≽ *Original Article* ≼

Increased Incidence of Cancer in Japanese Patients with Critical Limb Ischemia

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Objective: This multicenter observational study was conducted in order to investigate the incidence of cancer in patients with critical limb ischemia.
Materials and Methods: We prospectively investigated the incidence of cancer in 68 patients with critical limb ischemia over a two-year period. Patients underwent an intensive examination at enrollment, which included tumor marker levels and chest and abdominal computed tomography, as well as one- and two-year follow-up examinations. We compared the observed incidence of cancer with the expected incidence calculated from national cancer rates by the standardized incidence ratio (SIR).
Results: The majority (83.6%) of the patients were men, and 92.5% of the patients had a peripheral arterial disease that was classified as Fontaine stage III or IV. During enrollment, newly diagnosed cancers were detected in seven patients. Four additional cancers were detected during the follow-up period. All of the detected cancers were asymptomatic. We observed an increased risk of cancer (SIR, 4.04; 95% confidence interval, 1.31–9.42) in patients with critical limb ischemia.
Conclusion: This study suggests that critical limb ischemia is associated with an increased risk of cancer. Our findings should be taken into serious consideration by future investigators considering the use of therapeutic angiogenesis.

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Introduction

Critical limb ischemia (CLI) is a severe form of peripheral arterial disease that results in markedly reduced blood flow to the lower extremities. Although revascularization is considered to be the optimal treatment for CLI, large surveys suggest that only half of CLI patients will undergo some type of revascularization and that 40% of patients who do not undergo effective revascularization will lose their leg(s) within six months.[1]

Therapeutic angiogenesis using angiogenic cytokines or bone marrow mononuclear cells is being investigated as a potential treatment.[2–10] It is expected to serve as a useful option, especially for CLI patients who are not good candidates for any form of revascularization. The goal of therapeutic angiogenesis is the growth and proliferation of collateral vessels in the ischemic tissue. Theoretically, therapeutic angiogenesis can promote unexpected adverse angiogenesis or pathogenic angiogenesis, resulting in an increased incidence of cancer. Consequently, past clinical studies of therapeutic angiogenesis excluded patients with a previous or current history of cancer. In those cases, the incidence of cancer was used as a clinical endpoint to evaluate the safety of the treatment.[6,9,10] Although the incidence of cancer is an important concern with therapeutic angiogenesis, the incidence of cancer unrelated to this treatment in CLI patients remains unknown.

Materials and Methods

Patients

Nineteen surgical departments in Japan participated in this study. Patients were required to be older than 40 years, to have been diagnosed with CLI at the first consultation, and to have agreed to participate in this study within 12 weeks after their first consultation. We excluded patients with a previous or current history of cancer or a contraindication for the contrast agent. Written informed consent was obtained from all subjects, ensuring anonymous participation. This study was approved by an ethics committee at each institution. Sixty-eight CLI patients were enrolled in the study between September 7, 2007 and January 18, 2011. The follow-up period was two years.

Cancer detection

Patients underwent examinations for cancer detection at enrollment and one and two years following enrollment. Examinations included tumor marker levels (CEA, CA19-9, PIVKA-2, DUPAN-2, Elastase, PSA-ACT, CA125, SCC), chest and abdominal computed tomography with contrast agent, fecal occult blood, uric occult blood, and gastroscopy. Female patients also underwent mammography and cytology of the cervix.

Statistical analysis

We calculated the age-related standardized incidence ratio (SIR) and treated patients with CLI as a cohort. The number of expected cancer cases was computed using the national cancer rates in Japan as the standard. We adjusted for sex and age in five-year age groups. The SIR and the corresponding 95% confidence intervals (CIs) were calculated using the standard method of dividing observed cases by expected cases and the standard error. To investigate the risk factors for cancer, patients' characteristics were entered into the Cox univariate analysis. The factors found to be significant in the univariate analysis and sex were entered into the multivariate Cox regression analysis. Survival curves were plotted using the Kaplan–Meier method. Statistical analyses were performed with SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Table 1 presents patient characteristics and the extent of their peripheral arterial disease at enrollment. The majority of the 68 patients (83.6%) were men. The median age was 70, and the median disease duration was 242 days. Current smokers accounted for 44.8% of patients, while 35.8% had a history of smoking and 25.4% had a family

Table 1 Patient characteristics at the time of enrollment (N=68)

Characteristics	
Male (%)	83.6
Age (y)*	70.0 (45.0–89.0)
Disease duration (d)*	242.0 (15.0–3687.0)
Smoking status (%)	
Current	44.8
Past	35.8
None	11.9
Family history of cancer (%)	25.4
Medical history (%)	
Hypertension	80.6
Dyslipidemia	26.9
Diabetes	65.7
Coronary artery disease	29.9
Cerebrovascular disease	22.4
Fontaine classification (%)	
1	4.5
2a	0.0
2b	3.0
3	13.4
4	79.1
Ankle–brachial index*	0.47 (0.00–1.25)
History of revascularization (%)	67.2
History of major or minor amputation (%)	26.9

*Data are expressed as median (minimum–maximum).

