	実数	割合，%
1）施設区分		
診療所（無床）	80	(65)
診療所（有床）	26	(21)
200床未満病院	15	(12)
200床以上病院	3	(2)
2）標榜科（複数回答）		
内科	94	(76)
心療内科	6	(5)
精神科	6	(5)
神経科	9	(7)
呼吸器科	27	(22)
消化器科（胃腸科）	41	(33)
循環器科	31	(25)
アレルギー科	12	(10)
リウマチ科	8	(6)
小児科	39	(31)
外科	24	(19)
整形外科	22	(18)
形成外科	4	(3)
美容外科	1	(1)
脳神経外科	6	(5)
呼吸器外科	1	(1)
心臓血管外科	2	(2)
小児外科	1	(1)
皮膚科	10	(8)
泌尿器科	6	(5)
性病科	1	(1)
肛門科	6	(5)
産婦人科	5	(4)
眼科	15	(12)
耳鼻咽喉科	8	(6)
気管食道科	2	(2)
リハビリテーション科	27	(22)
放射線科	12	(10)
麻酔科	7	(6)
3）所在地		
東部Ⅰ	57	(46)
東部Ⅱ	23	(19)
西部Ⅰ	14	(11)
西部Ⅱ	5	(4)
南部Ⅰ	20	(16)
南部Ⅱ	3	(2)

