささえるという部分では時には主婦（夫）の役割を代行したり、家族の代わりができる。でも医療がからんでくると、命に直結するので介護の個の人は怖くなってくると思うんです。そこに看護師がいるといいのではないでしょうか。

川越 私は、どこまでをホームヘルパーにお願いするかという時の基準は「安全性」の一点しかないと思っています。たとえば、吸引という行為が安全かどうか、その患者さんの状態やホームヘルパーの訓練度によって違ってきます。で

在宅医療での医師の役割は「決断」と「責任」です。

かわごえ・しょうへい●1966年生まれ。東京医科歯科大学医学部卒業。国家公務員共済組合虎の門病院レジデントおよび血液科医員、特定医療法人財団健和会柳原病院内科医長を経て、99年、千葉県松戸市にあおそら診療所開設。現在、あおそら診療所上本郷院長、東京医科歯科大学臨床助教授、虎の門病院非常勤嘱託、日本内科学会認定内科専門医。

診療より訪問看護のほうを、まだまだ「特別」のように感じているのかもしれませんね。

「どう死にたい」まで希望をはっきり伝えよう

聡田　川越先生の診療所では現在、在宅ターミナルケアの方は、どれぐらいいますか？

川越　末期がんの方は5%ぐらい。あとは長期慢性疾患の方で、皆さんいろいろな病態を抱えておられるので、繰り返しになりますが、生活全体を支える仕事のほうが大

看護師の力は在宅医療に不可欠だね。

むらまつ・ぜいこ●1947年生まれ。日本赤十字中央女子短期大学卒業。筑波大学大学院カウンセリング専攻。日本赤十字医療センターICU看護婦長、日本赤十字看護大学兼任講師などを経て、86年、日本で最初の「関業ナース」として在宅看護研究センター設立。著書に「その時は家で 関業ナースがゆく」（日本看護協会出版会）などがある。

「自分らしく生きる」ために私たちは全力をつくします。

切だなと感じています。

村松　私のところでは約8割が、がんの方です。他で断られたり夜間来てもらえなかったなどの理由で、依頼があります。自分らしい生き方を望んだ結果、家で逝きたいとはっきりおっしゃる方が多くなってきました。

聡田　ご家族やご本人が「最期まで在宅で」と希望する場合、最初にどこに相談したらいいですか。

村松　病院のソーシャルワーカーや在宅医療連携室の看護師、地域の訪問看護ステーションやケアマネジャーに相談してもいいし、地域包括支援センターでもいい。とにかくどこかに連絡さえすれば、しかるべきところを紹介してもらえます。その際、生活へのこだわりの部分をきちんと聞いてくれるところ、こちらの要望をちゃんと聞いてくれているなという人に、自分の思いを伝えてほしいと思います。

聡田　技術的な問題だけでなく、「こんな人に来てほしい」というこだわりでもいいし、「こういうふうに最期まで生きたい」という生き方へのこだわりでもいいんだね。

村松　ええ。お金を払うのは患者さんの側なのですから、無口な人がいいとか、お話し好きの人など、来てほしい人のタイプまではっきり伝え、合わなかったら変えるかもしれないと言ったほうがいいです。

聡田　でも、普通の人は、なかなか「この人、いやだから変えてください」とは言えないと思うけどなあ。

川越　患者さんとそのご家族とは、本当はその場にいない医師や看護師へのグチまで言えるような関係が築ければいいですね。今後保険では、本来はケアマネジャーはそこまで要望を吸い上げてケアプランを立てることになっているのですが、当のケアマネジャーに対する不満というのもあるわけで、言うほど簡単ではないのでしょう。

村松　医師に対する不満を聞くことはありますね。当然だろうなと思う時も正直ありますが、看護師が医師との関係をきちんと築いておけば、「先生、患者さんがこういうことをおっしゃっているので」とお話しできます。

まだまだ報われない訪問看護師の仕事

聡田　在宅医療にかかわるプロとして、今の制度に対する不満や、逆にここ数年でこんなに良くなったという点などはありますか。

村松　在宅ターミナルケアの場合、途切れ途切れの複数回の訪問は保険で認められていますが、長時間のサービスが制度的に認められていない。看護は必要な時に行けなければ意味がないし、不安になったり苦痛になった時に、長時間いられればいいのですが、時間の

使い方の点でまだまだ課題が多いですね。それでも私たちは、「とにかく最期まで本物の看護をしよう」と励まし合っているわけですが、正直言って、経営のことが頭にちらつくような看護の仕事はもうしたくないですね。

それに在宅で看取る場合、医師は最期に来て診断書を作成すれば点数がつきますが、私たちは死に装束や死に化粧をしてさしあげたり、パニックになって泣き叫ぶご家族を支えても、亡くなられてしまうとなんの加算もないんです。30年やってきても、国の制度としては寂しいものを感じています。

川越　介護保険の前提は、できるだけ在宅でということですから、「在宅」という言葉を聞いたことがある方が増えていると感じますね。ほんの数年前に比べても、家で療養する、家で最期を迎えることも可能なんだということが少しは知られてきていると思います。

鎌田　在宅医療の中心は、やっぱり訪問看護なんだよね。医師の役割は絶対必要なんだけど、患者と接している時間は少なく、訪問看護が中心になって初めて在宅医療が成りするのに、当の看護師が大事にされないのはおかしいね。

川越　在宅医療は今後も広がっていくのは間違いないと思いますが、いくら在宅診療の数を図が強くても、医師だけでは不可能です。医師を支える看護師が地域にしっかり根づく環境を整えて、訪問看護ステーションも、綿になって全国に広がっていかないと、在宅医療は支えられません。そのためにも、訪問看護師の仕事ももっと評価されるべきですね。

村松　若い先生にそう言っていただくと、本当に心強いです。

医師にもの言える専門職が増えてほしい

川越　在宅医療は、地域のなかで今まではバラバラに点在していた専門職が、制度にネットワークを組んで仕事をするわけですが、たとえば医師が訪問看護ステーションの方と顔を合わせたこともなく、指示書1枚の紙のやりとりだけでは本当にいい仕事ができるわけがないと感じています。うちの場合は月に1度、連携ステーションのスタッフと合同カンファレンスを行っていますし、医師の携帯電話の番号を全部知らせていて、「病院の内線電話と同じ感覚で使ってください」と伝えています。

鎌田　それはなかなかできないことですね。日本の医療のなかで、医師は歴史的にそういうことをやってこなかった。特に介護の職種の人は医療職にはなかなか電話できないですよね。

村松　ケアマネジャーもホームヘルパーも、医師には言いにくいといいますね。

川越　介護保険の居宅療養指導は、今年から報告書を書かないと算定できなくなったので、うちも4月から書式をつくって毎回の診療報告書をケアマネジャーに送るようになりました。そうしたら、その内容についてケアマネジャーからかなり頻繁に連絡をいただくようになったんです。ということは、今まではこちらからの積極的な連絡が不足していたんだなと反省しています。

鎌田　そうやって制度が整備されることによって連携が進んだというのは、前進だね。お二人とも、在宅医療は24時間365日支えられなければと著書などでおっしゃっていますが、過酷な仕事ですね。

村松　過酷です。年末年始もないですから。在宅ターミナルケアの方は夜間、医師よりも看護の手を要求してきます。特に看取る時は、患者も家族も私たちもエネルギーを出しきるんです。それでやっと「ありがとう」の言葉をいただけるんですが、それをやっている看護師たちには、もっといろいろな面で余裕をあげたいと思います。

鎌田　なぜ24時間にこだわるんだろう？

村松　夜は、家族がすごく大変ですよ。ご本人にとっても、周りが寝静まると、自分はもうこのまま死ぬんじゃないかと、一番不安が強くなる時間帯なんです。そんな時に、看護師さんに来てほしいとおっしゃる人に「行けません」とは言えません。

川越　僕らは地域が病棟だと思ってやっています。病院の病棟で仕事をしていた時は、ナースコールが鳴ればまず看護師が駆けつけて状況を把握し、医師に相談し、夜間であっても対応していたわけです。在宅になっても、たまたま家が病棟のベッドと考えればいいんです。でも、それを看ているのは医療の基礎知識のないご家族なので、今すぐ医師に連絡すべきか、たいしたことがないのかの判断ができない。そのため、たとえたいしたことがなくても、「ご家族が不安に思った時が、相談すべき時ですよ」と伝えています。そうした不安を支えてあげられないと、家で療養するという前提が崩れてしまうという気がします。

公的制度を使えば高額にはならない

鎌田　一般の人が在宅で療養を望む時、費用の問題は大きいと思いますが、在宅医療はどれぐらいお金がかかるんでしょう。

村松　週2～3回の定期的な訪問で、75歳以上の高齢者なら一割負担で1万円ぐらいですが、要介護度や頻度、訪問時間によって異なってきます。

川越　医師の場合は月に2回訪問するのが一般的で、医療保険での一割負担で月に6000円ぐらいです。

鎌田　費用という視点から見ると、思ったよりはかからないんだね。

最後に、お一人は、在宅医療にかかわったことへの後悔はない？特に川越先生は、最先端の高度医療に携わっていて、そこから在宅医療に転身したことは、すごく勇気のいることだったと思うのですが。

川越　先端医療の現場で、次第に自分の目指しているものとのギャップが生じてきて、自分の居場所はそこだけじゃないと考えて仲間とつくった診療所ですから。この8年、楽しいというと語弊はあるかもしれませんが、やりがいの持てる現場に携われて、醍醐味を感じています。

村松　私も大変ですけど後悔はないです。30年前に携わった患者のご家族から今でもお手紙をいただいたり電話をいただくと、人間同士の心に触れる仕事なんだと実感できますし、多くの人の心に支えられている喜びを感じますね。

鎌田　患者さんのほうも、病院で治療や看護をうけるよりも、在宅でいい医療や看護に出合えれば幸せということでしょうね。今日はありがとうございました。

International Journal of
HEMATOLOGY

Prevalence of Anemia among Healthy Women in 2 Metropolitan Areas of Japan

Eiji Kusumi,[a] Midori Shoji,[b] Shigeyuki Endou,[c] Yukiko Kishi,[d] Taro Shibata,[e] Naoko Murashige,[f] Tamae Hamaki,[g] Tomoko Matsumura,[a] Koichiro Yuji,[h] Akiko Yoneyama,[e] Masahiro Kami[a]

[a]Division of Exploratory Research, The Institute of Medical Science, The University of Tokyo, Tokyo; [b]Health Screening Center, Yuai Memorial Hospital, Ibaraki; [c]Department of Clinical Laboratory, Toranomon Hospital, Tokyo; [d]Division of Regenerative Medicine, Jichi Medical School, Tochigi; [e]Statistics and Cancer Control Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo; [f]Office for Life-Style Related Disease Control, Ministry of Health, Labour and Welfare, Tokyo; [g]Division of Transfusion Medicine, Tokyo Metropolitan Fuchu Hospital, Tokyo; [h]Department of Internal Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Received April 26, 2006; received in revised form May 29, 2006; accepted June 13, 2006

Abstract

Anemia is common among young women, and iron deficiency is one of the leading causes. In Europe and the US, the iron fortification of flour increased oral iron intake and decreased anemia prevalence from 30% to 10%. The National Nutrition Survey in Japan revealed that anemia prevalence among young Japanese women is increasing; however, no nationwide preventive policy has been aimed at iron deficiency anemia. The endpoint of this study was the estimation of anemia prevalence among healthy Japanese woman, based on a large sample size. We collected data from the consecutive check-up examination records of apparently healthy women (n = 13,147). We defined hemoglobin lower than 12 g/dL as anemia, hemoglobin lower than 10 g/dL as severe anemia, and a mean corpuscular volume lower than 80 fl as microcytic anemia. Of the 13,147 persons, anemia was identified in 2331 (17.3 %), and severe and microcytic anemia in 438 (3.3 %) and 700 (5.2 %), respectively. Among women younger than 50 years, anemia was identified in 22.3 %, and 25.2 % of them had severe anemia. In conclusion, the prevalence of anemia and severe anemia among young women is high in Japan. Some action needs to be considered to improve women's quality of life.

