Imaging angiogenesis using $^{99m}$Tc-MAA scintigraphy in patients with peripheral artery disease

Short title: Takagi: Diagnostic examination for angiogenesis.

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Subject Categories: Drug safety, SPECT, Vascular
ABSTRACT

One problem of vascular angiogenesis therapy is the lack of reliable methods for evaluating blood flow in the microcirculation. We aimed to assess whether 99mTc-macroaggregated albumin perfusion scintigraphy (99mTc-MAA) predicts quantitated blood flow after therapeutic angiogenesis in patients with peripheral artery disease (PAD).

MATERIALS AND METHODS Forty-six patients with PAD were treated with bone marrow mononuclear cell implantation (BMCI). Before and 4 weeks after BMCI, blood flow was evaluated via transcutaneous oxygen tension (TcPO2), ankle-brachial index (ABI), intravenous 99mTc-tetrofosmin perfusion scintigraphy (99mTc-TF), and intraaortic 99mTc-MAA.

RESULTS Four weeks after BMCI, TcPO2 improved significantly (20.4 ± 14.4 to 36.0 ± 20.0 mmHg, p<0.01), but ABI did not (0.65 ± 0.30 to 0.76 ± 0.24, p=0.07). Improvement in 99mTc-TF count (0.60 ± 0.23 to 0.77 ± 0.29 count ratio/pixel, p<0.01) and 99mTc-MAA count (5.21 ± 3.56 to 10.33 ± 7.18 count ratio/pixel, p=0.02) was observed in the foot region but not the lower limb region, using both methods. When these data were normalized by subtracting the pixel count of the untreated side, the improvements in 99mTc-TF count (-0.04 ± 0.26 to 0.08 ± 0.32 count ratio/pixel, p=0.04) and 99mTc-MAA count (1.49 ± 3.64 to 5.59 ± 4.84 count ratio/pixel, p=0.03) in the foot remained significant. 99mTc-MAA indicated that the newly developed arteries were approximately 25 μm in diameter.

CONCLUSIONS BMCI induced angiogenesis in the foot, which was detected using 99mTc-TF and 99mTc-MAA. 99mTc-MAA is a useful method to quantitate blood flow, estimate vascular size, and evaluate flow distribution after therapeutic angiogenesis.

**KEY WORDS** angiogenesis, bone marrow mononuclear cells, peripheral artery disease, 
radionuclide imaging, sensitivity and specificity
INTRODUCTION

Peripheral artery disease (PAD), which is mainly due to atherosclerosis, has a poor prognosis (1) and is increasingly becoming a worldwide problem (2). Recently, the therapeutic focus has shifted to regenerative techniques that induce angiogenesis. Human bone marrow is a diverse reservoir for several progenitor cell populations, including endothelial progenitor cells (EPCs) (3). The use of progenitor cells (stem cells) for cell-based regenerative therapy is promising because of their high proliferative capacity and multilineage differentiation potential and also because of their contribution to angiogenesis and/or arteriogenesis (4) through the recruitment of EPCs (5), and their functionality and secretion of growth factors (6) and cytokines (7-9) that promote cell survival.

Thus, many approaches to therapeutic angiogenesis have been investigated (10-13). We have investigated therapeutic angiogenesis using bone marrow mononuclear cell implantation (BMCI) and have accumulated clinical evidence for different populations (12, 14, 15). To resolve the issue of the lack of a reliable technique for blood flow analysis at the microcirculation level, one of the aims of this study was to establish a method for analysis of angiogenesis by quantitative radionuclide determination in order to evaluate blood flow in terms of the target-to-background ratio (TBR), which references the brain count as a control, as we previously reported (12, 14, 15). A secondary aim was to confirm the location of improved blood flow using the radioisotope examination. Previous reports have evaluated the localization of perfusion using $^{99m}$technetium-macroaggregated albumin ($^{99m}$Tc-MAA) (16-18). However the results are difficult to interpret. A third aim was to estimate the sizes of new arteries that are smaller than visible arteries (<200 μm). In this context, we hypothesized that radionuclide assessment after BMCI would be advantageous. $^{99m}$Tc-MAA has been used almost universally as the perfusion agent for lung scintigraphy. After intravascular injection of $^{99m}$Tc-MAA, radioisotope particles are lodged in the capillaries and pre-capillary arterioles of the target vessels in proportion to perfusion (19), and this
is less affected by inflammation. $^{99m}$Tc-MAA perfusion scintigraphy is a suitable method to confirm the presence of angiogenesis-related microvascular blood flow. In this investigation, we performed a quantitative analysis of perfusion, determined blood flow distribution, and estimated vessel size after BMCI using $^{99m}$Tc-MAA perfusion scintigraphy, and also estimated the diagnostic accuracy.

