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ORIGINAL ARTICLE

Transcutaneous oxygen pressure as a surrogate index of lower limb amputation

Hiroomi NISHIO [1], Kenji MINAKATA [1] *, Atsushi KAWAGUCHI [2], Motoyuki KUMAGAI [1], Takafumi IKEDA [3], Akira SHIMIZU [3], Masayuki YOKODE [4], Satoshi MORITA [2], Ryuzo SAKATA [1]

[1]Department of Cardiovascular Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]Department of Biomedical Statistics and Bioinformatics, Institute for Advancement of Clinical and Translational Science, Kyoto University Hospital, Kyoto, Japan; [3]Department of Experimental Therapeutics, Institute for Advancement of Clinical and Translational Science, Kyoto University Hospital, Kyoto, Japan; [4]Department of Clinical Innovative Medicine, Institute for Advancement of Clinical and Translational Science, Kyoto University Hospital, Kyoto, Japan

*Corresponding author: Kenji Minakata, Department of Cardiovascular Surgery, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto, 606–8507, Japan. E-mail: minakata@kuhp.kyoto-u.ac.jp

ABSTRACT

INTRODUCTION: A large number of clinical trials of therapeutic angiogenesis in patients with critical limb ischemia have been conducted in recent years. However, limb amputation, which is used as a primary endpoint in such studies, is not often required in Japan, which can make it difficult to carry out related clinical trials. Transcutaneous oxygen pressure ($TcPO_2$) is widely used to evaluate the severity of limb ischemia, to decide the level of amputation, and to predict wound healing after limb amputation. The aim of the present study was to elucidate whether $TcPO_2$ can be a surrogate index of limb ischemia, and to define an appropriate cutoff value for wound healing after limb amputation using meta-analysis.
EVIDENCE ACQUISITION: A computer search was performed to identify studies describing the association between $TcPO_2$ and limb ischemic events. From these, studies focused on wound healing after limb amputation were combined and analyzed.
EVIDENCE SYNTHESIS: Eleven studies were identified for inclusion in this analysis. The analysis demonstrated that $TcPO_2$ 20 mmHg was a valid cutoff value for limb amputation and $TcPO_2$ 30 mmHg would be an appropriate value for wound healing after limb amputation.
CONCLUSIONS: $TcPO_2$ of 20 and 30 mmHg were considered appropriate cutoff values for limb amputation and wound healing after amputation, respectively.

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Key words: Lower extremity - Amputation - Blood gas monitoring, transcutaneous - Wound healing - Meta-analysis.

Introduction

Peripheral arterial disease (PAD) in the lower limbs is associated with stenosis or occlusion in the arteries of the lower limbs and appears as one of the conditions of systemic arteriosclerosis. Clinically, it is characterized by intermittent claudication and induces rest pain and ulceration as the disease condition progresses. Treatment of this disease includes improvement of lifestyle through smoking cessation, exercise and diet therapies, and treatment of underlying diseases, such as diabetes mellitus, dyslipidemia, and hypertension. In addition, when the disease is complicated by severe stenotic lesions, percutaneous transluminal angioplasty or surgical revascularization is indicated. However, some patients have severe ischemic lesions, such as diffuse stenosis below the knee or peripheral arteriolar lesions, for which angioplasty and surgical revascularization are not effective or indicated; if these severe ischemic lesions are associated with refractory gangrene or uncon-

trollable pain, the only effective treatment option is amputation of the diseased limb. A report from Europe and the USA reported an approximately 30% rate of lower limb amputation one year after the diagnosis of severe lower limb ischemia.[1] Thus, the development of novel treatment methods for conservation and functional improvement of diseased limbs is urgently needed. The development of angiogenic therapy using regenerative medicine is advancing in Japan, and is attracting substantial attention with high expectations. To date, global clinical trials of new therapies have used so-called hard endpoints, such as amputation of diseased limbs or death, as efficacy endpoints. The incidence rate of lower limb amputation is reportedly 120 to 500 per 1,000,000 population in Europe and the USA, whereas in Japan, the incidence rate is much smaller and is estimated to be 30 per 1,000,000 population.[1, 2] Therefore, in Japan, it is extremely difficult to set an appropriate and feasible number of patients to be enrolled in a study using the rate of lower limb amputation after treatment as a primary endpoint for development of new treatment methods for severely ischemic limbs. On the other hand, the evaluation of function of blood capillaries that directly reflects severe lower limb ischemia, i.e., the measurement of transcutaneous oxygen pressure ($TcPO_2$), has been actively conducted for the purpose of severity classification of ischemic limbs, assessment of amputation level and treatment efficacy since the 1980s. Many studies have reported the relationship between $TcPO_2$ and lower limb amputation.[3, 4] In this meta-analysis, we investigated whether $TcPO_2$ can serve as a surrogate index for avoiding lower limb amputation, and determined the $TcPO_2$ values that would affect postamputation wound healing, by setting multiple cutoff values.