Int J Hematol. 2006;84:217-219. doi: 10.1532/IJH97.06097

Key words: Iron deficiency; Erythropoietin; Hematological abnormalities; Hemoglobin; Mean corpuscular volume (MCV); Thrombocytopenia; Anemia in the elderly; Women's health; Iron fortification

1. Introduction

Anemia is common among young women. The National Health and Nutrition Examination Survey (NHENES) revealed that an insufficient iron intake was one of the leading causes of anemia in the US. In Europe and the US, the iron fortification of flour increased oral iron intake, and the prevalence of anemia consequently decreased from 30% to 10% [1].

Correspondence and reprint requests: Kusumi Eiji, MD, Division of Exploratory Research, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan; 81-3-6409-2068; fax: 81-3-6409-2069 (e-mail: kusumi-tora@umin.ac.jp).

There are 3 epidemiological studies on anemia among Japanese women [2-4]. Uchida et al studied abnormal iron metabolism among 3015 women from 1981 to 1991 [2]. The lifestyle at the time of the study, more than 20 years ago, was probably different from the present one. The authors did not report the prevalence of anemia. Maeda et al studied chronological changes in the prevalence of anemia in junior and senior high school students between 1966 and 1997 [3]. They did not report anemia prevalence among the population except for junior and senior high school students. The only epidemiological study on anemia among Japanese women after the 1990s was the National Nutrition Survey in Japan (NNSJ) by the Ministry of Health, Labour and Welfare [4]. The study mainly included elderly women; only 37% were younger than 50. There are insufficient epidemiological data on anemia among young Japanese women.

We investigated the prevalence of anemia in Japanese women, mostly young women, collecting data from the medical records of check-up examinations for apparently healthy people and the staff of Toranomon Hospital and Yuai Memorial Hospital.

2. Material and Methods

2.1. Data Collection

We collected data from the consecutive check-up examination records of apparently healthy women in different age groups in Toranomon Hospital (between January 2002 and March 2005; n = 8265) and Yuai Memorial Hospital (between February 1998 and February 2005; n = 5153).

2.2. Definitions

We defined hemoglobin lower than 12 g/dL as anemia, hemoglobin lower than 10 g/dL as severe anemia, and a mean corpuscular volume lower than 80 fl as microcytic anemia. Complete blood cell counts were analyzed using routine blood counting analyzers (XE-2100; Sysmex, Kobe, Japan in Toranomon Hospital and Coulter Gen-S; Beckman Coulter, Fullerton, CA, USA in Yuai Memorial Hospital).

2.3. Objectives and Statistical Analysis

This study aimed to estimate the prevalence of anemia, severe anemia, and microcytic anemia among healthy Japanese women, and to evaluate the association between these variables and age. The Fisher exact test was used for univariate analysis. A P value of less than .05 was considered significant. All analyses were performed with the statistical software JMP (version 5.01; SAS Institute, Cary, NC, USA).

3. Results

3.1. Prevalence of Anemia, Severe Anemia, and Microcytic Anemia

The median age was 47 years (range, 11-87 years). Anemia was diagnosed in 2331 (17.3%), including severe anemia in 438 (3.3%) and microcytic anemia in 405 (3.0%) (Table 1).

3.2. Age-Specific Prevalence of Anemia

Table 2 and Figure 1 present the age-specific prevalence of anemia. The prevalence of anemia was high among those in their 20s to 40s, and tended to decrease above 50 years. The median hemoglobin levels in each age group were strongly correlated with the prevalence of anemia (Figure 1, R = 0.96). The prevalence of severe anemia and the median hemoglobin levels in each age group were also positively associated (R = 0.80).

3.3. Platelet and White Blood Cell Counts

White blood cell and platelet counts are tabulated in Table 2.

Table 1.
Characteristics of Women Included in the Study

	Median (range)
Age	47 (11-87)
Toranomon Hospital/Yuai Memorial Hospital	8265/5153
Hemoglobin, g/dL	13.0 (4.4-17.7)
Red blood cell count, $\times 10^9$/L	4.52 (1.98-6.03)
Hematocrit, %	39.0 (17.4-53.4)
Mean corpuscular volume, fl	91.2 (54.0-116.6)
Mean corpuscular hemoglobin concentration, g/dL	33.2 (24.3-37.9)
White blood cell count, $\times 10^9$/L	6.3 (1.9-17.0)
Platelet count, $\times 10^9$/L	243.0 (100.0-792.0)
Anemia prevalence, %	2331 (17.3)
Severe anemia prevalence, %	438 (3.3)
Mircocytic anemia prevalence, %	405 (3.0)

4. Discussion

In the present study, the prevalence of anemia was 17.3%. Of the anemic women, 18.7% had severe anemia and 17.3% microcytic anemia. The high prevalence of anemia in Japan is a significant clinical issue; the situation is similar to that in other Asian countries and Northern Europe, where no food products are fortified with iron [5,6].

The prevalence of anemia in those under 50 was 22.3%. It was as high as 25.8% in those aged 40-49 years; of those with anemia in that age group, 25.2% had severe anemia and 25.6% microcytic anemia. In contrast, the prevalence of anemia in those aged 50 and older was 11.2%, which was lower than that in younger women. The high prevalence among those aged 40-49 years in the present study is consistent with the previous reports [7], suggesting that anemia in this age group is due to a loss of iron from menstruation and menorrhagia.

The present study suggests that the prevalence of anemia is increasing among young Japanese women. Although there are few reports on chronological changes in the prevalence of anemia among Japanese women, compared with the results of the NNSJ among women aged 30-49 in 1990, our findings suggest that the prevalence of anemia has risen from 20% to 24% [4]. Maeda et al showed an increase in the prevalence of anemia among Japanese female adolescents [3]. The national average of oral iron intake has decreased from 10.8 mg/day in 1975 to 8.1 mg/day in 2003, and the average oral iron intake among women aged 18-29 was 7.0 mg/day in 2003 [4]. A possible cause of decreased iron intake is the popularity of weight-loss diets among young Japanese women, and increased iron loss may be due to an increase in menorrhagia, although the definitive cause remains unknown. A more detailed study is necessary regarding the causes of anemia in young Japanese women. In contrast, the prevalence of anemia in the elderly in the present study is equivalent to that of the NNSJ in 1990 [4]. The observation suggests that the causes of anemia in menopausal women are different from those in young women, probably being related to aging and various medical conditions [8-10]. There have been few studies on the causes of anemia among the elderly, and further study is awaited.

Table 2.
Complete Blood Count and Anemia Prevalence According to Age*

	10-19 y	20-29 y	30-39 y	40-49 y	50-59 y	60-69 y	≥ 70 y
Number of women included	121	1896	2157	3276	3704	1785	478
Hemoglobin, g/dL	13.0 (8.7-15.7)	12.9 (5.5-17.1)	12.8 (4.4-16.2)	12.8 (5.4-15.8)	13.1 (6.0-17.4)	13.2 (8.4-17.7)	13.1 (8.4-15.6)
Red blood cell count, $\times 10^{12}$/L	4.48 (3.68-5.47)	4.90 (2.00-5.94)	4.59 (2.57-5.67)	4.65 (2.80-5.63)	4.50 (2.20-5.68)	4.40 (3.00-6.03)	4.25 (3.07-5.01)
Hematocrit, %	39.1 (29.2-46.2)	38.6 (17.4-49.2)	38.4 (18.1-47.2)	38.5 (20.8-47.2)	39.5 (21.9-52.0)	39.7 (26.7-53.4)	39.5 (28.8-46.7)
Mean corpuscular volume, fl	88.0 (64.0-98.0)	90.0 (57.0-105.4)	90.4) (59.0-116.6	90.7 (58.0-109.0)	92.0 (54.0-112.1)	93.0 (71.9-104.8)	93.6 (73.6-103.3)
Mean corpuscular hemoglobin concentration, g/dL	33.2 (28.3-35.4)	33.3 (26.7-37.9)	33.2 (24.3-35.8)	33.1 (24.5-36.3)	33.2 (24.4-36.4)	33.2 (30.6-35.8)	33.1 (28.2-35.3)
White blood cell count, $\times 10^9$/L	5.9 (2.4-17.0)	9.2 (2.4-12.7)	7.4 (2.1-14.0)	7.0 (2.2-14.4)	5.6 (1.9-11.4)	5.3 (2.3-11.6)	5.3 (2.1-12.1)
Platelet count, $\times 10^9$/L	263.0 (135.0-578.0)	244.0 (94.0-501.0)	247.0 (50.0-572.0)	252.0 (47.0-649.0)	240.0 (100.0-610.0)	232.0 (56.0-792.0)	230.0 (26.0-426.0)
Anemia prevalence, %†	15.7	18.0	21.1	25.8	12.8	7.7	11.5
Severe anemia prevalence, %	3.3	1.9	3.8	6.5	2.6	0.2	0.8
Microcytic anemia prevalence, %	9.1	2.2	4.3	6.6	1.1	0.1	0.4

*Data are written as median value (range).
†Anemia prevalence includes severe anemia.

The present study showed that anemia is a significant issue among young Japanese women, although the interpretation requires caution. First, the study subjects were health-conscious women who resided in a metropolitan area and came for check-up examinations at the two hospitals, suggesting the possible existence of a selection bias. Second, since no data are available on serum chemistries, symptoms, and physical examination regarding iron metabolism, we cannot assess the causes of anemia based on the present study. Last, the numbers of women varied between the different age groups. Any future study should include equal numbers of women for a more precise analysis. A prospective, nationwide study is awaited, to assess the prevalence of anemia in a larger sample size.