MATERIALS AND METHODS

Study Design and Participants

We enrolled 46 consecutive patients (age 59.8 ± 13.7 years, male 63%) with arteriosclerosis obliterans or thromboangiitis obliterans from 2011 September to 2012 December who had rest pain or ischemic ulcer (Fontaine class 3 to 4, Rutherford class III-4 to III-6) for more than 3 months under standard treatment but were not eligible for bypass surgery or endovascular catheter treatment. Exclusion criteria were: active infection confirmed by blood examination or the presence of osteomyelitis; no evidence of angiologic stenosis; vascular surgery within the previous 30 days; active malignancy as determined by endoscopy, tumor marker, or fecal occult blood testing or history of cancer treatment within the past 5 years; untreated proliferative diabetic retinopathy; current smoker; addiction to alcohol or any other drug; evidence of viral infection (HBV, HCV, HIV); complications of any serious disease affecting the patient’s general condition, such as organic brain disease or heart, lung, kidney, or liver failure; and inability to participate in radionuclide imaging studies before and after the BMCI. This study was performed at the Nippon Medical School Hospital and was approved by the Institutional Review Board (ethical committee of Nippon Medical School), and all patients provided written informed consent. The protocol was registered with the University Hospital Medical Information Network-Clinical Trial Registry (UMIN-CTR), which is accepted by the International Committee of Medical Journal Editors (No. UMIN000006166).
Bone Marrow Mononuclear Cell Implantation

Bone marrow mononuclear cells were injected in the calf and foot muscles of the ischemic limbs as previously described in detail (14, 15). Briefly, bone marrow (400 to 600 mL) was collected from the bilateral iliac bones under general anesthesia. The mononuclear cell fraction was sorted, and 60 to 100 mL of the cell suspension was processed by a cell separator (AS-TEC 204, Fresenius Kabi). As bone marrow aspirates were being processed, necrotic tissue was surgically debrided under sterile conditions. Thereafter, the cell suspension (1 mL/point) was injected intramuscularly. Cell injection was performed based on guidance from a marked transparent overlay (figure), which helped to ensure that cells were evenly injected, at 1 mL per site, into the entire ischemic area of the below-knee muscles. Finally, skin grafting was performed to cover the ulcers unless there was spontaneous epithelialization of the wound margin.

Flow cytometry was performed with the bone marrow cells for quality analysis of the mononuclear cell count, including endothelial progenitor cells (EPCs), as described previously (20). Briefly, EPCs were analyzed for the expression of CD34, CD45, CD133, and vascular endothelial growth factor receptor-2 (VEGFR-2) using four-color flow cytometry (FACSCalibur; BD Biosciences). Samples were incubated with anti-CD34 FITC (Beckman Coulter Inc.), anti-CD45-PerCP (BD Biosciences), anti-CD133/2 (293C3)-APC (Miltenyi Biotec GmbH), and phycoerythrin-conjugated anti-VEGFR-2 (R&D Systems, Inc.) for 40 minutes at 4°C, followed by erythrolysis via the addition of a lysing reagent, and then washed once with 0.2% phosphate buffered saline with bovine serum albumin. CD34+ cells were analyzed using sequential gating strategies. A CD45 versus side scatter dot plot was set to include all CD45+ events and CD45+ events were set to include all nucleated white blood cells and to exclude red blood cells, nucleated red blood cells, platelets, and other cellular debris, which do not express CD45. C45+ cells were gated on a forward scatter versus
side scatter dot plot to confirm the mononuclear cell fraction. Mononuclear cells formed a cluster with low side scatter and low to intermediate forward scatter. CD34+ and CD45dim cells in the mononuclear cell fraction were gated on a forward scatter versus side scatter dot plot to obtain a cluster of true CD45dim CD34+ cells. True CD45dim and CD34+ events were displayed on a CD133 versus VEGFR-2 dot plot, and then the resulting population was examined for the dual expression of VEGFR-2 and CD133. CD45dim/CD34+/CD133+/VEGFR-2+ cells were enumerated in the upper right quadrant of the plot. At least 2,000,000 events were measured in the CD45+ gate. Data were analyzed using CELLQuest (BD Biosciences). The EPC values were defined as the percentage of CD34+, CD45dim, CD133+, and VEGFR-2+ cells per CD34+CD45dim cells fraction.