Evidence acquisition

Reports that satisfied the following five inclusion criteria were evaluated for this study: (1) patients had lower limb ischemia; (2) $TcPO_2$ values were measured, and endpoints associated with lower limb amputation were included; (3) the site of lower limb amputation was other than the toe; (4) the cutoff value was set or could be set; and (5) the reports were published in English. The study data were collected by searching PubMed for clinical research papers up to February 2015. "Limb ischemia" and "transcutaneous oxygen pressure" were used as search keywords. A manual search was also performed at the same time. The measured $TcPO_2$ values were classified using 4 cutoff values of 10 mmHg, 20 mmHg, 30 mmHg, and 40 mmHg, and meta-analyses of the diagnostic odds ratio (DOR) were performed for individual cutoff values. The DOR were integrated using the DerSimonian and Laird method, a random effects model.[5] I2 values and corresponding p values were calculated to evaluate homogeneity. The statistical significance level was set at $P<0.05$. The meta package (version 4.1-0) of the statistical analysis software R (version 3.1.3) was used.[6, 7] In addition, in order to further investigate appropriate cutoff values, a meta-analysis of sensitivity and specificity was performed. The integration of sensitivity and specificity was performed using a bivariate generalized mixed model approach.[8] Furthermore, the mean value of integrated sensitivity and specificity ([sensitivity + specificity]/2) was also calculated. The NLMIXED procedure of the statistical analysis software SAS (version 9.3, SAS Institute Inc., Cary, NC, USA) was used. All statistical analyses were performed by biostatisticians (AK and SM).

Evidence synthesis

The electronic search extracted 259 reports, and 5 reports that satisfied the inclusion criteria were selected. In addition, 8 reports that satisfied the inclusion criteria by manual search were added; 2 reports that had included the same patients were excluded. Finally, 11 reports were chosen for analysis (Table I).[9-19] The reports for which analysis of individual $TcPO_2$ cutoff values could be performed included 9 for 10 mmHg, 10 for 20 mmHg, 9 for 30 mmHg, and 11 for 40 mmHg. It was difficult to distinguish patients in the report by Bacharach *et al.*,[19] and the report was excluded from the analyses at cutoff values of 10 mmHg and 30 mmHg. The endpoint for all reports was defined as healing failure after lower limb amputation. The total number of patients investigated was 534, and the number of events was 96 (17.9%). The studies by Katsamouris *et al*.[12] and Bacharach *et al*.[19] did not report the outcome by amputation site. Slagsvold *et al*.[18] reported that no events occurred in 4 patients who underwent through-knee amputation but that 3 events (15.0%) occurred in 20 patients who underwent below-knee (BK) amputation. The number of patients by amputation level and

TABLE I.—*Description of studies included in this analysis.*

Study	Design	Sample size	Endpoint	Event (%)
Burgess (1982)[9]	Prospective cohort	37	Healing failure after BK amputation	7 (18.9)
Dowd (1983)[10]	Prospective cohort	14	Healing failure after AK amputation	0 (0.0)
		5	Healing failure after BK amputation	2 (40.0)
		3	Healing failure after foot amputation	2 (66.6)
Ratliff (1984)[11]	Prospective cohort	17	Healing failure after AK amputation	1 (5.8)
		33	Healing failure after BK amputation	5 (15.1)
Katsamouris (1984)[12]	Prospective cohort	29	Healing failure after amputation	5 (17.2)
Harward (1985)[13]	Prospective cohort	17	Healing failure after AK amputation	8 (47.0)
		55	Healing failure after BK amputation	3 (6.0)
Wagner (1988)[14]	Prospective cohort	65	Healing failure after BK amputation	10 (15.3)
Wyss (1988)[15]	Prospective cohort	31	Healing failure after foot amputation	8 (25.8)
Kram (1989)[16]	Prospective cohort	40	Healing failure after BK amputation	7 (17.5)
Falstie-Jensen (1989)[17]	Prospective cohort	16	Healing failure after AK amputation	4 (25.0)
		58	Healing failure after BK amputation	10 (17.2)
Slagsvold (1989)[18]	Prospective cohort	24	Healing failure after amputation	3 (12.5)
Bacharach (1992)[19]	Retrospective cohort/chart review	90	Healing failure after amputation	21 (23.3)

AK: above-knee; BK: below-knee.