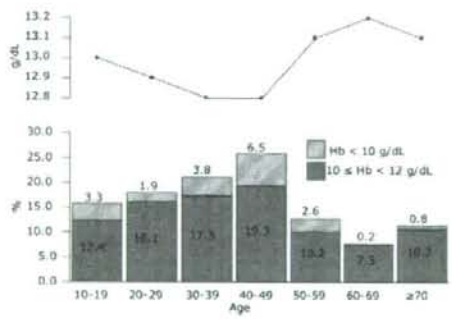

Figure 1. Prevalence of anemia and median hemoglobin levels according to age groups.

The high prevalence of anemia in young Japanese women is a significant clinical issue. In many cases, the causes are probably insufficient iron intake and iron deficiency due to iron loss. Anemia is likely to adversely affect young women's health. Nationwide consideration and an epidemiological approach are necessary.

References

1. Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. *JAMA*. 1997; 277(12):973-976.
2. Uchida T, Kawachi Y, Sakamoto Y, et al. Prevalence and pathogenesis of iron deficiency in Japanese women (1981-1991). *Rinsho Ketsueki*. 1992;33(11):1661-1665.
3. Maeda M, Yamamoto M, Yamauchi K. Prevalence of anemia in Japanese adolescents: 30 years' experience in screening for anemia. *Int J Hematol*. 1999;69(2):75-80.
4. National Nutrition Survey in Japan. http://www.mhlw.go.jp/bunya/kenkou/eiyou-chosa2-01/index.html.
5. Malhotra P, Kumari S, Kumar R, Varma S. Prevalence of anemia in adult rural population of north India. *J Assoc Physicians India*. 2004;52:18-20.
6. Mehta BC. Iron deficiency amongst nursing students. *Indian J Med Sci*. 2004;58(9):389-393.
7. Uchida T, Yoshida M, Sakai K, et al. Prevalence of iron deficiency in Japanese women. *Nippon Ketsueki Gakkai Zasshi*. 1988;51(1):24-27.
8. Artz AS, Fergusson D, Drinka PJ, et al. Mechanisms of unexplained anemia in the nursing home. *J Am Geriatr Soc*. 2004;52(3):423-427.
9. Ioannou GN, Rockey DC, Bryson CL, Weiss NS. Iron deficiency and gastrointestinal malignancy: a population-based cohort study. *Am J Med*. 2002;113(4):276-280.
10. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood*. 2004;104(8):2263-2268.

Available online at www.sciencedirect.com
ScienceDirect
Transplant Immunology 18 (2007) 115–121
Transplant Immunology
www.elsevier.com/locate/trim

Role of soluble tumor necrosis factor-related apoptosis-inducing ligand concentrations after stem cell transplantation

Shosaku Nomura [a,*], Kazuyoshi Ishii [a], Norihito Inami [b], Nobuhiko Uoshima [c], Hiroyuki Ishida [d], Takao Yoshihara [d], Hitoshi Kitayama [e], Kunio Hayashi [e]

[a] Division of Hematology, Kishiwada City Hospital, 1001, Gakuhara-cho, Kishiwada, Osaka 596-8501, Japan
[b] Second Department of Internal Medicine, Kansai Medical University, Moriguchi, Osaka, Japan
[c] Department of Internal Medicine, Matsushita Memorial Hospital, Moriguchi, Osaka, Japan
[d] Department of Pediatrics, Matsushita Memorial Hospital, Moriguchi, Osaka, Japan
[e] Department of Internal Medicine, Hoshigaoka Koseinennkinn Hospital, Hirakata, Osaka, Japan

Received 5 May 2007; accepted 21 May 2007

Abstract

Although stem cell transplantation (SCT) is being used for hematopoietic reconstitution following high-dose chemotherapy for malignancy, it involves certain serious transplant-related complications such as graft-versus-host disease (GVHD). Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) plays important roles in regulating cell death, immune response, and inflammation. However, the role of soluble TRAIL (sTRAIL) after SCT is poorly understood. In this study, 42 patients underwent SCT; 22 patients received allogeneic SCT, while the remaining 20 received autologous SCT. In these patients, levels of sTRAIL, cytokines, and soluble factors were measured by enzyme-linked immunosorbent assay (ELISA). In addition, a basic study of the generation of endothelial cell-derived microparticle (EDMP) by TNF-α and soluble Fas ligand (sFasL) was conducted. sFasL and EDMP exhibited significant elevation in the early phase (2–3 weeks) after SCT. In addition, the elevation of IL-6, TNF-α, and sIL-2R after allogeneic SCT was observed. EDMP also exhibited changes similar to sFasL. The patients with high sTRAIL exhibited significant decrease of sFasL and EDMP as compared with those without high sTRAIL. TNF-α and sFasL induced an increase in procoagulant and apoptotic markers in endothelial cells, and EDMP shedding was observed. Furthermore, sTRAIL inhibited the EDMP elevation caused by TNF-α and sFasL. The apoptotic markers such as sFasL and sTRAIL exhibited particular changes after SCT. Our results suggest that sTRAIL generation after allogeneic SCT relates to the prevention of GVHD.

Keywords: TRAIL; GVHD; Stem cell transplantation; sE-selectin; EDMP

1. Introduction

Stem cell transplantation (SCT) involves some serious transplant-related complications [1,2], such as graft-versus-host disease (GVHD), and vascular disorders, such as veno-occlusive disease (VOD), pulmonary vasculopathy, thrombotic microangiopathy (TMA), and capillary leak syndrome [3–5]. Although the complex pathophysiology of acute GVHD involves the conditioning regimen, cytokines, nitric oxide, and non-T effector cells, the cytolytic activity of donor T-cells is essential for the development of GVHD activity [6,7]. The cytolytic activity of cytotoxic T-lymphocytes (CTLs) is primarily mediated through certain effector mechanisms such as the Fas/FasL and perforin/granzyme pathways [8,9]. Interaction of FasL, expressed on the CTL cell surface, with the Fas receptor on the target cell membrane results in the initiation of the Fas cell death pathway [10]. Recent accumulating evidence indicates that the Fas/FasL system is implicated in the pathogenesis of acute GVHD [7,11–13].

Cellular microparticles are fragments that shed almost spontaneously from the plasma membrane blebs of virtually all cell types when subjected to a number of stress conditions [14,15]. In addition, these microparticles have more recently been shown to reflect in vitro cell stimulation, and testify to cellular activation and/or tissue degeneration occurring in vivo under various pathophysiologic conditions [14,15]. Thus, there is a

* Corresponding author. Tel.: +81 72 445 1000; fax: +81 72 532 1113.
E-mail address: shosaku-n@mbp.ocn.ne.jp (S. Nomura).

0966-3274/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.trim.2007.05.012

possibility that the cellular microparticles exhibit a dynamic change after SCT [16]. In contrast, diagnosing vascular complications in patients undergoing SCT is challenging, and damage to endothelial cells is regarded as the common feature of these complications [17,18]. Furthermore, endothelial damage, perpetuated by $CD8^+$CTL, has been linked to GVHD which is described in the skin and gut [18–22].

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)/Apo-2L is a member of the TNF family of cytokines, which are structurally related proteins playing important roles in regulating cell death, immune response, and inflammation [23]. TRAIL is a type II membrane protein, which can be proteolytically cleaved to a soluble form [24], as previously shown also for TNF-α and CD95 (Apo-1/Fas). The unique feature of TRAIL, as compared with other members of the TNF family, is its ability to induce apoptosis in a variety of malignant cells both in vitro and in vivo, displaying minimal toxicity on normal cells and tissues [25,26]. TRAIL interacts with four cellular receptors that form a distinct subgroup within the TNF receptor superfamily. TRAIL receptor 1 (TRAIL-R1 or DR4) and TRAIL receptor 2 (TRAIL-R2 or DR5) have cytoplasmic death domains and signal for apoptosis and NF-κB [27–29]. Two additional receptors, TRAIL receptor 3 (TRAIL-R3 or DcR1) and TRAIL receptor 4 (TRAIL-R4 or DcR2), are homologous to DR4 and DR5 in their cysteine-rich extracellular domain, but they lack intracellular death domains and apoptosis-inducing capability [30,31]. It has been shown that endothelial cells express TRAIL-R3 and TRAIL-R4 [30,32], further suggesting the relationship between TRAIL and endothelial function. Furthermore, there are several indications that TRAIL could be involved in the pathophysiology of autoimmune diseases [33–35]. However, the role of soluble TRAIL (sTRAIL) after SCT is poorly understood.

We measured and compared levels of sTRAIL, cytokines, and soluble factors in patients undergoing SCT. The results suggested that sTRAIL plays a unique role after SCT.

2. Materials and methods

2.1. Subjects

The subjects were 42 patients who underwent SCT between June 2001 and May 2006 at the institution of residence. In all, 22 patients received allogeneic SCT, while the remaining 20 received autologous SCT (Table 1). The 10 male and 12 female allogeneic SCT patients ranged in age from 6 to 68 years (median: 31 years), and the 12 male and 8 female autologous SCT patients ranged in age from 36 to 67 years (median: 51 years). Patient diagnoses consisted of 4 acute myeloid leukemia, 5 acute lymphoblastic leukemia, 2 chronic myeloid leukemia, 3 acute promyelocytic leukemia, 14 non-Hodgkin's lymphoma, 6 multiple myeloma, and 8 others. Conditioning applied was: total body irradiation for 13 and non-total body irradiation for 29. For allogeneic SCT, prophylaxis included cyclosporine for 19 patients with GVHD. The donor sources were 6 bone marrow transplantations, 10 peripheral blood stem cell transplantations, and 6 cord blood transplantations. Twenty-four patients received filgrastim and 18 received lenograstim. Written informed consent was obtained from all the patients.

2.2. Cytokine evaluation

Blood samples from each patient were collected into plastic tubes and immediately centrifuged to obtain serum. The serum was divided into aliquots and frozen at −30 °C until use. As a positive control, recombinant products were used in each assay, as well as standard solutions provided with the commercial kits. Human TNF-α, IFNγ, IL-4, and IL-6 ELISA kits were purchased from BioSource International, Inc. (Camarillo, California, USA). Serum levels of cytokines were measured according to the 'manufacturers' instructions. Normal ranges were as follows: TNF-α: 5–20 pg/ml, IFNγ: 0–12.5 pg/ml, IL-4: 0–3.5 pg/ml, and IL-6: 0.2–4.5 pg/ml.

