cooperation. In this study, we constructed the actual fundamental infrastructure needed for local medical EHR, and carried out EHR projects in several regions of Japan [6], and during this study we found that it will be impossible to gain a comprehensive grasp of patient medical information at the national level only because no solution to the problems caused by movement of patients. Of course, we cannot use national identified number in Japan, so it make the solving problems more difficult.

Therefore when considering the future development of EHR, a mechanism for consolidating local-level medical information on the national level, as well as functions for data compatibility and other purposes, will be needed.

In this study, we constructed a mechanism for a medical cooperation system which links the EHR systems of individual regions and is able to create a one-patient, one-record system on the national level. In this paper, we will provide a report of this mechanism and of the 4-year operational trial.

Methods

As we discuss a mechanism for medical cooperation between regions, we will first describe the current conditions of inter-regional medical cooperation in Japan.

Local-level EHR

Many regions in the world have created EHR systems for managing patient medical data within that region, and many projects have been launched using these systems as hubs for coordinated health care and the provision of medical record [1, 7, 8]. For these purposes, it is necessary to ensure safe routes of information between the medical institutions and the system, and also to create a mechanism that allows patients to safely view medical data via the internet, which penetration rate is 78.0% in Japan [9]. The formulation and operation of an open standard for exchanging medical data from a wide variety of medical records are also important [10]. In Japan as well, there are many EHR systems operating in individual regions. In these cases, the systems are operated in a way that makes best use of the unique characteristics of each region. Data exchange is accomplished in a variety of ways, including direct connections to the hospital information systems of large scale hospitals, and exchange using MML (Medical Markup Language) [11, 12] or HL7 (Health Level 7) [13]. Because it is the local governments which are directly faced with a need for health care cooperation in the region, in many cases the systems are operated under the leadership of the local governments, and currently it is difficult to carry out activities that span multiple regions.

Construction of a mechanism for wide-area medical cooperation

As described above, attempts to integrate local EHR systems and carry out services over a wide area face a number of problems. One is data-level integration. Although some believe that collecting data using a single unified format is sufficient, this approach is not practical when one considers the current conditions in which many independent local EHR systems are operating, using various formats. The solution is data conversion (mapping) on the content level between different data structures.

Another problem is fragmentation among EHRs because of lack of national level patient's identification. It is thought that this problem can be resolved by assigning an internal upper-level ID at upper-level sites in place of the unique IDs used on the local level, and to assign the local IDs to these upper-level IDs (essentially assigning them to an upper-level directory structure) [14]. Following is a description of data mapping and the upper-level directory structure.

Data mapping

Absorbing differences in data structures can be accomplished by constructing a mechanism for XML (eXtensible Markup Language) data mapping. Figure 1 shows a concept diagram of XML data mapping. A document has a format showed in the left-hand side while another document has a format showed in the right-hand side. For example, the document on the left-hand side in Fig. 1 defines the patient ID as <ID>, while the document on the right-hand side defines it as <SocNum>. If these two are considered equivalent, they can be mapped so that they can be converted back and forth. In the same way, <given name> and <first name> is another example that is often seen. If the XML label and the data indicated by that label have the same code system, they can also be mapped. For example, if <disease> in the left-hand document contains an ICD-10 code, then it can be converted to the <ICD code> in the right-hand document.

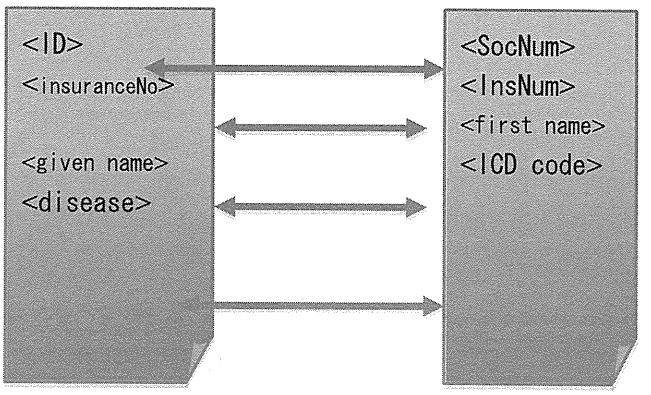


Fig. 1 XML data mapping

There have been reports of cases in which XML data mapping is used for bidirectional data conversion between EMR and EHR [15], and commercially products such as Asteria from the Infoteria Corporation (Japan) [16] and Rhapsody™ from Orion Health (New Zealand) [17] have been marketed as middleware intended for medical use. Use of these sorts of products makes data compatibility possible.

Upper-level directory structure

On the national level, if it is possible to issue and use a unique patient ID to each citizen on the national level, then such IDs can be used. However in many countries including Japan, use of these IDs in EHR is difficult. If a person is issued different patient IDs by multiple local EHR systems, it is necessary to understand that these different patient IDs actually indicate the same person. For this purpose, when a certain local EHR system issues a patient ID, an authorized organization can issue an upper-level patient ID for the national level, and can manage the links between patient IDs in multiple local EHR systems [18]. Using this mechanism, when a search for user data is performed using any local EHR system patient ID, it is possible to send a search request to other local EHR systems by means of the upper-level patient ID and return complete and integrated search results. Figure 2 shows a concept diagram of this process.

Actual system

In this study, we constructed a nationwide-capable EHR directory service (super site), with named "Super Dolphin" that includes the XML data mapping and upper-level directory structure described earlier, and verified that it is possible to link multiple local EHR systems together. Specifically, the subjects were two regions of Japan (Miyazaki and Kyoto) where EHR systems are actually operating. These two local EHR systems were connected to the super site that we constructed, and this super site was given the name "Super Dolphin". The NPO Japan Medical Network Association, which was established since 2005 to implement nationwide EHR manages this super site [19].

Table 1 is an overview of the two local EHR systems which were the subjects of this test.

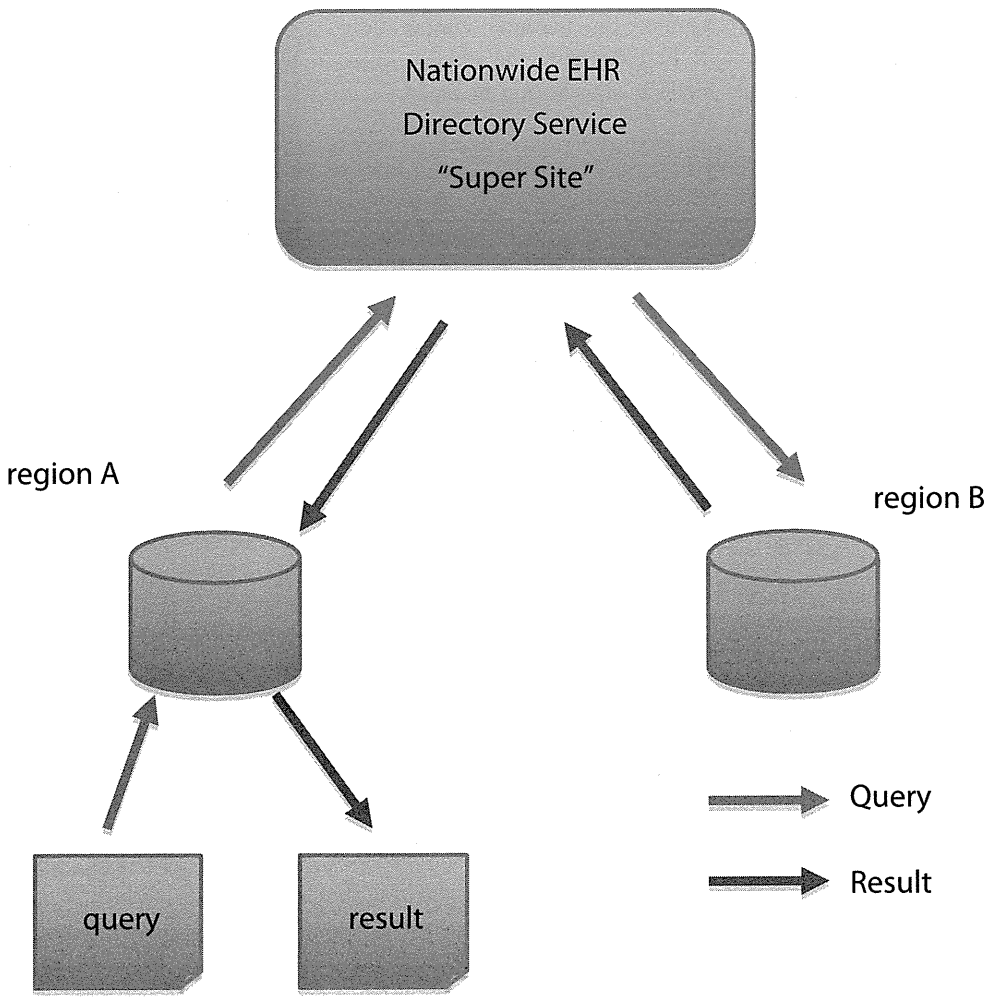


Fig. 2 Concept diagram of upper-level directory structure

Table 1 Test conditions

1) Local EHR systems and using data formats
Miyazaki EHR system (haniwa): Using MML2.3
Kyoto EHR system (maiko): Using MML3.0 (CDA* rel.1 compliant)
2) Upper-level site: Super Dolphin
3) Paths: The two local EHR systems and Super Dolphin were connected by Japan Gigabit Network version2 (JGN2**)
The internet is used for the communications route from the medical institutions to the center server