**Clinical Assessment**

The visual analogue pain scale (VAS, mm) and maximum walking distance were evaluated. The VAS scored maximal pain as 100 and minimal pain as 0. The maximum walking distance was determined using a standard treadmill test. Specialized personnel supervised the exercise tests. All patients were asked to walk on a treadmill at a speed of 3.1 km/h for a maximum of 5 min. No inclining plane protocol was used. Patients were encouraged to finish the whole test, but it was stopped when the patient was unable to walk further. The time and walking distance until the occurrence of leg pain and the total walking time and distance were recorded. Several parameters were evaluated to quantify recovery of local blood flow. The ankle-brachial index (ABI) (Omron Healthcare Co. Ltd.) was measured using standard methods and calculated as the ratio of ankle to brachial pressure. Tissue oxygen content was measured as transcutaneous oxygen tension (TcPO2) with a TCM 400 monitor (Radiometer, Inc.). The transducer was placed on the dorsum of the ischemic limb and warmed to 43.5°C to increase skin permeability to oxygen.
molecules at the measurement site. Consistent TcPO$_2$ data was collected for approximately 20 min with the patient resting in a supine position and breathing room air.

$^{99mTc}$-TF radionuclide imaging was performed on 45 patients using a previously reported method ($^{14}$, $^{15}$). $^{99mTc}$-TF (740 MBq, total volume 1.25ml) was injected intravenously. The $^{99mTc}$-TF data was used to enable comparison and examination of $^{99mTc}$-MAA scintigraphy. $^{99mTc}$-MAA imaging analysis was performed for 11 patients who successfully underwent angiography. All other patients did not undergo angiography were excluded from the $^{99mTc}$-MAA study. $^{99mTc}$-MAA scintigraphy was performed as follows: a pig-tail catheter was placed in the descending aorta below the renal artery and just above the iliac bifurcation and 296 MBq of commercial $^{99mTc}$-MAA (Nihon Medi-Physics Co., Ltd.) was injected into the artery in an antegrade fashion. The $^{99mTc}$-MAA consisted of 1.7 mg of human serum macroaggregated albumin (24×$10^4$ particles, total volume 19.8 ml) in which 95% of the $^{99mTc}$ was bound to the MAA.

Data were acquired in a 256×1024 matrix on the 140 KeV photopeak of $^{99mTc}$. Approximately 12 min after injection of the radiotracer, whole-body scintigraphy was performed with the patient in a prone position in both anterior and posterior projections with a dual-head large field-of-view gamma camera (ADAC Vertex, Philips). Each head of the gamma camera was equipped with a low-energy, high-resolution collimator. The scan speed was 120 mm/min and the image acquisition time was approximately 15 min. The data were averaged for quantitative analysis. To analyze the data acquired from $^{99mTc}$-TF and $^{99mTc}$-MAA scintigraphy, regions of interest (ROI) of equal surface area were drawn around the appropriate muscle group (calf muscles and foot) in the anterior and posterior projections. After the radionuclide count within the ROI was determined, intracranial (brain) uptake was calculated as the background. The target-to-background ratio (TBR) was defined as the average counts per pixel in each muscle/the average counts per pixel in the brain.

Examinations were performed before and at 4 weeks after therapy. To avoid bias, the actual change
in blood flow was analyzed by subtracting the pixel count of the untreated limb from that of the treated limb. Granulometric analysis of $^{99m}$Tc-MAA microparticles was performed by a laser diffraction particle size analyzer (Sald-7000, Shimadzu Scientific Instruments), and the diameter was visually confirmed under a microscope after staining with a 1% 4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein solution. Samples from 3 lots were analyzed to determine these measurements and averaged. Granulometric analysis was performed by FujiFilm RI Pharma Co., Ltd. All data were collected retrospectively.

Patients were monitored for major adverse cardiovascular events until 4 weeks after BMCI. These were defined as all-cause mortality, non-fatal myocardial infarction (including silent myocardial infarction), stroke, acute coronary syndrome, and endovascular or surgical intervention on the coronary or leg arteries. In addition, patients were followed for one year to monitor for amputation-free limb survival and major adverse events.