Table 1
Clinical profiles of SCT patients

Characteristics	Allogeneic SCT	Autologous SCT
Gender		
Male/Female	10/12	12/8
Age (years) median (range)	31 (6–68)	51 (36–67)
Diagnosis		
Leukemia	AML: 4 APL: 3 ALL: 5 CML: 2	
Malignant lymphoma	DLBC: 1	DLBC: 8 FCL: 5
Others	AA: 1 MDS: 5 Renal cancer: 1	MM: 6 Lung cancer: 1
Conditioning		
TBI	CY: 5	L-MAP: 3 Flu/L-PAM: 5
Non-TBI	Flu: 3 Flu, Bu: 5 Flu, L-PAM: 4	VP-16, CY: 4 MCNU, IFO, CBDCA, VP-16: 8 MCNU, L-PAM, Ara C, VP-16: 5
Donor source	BMT: 6 PBSCT: 10 CBT: 6	
G-CSF		
Filgrastim	14	10
Lenograstim	8	10

AML: acute myeloblastic leukemia; APL: acute promyeloblastic leukemia; ALL: acute lymphoblastic leukemia; CML: chronic myeloblastic leukemia; DLBC: diffuse large B cell lymphoma; FCL: Follicular cell lymphoma; AA: aplastic anemia; MDS: myelodysplasia syndrome; MM: multiple myeloma; TBI: total body irradiation; CY: cyclophosphamide; Flu: fludarabine; L-PAM:melphalan; VP-16: etoposide; IFO: ifosfamide; CBDCA: carboplatin; BMT: bone marrow transplantation; PBSCT: peripheral blood stem cell transplantation; CBT: cord blood transplantation.

2.3. Measurement of sFasL, sTRAIL, sIL-2R, sVCAM-1 and sE-selectin

sFasL, sTRAIL, sIL-2R, sVCAM-1, and sE-selectin ELISA kits were purchased from BioSource International Inc. For measurement of sFasL, sTRAIL, sIL-2R, sVCAM-1 and sE-selectin in serum, all the kits were used according to the manufacturers' instructions. Normal ranges were as follows: sFasL: 0.02–0.14 ng/ml, sTRAIL: 100–500 pg/ml, sIL-2R: 150–450 IU/ml, sVCAM-1: 395–714 ng/ml, and sE-selectin: 23.0–79.2 ng/ml.

2.4. Assessment of endothelial cell-derived microparticle (EDMP)

EDMPs were detected using a previously reported method with some modifications [36]. A 10-μl aliquot of washed intact platelets (3×10^8/ml) was added to the plasma, and the mixture was incubated for 30 min in dark at room temperature, with FITC-labeled Annexin V (FITC-Ann V) and phycoerythrin (PE)-labeled CD51 ($\alpha v\beta 3$) to detect EDMP. The samples were diluted 1:10 with HEPES-Tyrodes buffer containing 5 mmol/l EGTA and analyzed using the Ortho Cytoron Absolute Analyzer (Ortho Diagnostic Systems, Inc., Tokyo, Japan), set to detect only the particles bound to FITC-labeled Annexin V and PE-labeled CD51. This method was designed to ensure the detection of only procoagulant EDMP. The concentrations of these microparticles were then calculated per μl of the whole blood.

2.5. Activation of endothelial cells

Endothelial cells isolated from freshly obtained human umbilical cord veins were cultured according to the method of Jaffe et al. [37]. Second-passage cells

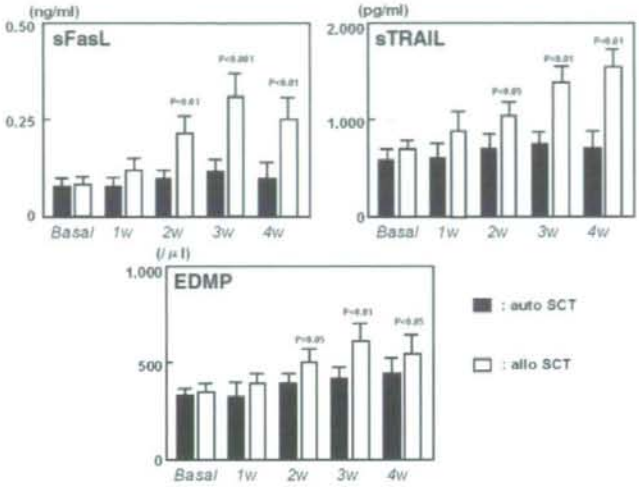

Fig. 1. Changes of apoptotic markers and EDMP in patients undergoing allogeneic and autologous SCT. Blood samples were obtained on days 0 (basal), 7 (1 weeks), 14 (2 weeks), 21 (3 weeks), and 28 (4 weeks) after the transplantation. Error bars show standard error. Student's *t*-test was used for statistical comparisons.

were grown to confluence in 25 cm2 culture flasks (3–4 days). The cells were subcultured in 12-well plates containing M-199 with growth supplement, fetal bovine serum, and heparin, after which they were washed once with 10-mM EDTA in phosphate-buffered saline to remove calcium-dependent binding proteins such as vitamin K-dependent coagulation factors. EDTA was subsequently removed by three washes with 0.4% fetal bovine serum albumin. Then, the cells were incubated at 37 °C with 10 ng/ml of TNF-α (BioSource International Inc.), 20 ng/ml of sFasL (Alexis Biochemicals, San Diego, CA), or 100 ng/ml of sTRAIL (BioSource International Inc.) in M-199, 0.4% BSA. Samples of the cells containing FITC-labeled anti-annexin V or PE-labeled anti-APO 2.7 antibodies (MBL Inc., Nagoya, Japan) were added to Falcon tubes and analyzed using the Ortho Cytoron Absolute Analyzer.

2.6. Confocal laser scanning microscopy

Microwells were prepared by attaching a Flexiperm chamber (Heraeus Instruments, Osterode, Germany) onto a cover glass. Samples of each cell line containing FITC-labeled anti-CD9 antibody (NNKY1-19)[38] were added to the microwells and incubated for the indicated times. For each sample, 6 or 8 optical sections separated by 0.5-μm steps were recorded via a Carl Zeiss Plan-Apo 63×1.4 objective. Differential interference contrast (DIC) images and fluorescent confocal (FC) images were obtained simultaneously, and every picture was a combination of four accumulated frames. FITC fluorescence was detected at an excitation wavelength of 488 nm with a barrier filter at 500 nm. Individual images were exported to Adobe Photoshop, and the slides were printed using Fujix Pictrography 3000 (Fuji Photo Film, Tokyo, Japan).

2.7. Statistical analysis

Results are shown as the mean±standard deviations. Student's *t*-tests were used for statistical comparisons. Linear regression analysis was used to compare sTRAIL and other factors. A p value <0.05 was considered statistically significant.

3. Results

3.1. SCT-related complications

Twenty-two patients who received allogeneic SCT developed acute GVHD (grade I, 11; grade II, 8; grade III, 2; grade IV, 1). Three patients (grade III and IV) who received allogeneic SCT had severe complications and died after the procedure. Another who had received autologous SCT suffered from severe sepsis. Therefore, three patients – two from those who received allogeneic SCT and one who received autologous SCT – were excluded from the analysis of the present study.

3.2. Changes in sFasL, sTRAIL and EDMP

Fig. 1 shows the changes in sFasL, sTRAIL, and EDMP levels after SCT. The level of sFasL in the group that received allogeneic SCT peaked within 3 weeks (0.31±0.06 ng/ml, $p<0.001$) and then decreased. The level of sTRAIL in allogeneic SCT continued to increase for up to 4 weeks (2 weeks, 1012±89 pg/ml, $p<0.05$; 3 weeks, 1376±72 ng/ml, $p<0.01$; 4 weeks, 1589±143 ng/ml, $p<0.01$). The level of EDMP showed the same tendency as sFasL (allogeneic SCT: 2 weeks, 497±51/μl, $p<0.05$; 3 weeks, 597±85/μl, $p<0.01$, 4 weeks, 515±56/μl, $p<0.05$). In contrast, level of sFasL, sTRAIL, and EDMP in the

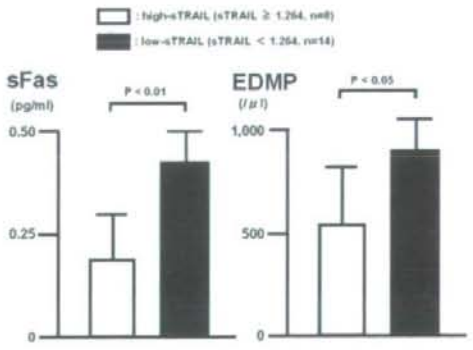

Fig. 2. sFasL and EDMP levels in patients after allogeneic SCT with and without high-sTRAIL. Values presented as mean±standard error.

Table 2
Changes of cytokines and soluble factors with SCT

Cytokine/factor	Before SCT	After SCT			
		1 week	2 weeks	3 weeks	4 weeks
Allo SCT					
IFNγ (pg/ml)	9.4±0.5	11.6±0.7	13.1±0.5	14.2±1.1*	14.8±0.9**
IL-4 (pg/ml)	5.5±0.4	5.4±0.6	5.8±0.3	5.9±0.4	5.5±0.6
TNF-α(pg/ml)	12.2±0.8	16.5±1.2	24.4±1.8*	28.3±5.9**	23.8±1.7*
IL-6 (pg/ml)	21.1±6.2	34.8±6.7	51.9±14.1**	49.8±10.5*	47.4±15.2*
sIL-2R (IU/ml)	729±62	753±72	1029±68*	1129±55*	1331±97**
sVCAM-1(pg/ml)	962±65	1133±74	1362±94*	1577±92**	1612±121**
sE-selectin(ng/ml)	66.7±3.9	72.1±4.6	90.8±4.8*	117.2±5.7**	128.5±7.4**
Auto SCT					
IFNγ (pg/ml)	6.3±0.9	6.1±0.8	5.8±0.6	5.9±0.5	6.3±0.9
IL-4 (pg/ml)	3.5±0.5	3.7±0.6	3.4±0.7	3.1±0.7	2.9±0.8
TNF-α(pg/ml)	8.7±1.2	10.1±1.4	14.9±1.8*	10.5±1.1	9.7±0.8
IL-6 (pg/ml)	12.3±2.2	38.7±22.4*	42.6±12.7*	19.6±5.3	15.9±4.5
sIL-2R (IU/ml)	855±149	1455±364*	1427±152*	1163±168	1037±114
sVCAM-1 (pg/ml)	1,214±85	1161±121	1447±64*	1249±95	1124±121
sE-selectin (ng/ml)	84.5±10.6	79.8±4.8	91.5±10.4*	119.2±13.3*	94.7±9.5

Data represent means±standard error. TNF-α: tumor necrosis factorα; IFNγ: interferon γ; IL-4: interleukin 4; IL-6; interleukin 6 sIL-2R: soluble interleukin-2 receptor; sVCAM-1: soluble vascular cell adhesion molecule-1; sE-selectin: soluble E-selectin.

recipients of autologous SCT did not show significant changes. There were no significant correlations between sTRAIL, sFasL, and EDMP in the recipients of allogeneic SCT.