*CDA** clinical document architecture

JGN2** is research and development testbed network operated by the National Institute of Information and Communications Technology (NiCT) in Japan

Both the Miyazaki and Kyoto EHR systems are EHR systems that were constructed within the framework of the Dolphin Project [2]. The Dolphin Project was proposed by Yoshihara et al. in 1997 [11], and took its first step toward becoming reality in 2000 as a R&D project of the Ministry of Economy, Trade and Industry, Japan. Subsequently, experimental EHR services were launched in two regions, Kumamoto [20] and Miyazaki [21], in December 2001 and remain in use today. Later, full-scale projects aimed at providing practical services were launched in Tokyo [22], Kyoto [23], and other major cities.

The framework of the Dolphin Project involves integrated management of the medical data stored in the EHR system central server under a certain level of security. This allows medical practitioners to centrally view the medical data of patients who have concluded treatment agreements, and allows coordinated medical care. Patients can also view their own medical data (electronic record disclosure) and can enter symptoms and other information into their own records. The central server is connected to clinics, hospitals, laboratory test services, pharmacies, home nursing-care stations, and other facilities, which can send information such as past histories, laboratory results, letters of introduction, and discharge summaries. This information is all integrated and stored for each patient. In addition to sharing of local treatment data, this information is also used as a backup for the record data of each medical institution. In the Dolphin Project, the data of each medical institution is sent to the central server using MML, HL7, or other data format and is stored by the server in a database. A web interface is provided to the patients and medical practitioners. At present, each region is currently operating an original system utilizing the above basic design but making use of the local characteristics. The scale of each local project is as shown below (Table 2).

In this study, each patient is issued a unique patient ID in the local EHR system where person wants to receive service. Using this ID, the patient is able to view pateient's own medical information within the region. When a patient wants to view his/her own medical information from another region, by linking the patient IDs from multiple local EHR systems, Super Dolphin allows medical information from different regions to be viewed.

When a search for medical information is performed on the Miyazaki or Kyoto system, first a query is sent to the database of that local EHR system using the patient ID as the key. At the same time, the local EHR system sendsa query to Super Dolphin to check whether or not that patient ID is linked with patient IDs in other regions. As a result of this query, if the patient ID is found to be linked to an EHR system patient ID in another system, Super Dolphin uses this link information to request a search. The obtained data is converted to the data structure used by the data center which sent the request, and displayed. Communications between each EHR system and the super site utilize a local area network that uses the JGN2 network (Japan Giga Network version2) [24] provided jointly by the Ministry of Internal Affairs and Communications (MIC) and by NiCT. For local EHR systems and users, communication uses SSL with security functions utilizing Certification Authorities. The overall configuration is shown in Fig. 3.

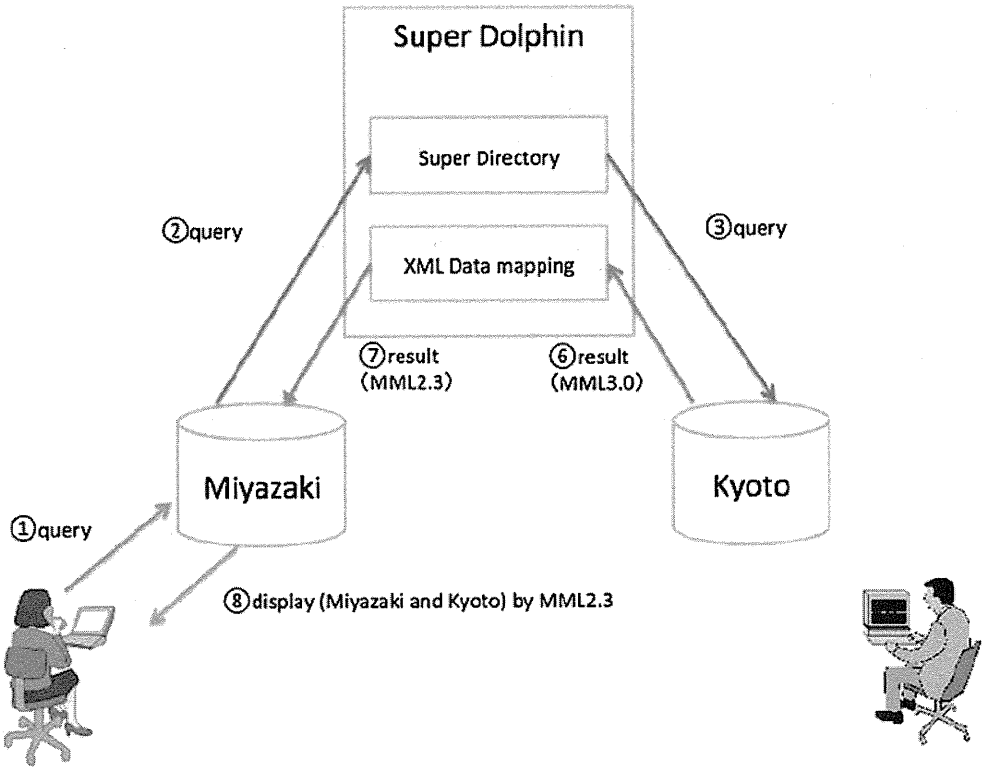
Results

Figure 4 shows an example of the results from display of patient medical data.

Table 2 The scale of each local project

	Miyazaki	Kyoto
Registered patients	1078	1,100
Registered medical institutions	84	5
Registered physicians	478	2,000
Monthly views (physicians)	185	100
Monthly views (patients)	60	2,000
No. of documents sent (text)	1,600,000	7,000,000
No. of documents sent (images)	85,000	86,000
Year started	2002	2007