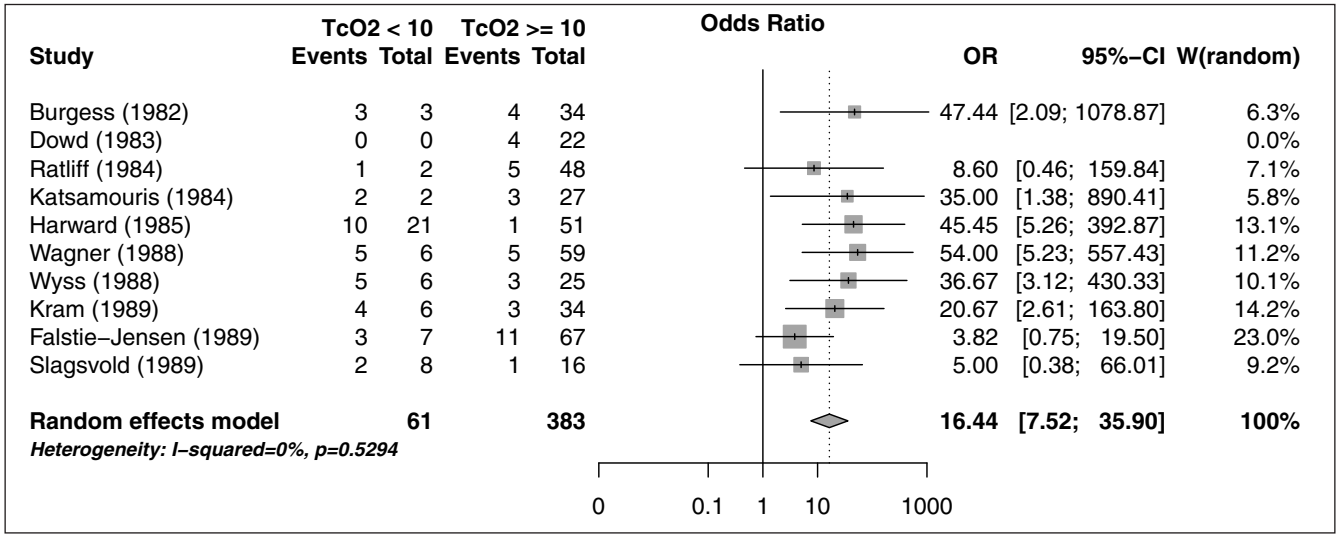

Figure 1.—Meta-analysis of DOR. $TcPO_2$ cutoff value is 10 mmHg.

the number of events (%) were as follows: above-knee (AK) amputation, 64 patients, 13 events (20.3%); BK amputation, 313 patients, 47 events (15.0%); and foot amputation, 34 patients, 10 events (29.4%).

The integrated DOR and 95% confidence intervals (CI) are shown in Figures 1 to 4.[9-19] The DOR for cut-off values of 10 mmHg, 20 mmHg, 30 mmHg, and 40 mmHg were 16.44, (95% CI: 7.52-35.9), 14.58 (95% CI: 5.97-35.6), 9.16 (95% CI: 3.52-23.80), and 8.46 (95% CI: 3.15-22.73), respectively. Thus, the DOR value was the highest when the cutoff value was 10 mmHg. With regard to homogeneity, the I2 value and P-value were 0% (P=0.5294), 42.4% (P=0.0752), 41.3% (P=0.0917), and 46% (P=0.0471) for the cutoff values of 10 mmHg, 20 mmHg, 30 mmHg, and 40 mmHg, respectively. Thus, the homogeneity was high with the cutoff value of 10 mmHg, and moderate with other cutoff values. Significant heterogeneity was suggested only when the cutoff value was 40 mmHg.