Fig. 2 shows the changes in levels of sFasL and EDMP at 4 weeks in the recipients of allogeneic SCT with elevated sTRAIL levels. sFas and EDMP levels at 4 weeks in allogeneic SCT with elevated sTRAIL (greater than the mean+two standard deviations of basal levels) were used for the analysis. Eight patients had high sTRAIL levels (sTRAIL>1264 pg/ml). The patients with high sTRAIL exhibited significant decrease of sFasL and EDMP as compared with those without high sTRAIL.

3.3. Changes in serum cytokines and soluble factors

The level of cytokines and soluble factors before and after SCT is shown in Table 2. IFNγ levels were found to be significantly higher 3 weeks after allogeneic SCT than beforehand, while IL-4 and IFNγ levels after autologous SCT remained almost unchanged. In contrast, TNF-α, IL-6, sIL-2R, sVCAM-1, and sE-selectin exhibited a significant elevation after both autologous and allogeneic SCT, although the changes after autologous SCT were temporary. Levels of sIL-2R, sVCAM-1, and sE-selectin after allogeneic SCT continued to increase for up to 4 weeks. Table 3 shows the relationship between sFasL or sTRAIL and cytokines/soluble factors in allogeneic SCT. sFasL levels correlated positively with TNF-α, IL-4, IL-6, sIL-2R, sVCAM-1, and sE-selectin. In contrast, sTRAIL levels correlated negatively with sIL-2R, sVCAM-1, and sE-selectin.

Table 3
Relationship between sFasL, sTRAIL, and cytokines/soluble factors in allogeneic SCT

	sFasL (allogeneic SCT)		sTRAIL (allogeneic SCT)	
	r	*p* value	*r*	*p* value
TNFα	0.2931	<0.01	−0.1925	N.S.
IFNγ	−0.1242	N.S.	0.1301	N.S.
IL-4	0.2194	<0.05	0.1455	N.S.
IL-6	0.2287	<0.05	−0.1882	N.S.
sIL-2R	0.3325	<0.01	−0.2478	<0.05
sVCAM-1	0.2946	<0.01	−0.2451	<0.05
sE-selectin	0.3842	<0.01	−0.3317	<0.01

Statistically significant *p* values are underlined.

3.4. Relationship between sTRAIL, sFasL, and EDMP in activated endothelial cells

Fig. 3 shows endothelial cell activation and EDMP shedding caused by TNF-α and sFasL stimulation. TNF-α and sFasL increased binding of the procoagulant marker annexin V and the apoptotic marker APO2.7. Fig. 4 shows the release of EDMP from endothelial cells caused by TNF-α and sFasL, detected by flow cytometry using FITC-

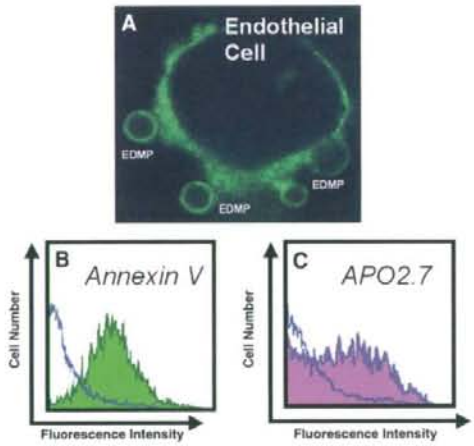

Fig. 3. Confocal microscopic images of the immunoreactivity and flow cytometric analysis of activated endothelial cells. Activated endothelial cells were stained with FITC-conjugated anti-CD9 antibody (A). In flow cytometric analysis, endothelial cells were stained using FITC-labeled Annexin V and PE-APO2.7 (B & C). Blue histogram is the control (unstimulated) (B & C).

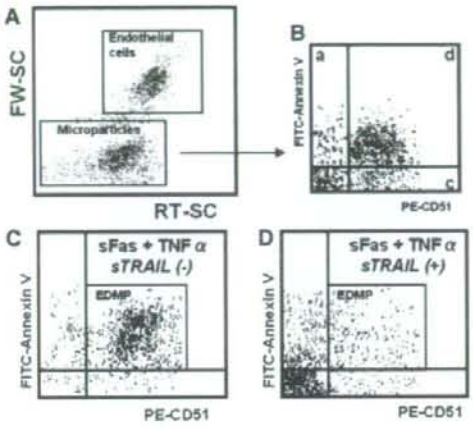

Fig. 4. Flow cytometric analysis of EDMP. Only particles positive for FITC-labeled Annexin V and PE-CD51 were gated to distinguish endothelial cells and EDMP from electric noise (A and B). EDMPs were elevated by stimulation of sFasL and TNF-α (C), and this elevation was inhibited with the addition of sTRAIL (D). Representative data from one of the five independent experiments are shown.

labeled Annexin V and PE-labeled CD51 (Fig. 4A, B). TNF-α and sFasL promoted the release of EDMP (Fig. 4C); however, this effect was inhibited by the addition of sTRAIL (Fig. 4D).

4. Discussion

TRAIL is considered to be the peculiar molecule that triggers apoptosis through interaction with the death receptor [39]. Many studies have shown that both the membrane-bound and the soluble extracellular domain of TRAIL can induce apoptosis in a wide variety of tumor cell lines without affecting most of the normal cells [25,26,40,41]. Furthermore, repeated administration of recombinant and biologically active sTRAIL can induce tumor cell apoptosis, suppress tumor progression, and improve survival in tumor-bearing mice [25,26,40]. Some studies suggest, however, that sTRAIL could not induce apoptosis in normal cells [39,42]. On the other hand, SCT is an established method for treating various hematological diseases, and it also provides an opportunity to trace the process of hematopoietic reconstitution in vivo. Although many cytokines are known to control the process of hematopoiesis, the role of TRAIL in this process is not well understood.

In the present study, several apoptotic markers were measured before SCT and serially after SCT. sFasL and EDMP exhibited significant elevation in the early phase (2 or 3 weeks) after SCT (Fig. 1). Cytokines and soluble factors were also measured.

It has previously been reported that certain cytokines and soluble factors are useful for the diagnosis of GVHD after allogeneic SCT. Proinflammatory cytokines including IFNγ, IL-6, and TNF-α are important mediators and regulators of GVHD [43,44]. sIL-2R appears to be a convenient marker for the detection of acute GVHD[45]. In the present study, the involvement of apoptotic markers in the elevation of IL-6, TNF-α, and sIL-2R after allogeneic SCT appeared to be important, since sFasL exhibited the same changes as these cytokines and soluble factors [46–48]. This suggests the possibility that sFasL plays a role in GVHD after allogeneic SCT [11–13,49].

EDMP also exhibited changes similar to those of sFasL. It is reported that EDMP exhibit a dynamic change after SCT [16]. In particular, the increase of EDTA in the TMA/thrombotic thrombocytopenic purpura (TTP) case was quite remarkable [16,50]. In the present study, TNF-α and sFasL induced the increase of procoagulant and apoptotic markers in the endothelial cells (Fig. 3). In addition, EDMP shedding was also observed in this experiment (Fig. 3). Therefore, it is possible to associate EDMP with apoptosis.

Although the molecular pathogenesis of GVHD remains to be uncovered, there is a general agreement that infiltrating T lymphocytes play a central role [51–53]. Some studies have suggested that the expression of TNF and TRAIL can also contribute to CTL acytotoxicity [54,55]. Endothelial cells are targets of CTL [22], and Li et al. [56] reported that TRAIL induces apoptosis in human endothelial cells. However, several other reports showed that TRAIL promotes the survival of human vascular endothelial cells, since endothelial cells have decoy receptors such as TRAIL-R3 and-R4 that protect cells from apoptosis [57–60]. In addition, some reports exhibited that TRAIL inhibits activation of antigen-specific T-cells via blockade of cell cycle progression, and results in preventing GVHD [61–63].

In the present study, the patients with high sTRAIL exhibited significant decrease of sFasL and EDMP as compared with those without high sTRAIL (Fig. 2). Furthermore, sTRAIL inhibited EDMP elevation caused by TNF-α and sFas (Fig. 4). Our results support previous reports regarding the preventive effect of sTRAIL on GVHD. When lymphocyte or monocyte are activated after SCT, they could cause the generation of sTRAIL. In this manner, sTRAIL generation after allogeneic SCT makes it possible to control GVHD. However, further examination will be necessary to establish the exact mechanism of control.

In conclusion, we measured and compared levels of cytokines and soluble factors in patients undergoing SCT. The apoptotic markers such as sFasL and sTRAIL exhibited particular changes after SCT. Our results suggest that sTRAIL generation after allogeneic SCT relates to the prevention of GVHD.

Acknowledgments

This work was partly supported by a grant from the Japan Foundation of Cardiovascular Research and by a Research Grant for Advanced Medical Care from the Ministry of Health and Welfare of Japan.

References

[1] Jutmer CA, To LB, Ho J, Bardy PG, Dyson PG, Haylock DN, et al. Early lympho-hematopoietic recovery after autografting using peripheral blood stem cell in acute nonlymphoblastic leukemia. Transplant Proc 1998;20(1):40–2.

[2] Roberts MM, To LB, Gillis D, Mundy J, Rawing C, Ng K, et al. Immune reconstitution following peripheral blood stem cell transplantation,