Measurement date is 30 Oct 2010



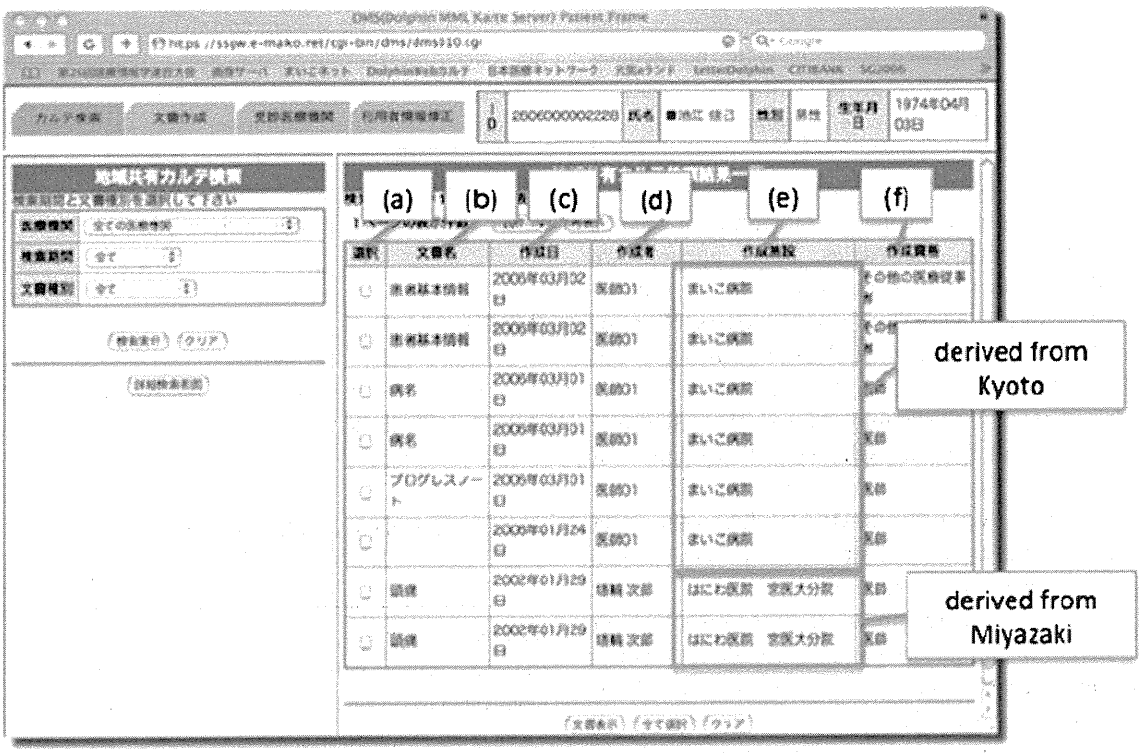


Fig. 3 Overall super dolphin configuration

Fig. 4 Patient medical data

The medical data is organized into lists that are based on the MML structure and that include disease name information, laboratory information, and progress reports. The fifth column in the list indicates the medical institution where the patient was treated. "Maiko Hospital" in this column indicates a Kyoto area medical institution and data which was uploaded to the Kyoto EHR system in CDA rel.1 (MML3.0) format. On the other hand the data of "Haniwa hospital" was uploaded to Miyazaki EHR system in MML2.3 format. In this way, this super site is able to merge and display data from EHR systems in different regions.

Observations

In this study, we considered, and verified by testing, a mechanism for integrating local EHR systems and providing medical cooperation that spans multiple regions. We constructed a super site (Super Dolphin) with data mapping functions for the purposes of matching patient IDs from different regions using an upper-level directory structure, and of compatibility between different data structures. Japan Medical Network Association was established as the operating body for operation of this super site. Although the upper-level directory structure is simple, it is highly universal and is expected to provide large benefits for medical cooperation between regions within a country and with regions where a unique national-level ID cannot be used. In this study, we also succeeded in mapping between different data structures. As described before, in this case conversions were only performed in one-to-one combinations (MML2.3 and CDA rel.1(MML3.0)). Naturally in order to use this mechanism to handle a broad range of clinical data in a versatile manner, the development of a correspondence table for the various standards will be necessary. In this case, determining how to coordinate the different levels of detail in the information may be a larger problem than mapping. However, the structures of the minimum necessary data that needs to be recorded for medical purposes do not differ greatly, and we believe that there will be no serious problems. In the future, we intend to increase the number of local EHR systems which participate in this super site and verify the effectiveness of this fundamental infrastructure, working towards achieving a national-level EHR.

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Original Article

Prediction of Limb Salvage after Therapeutic Angiogenesis by Autologous Bone Marrow Cell Implantation in Patients with Critical Limb Ischemia

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Purpose: **Despite advances in therapeutic angiogenesis by bone marrow cell implantation (BMCI), limb amputation remains a major unfavorable outcome in patients with critical limb ischemia (CLI). We sought to identify predictor(s) of limb salvage in CLI patients who received BMCI.**

Materials and Methods: **Nineteen patients with CLI who treated by BMCI were divided into two groups; four patients with above-the-ankle amputation by 12 weeks after BMCI (amputation group) and the remaining 15 patients without (salvage group). We performed several blood-flow examinations before BMCI. Ankle-brachial index (ABI) was measured with the standard method. Transcutaneous oxygen tension ($TcPO_2$) was measured at the dorsum of the foot, in the absence (baseline) and presence (maximum $TcPO_2$) of oxygen inhalation. ^{99m}technetium-tetrofosmin (^{99m}Tc-TF) perfusion index was determined at the foot and lower leg as the ratio of brain.**

Results: **Maximum $TcPO_2$ ($p = 0.031$) and ^{99m}Tc-TF perfusion index in the foot ($p = 0.0068$) was significantly higher in the salvage group than in the amputation group. Receiver operating characteristic (ROC) curve analysis identified maximum $TcPO_2$ and ^{99m}Tc-TF perfusion index in the foot as having high predictive accuracy for limb salvage.**

Conclusion: **Maximum $TcPO_2$ and ^{99m}Tc-TF perfusion index in the foot are promising predictors of limb salvage after BMCI in CLI.**

Key words: critical limb ischemia, bone marrow cell implantation, limb salvage, ^{99m}technetium-tetrofosmin perfusion scintigraphy, transcutaneous oxygen tension

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Introduction

Peripheral arterial disease (PAD) is a progressive illness primarily due to atherosclerosis. It also occurs in Buerger's disease and collagen disease involving vasculitis in small and medium-sized arteries. The most severe manifestation is termed "critical limb ischemia (CLI)," as demonstrated by ischemic rest pain and/or loss of tissue integrity, including skin ulceration and gangrene.[1)] PAD relates to a worse long-term outcome[2)]; furthermore, only 45% of CLI patients were free from limb amputation or

death within a year after diagnosis.[1] The current goals of management of CLI are to relieve ischemic pain, heal ischemic ulcers, and prevent major limb amputation, in addition to reducing cardiovascular mortality.

Recent advances in surgical and percutaneous revascularization have led to better treatment options for patients with CLI.[3] Notably, revascularization is not suitable in a considerable proportion of patients (10–15%) ("no-option patients"). Of these patients, more than 40% would require a major limb amputation, and 20% would die within 6 months.[4] For such "no-option patients," therapeutic angiogenesis by autologous bone marrow cell implantation (BMCI) has emerged as a novel strategy in an attempt to improve blood perfusion at the ischemic site, and evidence has accumulated that BMCI is a safe and effective treatment for CLI.[5,6]

Because the clinical condition of patients with CLI changes with time, repeated blood flow examinations are necessary. Thus, it is important to establish non-invasive and convenient examinations to detect blood flow. The ankle-brachial index (ABI) is convenient and the most utilized method to diagnose PAD, and transcutaneous oxygen tension ($TcPO_2$) is one of the commonly used noninvasive examinations reflecting local arterial blood flow.[7,8] Amann et al. reported that $TcPO_2$ is a useful measurement for evaluating blood flow recovery in patients with CLI who underwent BMCI.[9] In a previous investigation, we demonstrated that ^{99m}technetium-tetrofosmin (^{99m}Tc-TF) perfusion scintigraphy is useful for the assessment of quantitavive blood flow after BMCI.[5] However, among these non-invasive examinations of blood flow, predictors of limb salvage in patients with CLI subjected to BMCI have not been established.

The aim of this study was to evaluate the usefulness of these non-invasive measurements of blood flow as predictors of limb salvage after BMCI in patients with CLI.

Methods

Patient selection

We enrolled 20 consecutive patients who gave their written informed consent and who met the following criteria: a) atherosclerotic PAD or Buerger's disease with total occlusion or severe stenosis of leg arteries below popliteal artery diagnosed by digital subtraction angiography, b) CLI with Rutherford classification II-4 to IV-6 (Fontaine class 3 or 4) unsuitable for peripheral catheter intervention or bypass surgery, c) continuous ischemic symptoms for more than 6 months despite other conventional treatments, d) no evidence of malignant disorder during the past 5 years and no proliferative diabetic retinopathy, e) male or female aged 20–79 years. Preoperative screening was performed as described previously,[5] and the therapeutic decision was made by the advisory committee (consisting of cardiologists, vascular surgeons, plastic surgeons, radiologists and anesthesists).

At 12 weeks of follow up after BMCI, the patients were retrospectively divided into a salvage group (n = 15) and an amputation group (n = 4). Major amputation was defined as amputation above the ankle joint,[3] and limb salvage was defined as avoidance of major amputation. We performed partial amputation of the toes for complete necrosis or local osteomyelitis. One patient was excluded from this study because he died of congestive heart failure 60 days after BMCI.

The medical ethics committee of Nippon Medical School reviewed and approved this clinical trial.