表1. 調査対象

複数の標榜科を有する医療機関について重複して集計した。所在地は2次医療圏別に集計した。県庁所在地である徳島市は東部Ⅰ医療圏である。

C．研究結果

調査結果を図1から図6に示す。

調査医療機関の1ヶ月間の外来患者数を図1に集計した。患者数100から1000人/月が50%を占めた。

悪性疾患の患者割合は67%の施設で1から10%であった。同割合が30%を超える医療機関はなかった。

1ヶ月間の紹介患者数を図3に示す。1から10人で全体の56%を占めた。

紹介先医療機関の診療科について複数選択方式で調査しその回答数を図4に示す。上位を消化器科（胃腸科）、循環器科、脳神経外科が占めた。

中核医療機関から診療所への紹介を逆紹介と定義し、その患者数を調査した（図5）。調査医療機関の55%が1ヶ月間の逆紹介患者数は1から5人であった。

逆紹介における紹介元医療機関の属性を図6に示す。3次医療機関及び中重症対応医療機関からの紹介が72%であった。

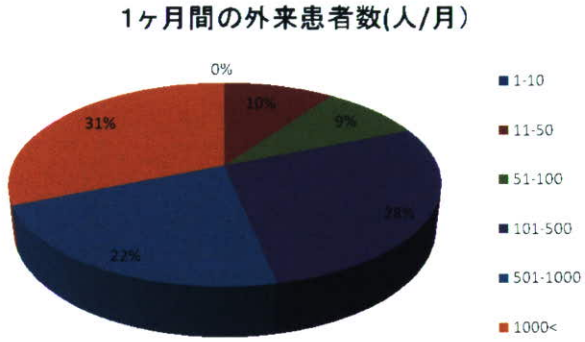

図1. 調査医療機関の1ヶ月間の外来患者数

患者数を凡例の如く集計し、その割合をグラフに示した。

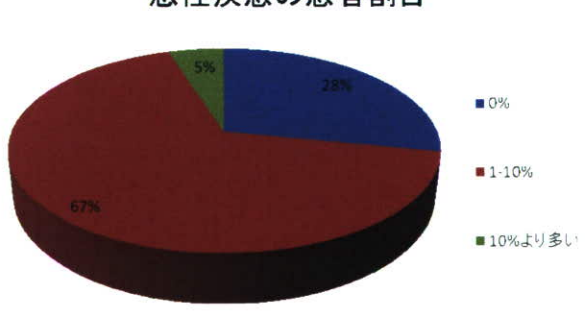

図2. 調査医療機関の悪性疾患の患者割合

悪性疾患の患者割合を凡例の如く集計し、その割合をグラフに示した。30%を超える医療機関はなかった。

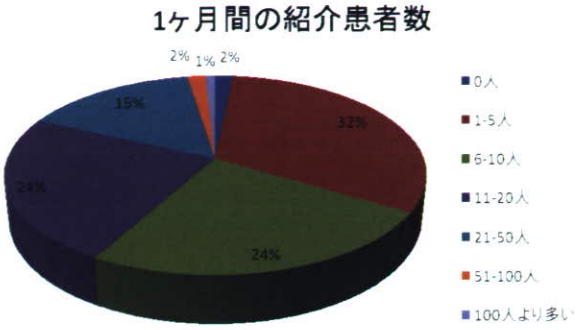

図3. 調査医療機関の1ヶ月間の外来患者数

1ヶ月間の外来患者数を凡例の如く集計し、その割合をグラフに示した。

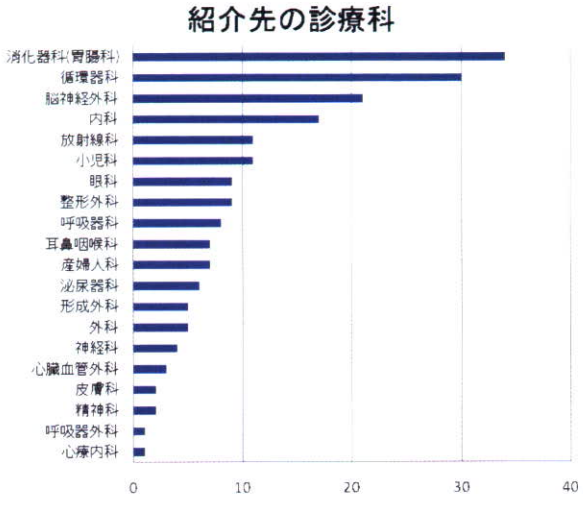

図4. 紹介先の診療科

紹介先診療科について複数選択で調査し、その回答数を図示した。

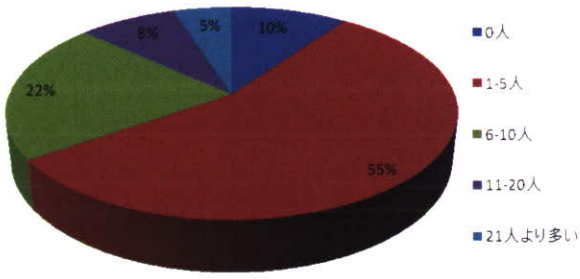

図5. 1ヶ月間の逆紹介患者数

1ヶ月間の逆紹介患者数を凡例の如くに集計し、その割合を図示した。

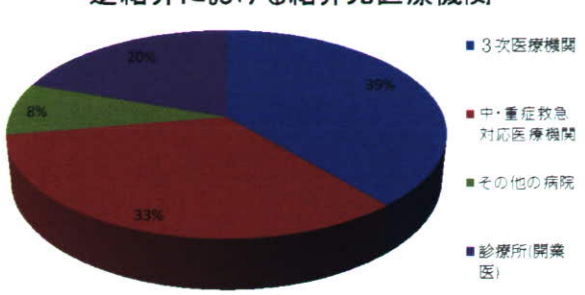

図6. 逆紹介における紹介元医療機関の属性

逆紹介における紹介元医療機関を凡例の如くに集計し、その割合を図示した。

D. 考察

地域医療における患者紹介の実態を、研究の先行している徳島県において実施した。調査対象の86%は診療所であり、今年度調査の目的である、紹介元医療機関サイドからの研究が施行されたと考えられよう。

図2に示されるように診療所レベルでの診療において、悪性疾患の患者割合は低い傾向である。このことは、悪性疾患の診療には高度な医療資源が必要であり、がん医療に関して地域医療連携を推進する際、診療リスクに応じて医療資源を適正に配分する必要性を示唆している。

また、図3と図5を比較することにより、中核医療機関に患者数が増加する傾向であることが分かる。即ち、逆紹介患者数が紹介患者数に比して少ない傾向にある。中核医療機関に勤務する医師の負担がますます増加するという悪循環が示唆される。

逆紹介元の医療機関は予想されるように高次医療機関が多くの割合を占めたが、診療所間の逆紹介も一定の割合を有することが注目される。

高度化するがん診療を国民に広く均てん化するためには、患者紹介が円滑に行われることが重要であるが、今回調査からは、患者紹介が診療所から高次医療機関への紹介に偏在していることが明らかとなった。なお、患者紹介に関連する因子については中村利仁氏の分担研