**Statistical Analysis**

Exact 2-sided 95% confidence intervals (CIs) were evaluated using a binominal distribution. The association between BMCI and the untreated side was examined using two-way repeated measures ANOVA. Within-treatment analyses of changes were performed using a Wilcoxon rank sum test. Cohen’s $\kappa$ coefficient analysis was used to test for better-than-chance agreement between two observers (radiologists). For intra-observer comparisons, at least two examinations from each patient were assessed by each observer, and the observers were blinded to the patient's clinical condition.

Considering the high rate of limb amputations in the non-treatment group, untreated limbs in treated patients were used as controls. The sensitivity and specificity of radioisotope detection were calculated with TcPO$_2$ threshold against limb amputation as the standard and used to determine
likelihood ratios. For a positive change in the TBR, the likelihood ratio was calculated as sensitivity / 1 – specificity; for a negative change in the TBR, the likelihood ratio = 1 – sensitivity / specificity. A 95% CI was calculated for every measure. The amputation-free limb survival rate and the occurrence of major adverse events within the one-year follow-up period were assessed using a chi-squared analysis. A value of p<0.05 was considered statistically significant. All statistical analyses were performed using SPSS statistics ver. 20 software (IBM Corp.).

RESULTS
The baseline characteristics of the study participants are shown in Table 1. Improvements were seen in the indicators of clinical effectiveness. VAS decreased significantly, from 79.7±23.8 at baseline to 9.2±12.0 at 4 weeks after BMCI (p<0.01), and maximum walking distance increased significantly, from 94.3±78.5 to 258.0±141.1 m (p<0.01) (Figure 1). Skin perfusion increased significantly 4 weeks after BMCI, as indicated by a change in TcPO2 from 20.4±14.4 at baseline to 36.0±19.8 mmHg at 4 weeks (p<0.01), whereas the ABI did not change significantly (0.65±0.30 to 0.76±0.24, p=0.07) (Figure 2). ABI in the non-treated leg indicated no significant changes (0.86±0.28 to 0.89±0.27, p=0.65).

In the assessment of angiogenesis by radionuclide imaging (representative images are shown in Figure 3), significant increase in the $^{99m}$Tc-TF score was seen in the foot region both the treated limb (0.60±0.23 to 0.77±0.29 count ratio/pixel, p<0.01, Figure 4A) and the untreated limb (0.64±0.20 to 0.70±0.24 count ratio/pixel, p=0.04, Figure 4A). The scores were changed significantly in the lower leg region at 4 weeks after BMCI(treated limb 1.03±0.27 to 1.18±0.38, p<0.01; untreated limb 1.01±0.28 to 1.16±0.34, p<0.01, Figure 4B). On the other hand, the $^{99m}$Tc-MAA score was improved significantly in the foot region of the treated limb at 4 weeks after treatment (5.21±3.56 to 10.33±7.18 count ratio/pixel, p=0.02, Figure 4C) compared with the
untreated side (3.82±1.63 to 6.05±6.07, p=0.33, Figure 4C), but not in the lower leg region (treated limb 5.28±3.63 to 11.79±10.22, p=0.09; untreated limb 6.17±4.40 to 10.85±8.90, p=0.09, Figure 4D). The interaction between the BMCI-treated side and the untreated side were compared (Figure 4). There were significant interactions in the TBR in the foot region for both $^{99m}$Tc-TF (p=0.01, two-way repeated measures ANOVA between the two regions, Figure 4A) and $^{99m}$Tc-MAA (p=0.01, two-way repeated measures ANOVA between the two regions, Figure 4C). However, no interaction was observed in the lower leg region for $^{99m}$Tc-TF (p=0.94, two-way repeated measures ANOVA between the two limbs, Figure 4B) or $^{99m}$Tc-MAA (p=0.31, two-way repeated measures ANOVA between the two regions, Figure 4D). The net gain in blood flow (Figure 5) was calculated by subtracting the pixel count of the untreated limb from that of the treated limb and is expressed as a normalized score. The normalized $^{99m}$Tc-TF score in the foot region improved significantly after BMCI (-0.04±0.26 to 0.08±0.32, p=0.04, Figure 5A) but no improvement was observed in the lower leg region (0.01±0.20 to 0.01±0.26, p=0.97, Figure 5B). In addition, the normalized $^{99m}$Tc-MAA score in the foot region improved significantly (1.49±3.64 to 5.59±4.84, p=0.04, Figure 5C) but no significant changes were observed in the lower leg region (-0.17±4.36 to 1.55±5.14, p=0.45, Figure 5D). There was no difference in TBR associated with disease type.