Cell preparation and implantation

Bone marrow cell implantation was performed as described previously.[5] In brief, approximately 500 ml of bone marrow fluid was aspirated from the bilateral iliac bones of the patient under general anesthesia and collected in a sterile bag containing heparinized (20,000 units) RPMI 1640 medium (GIBCO, Grand Island, NY). The mononuclear cell fraction was sorted using an AS TEC 204 blood cell separator (Fresenius Kabi, Bad Homburg, Germany) and processed to obtain a final volume of about 70 ml. Finally, we injected the mononuclear cell suspension (1 ml/point) intramuscularly into the ischemic regions.

Assessment of limb ischemia

ABI was determined as the ratio of ankle systolic blood pressure to brachial systolic blood pressure, with both measured using an automatic device (PWV / ABI; Colin, Ltd., Komaki, Japan). The device simultaneously measured bilateral brachial and ankle blood pressure by the modified oscillometric pressure sensor method. The higher brachial pressure on either the left or right was used for calculating ABI. It was not possible to assess ABI in one patient in the salvage group because of cuff pain.

$TcPO_2$ was measured by a TCM 400 (RADIOMETER Inc., Tokyo, Japan). The sampling site was carefully selected so as not to overlie a bony prominence, superficial vessel, or pulse site. After wiping the skin with alcohol, we placed a transducer on the dorsum of the ischemic limb and warmed the skin to 43.5 C to increase the

permeability of the skin to oxygen molecules at the measurement site. With the patient resting in the supine position, we acquired baseline $TcPO_2$ at ambient conditions for about 20 min. Then, we repeated the acquisition, this time at 5 L/min pure oxygen inhalation for 5 min, to determine the $TcPO_2$ value in response to an oxygen challenge (maximum $TcPO_2$).

^{99m}Tc-TF perfusion scintigraphy was performed as described previously.[10] Simply, approximately 10 min after intravenous injection of ^{99m}Tc-TF (555–740 MBq), we preformed whole-body scintigraphy of the patient in the prone position, for both anterior and posterior projections, with a dual-head large field-of-view gamma camera (Vertex, ADAC). Each head of the gamma camera was equipped with a high-resolution, low-energy collimator. Scan speed was 12 cm/min. Image acquisition time was approximately 15 min. Data were acquired in a 512 × 1024 matrix and a 2096 window centered on the 140 keV photopeak of ^{99m}Tc. Both the anterior and posterior views were used for the quantitative analysis. Regions of interest (ROI) of equal size were drawn around the lower leg (region from knee to ankle) and the foot (region from ankle to toes) in the anterior and posterior projections (muscle uptake; M).[10] Additionally, intracranial uptake (brain uptake; B) was obtained and used as background. ^{99m}Tc-TF perfusion index was defined as muscle-to-brain (M/B) ratio of averaged counts per pixel.[5] Two patients in the salvage group did not undergo ^{99m}Tc-TF perfusion scintigraphy.

Statistical analysis

All data are presented as mean ± SD. Intergroup comparisons were made with Student's t test or chi-squared test. Receiver operating characteristic (ROC) curves were constructed to evaluate the predictive accuracy of examinations. Areas under the ROC curves and their 95% confidence intervals (CI) were calculated and compared using a nonparametric test. Then, the best cut-off value for each measurement to predict limb salvage was determined as the one that minimized the distance to the ideal point (sensitivity = specificity = 1) on the ROC curve.[11] Time to major limb amputation was compared between the two groups, divided by the cut-off value using Kaplan-Meier analysis with the log-rank test. A value of $p < 0.05$ was considered to be statistically significant.

Results

Clinical characteristics

The overall limb salvage rate in this study was 79%. The reasons for major amputation were uncontrolled infection (n = 3) and acute limb ischemia (n = 1). The clinical characteristics of the limb salvage and amputation groups are summarized in **Table 1**. The major cause of CLI was atherosclerotic PAD, but five patients with Buerger's disease were included in the salvage group. There was no significant difference between the two groups in age, sex, Rutherford classification (Fontaine class), prevalence of smoking, hypertension, diabetes and hemodialysis. The use of aspirin, cilostazol, anti-hypertensive drugs, statins and insulin did not significantly differ between the two groups. Laboratory data before BMCI were also comparable between the two groups; however, LDL-cholesterol and estimated GFR were significantly higher in the salvage group than in the amputation group ($p < 0.05$). The number of collected bone marrow mononuclear cells was $6.0 \pm 3.5 \times 10^9$ in the salvage group and $4.9 \pm 1.1 \times 10^9$ in the amputation group, with no significant difference between the two groups.

Perfusion parameters prior to BMCI

Fig. 1 shows ABI within one week before BMCI. There was no significant difference in ABI between the two groups (0.71 ± 0.34 in salvage group vs. 0.59 ± 0.63 in amputation group, $p = 0.59$). In **Fig. 2**, $TcPO_2$ values in the absence (baseline) or presence (maximum) of pure oxygen inhalation were compared. Although the difference in baseline $TcPO_2$ between the two groups was not significant (25 ± 14 vs. 13 ± 15 mmHg, $p = 0.14$) (**Fig. 2A**), maximum $TcPO_2$ was significantly higher in the salvage group than in the amputation group (42 ± 24 vs. 12 ± 14 mmHg, $p < 0.05$) (**Fig. 2B**). **Fig. 3** shows a representative image of ^{99m}Tc-TF perfusion scintigraphy, and perfusion index at the foot (ankle to toes), and lower leg (knee to ankle) regions were calculated. The perfusion index in the lower leg region was comparable between the two groups (0.98 ± 0.16 vs. 0.98 ± 0.10, $p = 0.94$) (**Fig. 4A**). In contrast, tissue perfusion of the foot region was estimated to be significantly greater in the salvage group than in the amputation group (0.84 ± 0.25 vs. 0.42 ± 0.14, $p = 0.0068$) (**Fig. 4B**).

Cut-off values for limb salvage

The area under the ROC curve was 0.85 [95% CI 0.66–1.05; $p = 0.036$] for maximum $TcPO_2$ and 0.94 [95%

Table 1 Clinical characteristics

	Limb Salvage group (n = 15)	Major Amputation group (n = 4)	*p* value
Age (years)	57 ± 14	68 ± 8.3	0.17
Male / Female (%)	11 / 4	2 / 2	0.77
PAD / Buerger's disease (%)	10 / 5	4 / 0	0.48
Fontaine class	3.8 ± 0.4	4 ± 0	0.36
(Rutherford classification)	II-4 to IV-6	III-5 to IV-6	
Smoking (%)	13 (87)	2 (50)	0.36
Hypertension (%)	8 (53)	3 (75)	0.83
Diabetes mellitus (%)	7 (47)	3 (75)	0.66
Hemodialysis (%)	3 (20)	2 (50)	0.57
Aspirin (%)	6 (40)	3 (75)	0.5
Cilostazol (%)	7 (47)	1 (25)	0.83
ACE-I/ARB (%)	8 (53)	4 (100)	0.26
Calcium channel blocker (%)	5(33)	3(75)	0.35
β-blocker (%)	3 (20)	1 (25)	0.64
Statin (%)	6 (40)	2 (50)	0.83
Insulin (%)	3 (20)	0 (0)	0.84
Fasting glucose (mg/dl)	107 ± 26.4	135 ± 88.0	0.28
HbA1c (%)	5.6 ± 0.7	5.6 ± 1.1	0.96
HDL-cholesterol (mg/dl)	41 ± 11	33 ± 3.9	0.17
LDL-cholesterol (mg/dl)	97 ± 26	54 ± 12	<0.01
Triglyceride (mg/dl)	115 ± 78.6	157 ± 149	0.45
Serum creatinine (mg/dl)	2.2 ± 3.1	6.1 ± 4.7	0.06
eGFR (mL/min per 1.73 m^2)	62 ± 41	14 ± 14	<0.05
CRP (mg/dl)	2.0 ± 2.4	1.0 ± 0.5	0.43

Data are shown as mean ± SD or number (%).
eGFR (mL/min/1.73 m^2) = 194 × Serum creatinine $^{-1.094}$ × Age$^{-0.287}$ (Female × 0.739)
PAD, peripheral artery disease; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin 1 receptor blocker; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein

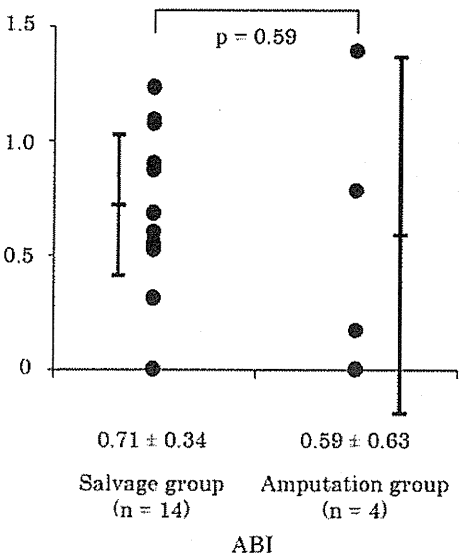

Fig. 1 Ankle brachial index before bone marrow cell implantation.