autologous bone marrow transplantation and allogeneic bone marrow transplantation. Bone Marrow Transplant 1993;12(5):469–75.
[3] Rowbottom AW, Riches PG, Downie C, Hobbs JR. Monitoring cytokine production in peripheral blood during acute graft-versus-host disease following allogeneic bone marrow transplantation. Bone Marrow Transplant 1993;12(6):635–41.
[4] Testa S, Manna A, Porcellini A, Maffi F, Morstabilini G, Denti N, et al. Increased plasma level of vascular endothelial glycoprotein thrombomodulin as an early indicator of endothelial damage in bone marrow transplantation. Bone Marrow Transplant 1996;18(2):383–8.
[5] Nishida T, Hamaguchi M, Hirabayashi N, Haneda M, Terakura S, Atsuta Y, et al. Intestinal thrombotic microangiopathy after allogeneic bone marrow transplantation: a clinical imitator of acute enteric graft-versus-host disease. Bone Marrow Transplant 2004;33(11):1143–50.
[6] Krenger W, Hill GR, Ferrara JL. Cytokine cascades in acute graft-versus-host disease. Transplantation 1997;64(4):553–8.
[7] Schmaltz C, Alpdogan O, Horndasch KJ, Muriglan SJ, Kappel BJ, Teshima T, et al. Differential use of Fas ligand and perforin cytotoxic pathway by donor T-cells in graft-versus-host disease and graft-versus-leukemia effect. Blood 2001;97(9):2886–95.
[8] Kagi D, Vignaux F, Ledermann B, Burki K, Depraetere V, Nagata S, et al. Fas and perforin pathways as major mechanisms of T-cell-mediated cytotoxicity. Science 1994;265(5171):528–30.
[9] Lowin B, Hahne M, Mattmann C, Tschopp J. Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways. Nature 1994;370 (6491):650–2.
[10] Nagata S. Apoptosis by death factor. Cell 1997;88(3):355–65.
[11] Das H, Imoto S, Murayama T, Kajimoto K, Sugimoto T, Isobe T, et al. Levels of soluble Fas and FasL gene expression during the development of graft-versus-host disease in DLT-treated patients. Br J Haematol 1999;104 (4):795–800.
[12] Takada S, Hatsumi N, Saito T, Matsushima T, Sakura T, Tamura J, et al. Two cases of chronic graft-versus-host disease with elevated levels of soluble Fas ligand in serum. Am J Hematol 2000;64(2):133–6.
[13] Jaksch M, Uzunel M, Martinez Cangana G, Remberger M, Mattsson J. Increased of immune transcript in patients with acute GVHD after allogeneic stem cell transplantation. Bone Marrow Transplant 2003;31(3): 183–90.
[14] Freyssinet JM. Cellular microparticles: what are they bad or good for? J Thromb Haemost 2003;1(7):1655–62.
[15] Jy W, Horstmann LL, Jimenez JJ, Ahn YS, Biro E, Niewland R, et al. Measuring circulating cell-derived microparticles. J Thromb Haemost 2004;2(10):1842–51.
[16] Nomura S, Ishii K, Kanazawa S, Inami N, Uoshima N, Ishida H, et al. Significance of elevation in cell-derived microparticles after allogeneic stem cell transplantation: transient elevation of platelet-derived microparticles in TMA/TTP. Bone Marrow Transplant 2005;36(10):921–2.
[17] Dickinson AM, Wang XN, Sviland L, Vyth-Dreese FA, Jackson GH, Schumacher TN, et al. I situ dissection of the graft-versus-host activities of cytotoxic T-cells specific for minor histocompatibility antigens. Nat Med 2002;8(4):410–4.
[18] Woywodt A, Scheer J, Hambach L, Buchlolz S, Ganser A, Haller H, et al. Circulating endothelial cells as a marker of endothelial damage in allogeneic hematopoietic stem cell transplantation. Blood 2004;103(9):3603–5.
[19] Dumler JS, Beschorner WE, Farmer ER, Gennaro KADi, Saral R, Santos GW. Endothelial-cell injury in cutaneous acute graft-versus-host disease. Am J Pathol 1989;135(6):1097–103.
[20] Marelli-Berg FM, James MJ, Dangerfield J, Dyson J, Millrain M, Scott D, et al. Cognate recognition of the endothelium induces HY-specific CD8+ T-lymphocyte transendothelial migration (diapedesis) in vivo. Blood 2004;103(8):3111–6.
[21] Ertault-Daneshpouy M, Leboeuf C, Lemann M, Bouhidel F, Ades L, Gluckman E, et al. Pericappillary hemorrhage as criterion of serve human digestive graft-versus-host disease. Blood 2004;103(12):4681–4.
[22] Kummer M, Lev A, Reiter Y, Biedermann BC. Vascular endothelial cells have impaired capacity to present immunodominant, antigenic peptides: a mechanism of cell type-specific immune escape. J Immunol 2005;174 (4):1947–53.
[23] Baker SJ, Reddy EP. Transducers of life and death: TNF receptor superfamily and associated proteins. Oncogene 1996;12(1):1–9.
[24] Mariani SM, Krammer PH. Differential regulation of TRAIL and CD95 ligandin transformed cells of the T and B lymphocyte lineage. Eur J Immunol 1998;28(3):973–82.
[25] Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M, et al. Tumoricidalactivity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nat Med 1999;5(2):157–63.
[26] Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, et al. Safety and antitumor activity of recombinant soluble Apo 2 ligand. J Clin Invest 1999;104(2):1551–15562.
[27] Pan G, O'Rourke K, Chinnaiyan AM, Gentz Rebner R, Ni J, Dixit VM. The receptor for the cytotoxic ligand TRAIL. Science 1997;276(5309):111–3.
[28] Wu GS, Burns TF, McDonald ERR, Jiang W, Meng R, Krantz ID, et al. KILLER.DR5 is a DNA damage-inducible p53-regulated death receptor gene. Nat Genet 1997;17(2):141–3.
[29] Schneider P, Thome M, Burns K, Bodmer JI, Hofmann K, Kataoka T, et al. TRAIL receptors 1 (DR4) and 2 (DR5) signal FADD-dependent apoptosis and activate NF-κB. Immunity 1997;7(6):831–6.
[30] Sheridan JP, Marsters SA, Pitti PM, Gurney A, Skubatch M, Baldwin D, et al. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. Science 1997;277(5327):818–21.
[31] Mongkolsapaya J, Cowper AE, Xu XN, Morris G, McMichael AJ, Bel JL, et al. Lymphocyte inhibitor of TRAIL (TNF-related apoptosis-inducing ligand): a new receptor protecting lympnocytes from the death ligand TRAIL. J Immunol 1998;160(1):3–6.
[32] Zhang XD, Nguyen T, Thomas WD, Sanders JE, Hersey P. Mechanisms of resistance of normal cells to TRAIL induced apoptosis vary between different cell types. FEBS Lett 2000;482(3):193–9.
[33] Matsumura R, Umemiya K, Kagami M, Tomioka H, Tanabe E, Sugiyama T, et al. Expression of TNF-related apoptosis inducing ligand (TRAIL) on infiltrating cells and of TRAIL receptors on salivary glands in patients with Sjogren's syndrome. Clin Exp Rheumatol 2002;20(6):791–8.
[34] Matsuyama W, Yamamoto M, Higashimoto I, Oonakahara KI, Watanabe M, Machida K, et al. TNF related apoptosis inducing ligand is involved in neutropenia of systemic lupus erythematosus. Blood 2004;104(1):184–91.
[35] Lub-de Hooge MN, de Vries EGE, de Jong S, Bijl M. Soluble TRAIL concentrations are raised in patients with systemic lupus erythematosus. Ann Rheum Dis 2005;64(6):854–8.
[36] Nomura S, Shouzu A, Omoto S, Nishikawa M, Iwasaka T, Fukuhara S. Activated platelet and oxidized LDL induce endothelial membrane vesiculation: clinical significance of endothelial cell-derived microparticles in patients with type 2 diabetes. Clin Appl Thromb Hemost 2004;10(3): 205–15.
[37] Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins. J Clin Invest 1973;52 (11):2745–56.
[38] Ozaki Y, Satoh K, Kuroda K, Qi R, Yatomi Y, Yanagi S, et al. Anti-CD9 monoclonal antibody activates $p72^{syk}$ in human platelets. J Biol Chem 1995;270(25):15119–24.
[39] Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995;3(6):673–82.
[40] Kagawa S, He C, Gu J, Koch P, Rha SJ, Roth JA, et al. Antitumor activity and bystander effects of the tumor necrosis factorelated apoptosis-inducing ligand (TRAIL) gene. Cancer Res 2001;61(8):3330–8.
[41] Shi J, Zheng D, Liu Y, Sham MH, Tam P, Farzaneh F, et al. Overexpression of soluble TRAIL induces apoptosis in human lung adenocarcinoma and inhibits growth of tumor xenografts in nude mice. Cancer Res 2005;65 (5):1687–92.
[42] Jeremias I, Herr I, Bochler T, Debatin KM. TRAIL/Apo-2-ligand-induced apoptosis in human T cells. Eur J Immunol 1998;28(1):143–52.
[43] Ferrara JL, Cooke KR, Pan L, Krenger W. The immunopathophysiol of acute graft-versus-host-disease [review]. Stem Cells 1996;14(5):473–89.
[44] Schots R, Kaufman L, Van Riet I, Ben Othman T, De Waele M, Van Camp B, et al. Proinflammatory cytokines and their role in the development of major transplant-related complications in the early phase after allogeneic bone marrow transplantation. Leukemia 2003;17(6):1150–6.

[45] Grimm J, Zeller W, Zander AR. Soluble interleukin-2 receptor serum levels after allogeneic bone marrow transplantations as a marker for GVHD. Bone Marrow Transplant 1998;21(1):29–32.
[46] Liem LM, van Houwelingen HC, Goulmy E. Serum cytokine levels after HLA-identical bone marrow transplantation. Transplantation 1998;66 (7):863–71.
[47] Min CK, Lee MY, Min DJ, Lee DG, Kim YJ, Park YH, et al. The kinetics of circulating cytokines including IL-6, TNF-α, IL-8 and IL-10 following allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2001;28(10):935–40.
[48] Lee WY, Baek KH, Rhee EJ, Tae HJ, Oh KW, Kang MI, et al. Impact of circulating bone-resorbing cytokines on the subsequent bone loss following bone marrow transplantation. Bone Marrow Transplant 2004;34(1):89–94.
[49] Kanda Y, Tanaka Y, Shirakawa K, Yatomi T, Nakamura N, Kami M, et al. Increased soluble Fas-ligand in sera of bone marrow transplant recipients with acute graft-versus-host disease. Bone Marrow Transplant 1998;22(8):751–4.
[50] Jimenez JJ, Jy W, Mauro LM, Horstman LL, Soderland C, Ahn YS. Endothelial microparticles released in thrombotic thrombocytopenic purpura express von Willebrand factor and markers of endothelial activation. Br J Haematol 2003;123(5):896–902.
[51] Rus V, Svetic A, Nguyen P, Gause WC, Via CS. Kinetics of TH1 and Th2 cytokine production during the early course of acute and chronic murine graft-versus-host disease. J Immunol 1995;155(5):2396–406.
[52] Murai M, Yoneyama H, Harada A, Yi Z, Verstegaard C, Guo B, et al. Active participation of CCR5$^+$CD8$^+$T lymphocytes in the pathogenesis of liver injury in graft-versus-host disease. J Clin Invest 1999;104(1):49–57.
[53] Visentainer JE, Lieber SR, Persoli LB, Vigorito AC, Aranha FJ, de Brito Eid KA, et al. Serum cytokine levels and acute graft-versus-host disease after HLA-identical hematopoietic stem cell transplantation. Exp Hematol 2003;31(11):1044–50.
[54] Ware CF, Van Arsdale TL, Crowe PD, Browning JL. The ligands and receptors of the lymphotoxin system. Curr Top Microbiol Immunol 1995;198:175–218.
[55] Thomas WD, Hersey P. TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis in Fas ligand-resistant melanoma cells and mediates CD4 T cell killing of target cells. J Immunol 1998;161(5):2195–200.
[56] Li JH, Kirkiles-Smith NC, McNiff JM, Pober JS. TRAIL induces apoptosis and inflammatory gene expression in human endothelial cells. J Immunol 2003;171(3):1526–33.
[57] Zauli G, Pandolfi A, Gonelli A, Di Pietro R, Cuarnieri S, Ciabattoni G, et al. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) sequentially upregulates nitric oxide and prostanoid production in primary human endothelial cells. Circ Res 2003;92(7):732–40.
[58] Secchiero P, Gonelli A, Carnevale E, Milani D, Pandolfi A, Zella D, et al. TRAIL promotes the survival and proliferation of primary human vascular endothelial cells by activating the Akt and ERK pathways. Circulation 2003;107(17):2250–6.
[59] Secchiero P, Crallini F, di Lasio MG, Gonelli A, Barbarotto E, Zauli G. TRAIL counteracts the proadhesive activity of inflammatory cytokines in endothelial cells by down-modulating CCL8 and CXCL10 chemokine expression and release. Blood 2005;105(9):3413–9.
[60] Di Pietro R, Mariggio MA, Guarnieri S, Sancilio S, Giardinelli A, Di Silvestre S, et al. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) regulates endothelial nitric oxide synthase (eNOS) activity and its localization within the human vein endothelial cells (HUVEC) in culture. J Cell Biochem 2006;97(4):782–94.
[61] Song K, Chen Y, Goke R, Wilmen A, Sedel C, Goke A, et al. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is an inhibitor of autoimmune inflammation and cell cycle progression. J Exp Med 2000;191(7): 1095–104.
[62] Lunemann JD, Waiczies S, Ehrlich S, Wendling U, Seeger B, Kamradt T, et al. Death ligand TRAIL induces no apoptosis but inhibits activation of human (auto) antigen-specific T cells. J Immunol 2002;168(10):4881–8.
[63] Sato K, Nakaoka T, Yamashita N, Yagita H, Kawasaki H, Morimoto C, et al. TRAIL-transduced dendritic cells protect mice from acute graft-versus-host disease and leukemia relapse. J Immunol 2005;174(7):4025–33.