究書を参照されたい。

本研究の限界として、調査地域が限定されていることが挙げられ、同様な調査を他の地域においても実施したいと期待される。

E. 結論

地域医療における患者紹介を診療所サイドに着眼し調査を行った。診療所において悪性疾患を診療する頻度は低く、また、高次医療機関から診療所への逆紹介も少ない傾向を示した。高度医療の均てん化及び医療資源の適正配分という観点から、診療リスクに応じた患者紹介の円滑な推進が望まれる。その際に、診療所間の患者紹介が一定の割合を占めることは多様な患者紹介システムの構築の際に貢献すると考えられる。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

なし

2. 学会発表

なし

H. 知的財産権の出願·登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

厚生労働科学研究費補助金（がん臨床研究事業）
分担研究報告書

患者・紹介医への情報発信

分担研究者　松村　有子　東京大学医科学研究所探索医療ヒューマンネットワークシステム部門
研究協力者　瀧田　盛仁　東京大学医科学研究所附属病院内科
研究協力者　大石香奈子　東京大学医科学研究所探索医療ヒューマンネットワークシステム部門
研究協力者　大西　睦子　ハーバード大学病理部

研究要旨

円滑な患者紹介システムの構築には、患者・医療者双方に適切な情報伝達手段を検討することが重要である。他の分担研究者の報告を踏まえ、研究班会議を行い、医療情報の伝達に関して考察を行った。

研究が先行した徳島県において、研究成果を広く国民に還元するために、新聞紙上で研究結果を公表した。直ちに県民から反響があり、がん医療の均てん化に寄与したのものと考えられた。

A. 研究目的

本研究は円滑にがん臨床試験を展開するために必要な患者紹介システムの確立を目的としている。徳島県において先行研究が進行し、研究初年度に造血器悪性疾患に関する患者動態を明らかにした。また、今年度は紹介医の立場から患者紹介システムに関する調査を行った。（分担研究者の中村利仁氏及び竹内賢吾氏の研究報告書を参照されたい。）これらの調査研究からは、医療者間の医療情報共有のためのシステム構築が要請された。

一方で、患者に対する医療情報提供のネットワークづくりも重要な社会的課題である。がん診療の分野では国立がんセンターにがん診療情報に関する部門が設置され、そのホームページ（http://ganjoho.ncc.go.jp/public/index.html）は有用である。

そこで、本研究においても医療情報伝達手段について考察したので本稿にて報告する。

B. 研究方法

2008年2月に本研究班の班会議を開催し、分担研究者の研究報告を元に医療情報伝達手段について考察を加えた。また、研究が先行している徳島県において、本研究班の研究成果を新聞紙上で発表し、県民から得られた意見を論点整理した。

C. 研究結果

（1）医療と情報伝達手段

以下に情報伝達に関する本研究班の班会議の論点を整理する。

開業医を対象とした調査からは、一般国民のみならず開業医も、地域の医療資源に関する情報を強く求めていることが明らかとなった。適切な情報伝達を確立することは、がん臨床試験推進及び地域医療連携パス推進には必須事項である。

本研究では、患者動態研究のみならず具体的な解決策を実行・検証したい。

情報伝達手段について、迅速性・地域性・理解の容易性の軸で整理可能である（図1）。患者及び医師（特に主要な紹介医である開業医）に対する特徴的な情報伝達手段に関して年齢の軸で整理した（図2）。