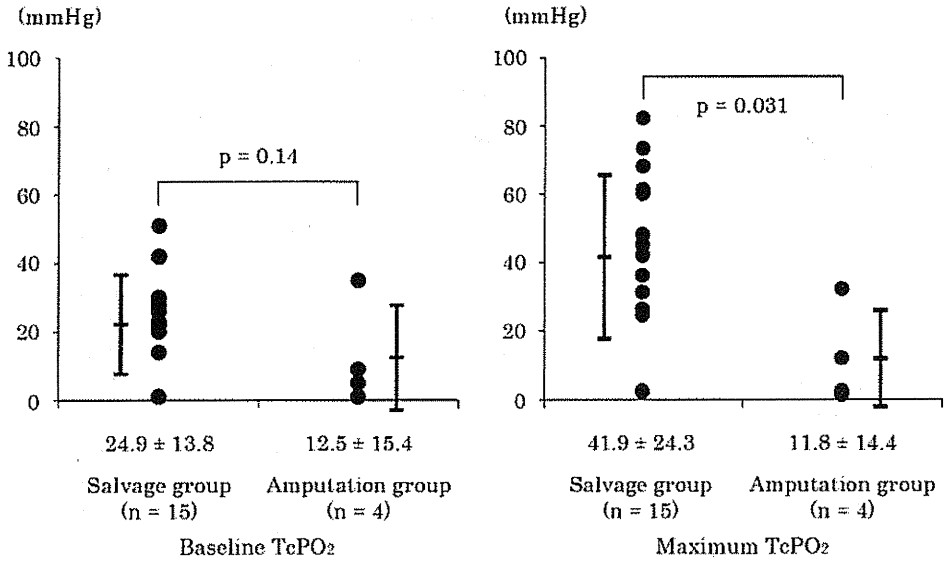

Fig. 2 Transcutaneous oxygen tension ($TcPO_2$) measurements at ambient conditions (A: baseline $TcPO_2$) and during 5 min of pure oxygen inhalation at 5 mL/min (B: maximum $TcPO_2$).

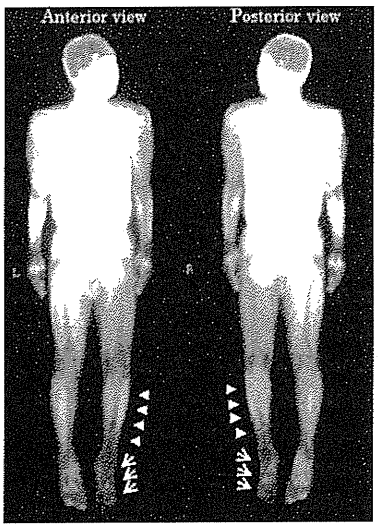

Fig 3 Representative image of ^{99m}Tc-TF perfusion scintigraphy. Arrow-heads indicate lower leg region, and arrows indicate foot region of ischemic leg.

CI 0.82–1.07; $p = 0.009$] for ^{99m}Tc-TF perfusion index in the foot region, suggesting high predictive accuracy of these parameters for limb salvage. The calculated best cut-off value was 18 mmHg for maximum $TcPO_2$ (sensitivity 0.87, specificity 0.75) and 0.65 for ^{99m}Tc-TF perfusion index in the foot region (sensitivity 0.77, specificity 1.0). **Fig. 5** illustrates major amputation-free survival when the patients were divided into two groups by these cut-off values. Patients with maximum $TcPO_2$ and ^{99m}Tc-TF perfusion index in the foot region above the cut-off value had a significantly higher limb salvage rate than those whose measurements were below the cut-off value (log-rank test, $p = 0.008$, $p = 0.006$, respectively) (**Fig. 5**).

Discussion

Because the major reason for amputation in CLI is an unhealed ulcer or gangrene, one of the important objectives of therapeutic angiogenesis is wound healing and prevention of ischemic ulcers. Therapeutic angiogenesis by BMCI is thought to improve the microcirculation, playing a crucial role in wound healing.[12] The possible mechanism is that bone marrow mononuclear cells consist of a variety of cell populations committed to vascular formation,[13] secretion of angiogenic cytokines,[14] and stimulation of muscle cells, promoting the secretion of angiogenic factors.[15]

In the present study, the mean value of baseline $TcPO_2$ of all patients was 22 ± 15 mmHg, which was almost identical to the cut-off value for limb amputation previously reported.[8,12,16] Even for patients in this critical condition, we achieved a 79% limb salvage rate at 12 weeks of follow up after BMCI, indicating that BMCI could be used as a powerful strategy for CLI to salvage limbs

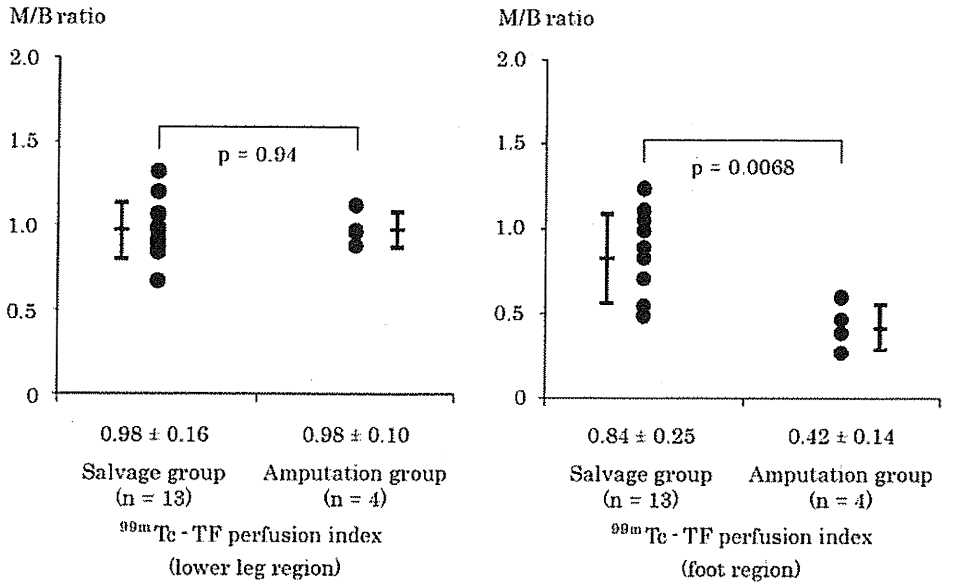

Fig. 4 Tissue blood flow estimated by ^{99m}Tc-TF perfusion scintigraphy (^{99m}Tc-TF perfusion index: expressed as muscle to brain [M/B] ratio of mean counts per pixel). Lower leg region (A) and foot region (B).

from major amputation.

In the present study, the value of $TcPO_2$ was significantly different between the two groups, only when taken during O_2 inhalation (maximum $TcPO_2$). Furthermore, the ROC curve analysis showed that maximum $TcPO_2$ is highly accurate in predicting limb salvage. It is reported that maximum $TcPO_2$ is a useful predictor of limb amputation in PAD,[17] and our data support this concept as well. The underlying mechanism is that maximum $TcPO_2$ could reveal the remaining potential of flow reserve which is masked under a normoxic condition, by increasing the arterial oxygen content.[18] Thus, maximum $TcPO_2$ rather than baseline $TcPO_2$ might represent the severity of ischemia more accurately.