Int J Hematol (2008) 87:236–238
DOI 10.1007/s12185-008-0049-8

LETTER TO THE EDITOR

Regional differences exist in allogeneic stem cell transplantation rates for acute leukemia

Yuji Miura · Takuhiro Yamaguchi · Takayuki Azuma · Tamae Hamaki · Yuko Kodama · Eiji Kusumi · Tomoko Matsumura · Toshihiko Nakamura · Masahiro Kami · Tsunehiko Komatsu

Received: 1 November 2007 / Revised: 21 December 2007 / Accepted: 26 December 2007 / Published online: 22 February 2008

Allogeneic stem-cell transplantation (allo-SCT) is a curative treatment for advanced hematologic malignancies, although it is frequently accompanied by severe complications and the practice is usually limited to experienced medical teams [1]. Regional differences in indications and management strategies for allo-SCT among physicians and institutions have been indicated; however, their existence and factors associated with them among physicians and institutions remain unknown. We examined the differences in allo-SCT rates for acute leukemia among prefectures and districts in Japan, and investigated the association between the differences and several possible factors. Japan's prefectures comprise the 47 administrative units of the country, and districts make up ten areas divided according to geographical and historical backgrounds: Hokkaido, Tohoku, Koshinetsu, Kanto, Tokai, Hokuriku, Kinki, Chugoku, Shikoku, and Kyushu.

Since 97% of the patients who underwent allo-SCT for acute leukemia from 2000 to 2004 were younger than 60 years in Japan [2], we assumed that allo-SCT was indicated in patients aged less than 60. We defined allo-SCT rates as the rate of the number of patients who underwent allo-SCT to that of patients with acute leukemia. We investigated the number of patients with acute leukemia aged less than 60 as a denominator and the number of those who underwent allo-SCT under the age of 60 as a numerator. We estimated the annual number of new patients with acute leukemia under the age of 60 in each prefecture and district, using the incidences of acute leukemia by age-groups in Japan [3] and the population by age-groups in each prefecture according to a census in 2004 [4]. We defined the number of treated patients in each district as the estimated number of patients with acute leukemia, while the number of patients in each prefecture was adjusted by patient migration to and from other prefectures [5]. The number of patients who underwent allo-SCT for acute leukemia under the age of 60 in each prefecture and district from 2000 to 2004 was obtained from the annual report in 2005 published by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) [2]. We assumed the percentage of acute leukemia to the primary diseases for which allo-SCT was indicated in each prefecture to be the same as the national average (83%) during the same time period [2], because JSHCT reports the total numbers of the primary diseases for which allo-SCT was indicated in Japan without the breakdown of data by prefecture. We investigated the association between the differences and several possible factors using the Spearman correlation coefficient (significance level 0.05). They included physicians per unit population [6], the numbers of

Y. Miura (✉) · T. Komatsu
Department of Hematology, Teikyo University Chiba Medical Center, 3426-3, Anesaki, Ichihara, Chiba 299-0111, Japan
e-mail: yujmiura@gmail.com

T. Yamaguchi
Department of Clinical Trial Data Management, Graduate School of Medicine, The University of Tokyo Hospital, Tokyo, Japan

T. Azuma
Department of Hematology, Tsukuba Memorial Hospital, Tsukuba, Japan

T. Hamaki · Y. Kodama · E. Kusumi · T. Matsumura · M. Kami
Division of Exploratory Research, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

T. Nakamura
Division of Social Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan

hematologists per unit population [7], the numbers of allo-SCT institutions per unit population [2], and incomes per person [8] in prefectures and districts.

The estimated number of patients who developed acute leukemia under the age of 60 years from 2000 to 2004 in Japan was 3,075 per year. The number of patients who underwent allo-SCT for acute leukemia under the age of 60 during this period was 1,633 per year. The allo-SCT rate in Japan was 53%.

The allo-SCT rates by district were high in the west and low in the east (Fig. 1a), with a maximum 2.1-fold difference (31 vs. 65%) [95% confidence interval (CI): 1.9 to 2.4-fold]. There was a trend between the allo-SCT rates by district and the numbers of hematologists per unit population ($r = 0.5627$, $P = 0.0963$), while no association was found between the numbers of physicians per unit population ($r = 0.4012$, $P = 0.2475$), allo-SCT institutions per unit population ($r = 0.4233$, $P = 0.2182$), or incomes per person ($r = 0.1255$, $P = 0.7298$).

The allo-SCT rates by prefecture are shown in Fig. 1. There was a maximum 17.9-fold difference (5.6 vs. 100%) (95% CI 11.0 to 29.3-fold). No prefectures in Tohoku or Koshinetsu districts had allo-SCT rates exceeding 60%. The allo-SCT rates by prefecture were significantly associated with the numbers of physicians, hematologists, and allo-SCT institutions per unit population [$r = 0.4354$ ($P = 0.0022$), $r = 0.5773$ ($P < 0.0001$), $r = 0.5255$ ($P = 0.0001$)]; these three factors were significantly associated with each other. There was no association between the allo-SCT rates by prefecture and incomes per person ($r = 0.1147$, $P = 0.4426$). The year of the foundation of medical faculties in each prefecture was associated with the allo-SCT rates (Fig. 2).

The present study demonstrated that the allo-SCT rates for acute leukemia were different among districts in Japan, with a maximum 2.1-fold difference. The results were comparable with previous reports from Europe; Gratwohl et al. [9] reported a maximum 2.8-fold difference in the numbers of allo-SCT per unit population in Western Europe. However, we need to be aware of differences in the backgrounds, when our study is compared with the report by Gratwohl et al. Their study on allo-SCT activities in different countries in Western Europe with varying medical administrative systems sharply contrasts to ours based on a single country managed by a single administrative system. Since each district constitutes an independent medical service area with negligible patient migration among districts in Japan [5], the present study suggests that factors other than administrative systems affect the difference in allo-SCT activities among districts. Identifying these factors and taking countermeasures are important for the spread of allo-SCT.

Our study suggested that the number of hematologists per unit population possibly affects the allo-SCT rates by district. This observation implies that education to increase the number of physicians who can manage allo-SCT is necessary for its dissemination. As education to increase numbers of physicians is limited to medical faculties at

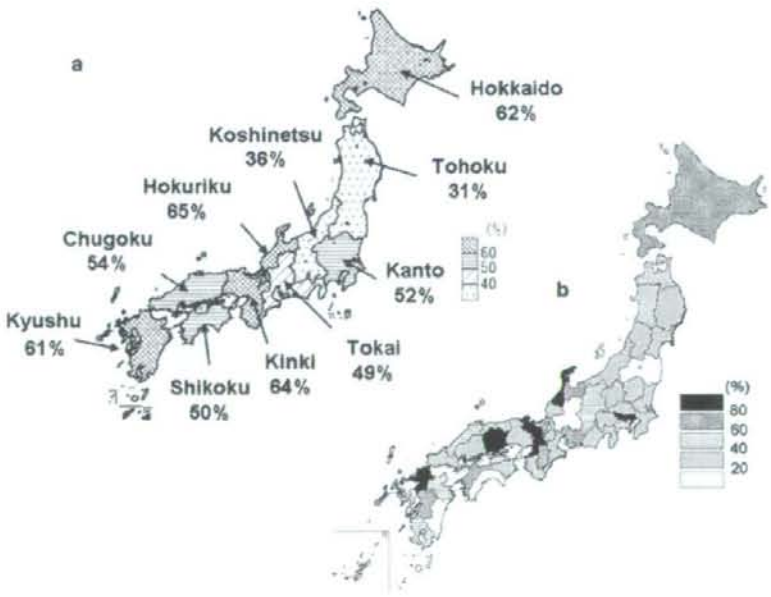

Fig. 1 **a** Allogeneic stem cell transplantation rates by district, **b** allogeneic stem cell transplantation rates by prefecture. The allogeneic stem cell transplantation rates were 62% in Hokkaido, 31% in Tohoku, 36% in Koshinetsu, 52% in Kanto, 49% in Tokai, 65% in Hokuriku, 64% in Kinki, 54% in Chugoku, 50% in Shikoku, and 61% in Kyushu

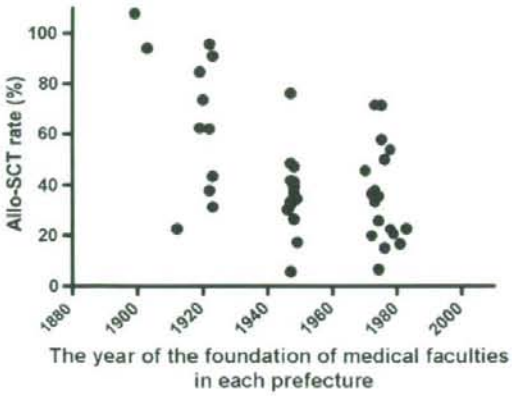

Fig. 2 Association of allogeneic stem cell transplantation rates by prefecture with the year of the foundation of medical faculties in each prefecture

universities and colleges in Japan, the number of physicians in a district is probably affected by the regulated number of students at medical faculties and the year of the foundation of medical faculties. The present study showed a significant association between the year of the foundation of medical faculties in each prefecture and the allo-SCT rates (Fig. 2). The allo-SCT rates by district which were high in the west and low in the east are probably explained by the history where medical faculties had been founded mostly in Western Japan since the Meiji Revolution in 1868. Depending on the migration of health care professionals seems insufficient to minimize the difference in medical services; focusing on the first step of physicians' education will be important. The present study also demonstrated no significant association between incomes per person and the allo-SCT rates by district. The national health insurance system which reduced the economical difference in access can explain the negative results in association between economical factors and the allo-SCT rates in Japan.