Regarding the reliability of the TBR assessment, the linear-weighted $\kappa$ values indicated moderate interobserver agreement (0.710) and intraobserver agreement of 0.667 for $^{99m}$Tc-TF, determined after rounding the numerical data to one decimal place. Above-amputation thresholds of TcPO$_2$ and improvement in TBR were assessed at the 4-week evaluation (chi squared test; p=0.64 for $^{99m}$Tc-TF and p=0.024 for $^{99m}$Tc-MAA). The sensitivity, specificity, positive predictive value, negative predictive value, and predictive accuracy for $^{99m}$Tc-TF and $^{99m}$Tc-MAA are shown in Table 2. The granulometric distribution of $^{99m}$Tc-MAA displayed a mean particle size of 25.83 $\mu$m, and 92.7% of particles were within 10 to 60 $\mu$m (Figure 6).
No adverse events occurred during the 4-week follow-up period. All patients had healed ulcers and all were able to use the affected foot at discharge. There were 3 major limb amputations and 1 death during the 1-year follow-up period (mean follow-up 319 days, 95% CI 291–347). The overall limb salvage rate was 93.5% (mean follow-up 317 days, 95% CI 287–348), and the major adverse cardiovascular event-free rate was 93.5% (mean follow-up 309 days, 95% CI 274–344 days). The cut off value of $\Delta^{99mTc}$-TF was 0.11 (determined by ROC curve), and Kaplan-Meier analysis ($p=0.005$ by Log Rank test) against leg amputation showed significant differences (supplementary figure 1). The cut off for $^{99mTc}$-MAA was not included because no leg amputation was observed in this group.

**DISCUSSION**

In this study, we identified a reliable means of quantitative analysis of therapeutic angiogenesis after BMCI. In order to reduce the effect of confounding variables that relate to bilateral limb condition, such as improvements in walking distance and nutritional status (Figure 1), normalized values were calculated by subtracting the untreated limb count (as an internal control) to confirm the clinical effects of BMCI, and the results showed statistically significant improvement in the foot region on both $^{99mTc}$-TF and $^{99mTc}$-MAA scintigraphy (Figure 5). We also examined regional recovery after BMCI by utilizing specific ROIs in the lower leg and foot region, and successfully showed the regional differences. Furthermore, the scores of both $^{99mTc}$-TF (reference diameter of average triclinic crystal structure size of 1.25 nm) (21) and $^{99mTc}$-MAA (reference diameter of average microsphere size of 25 µm) showed improvement at 4 weeks after BMCI. This is direct evidence that mature arterial growth was promoted in the ischemic foot area in the 4 weeks following BMCI. Blood flow analysis by skin perfusion pressure and TcPO$_2$ have also been useful parameters in several therapeutic angiogenesis protocols (12, 14, 15, 22, 23). $^{99mTc}$-TF scintigraphy
is a useful examination for detection of early-phase angiogenesis, as we have reported previously (14, 15). However, the specificity is lower than that of $^{99m}$Tc-MAA, which likely implies that a local inflammatory reaction, such as osteomyelitis or phlebitis (14, 15), results in a large standard deviation, even in the foot region, when compared with $^{99m}$Tc-MAA (Figures 4A and 5A).

Concerning the feasibility of the examination, $^{99m}$Tc-TF is useful because it can be performed at the outpatient screening, whereas $^{99m}$Tc-MAA requires intraaortic injection. However, $^{99m}$Tc-MAA can be performed at the time of diagnostic digital subtraction angiography.

Several limitations of this study deserve mention. Some of the ABI data were indicators of severity of disease. Most of the patients had calcified arteries that made it difficult to obtain correct pressure measurements or to detect ischemia localized only in the foot region. Also, other parameters, such as the skin perfusion pressure or toe pressure, could not be measured because of the location of the ulcers. Nonetheless, tissue ischemia was confirmed by several different modalities, consistent with our previous observations (15). The sample size of patients receiving $^{99m}$Tc-MAA scintigraphy was small, and data for patients with both ASO and TAO were included in the data analysis. Thus, it will be important to confirm these results with a large-scale investigation in a uniformly affected population.