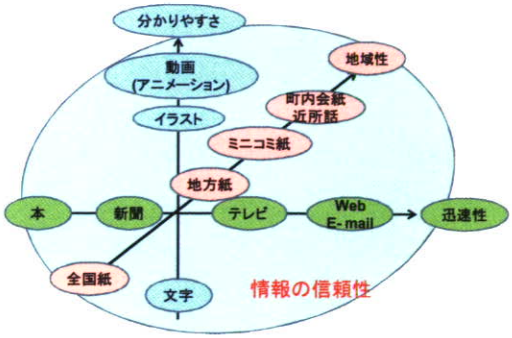

図1. 多様な情報伝達手段とその特徴

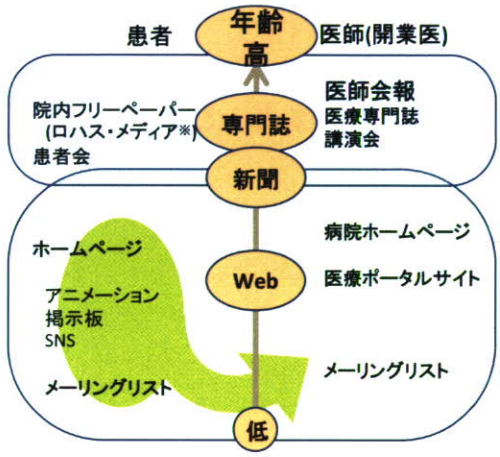

図2. 年齢層に応じた情報提供手段

※平成18年度厚生科研 第3次対がん総合戦略研究事業と共同研究

齋藤班：社会学・心理学等との連携による国民のリテラシー向上と患者の納得形成に関する研究

中田班：情報工学等の連携による国民・患者のリテラシー向上に関する研究

(2)　一般国民への研究成果の還元

がん診療が一般国民と密接に関係しているが故に、本研究班の研究成果を一般国民への還元することが極めて意義深い。当研究班の研究成果を整理し、インターネット上でその内容を公開した(http://expres.umin.jp/dynamic/)。また、研究が先行している徳島県において適切な情報伝達手段を検討したところ、費用対効果の観点から新聞紙面での公表が最も効果的であると判断し、平成20年2月24日に図3を発表した。

紙面に関する反響を表1に要約した。公表後1週間に研究事務局に28通の意見書が郵送された。回答者の年齢分布は14歳から91歳であった。回答者のうち女性が80%であった。新聞紙面に対する感想が64%、先進医療の地域格差に関する記述が29%を占めた。

回答者の年齢	分布	14－91歳
	中央値	55歳
回答者の性別	男性	20%
	女性	80%
内容	地域の医療資源について理解したなどの新聞紙面に関する感想	64%
	先進医療の地域格差に関する記述	29%
	その他	7%

表1. 新聞紙面に対する反響

紙面公表後1週間に研究事務局に寄せられた意見は28通であった。回答者の年齢・性別・記述内容を整理した。

D. 考察

がん先進医療の均てん化のためには、医療資源の高度化(ハード面)と患者・医療者双方の情報ネットワークシステムの醸成(ソフト面)の2つが、いわば「車の両輪」となって展開されなければならない。本研究班は患者紹介の実態を明らかにすることで、円滑な患者紹介システムの構築に寄与したいと考えている。

研究2年目として、これまでの中核医療機関サイドからの研究だけではなく、地元医療機関の立場に立脚して調査を行った。その際に、極めて重要な論点として、患者・医療者双方における有効な医療情報提供システムの構築が取り上げられた。

インターネット技術に代表されるように情報伝達手段は革新的に変化しつつある。図1に示されるように、各々の情報伝達手段には特徴を有し、その特徴にうまく適合した伝達内容や対象を選択することが望まれる。

例えば、対象者の年齢を軸に議論すると、図2に示されるが如くとなる。重要なことは、インターネットだけに捕らわれず多様な情報伝達手段を組み合わせることである。

本研究班の研究成果を公表する手段として、我々は研究の先行している徳島県において新聞を選択した。これは、公益性のある新聞社が徳島県に存在し、しかも世帯普及率が高く、従来型のパンフレット作成及びその配布を遙かにしのぐ効果が得られると判断したためである。

徳島県における新聞紙上での公表は費用対効果という側面でも他の方法より優れている。実際に、紙面に対する反響が続々と研究事務局に寄せられた。しかし、他の都道府県では、必ずしもこの方法が適切とは限らない。今後、他の地域においても適切な情報伝達手段を検討し、広く一般国民に研究成果を還元してゆく予定である。

E. 結論

医療情報を伝達する際には、情報伝達手段の特徴を十分に吟味し、適切な手段を組み合わせることが重要である。
研究が先行した徳島県において、研究成果を新聞紙面上で公表した。公表後、直ちに県民より反響があり、研究成果の国民への還元に一定の効果があったと考えられる。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