Integrated sensitivity and specificity are shown in

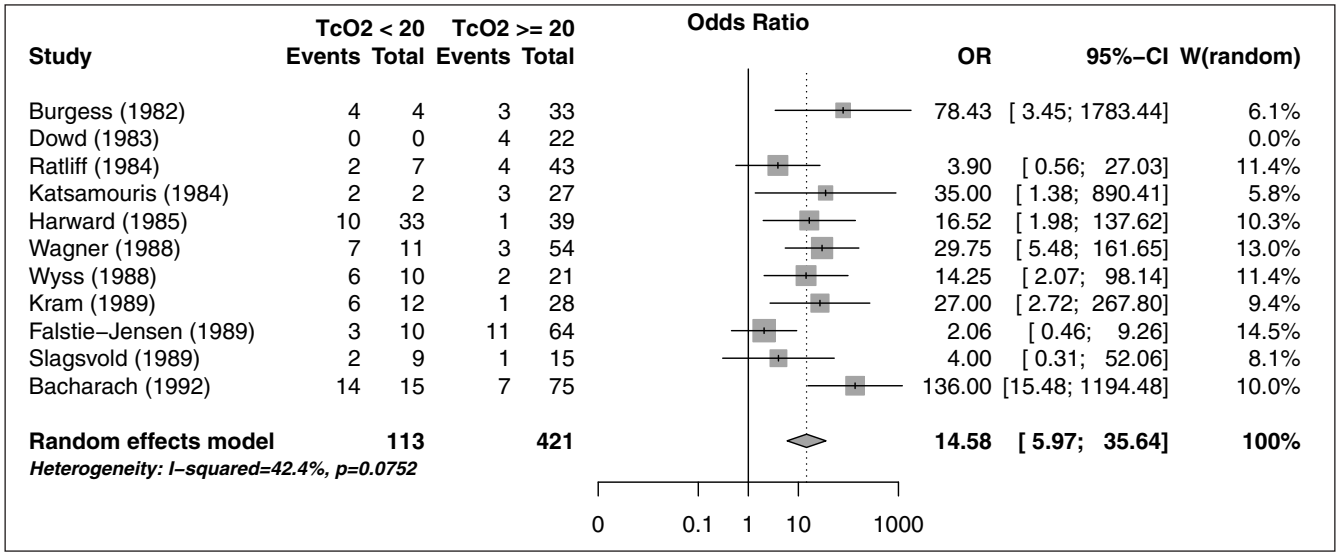

Figure 2.—Meta-analysis of DOR. TcPO$_2$ cutoff value is 20 mmHg.

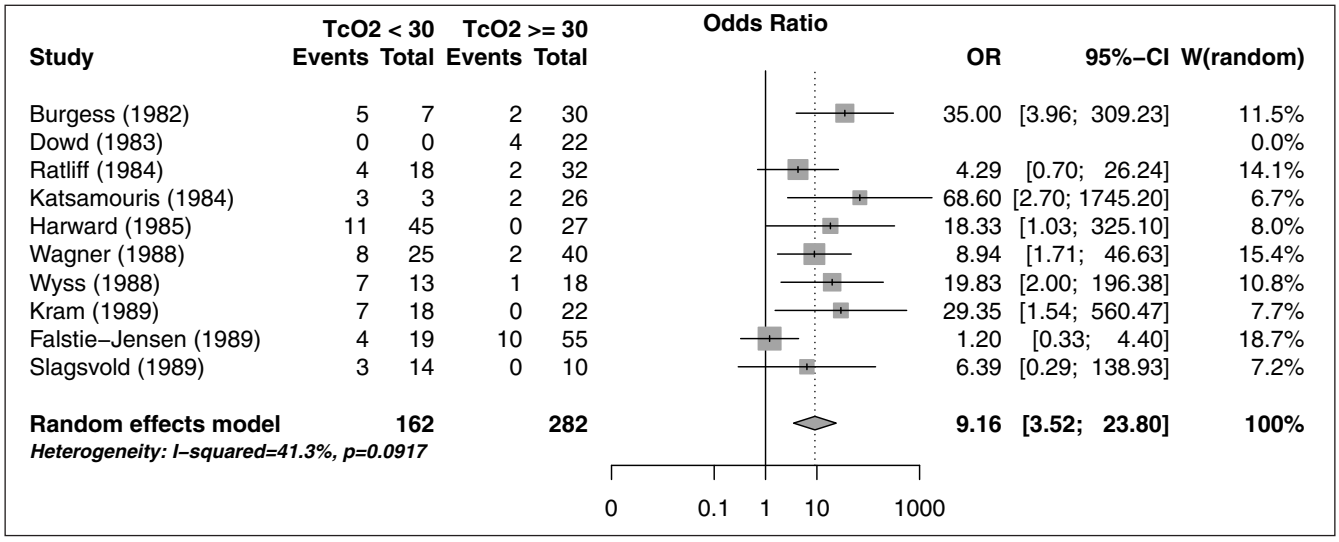

Figure 3.—Meta-analysis of DOR. TcPO$_2$ cutoff value is 30 mmHg.