The present study showed a maximum 14.6-fold difference in allo-SCT rates among prefectures in a single district. The observation suggests that a prefecture plays a central role in each district and that patients cross the prefectural border to the central prefecture to undergo allo-SCT. Interestingly, no central prefecture exists in Tohoku district, where the allo-SCT rate was the lowest. Since the Japanese capital had been located in Kinki district (West Japan) until the mid nineteenth century, mainly West Japan was developed. The weak economical platform and premature social infrastructure in East compared with West Japan may hamper the centralization of allo-SCT in Tohoku.

Our study has some limitations. While we used relatively accurate numbers of allo-SCT, we could not obtain actual data on the incidences of acute leukemia in prefectures and patient migration rates to and from other prefectures. We estimated the former based on incidences by age-groups and populations, and adjusted the latter with the rates of immigration and emigration of all patients, yet the allo-SCT rates reached 100% in some prefectures. The results suggest that patients with acute leukemia more often cross the prefectural border for allo-SCT than for regular treatments. Data collection by the registration of patients with acute leukemia and the further investigation of patient migration for allo-SCT are warranted.

In conclusion, the present study suggested that social systems including physicians' education largely affect the dissemination of complex medical treatments such as allo-SCT. To reduce the regional differences in allo-SCT by the centralization of allo-SCT institutes, improvement of the education system for physicians and social infrastructure also needs to be considered.

Acknowledgments The present research was funded by Health Labour Sciences Research Grant by the Ministry of Health, Labour and Welfare, Japan.

References

1. Matsuo K, Hamajima N, Morishima Y, et al. Hospital capacity and post-transplant survival after allogeneic bone marrow transplantation: analysis of data from the Japan Society for Hematopoietic Cell Transplantation. Bone Marrow Transplant. 2000;26:1061–7.
2. Japan Society for Hematopoietic Cell Transplantation. The Annual Report of Nationwide Survey 2005 page. Available at: http://www.jshct.com/report_2005/index.html. Accessed at 26 August, 2007.
3. Nomura K. Cancer statistics in Japan 2005. Tokyo: Foundation for Promotion Cancer Research; 2005.
4. Ministry of Internal Affairs and Communications, Statistics Bureau. The Population Estimates page. Available at: http://www.stat.go.jp/data/jinsui/2004np/zuhyou/05k16-10.xls. Accessed at 26 August, 2007.
5. Ministry of Health, Labour and Welfare. The MHLW Statistical database page. Available at: http://www.mhlw.go.jp/toukei/saikin/hw/kanja/05/01-07.htm. Accessed at 26 August, 2007.
6. Ministry of Health, Labour and Welfare. The MHLW Statistical database page. Available at: http://wwwdbtk.mhlw.go.jp/toukei/youran/data18k/2-48.xls. Accessed at 26 August, 2007.
7. The Japanese Society of Hematology. The Board and Committees page. Available at: http://www.jshem.or.jp/system/cnt_06.html. Accessed at 26 August, 2007.
8. Cabinet Office. The National Accounts (NA) and other related statistics page. Available at: http://www.esri.cao.go.jp/jp/sna/kenmin/h16/4_skenmin.xls. Accessed at 26 August, 2007.
9. Gratwohl A, Baldomero H, Labar B, et al. Evolution of hematopoietic stem cell transplantation in Eastern and Western Europe from 1990 to 2003. A report from the EBMT activity survey. Croat Med J. 2004;45:689–94.

Int J Hematol (2008) 87:484–489
DOI 10.1007/s12185-008-0087-2

ORIGINAL ARTICLE

Current status of development of anticancer agents in Japan

Tomohiro Morita · Akiko Hori · Hiroto Narimatatsu · Tetsuya Tanimoto · Masahiro Kami

Received: 21 November 2007 / Revised: 18 February 2008 / Accepted: 14 March 2008 / Published online: 29 April 2008

Abstract To investigate the current status of the development of anticancer agents in Japan, we examined the number of these agents developed after 1999, their target diseases, and the association between the number of approved agents and the number of patients with the diseases. The data were obtained via the Internet. Of the 487 agents approved from 1999 to April 2007, 84 were anticancer drugs. Of these 84, 46 were approved based on clinical trials and 38 were approved through the new drug application for off-label usages without clinical trials. The target diseases of the 46 agents approved through clinical trials were nonhematologic tumors in 29, hematologic malignancies in 13, and others in 4. Of the 38 approved through the new drug application for off-label usages, 31 were for nonhematologic tumors and 7 for hematologic malignancies. The number of approved anticancer agents for hematologic malignancies per unit patient population was 6.5-times as many as that for nonhematologic tumors. This study demonstrated that the situation regarding the development of anticancer agents differs among tumor types. The majority of anticancer agents developed target hematologic malignancies, while the newly developed anticancer agents have affected treatment strategies for solid tumors.

Keywords Approval · Cancer · PMDA · Off-label use

1 Introduction

The number of cancer patients is increasing with rapid population aging in Japan [1]. Despite the progress of medical technology, the prognosis of cancer remains insufficient. When cure or prolongation of survival cannot be expected through existing surgery and chemotherapy, patients strongly desire to be involved in trials of novel anticancer agents. Since anticancer agents with novel mechanisms such as molecular targeting agents and antibody drugs were developed, the development of chemotherapeutic agents for cancers which have not responded to conventional cytotoxic agents has been attempted [2]. The number of newly developed anticancer agents is, therefore, increasing [3].

The so-called drug-lag, the delay in the availability of drugs in Japan which are already available in Europe and/or the US, is a social concern in Japan [4]. One of the reasons that Japan lags behind Europe and the US in the approval of anticancer agents is that development by drug manufacturers in Europe and/or the US precedes that in Japan [4]. The Ministry of Health, Labour and Welfare initiated discussions at the Conference on Unapproved Drug Use in January 2005 to reduce this drug-lag. The Ministry requests drug manufacturers to rapidly complete clinical trials in Japan on drugs which are judged necessary by the conference. As of April 2007, the conference deals with 35 agents (ingredients), of which 21 are anticancer agents [5].

T. Morita (✉) · H. Narimatatsu · M. Kami
Division of Exploratory Research,
The Institute of Medical Science,
The University of Tokyo, 4-6-1, Shirokanedai,
Minato-ku, Tokyo 108-8639, Japan
e-mail: t.morita526@gmail.com

A. Hori
First Outpatient Division,
The National Cancer Center Hospital,
Tokyo, Japan

T. Tanimoto
Department of Laboratory Medicine,
The National Cancer Center Hospital, Tokyo, Japan

Hematologic malignancies have been the major target of developed anticancer agents, despite their low incidence. This is related to the easy access to tumor samples and the high response rates to anticancer agents compared with solid tumors. The development of molecular targeting agents and antibody drugs and the social changes in cancer treatment have led to advances in the development of novel anticancer agents for solid tumors; however, the actual condition remains unclear. We investigated the number of anticancer agents which were developed after 1999, the target diseases, and the association between the number of approved anticancer agents and the number of patients with the diseases.

2 Materials and methods

2.1 Definition of anticancer agents

We defined anticancer agents as drugs used for antitumor activities and excluded supportive treatments and drugs used for complications or adverse effects of treatments such as bisphosphonates. We defined molecular targeting agents as drugs which aim at the selective inhibition of the transformed phenotype or target specific molecular lesions within tumor cells, leading to improved cure rates with limited toxicity.

2.2 Information collection regarding approved anticancer agents

The generic names, effects, and year of approval of anticancer agents which were approved from 1999 to April 2007 were obtained from the homepages of the Pharmaceuticals and Medical Devices Agency (PMDA) [3] and Japan Pharmacists Education Center (JPEC) [6]. When an identical chemotherapeutic agent was approved for another use, we treated it as a separate agent.

2.3 Information collection regarding anticancer agents undergoing development

While information on anticancer agents undergoing development in order to apply for approval is not publicly available, the agents which were judged necessary for early development or approval application by the Conference on Unapproved Drug Use are listed in "Information on unapproved drugs in Japan [7]" on the homepage of the Cancer Information Center at the National Cancer Center. In the present study, we considered the agents listed on the homepage as of 15 April 2007 as the drugs which were in preparation for approval application in Japan and collected information on the generic names and expected target diseases of those agents.

2.4 Information collection regarding the number of cancer patients

Incidence data were obtained from the homepage of the Cancer Information Center at the National Cancer Center [8] and the numbers of patients with different tumors were estimated. The investigated tumors were classified into brain tumor, head and neck, esophageal, gastric, colorectal, breast, uterine, ovarian, prostate, renal, bladder, hepatic, biliary tract, pancreatic, skin, and lung cancers as well as other solid tumors, malignant lymphoma, leukemia, other hematologic malignancies, and others.

2.5 Objectives

The primary objective of the present study was to clarify the numbers of anticancer agents which were approved from 1999 to April 2007, developing agents as of April 2007, and the target diseases. The secondary objective was to investigate the method of approval of each drug and to evaluate the association between the number of approved anticancer agents and the number of patients with the diseases.

3 Results

3.1 Approved anticancer agents

Of 487 applications approved from 1999 to April 2007, 84 (17%) were anticancer agents. Of these 84, 46 were approved based on the data obtained from clinical trials for approval, and 38 were approved based on the new drug application for off-label usages without clinical trials in Japan. The annual numbers of approved applications for anticancer agents are shown in Fig. 1. The number of approved applications increased in 2005, when the administrative measures approved 25 applications for anticancer agents, which had been used off-label.

The target diseases regarding the 46 applications of anticancer agents approved based on clinical trials in Japan were nonhematologic tumors in 2, hematologic malignancies in 13, and others in 4. These four agents categorized in others were all related to preparative regimens for hematopoietic stem cell transplantation. Details of the approved applications are shown in Table 1. No agents were approved for ovarian, renal, bladder, and skin cancer. Of the 46 applications, 10 were molecular targeting agents, and 7 of them targeted hematologic malignancies. The Conference on Unapproved Drug Use was involved in four applications (pemetrexed disodium, bortezomib, temozolomide, and oxaliplatin).