なし

2. 学会発表

なし

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

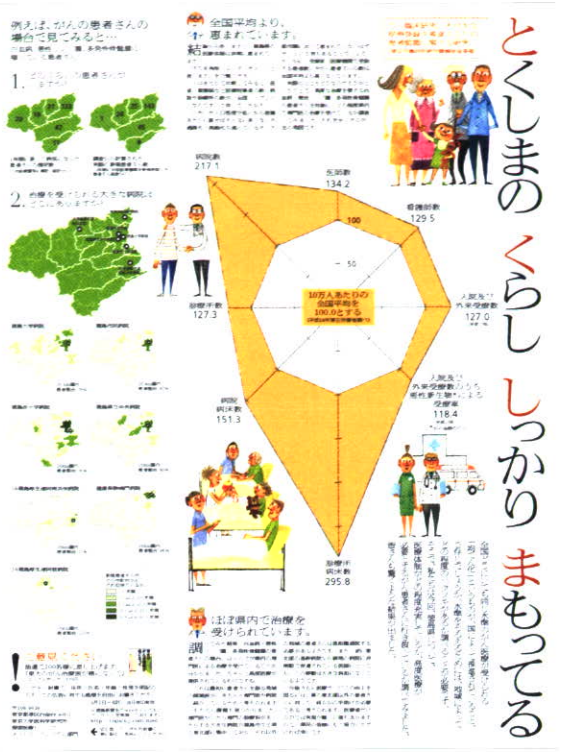

図3. 研究成果の一般国民への還元
研究が先行している徳島県において平成19年2月に研究成果を新聞紙面にて発表した。

Ⅲ．　研究成果の刊行に関する一覧

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Miura Y, Yamaguchi T, Azuma T, Hamaki T, Kodama Y, Kusumi E, Matsumura T, Nakamura T, Kami M, Komatsu T.	Regional differences exist in allogeneic stem cell transplantation rates for acute leukemia.	International Journal of Hematology			in press
Nomura S, Ishii K, Inami N, Uoshima N, Ishida H, Yoshihara T, Kitayama H, Hayashi K.	Role of soluble tumor necrosis factor-related apoptosis-inducing ligand concentrations after stem cell transplantation.	Transplant Immunology	18	115-121	2007
Kusumi E, Shoji M, Endou S, Kishi Y, Shibata T, Murashige N, Hamaki T, Matsumura T, Yuji K, Yoneyama A, Kami M.	Prevalence of anemia among healthy women in 2 metropolitan areas of Japan.	International Journal of Hematology	84	217-219	2006
宮腰　重三郎，大田　雅嗣．	疾患別にみた終末期・緩和ケアの実際	老年医学	44	1517-1524	2006
上　昌広	とくしまのくらし　しっかりまもってる	徳島新聞 (平成20年2月24日)	22966	11	2008

Ⅳ．　研究成果の刊行物、別刷り

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).

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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 (αvβ3) 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

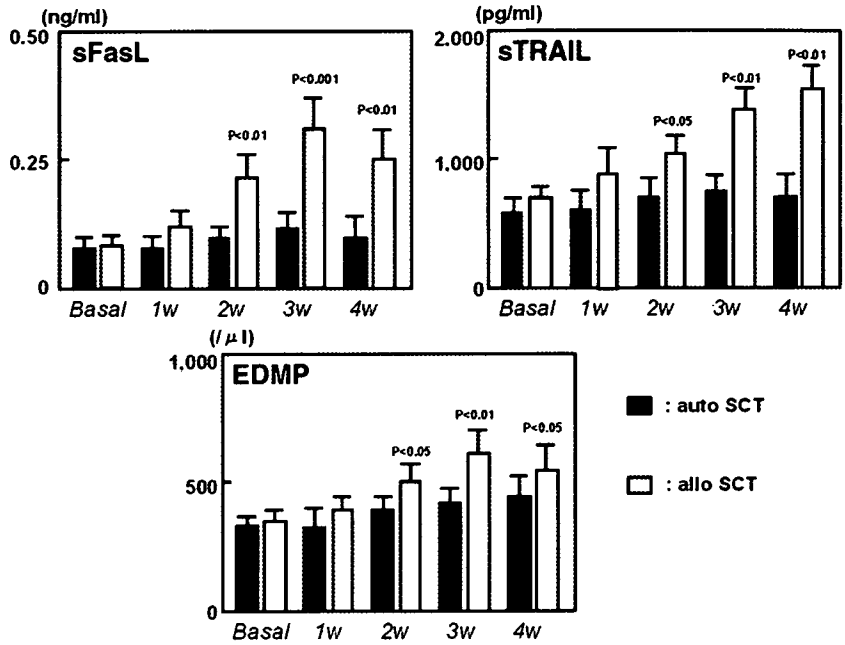

Fig. 1. Changes of apoptotic markers and EDMP in patients undergoing allogeneic and autologous SCT. Blood samples were obtained on days 0 (basal), 7 (1 weeks), 14 (2 weeks), 21 (3 weeks), and 28 (4 weeks) after the transplantation. Error bars show standard error. Student's *t*-test was used for statistical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