In our previous investigation, the ^{99m}Tc-TF perfusion index was shown to be a useful examination to evaluate the increase in blood flow of the microcirculation in response to BMCI.[5] The usefulness of ^{99m}Tc-TF is supported by the following mechanism: 1) ^{99m}Tc emits high energy photons, enhancing image quality, and the shorter half-life allows administration of a higher dosage,[19] 2) ^{99m}Tc represents both blood flow perfusion and cellular viability, because uptake and retention are dependent on cell membrane integrity and mitochondrial function.[19,20]

In the present study, we demonstrated that ^{99m}Tc-TF perfusion index in the foot region prior to BMCI was a significant predictor to divide patients into a salvage group and an amputation group. Furthermore, ROC curve analysis indicated ^{99m}Tc-TF perfusion index in the foot region to have high predictive accuracy.

We showed the cut-off values of maximum $TcPO_2$ and ^{99m}Tc-TF perfusion index in the foot region for limb salvage, which was determined by ROC curve analysis, and subsequent Kaplan-Meier analysis revealed that these cut off values estimated chronological changes in limb salvage. These findings provide important clinical precognition of responders to BMCI before treatment. Furthermore, to salvage the limbs of patients subjected to BMCI with values below the cut-off values, additional or alternative intervention may be required.

It was important to compare these cut-off values including the patients treated with conventional therapy. However, patients enrolled in this study were already scheduled to have a limb amputation, even before the consultation at our hospital. Thus, we could not randomize the patients to a BMCI treatment group versus a conventional treatment group, for ethical reasons. Even in such "no-option" patients, we encourage to find a possibility of limb salvage. Although clinicians who participate in the care of CLI sometime face an immediate decision of limb amputation, we believe that providing various treatment options for CLI would provide a better prognosis in CLI patients.

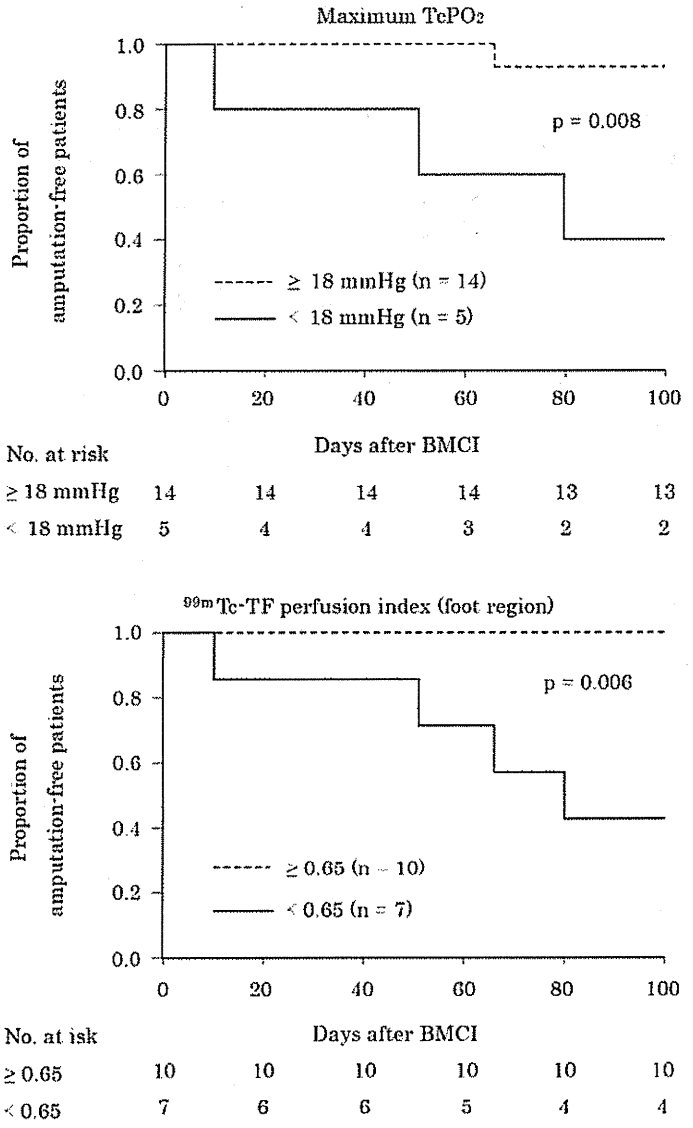

Fig. 5 Kaplan-Meier plots of time to major limb amputation dichotomized at the cut-off values of maximum $TcPO_2$ (A) and ^{99m}Tc-TF perfusion index in the foot region (B).

In the present study, there was no significant difference in ABI between the two groups. Although ABI is considered to be a useful method in the diagnosis of PAD, it does not represent blood flow of the microcirculation, which is an important factor in wound healing in CLI.[12] Besides, severe calcification causes a pseudo-normalized value, which may affect the accuracy of the evaluation of ischemic severity.

In summary, BMCI was performed in 19 CLI patients, and the limb salvage rate was 79%. Maximum $TcPO_2$ and ^{99m}Tc-TF perfusion index in the foot region are predictors of limb salvage after BMCI in CLI patients.

Study limitations

There are several limitations of the present study. First, this was a retrospective study with a small number of subjects. Therefore, multivariate analysis was not performed. For ROC curve analysis, the small number of outcomes made the curve rough. In addition, the cut-off values determined by this ROC curve analysis might be inadequate. Second, limb salvage was determined at 12 weeks of follow up after BMCI, which may be too short

a follow-up period. Third, blood concentration of LDL-cholesterol and estimated GFR at baseline were significantly higher in the salvage group than in the amputation group, suggesting that patient characteristics might also have influenced the outcome. The results of this study need to be confirmed in a large population.

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特集●再生医療の現状と将来

下肢血管再生治療

高木　元[*1]　宮本　正章[*1]　水野　杏一[*1]

要　約

虚血性の下肢病変は生活習慣病の増加に伴い有病率が増加している。実臨床では下肢切断の判断を迫られることもあり，切断適応の明確化が必要である。一方切断の有無にかかわらず下肢虚血患者の予後は悪く，この難治病態の解決策として下肢再生治療は開発された。臨床研究結果によりその長期予後成績も明らかになってきたが，下肢再生治療にも限界点が存在することが示唆されている。この点を認識し，さらなる下肢救済の可能性を広げる展望を考えたい。

Ⅰ．下肢疾患の特徴

下肢疼痛を生じる疾患の多くは整形外科領域であり初期加療されることが多いため，歩行の重要性と生命予後に関するエビデンスは整形外科領域で豊富である[1]。下肢虚血に関しては，わが国ではBuerger病（閉塞性血栓血管炎，TAO：Thromboangitis obliterans）が主な末梢閉塞性動脈疾患（PAD：peripheral artery disease）の原因であったが，近年では減少し，生活習慣や高齢化を背景に閉塞性動脈硬化症（ASO：arteriosclerosis obliterans）から生ずる虚血や壊疽などの下肢疾患の有病率が増加している。

Ⅱ．切断の判断

PAD患者の治療方針決定時，血行再建を行うか切断に踏み切るかの判断は極めて困難な決断である。一般には以下を考慮し判断が行われる[2]。1）血行再建術の技術的問題，2）創傷治癒の可能性（骨髄炎の存在や寝たきり等），3）全身合併症の有無。これらを十分に検討したうえで下肢温存の適応判断をすべきである。しかしPADでは大切断で歩行機能が損なわれると生命予後も悪化することが知られており[3]，可能な限り機能を温存することが望ましい。切断レベルの判断は非侵襲的検査にて簡便かつ迅速に行われるべきである。ABI，サーモグラフィー等がスクリーニングに有用であるが，重症では経皮酸素分圧検査（TcPO2），皮膚組織灌流圧，核医学検査など[4]が使用される。

Ⅲ．生命予後

PADの治療ガイドライン（TASC Ⅱ：Trans-Atlantic Inter-Society Consensus Ⅱ）によると，重症虚血肢（安静時疼痛，潰瘍，壊疽を伴う重症例，CLI：Critical Limb Ischemia）の生命予後は，発症から1年で75％生存，下肢切断を受けずに生存していた患者は45％にすぎず[5]，30％は大切断を受けている。さらに5年後の全生存率は30％と低率であり，CLIの予後は重篤である。CLI患者はさらに下肢切断2年後の歩行可能割合が40％と低率である。これは一般に交通外傷な