Table II. The mean values of sensitivity and specificity were 0.726 (95% CI: 0.640-0.811), 0.763 (95% CI: 0.686-0.841), 0.751 (95% CI: 0.671-0.830), and 0.714 (95% CI: 0.638-0.789) for the cutoff values of 10 mmHg, 20 mmHg, 30 mmHg, and 40 mmHg, respectively. Thus, the mean value of sensitivity and specificity was highest when the cutoff value was 20 mmHg, followed by 30 mmHg.

Discussion

PAD is an urgent challenge to address. The prevalence of PAD in the National Health and Nutrition Examination Survey in the United States was 4.3%.[20] In the last decade, the number of people with PAD increased by 23.5% globally.[21] PAD can progress to critical limb ischemia (CLI). Furthermore, 25% of patients

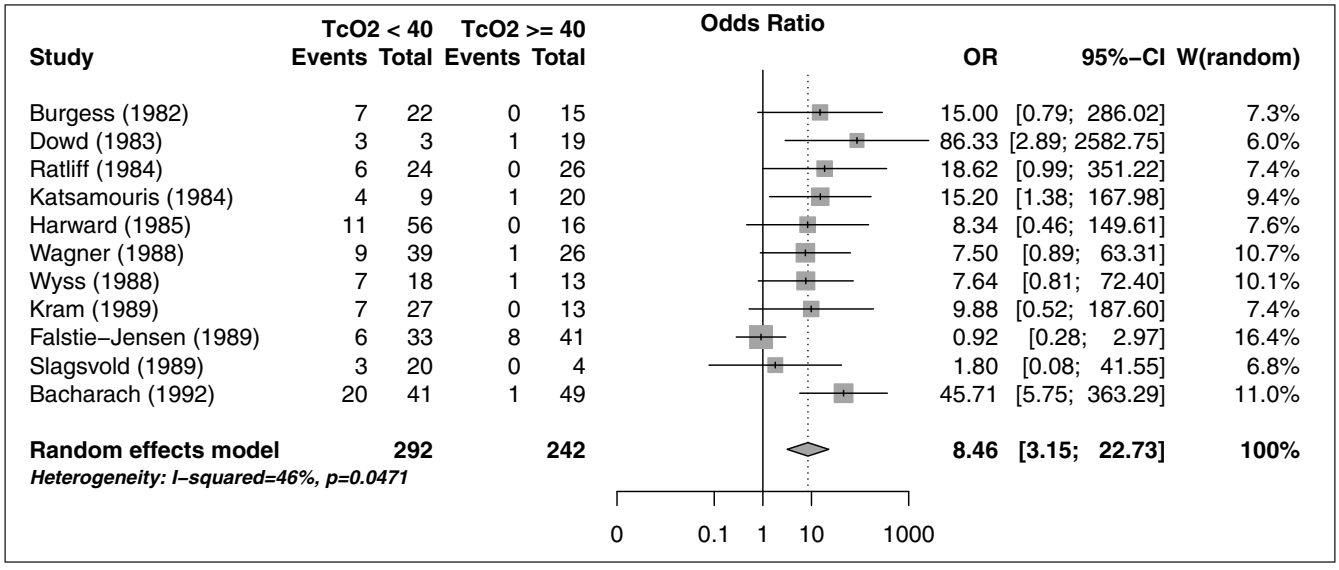

Figure 4.—Meta-analysis of DOR. $TcPO_2$ cutoff value is 40 mmHg.