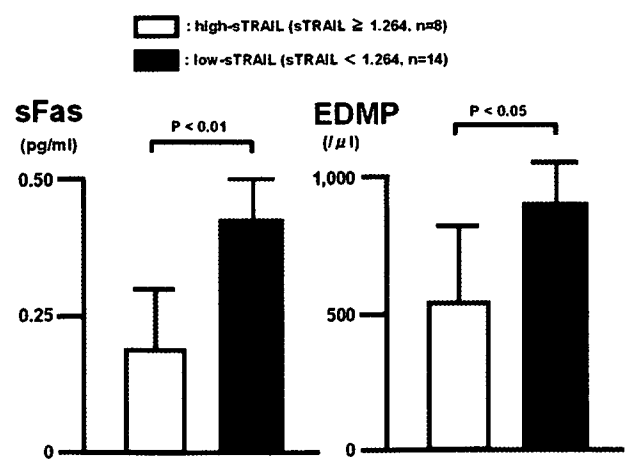

Fig. 2. sFasL and EDMP levels in patients after allogeneic SCT with and without high-sTRAIL. Values presented as mean±standard error.

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-

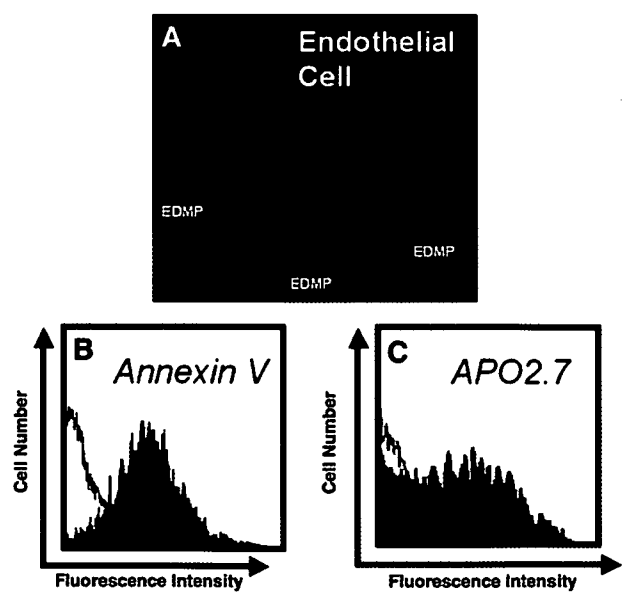

Fig. 3. Confocal microscopic images of the immunoreactivity and flow cytometric analysis of activated endothelial cells. Activated endothelial cells were stained with FITC-conjugated anti-CD9 antibody (A). In flow cytometric analysis, endothelial cells were stained using FITC-labeled Annexin V and PE-APO2.7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

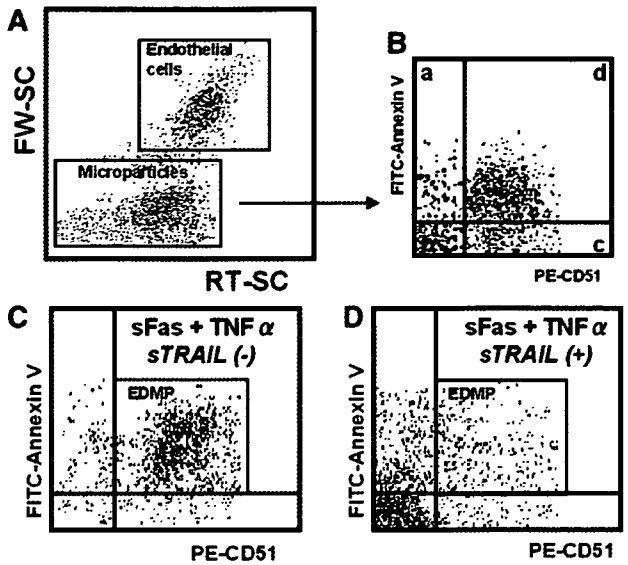

Fig. 4. Flow cytometric analysis of EDMP. Only particles positive for FITC-labeled Annexin V and PE-CD51 were gated to distinguish endothelial cells and EDMP from electric noise (A and B). EDMPs were elevated by stimulation of sFasL and TNF-α (C), and this elevation was inhibited with the addition of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

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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,[c] 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.