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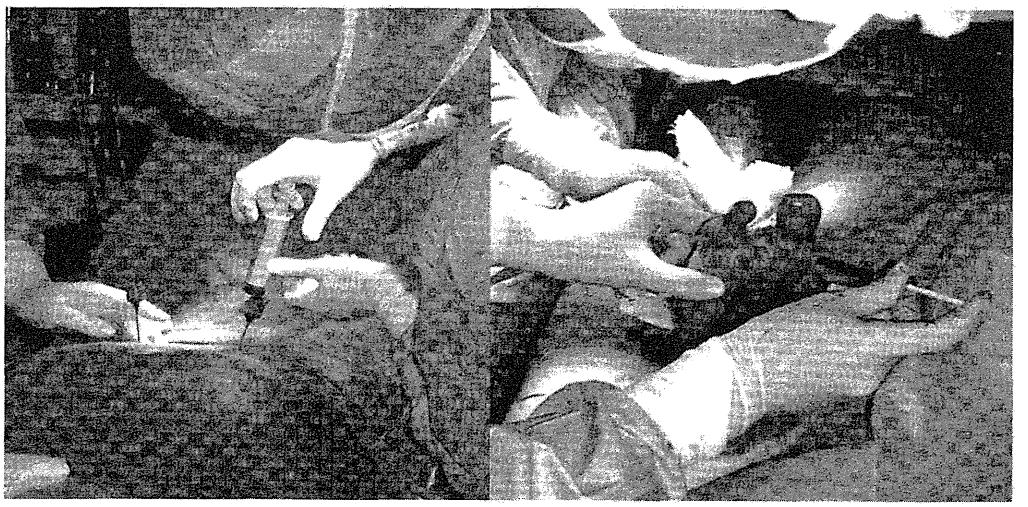

図1 われわれの施設で施行している自己骨髄細胞移植血管再生治療の方法
全身麻酔下に患者腸骨より400～600 mLの骨髄液採取を行い，Fresenius血液成分分離装置（AS TEC 204R）を用いた比重遠心分離法により骨髄単核球成分を分離・濃縮する。20～30 mLに濃縮した骨髄幹細胞溶液を，虚血部位に1.0 mL/箇所，60～100箇所筋肉内投与する。

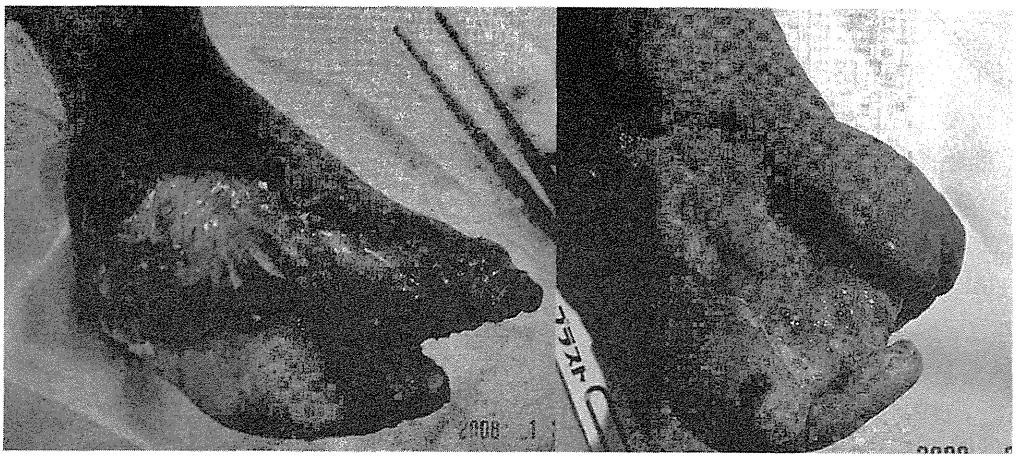

図2 血管再生治療により治癒した症例の術前（左）と術後（右）の写真

どの虚血以外の原因も含む下肢切断患者のリハビリ達成率が66～76%であることから考えると，虚血管理の難しさがうかがわれる。

Ⅳ．血管新生療法

CLIは，血流の低下による組織の壊疽や潰瘍を伴い通常の治療に抵抗性であるため，病態悪化を食い止める事が難しい症例が多い。血流増加を目的とした各種内科的治療（血管拡張薬，抗血小板薬）に加え，潰瘍治療に対する高気圧酸素療法や，血流増加を目的とした腰部交感神経節ブロックを併用するが，多くは重症であるため，入院の上速やかな検査（血管造影等）を行い，血管内カテーテル治療やバイパスグラフト手術の適応を判断する必要がある。いずれの加療も治療不可能か再発，難治性の最重症症例に対して血管新生治療が適応となる[4]。

方法は，自己骨髄幹細胞（**図1，2**）や末梢血幹細胞を用いる細胞治療と，増殖因子を使用する蛋白治療に加え，遺伝子治療まで幅広く臨床研究が行われている。

Ⅴ．有効性の根拠

受精卵や胚性幹細胞（embryonic stem cell：ES細胞）には劣るものの，自己複製能力をもつ幹細胞（stem cell）が成人の骨髄内に存在し，血管内皮前駆細胞（Endothelial Progenitor Cell：EPC）として末梢に動員され[6]，血管新生（An-

giogenesis）や，脈管発生（Vasculogenesis）に寄与していると考えられている。このEPCや各種サイトカインを含む自己骨髄液を採取し，単核球・血小板層を分離・濃縮し，自身の虚血患肢の筋肉内に注射する治療法が，世界初の細胞移植血管再生医療としてわが国で開発された[7]。移植された細胞はその後作業心筋や下肢骨格筋へ分化することが望まれたが，マウスの心筋研究では造血幹細胞は心筋細胞には分化しないと報告された[8, 9]。現在ではその有効性は移植された細胞より長期にわたり放出される増殖因子（b-FGF, VEGF），もしくは骨髄液中に存在する造血因子（顆粒球コロニー刺激因子：G-CSF，幹細胞因子：SCF, エリスロポエチン）などのサイトカインが，血管内皮前駆細胞の動員効果と併せ，血管新生や虚血部の細胞死を抑制する効果が報告されており[10]，造血因子や増殖因子などの蛋白質を治療応用した[11, 12]，もしく遺伝子を治療応用した研究も行われている。

Ⅵ. 血管新生療法成績

下肢虚血に対する自己骨髄細胞移植血管新生療法成績は，本邦のTACTスタディーをはじめとしてその有効性と長期予後が示されている[4, 7, 13~15]。末梢血幹細胞移植の効果も報告された[10]が，一方蛋白治療としてはG-CSF単独の効果はまだcontroversialである[16]。Isner等はVEGF遺伝子の効果を証明した[17]。徐放性b-FGF蛋白の有効性も示されている[11, 12]。これらの知見より，下肢血管新生療法としてのエビデンスは国内外で証明され，臨床治療法として十分応用可能なことから，特に細胞治療は先進医療として承認されている。

Ⅶ. 血管新生療法の限界

下肢再生治療は血管新生療法としての有効性が証明されたが，最終的には生命予後を改善することが目標である。適応疾患の多様性については，厚生省班研究等により，閉塞性動脈硬化症，TAOに加え，膠原病[18]，に対しても効果が証明され，可能性が示されている。

しかし下肢再生治療はあくまで局所治療であり，治療後に全身状態が改善することが生命予後改善には必要である。われわれは重症下肢虚血症例へ自己骨髄細胞移植血管新生療法を行った46症例での後ろ向き予後調査を行った。下肢切断をエンドポイントとした解析を行い，術後6ヵ月の時点における救肢率は87％であった。虚血以外の予後規定因子は，患者背景では血液透析患者が，全身状態指標としては，ヘモグロビン低値，アルブミン低値，コレステロール低値，クレアチニン高値の患者で有意に下肢切断が高率であった。一方治療後の血流指標は有意に改善していることから，血管再生治療の予後は局所の血流改善のみでは達成されないことを示している。これは血液検査より示唆される全身状態不良が強く関与していることを示している。骨髄細胞数の低値もまたリスクとなり得ると報告されており[19]，切断回避のためには全身の栄養状態の改善が不可欠である。栄養補助食品や，場合によっては中心静脈栄養や輸血を併用した全身管理に加え，食欲低下に関与するメンタルケアや疼痛管理等各科による連携治療体制が必要と考える。また重症化した原疾患である心腎脳疾患，糖尿病などの合併症を十分管理し，治療後も再発防止に努めることが重要である。

Ⅷ. 展　望

再生医療により多くの下肢が救われている一方で再生医療の現在の限界点も明らかになった。血管再生治療が血管内皮に与える好影響を示した報告もあり，局所の血流改善や疼痛軽減効果により歩行機能が改善されれば，全身への改善効果が期待できる。心疾患においてもリハビリによる予後改善効果は証明されており[20]，下肢再生治療は，急性期の切断回避の切り札としての意義があり，その後の全身管理による再発予防，歩行維持が長期間継続されて初めて予後改善に寄与すると考える。

…文　献……

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Abstract

Vascular Regenerative Therapy for Peripheral Artery Disease

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The prevalence of peripheral artery disease (PAD) is increasing with reference to the life style related disease. In order to improve worse prognosis of PAD, therapeutic vascular angiogenesis is promising. Researchers indicated the long term prognosis, and the evidence based specific limitations gave rise to further possibility of limb salvage.