**CONCLUSIONS**

Radionuclide determination of quantitative score, flow distribution, and vascular size with $^{99m}$Tc-MAA appears to be a useful method to evaluate functional blood flow recovery after BMCI. Thus, it may be reliable approach to confirm therapeutic angiogenesis in the clinical setting.

Furthermore, the radionuclide approach is applicable for other angiogenesis protocols such as those using growth factors, genes, or pluripotent stem cells.
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Disclosures

N Seki is an employee of FUJIFILM RI pharma Co., Ltd.

Trial Registration: UMIN-CTR: URL=http://www.umin.ac.jp/ctr/index-j.htm, No. UMIN000006166)
REFERENCES


Figure 1: Visual analogue scale and maximum walking distance

Graphs show significant improvement in the visual analogue scale score (A) and maximum walking distance (B) at 4 weeks after the therapy.
Figure 2: Transcutaneous oxygen tension (TcPO₂) and ankle-brachial index (ABI)

TcPO₂ (A) was significantly improved 4 weeks after the therapy. However, there was no significant change in the ABI (B).
Figure 3: Representative radioisotope images of angiogenesis using $^{99m}$technetium-tetrofosmin ($^{99m}$Tc-TF) and $^{99m}$technetium-macroaggregated albumin ($^{99m}$Tc-MAA) perfusion scintigraphy.

Panel A shows a representative $^{99m}$Tc-TF study from a patient who underwent BMCI in an ischemic left leg (left-hand image, baseline; right-hand image, 4 weeks after treatment). Panel B shows a representative $^{99m}$Tc-MAA study in a patient who underwent BMCI in an ischemic right leg (left-hand image, baseline; right-hand image, 4 weeks after treatment).
Figure 4: Quantitative radionuclide scores from treated and untreated limbs

Left panels indicate the foot region (A and C), and right panels indicate the lower leg region (B and D).

Upper panels (A and B) display the $^{99m}$technetium-tetrofosmin perfusion scintigraphy ($^{99m}$Tc-TF) score before and at 4 weeks after bone marrow mononuclear cell implantation (BMCI). There was significant improvement in the foot region of the BMCI-treated leg compared with the untreated limb (A), but not in the lower leg region (B). Lower panels (C and D) show the $^{99m}$technetium-macroaggregated albumin perfusion scintigraphy ($^{99m}$Tc-MAA) scores before and at 4 weeks after BMCI. There was significant improvement in the foot region of the BMCI-treated leg compared with the untreated limb (C), but not in the lower leg region (D).
Figure 5: Normalized radionuclide scores from treated and untreated limbs

The left panels show results from the foot region (A and C), and the right panels the lower leg region (B and D). Upper panels (A and B) display the $^{99m}$technetium-tetrofosmin perfusion scintigraphy ($^{99m}$Tc-TF) scores before and 4 weeks after bone marrow mononuclear cell implantation (BMCI). There was significant improvement at 4 weeks after BMCI in the foot region compared with the untreated limb (A), but not in the lower leg region (B). Lower panels (C and D) display the $^{99m}$technetium-macroaggregated albumin perfusion scintigraphy ($^{99m}$Tc-MAA) scores before and at 4 weeks after BMCI. There was significant improvement in the foot region after BMCI compared to the untreated limb (C), but not in the lower leg region (D).
Figure 6: $^{99m}$Tc-MAA particle size distribution

Particle size distribution patterns of $^{99m}$technetium-macroaggregated albumin ($^{99m}$Tc-MAA) microspheres.
### Table 1: Patient characteristics

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<td>Bone marrow cells (in total)</td>
<td>4.8±3.7×10⁶ (95% CI 3.4-6.2×10⁶)</td>
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<td>CD34+ cells</td>
<td>490.9±368 cells/µL</td>
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ASO=arteriosclerosis obliterans. TAO=thromboangiitis obliterans. CKD=chronic kidney disease. Data are shown as mean±SD or number (%) of patients.
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$^{99m}$Tc-TF = $^{99m}$technetium-tetrofosmin perfusion scintigraphy; $^{99m}$Tc-MAA = $^{99m}$technetium-macroaggregated albumin perfusion scintigraphy; AUC = area under the receiver-operator characteristic curve. *Values in parentheses are 95% confidence intervals.
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