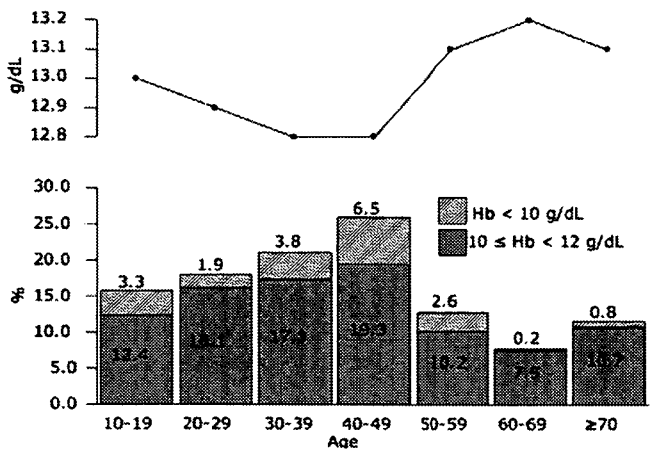

Figure 1. Prevalence of anemia and median hemoglobin levels according to age groups.

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.

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Geriat. Med. 44(11) : 1517～1524, 2006

特集 高齢者に有終の美を—終末期ケアと緩和ケア

5

疾患別にみた終末期ケア・緩和ケアの実際
2）がん

宮腰重三郎* 大田 雅嗣*

KEY WORD
高齢者悪性疾患
高齢者血液疾患
緩和・終末期ケア
対症療法的化学療法
患者学

POINT
- 高齢者悪性疾患の緩和・終末期ケアは，チーム医療が重要である．
- 高齢者血液疾患の緩和・終末期ケアは，他の固形がんのそれとは異なる．
- よりよい医療には，暗黙知を形式知する患者学が必要となる．

0387-1088/06/¥500/論文/JCLS

はじめに

高齢者社会に移行し，その死因として悪性疾患が第1位を占めるようになって久しい．また，悪性疾患の治療の進歩も著明であるが，その恩恵にあずかる症例は若年者に限られる場合も多いのが現状である．さらに高齢者特有の認知症，脳梗塞や心筋梗塞をはじめとする臓器障害を合併していることもまれではない．

本稿では，がんの緩和・終末期ケアに関して，特に血液悪性疾患を中心に記載したいと思う．

高齢者の悪性疾患の緩和・終末期ケアについて

悪性疾患に対する治療方法は，根治的療法として手術，放射線療法，化学療法およびその組み合わせが存在する．しかしそれらの治療が無効で，必ず“死”を迎えることがはっきりした時点からいわゆる“終末期”と定義するならば，その判断は極めて難しい．一般的に高齢者の場合，悪性疾患の進行速度が緩慢で，“終末期”が6カ月以上経過することは珍しくはない．また，経過が長くなれば高齢者特有の合併症，例えば，誤嚥性肺炎，心不全や中枢神経合併症など急性疾患の併発も少なくなく，その経過を予測することはさらに困難になる．一方，血液悪性疾患の場合は，高齢者だからといって，緩慢な経過を示すことは他の固形がんに比して稀で，加えて急性疾患(感染症や出血など)の合併が極めて高い特徴をもっている．さらに多くの血液悪性疾患に対して，治癒は目指さないものの，痛みや悪性疾患に伴う諸症状を軽減する目的で，化学療法や放射線療法を行うことがあり，その治療に伴う副作用が上乗せされることが多い．

終末期医療を施行するに当たり，若年者もそうであるように，悪性疾患を克服することはたやすいことではないが，考えられる治療法を模索する必要がある．多くの悪性疾患の治療方法は，レベルの高い臨床研究で確認された，いわゆるEBM(evidence based medicine)に沿った治

*みやこし しげさぶろう，おおた まさつぐ：東京都老人医療センター血液科