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◆特集／日本のフットケア・下肢救済に必要な医療

2．日本におけるフットケア・下肢救済医療 血管治療医の役割：血管再生医療

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Key Words：骨髄幹細胞(bone marrow stem cells)，血管再生治療(therapeutic angiogenesis)，末梢動脈疾患(peripheral arterial disease)，重症下肢虚血(critical limb ischemia)，^{99m}Tc-テトロホスミン血流シンチグラフィー(^{99m}Tc-TF perfusion scintigraphy)

Abstract　難病指定疾患であるBuerger病，閉塞性動脈硬化症(ASO)の治療抵抗性症例に対する自己骨髄幹細胞を用いた血管再生治療は，安全性に優れ有効性も証明され，現在わが国において先進医療に承認されている．さらに末梢血単核球細胞，末梢血幹細胞を使用した血管再生治療も先進医療に追加承認された．私共は，20～79歳までで現行のいかなる内科的・外科的治療でも治癒しない治療抵抗性末梢動脈疾患(PAD)であるBuerger病，ASO症例に対して疼痛除去，自立歩行による退院をprimary endpointとして，症例毎に再生医療適応評価委員会を開催した上で，自己骨髄幹細胞による血管再生治療を実施した．その結果現在まで58症例中52例(89.7%)において，このendpointを達成したが，外来通院中(当科および他院)に再生治療実施後2年以内に患肢大切断は2例増加し8例(13.8%)となった．膠原病症例中，特に進行性全身硬化症(PSS)症例は，手指症例も含めて7例全例に著効し，難治性潰瘍・壊疽の治癒が可能であった．そのため膠原病の中でも難治性潰瘍・壊疽を合併することが最も多いPSSに対して現在難治性疾患克服研究事業研究班としてデータをまとめ，厚生労働省に対して適応拡大申請中である．また，^{9m}Tc-TF血流シンチは，血管再生療法後の血流評価の半定量化，視覚化も可能であり，有効な指標になり得ることを報告した．

はじめに

糖尿病や末梢動脈疾患(Peripheral arterial disease；PAD)が増加し，その結果，現行の治療法に抵抗性の難治性足潰瘍・壊疽患者が急増している．この病態の特徴として，下肢血流の悪化と共に易感染性，創傷治癒遅延，さらに多施設・長期間の治療によりメチシリン耐性ブドウ球菌(Methicilin Resistance Staphylococcus Aureus；MRSA)，多剤耐性緑膿菌(Multiple Drug Resistance Pseudomonas Aueruginosa；MDRP)などの多剤耐性菌の繁殖が起こり，足趾潰瘍・壊疽から蜂窩織炎，骨髄炎，筋膜炎から患肢大切断へと至る症例が増加してきている．

これら重症例の治療には，血流改善は当然だが，潰瘍・壊疽の感染制御，創傷治癒の3要因を同時に総合的に治療することが必須となる．現行の保険診療の治療法を優先することは当然であるが，この治療抵抗性の難治性症例の包括的治療のため，私共は臨床部門の「再生医療科」として1)「自己骨髄幹細胞筋肉内投与による血管新生療法(先進医療承認)」，2)「DDS徐放化b-FGFによる血管新生療法(2008年内閣府スーパー医療特区分担研究課題)」，3)「マゴットセラピー(医療用無菌ウジ治療：株式会社バイオセラピーメディカルhttp://www.btmcl.com/を起業)」，4)「自己骨髄幹細胞浸透人工真皮による新しい組織再生法」，

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などを開発・臨床応用してきた．本稿では，私共が2002年4月より取り組んでいる重症下肢虚血(CLI)に対する血管再生治療の実際について述べる．

自己骨髄幹細胞，末梢血単核球，末梢血幹細胞による血管再生治療

1997年に成人末梢血中にも血管内皮前駆細胞(Endothelial Progenitor Cells；EPCs)が存在することが発見され[1]，血管発生に関して従来のAngiogenesis(血管新生)という概念に加え，Vasculogenesis(脈管発生)という新しい概念が生まれた[2]．この概念を活かしつつ，末梢血よりもさらに豊富にEPCsが存在し，各種サイトカインも豊富な骨髄より自己骨髄液を採取し(cytokine cocktail)，特殊な骨髄細胞分離装置を使用して単核球・血小板層を分離・濃縮し，自己の虚血患肢の筋肉内に注射する新治療法が，我が国で開発された．2000年6月よりPADであるBuerger病，閉塞性動脈硬化症(ASO)に対して臨床研究が開始され(TACT trial)，その有効性が報告された[3]．その後実施施設も増加し，安全性・有効性が確認され，我が国再生医療分野初の高度先進医療に承認された(現在は先進医療に名称変更，平成22年9月1日現在19施設の承認)．その後，骨髄液採取に対する全身麻酔，さらに採取時の腹臥位による全身循環系への影響などを考慮し，意識下で末梢血より全身循環している単核球細胞層を採取する「自己末梢血単核球移植による血管再生治療」も6施設，そしてG-CSF(Granulocyte colony stimulating factor)を使用した「末梢血幹細胞による血管再生治療」も6施設が先進医療に承認されている．

適応基準

これら骨髄・末梢血血管再生治療は，いずれもFontaine分類Ⅲ，Ⅳ度で現行のByPass手術やPTA(percutaneous transluminal angioplasty)などの治療法では治癒不可能な重症の治療抵抗性症例のみを適応としており，実施施設間での詳細な適応基準に多少の違いはあるにせよ膝関節下の下腿PAD症例を適応としている．TASC Ⅱ(Trans-Atlantic Inter-Society Concensus Ⅱ)における推奨事項37：大腿膝窩動脈病変の治療において，TASC AおよびD病変に対する治療として，血管内治療はA型病変に対する第一選択治療であり，手術療法はD型病変に対する第一選択治療であるgrade Cとしており，さらに膝窩動脈以下の血管内治療は通常，救肢が適応となるが，この部位におけるICに対する血管内治療とバイパス術とを比較したデータはなく，CLIや膝窩動脈以下の閉塞のある例において，内科的併存症があり，また血管形成術によりin-line流路が再形成される可能性のある場合では，PTAを推奨するエビデンスが増加している．しかし，PTAでは現在我が国の健康保険適応はなく，高い再閉塞，再狭窄率を考慮すると骨髄であれ，末梢血であれ血管再生治療は，明らかな治療法のないTASC Ⅱガイドラインを超えた第3の治療法になる可能性が高い(Beyond TASC Ⅱ)．ただし，このような治療抵抗性の重症症例にTASC Ⅱの求める無作為大規模臨床試験は倫理上実施しにくく，血管再生治療のエビデンスレベルは，grade Cに止まっている．さらに血管再生治療は，筋肉内投与であるが，注入された骨髄，末梢血幹細胞が静脈系よりさらに大循環系に移行する可能性を考えると，悪性新生物を有する症例での増悪，再発促進の可能性を現在では完全に否定しきれず，実施症例は，悪性新生物がないか，治療後5年以上経過した症例，そして未治療の増殖型糖尿病網膜症の存在がないことを確認する必要がある．

術前検査および評価

①生化学的検査(HbA1cを含む)，②悪性腫瘍チェック(腫瘍マーカー：CEA，CA19-9，AFPなど)，③心臓冠動脈造影(CAG)，④上部消化管内視鏡検査を必須術前検査としており，さらにevaluation studyとして前，および4週後に①VAS(visual analog scale)を用いた痛み測定，②DSA(digital subtraction angiography)：高度腎機