TABLE II.—*Meta-analysis of sensitivity and specificity.*

Cutoff	Label	Estimate	Lower	Upper
10mmHg	Sensitivity	0.511	0.321	0.701
	Specificity	0.940	0.899	0.982
	Average	0.726	0.640	0.811
20mmHg	Sensitivity	0.632	0.468	0.796
	Specificity	0.895	0.826	0.964
	Average	0.763	0.686	0.841
30mmHg	Sensitivity	0.776	0.609	0.942
	Specificity	0.726	0.605	0.846
	Average	0.751	0.671	0.830
40mmHg	Sensitivity	0.881	0.784	0.977
	Specificity	0.547	0.404	0.689
	Average	0.714	0.638	0.789

Lower and upper: lower and upper limit of each 95% confidence interval.

with CLI die, and 30% of them undergo amputation in a year after the diagnosis is made.[1] Only 25% of them survive asymptomatically without undergoing amputation. At initial presentation, 1-3% of patients with PAD are in the CLI stage. Patients with CLI have chronic rest pain, ulcers, or gangrene attributable to objectively proven PAD. It is not easy to make the diagnosis of CLI because expertise is required to establish the diagnosis, and there is no complete consensus regarding the diagnostic criteria. In the Trans-Atlantic Inter-Society Consensus II (TASC-II), an international guideline on peripheral arterial disease, a need for objective tests, including ankle pressure, toe pressures and $TcPO_2$, is emphasized in addition to clinical symptoms in making a diagnosis of CLI.[1] The critical level is set below 50 mmHg of toe pressures or 30 mmHg of $TcPO_2$. Moreover, the guideline published by the *European Society for Vascular Surgery* in 2011 strongly encourages the measurement of forefoot $TcPO_2$ in vascular clinics because it provides functional information about tissue perfusion and skin viability.[22]

$TcPO_2$ is a measure of partial oxygen pressure in the skin and serves as an index of skin microcirculation. The oxygen molecules that diffuse from the warmed skin blood capillaries are measured by electrodes affixed to the skin. $TcPO_2$ has mainly been used in research facilities since the 1980s, but has clinically been used in the diagnosis of ischemia, prediction of lower limb amputation, selection of amputation site, and prediction of healing of the amputation site. A normal $TcPO_2$ value in the lower limb in the supine position is ≥40 to 50 mmHg. A $TcPO_2$ value of <40 mmHg indicates lower limb ischemia, and a value of ≤20 to 30 mmHg indicates severe ischemia.[23] For lower limb ischemia, subjective endpoints include clinical symptoms, such as pain at the lesion due to ischemia, presence or absence of ulcers, and walking distance (Rutherford classification, Fontaine classification, etc.); objective markers include $TcPO_2$, Ankle-Brachial Index (ABI), Toe-Brachial Pressure Index (TBI),

and skin perfusion pressure (SPP) measured with a laser Doppler flowmeter. Because the ABI and TBI represent the blood pressure ratios of relatively large blood vessels, their relationships to severe pathology in ulcerated ischemic limbs are not clear. Both $TcPO_2$ and SPP are useful for the prediction of wound healing, in addition to the assessment of severity.[24] Although the measurement of $TcPO_2$ is noninvasive, the measurement of SPP may exacerbate pain in severely ischemic limbs because it requires pressurization. Although a study reported that SPP predicts wound healing better than $TcPO_2$,[25] there are cases in which the measurement of SPP may be avoided because of pain. In TASC II the $TcPO_2$ is listed as an objective endpoint, and laser Doppler flowmetry is used "for research purposes." Thus, $TcPO_2$ may be considered a more general index. However, $TcPO_2$ measurement requires examiners with a certain level of experience in setting measurement conditions, for example, and takes a longer time to perform than other tests. In addition, the test is not reimbursed by national health insurance in Japan. Therefore, it is difficult for general clinicians to use.

With regard to the method of analysis, there are multiple indexes to be integrated. In this study, we used the DOR, which allows evaluation in an integrated fashion and which is used in many clinical studies. In the present study, we used a random effects model; however, although the data are not shown, a similar result was obtained using a fixed effects model. The DOR was insufficient for determination of appropriate cutoff values; therefore, the integration of sensitivity and specificity was additionally performed. Thus, the most appropriate cutoff value that allows the healing of amputation stumps while avoiding unnecessary amputation would be approximately 20 mmHg. However, because definitive postamputation healing is desirable, a higher cutoff value might actually be more appropriate. Thus, we considered $TcPO_2$ of 30 mmHg as appropriate. The endpoints of this analysis were set for wound healing after amputation and not for amputation of diseased limbs per se. However, because the final decision as to whether to perform amputation is made based on the status and time course of refractory gangrene and/or uncontrollable pain in each patient, wound healing after amputation is indirectly reflected in the decision-making for amputation. In fact, the guideline published in 2011 describes a $TcPO_2$ value of $\leq$35 mmHg on the dorsum of the foot as a risk factor for major lower limb amputation or death (Grade B).[22] Although the number of reports used in the present study was small, the results support the abovementioned guideline and are of great significance.

In recent years, clinical trials for therapeutic angiogenesis in patients with CLI who are not suitable for bypass surgery or endovascular therapy have been conducted. Some of them showed promising results. Gene therapy comprises introducing growth factors, including vascular endothelial growth factor, hepatocyte growth factor (HGF) or fibroblast growth factor (FGF), intramuscularly or intraarterially using viral vectors or plasmids.[26-28] Moreover, Kumagai *et al*. reported the effectiveness of recombinant FGF combined with biodegradable gelatin hydrogel in CLI.[29] Meanwhile, in trials addressing cell transplantation, angiogenesis is facilitated with the differentiation of transplanted stem cells or progenitor cells into endothelial cells, vascular smooth muscle cells, or pericytes.[30-32] In terms of endpoints, it is preferable to adopt amputation because the goal of therapeutic angiogenesis is to avoid amputation. Recent clinical trials of therapeutic angiogenesis for PAD, however, show that amputation is not always an endpoint. In these trials, safety is often a primary endpoint, and amputation is sometimes a primary or secondary one. Although a definite endpoint other than amputation has been sought, none has been specified. Subjective markers in these trials include peak walking time, pain scale, or quality of life, and objective markers include TBI, $TcPO_2$, or ABI. Although ABI is prevalent and considered the first measurement in making a diagnosis of PAD, some studies reported that ABI did not increase despite an improvement in symptoms.[28] The reason is that therapeutic angiogenesis is effective at the microcirculatory level, and that the target vessel in ABI measurements is relatively large. On the other hand, there are many trials employing $TcPO_2$ as an endpoint.[27, 29-32] Powell *et al*. conducted the HGF-STAT trial to determine the effect of HGF plasmid on safety and limb tissue perfusion in patients with CLI.[27] In the trial, $TcPO_2$, which was one of the primary endpoints, increased at 6 months in the high-dose treatment group compared with the other groups. Although the value of $TcPO_2$ is known to be variable at measurement,[3] Powell *et al*. addressed the variability by measuring it twice before patient enrollment.[27] Considering that $TcPO_2$ can directly evaluate microcirculation, $TcPO_2$ is a suitable marker in clinical trials of therapeutic angiogenesis.

In Europe and the USA, the rates of lower limb amputation and mortality are often used as primary endpoints in studies of CLI treatment. In the TAMARIS trial, a clinical trial of therapy transfecting a gene that codes for FGF, 525 patients with CLI from 30 countries were investigated, with time to major lower limb amputation and mortality rate as primary endpoints.[26] On the other hand, because of epidemiological and economic reasons, such as differences in the incidence rate and severity of arteriosclerotic diseases, geographical location, race, and the health insurance system, the number of lower limb amputations due to ischemia in Japan is much smaller than that in other countries.[1, 2] Therefore, it is very difficult to use the rates of lower limb amputation and mortality as primary endpoints in similar clinical studies in Japan. $TcPO_2$ is described as the best noninvasive test for the quantification of ischemia and the prediction of outcome in the guideline published by the *European Society for Vascular Surgery* in 2011,[22] and is an objective endpoint for CLI in TASC II.[1] In addition, many studies have reported that $TcPO_2$ is useful for limb salvage and prediction of healing after amputation.[3, 4] Furthermore, lower limb angiogenic therapy has recently been actively studied with $TcPO_2$ as a primary endpoint as mentioned above.[27, 29-31] This data suggests that $TcPO_2$ can be a surrogate index of lower limb amputation and can serve as an important index that could affect wound healing after amputation.

Limitations of the study

There are several limitations that need to be addressed. First, the number of reports used in the present study is small. Second, the present study does not directly deal with the association between $TcPO_2$ and necessity for amputation because this study selected reports describing the association between $TcPO_2$ and wound healing after limb amputation. Third, this study integrated the outcomes from miscellaneous etiology. Moreover, there is not consistency among the selected reports regarding sites of $TcPO_2$ measurement.

Conclusions

A $TcPO_2$ of 20 mmHg is a surrogate index of lower limb amputation, and a $TcPO_2$ of 30 mmHg is an appropriate index that affects wound healing after amputation.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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