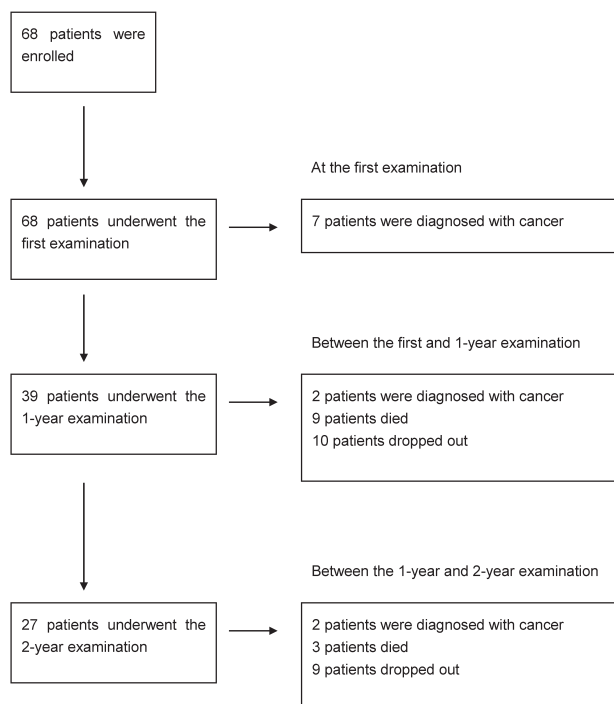**Fig. 1** The number of patients from enrollment to the two-year examination.

Table 2 Results of the univariate Cox analysis

Variables	Hazard ratio	95%CI	P value
Sex	0.610	0.13–2.91	0.54
Age	1.090	1.01–1.18	0.04
Disease duration	1.359	0.39–4.68	0.63
Family history of cancer	1.200	0.31–4.66	0.79
Medical history of			
Hypertension	0.430	0.13–1.48	0.18
Dyslipidemia	0.240	0.03–1.87	0.17
Diabetes	0.560	0.17–1.85	0.34
Coronary artery disease	0.220	0.03–1.71	0.15
Cerebrovascular disease	3.770	1.13–12.52	0.03

CI: confidence interval

Table 3 Results of the multivariate Cox analysis

Variables	Hazard ratio	95%CI	P value
Sex	0.859	0.18–4.19	0.85
Age	1.101	1.01–1.20	0.03
Medical history of cerebrovascular disease	4.376	1.27–15.05	0.02

CI: confidence interval

Table 4 Causes of death

Condition	No. of patients
Sepsis	3
Heart failure	2
Pulmonary embolism	1
Acute coronary syndrome	1
Hyperkalemia	1
Intestinal bleeding	1
Pneumonia	1
Renal failure	1
Unknown/sudden death	1

history of malignancy. Patients' medical histories included hypertension, dyslipidemia, diabetes, coronary artery disease, and cerebrovascular disease (**Table 1**). Although all patients were diagnosed with CLI at the first consultation, some patients had undergone revascularization before enrollment. The majority of their peripheral arterial diseases (92.5%) were classified as Fontaine stage III or IV. Among the patients, 67.2% had a history of revascularization and 26.9% had a history of major or minor amputation. No patients had experienced therapeutic angiogenesis.

At the baseline, newly diagnosed cancer was detected in seven of the 68 patients. Thirty-nine patients underwent the one-year examination. Between the initial examination and the one-year examination, two patients were diagnosed with cancer, nine patients had died, and ten patients dropped out from the follow-up. Twenty-seven patients underwent the two-year examination. Between the one-year examination and the two-year examination, two patients were diagnosed with cancer, three patients had died, and nine patients dropped out from the follow-up (**Fig. 1**). All of the cancers detected were asymptomatic. Three gastric cancers, a hepatocellular carcinoma, a lung cancer, a pancreatic cancer, and a uterine cancer were detected in the first examination. A skin cancer and a hepatocellular carcinoma were detected between the initial examination and the one-year examination. A tongue cancer and a sarcoma in the buttocks were detected between the one-year and the two-year examinations. The SIR calculated from

national cancer rates in Japan was 4.04 with a 95%CI of 1.31–9.42. **Tables 2** and **3** show the results of the univariate and multivariate Cox regression analyses. The ages of the patients and their medical history of cerebrovascular disease were independent risk factors for cancer. Because there was no incidence of cancer in patients who had never smoked, we evaluated the influence of smoking on cancer incidence with a pooled logistic analysis instead of the Cox regression analysis. The pooled logistic analysis showed that patients who were current or past smokers had an odds ratio of 1.847 (95%CI, 0.380–infinity) for developing cancer.

Twelve patients died during the follow-up period. The cumulative survival rates with a 95%CI were 0.82 (0.69–0.90) at one year and 0.797 (0.66–0.88) at two years. The causes of death are shown in **Table 4**. None of the patients died as a result of cancer.

Discussion

There has been a rapid development in therapeutic angiogenesis using angiogenic cytokines or bone marrow mononuclear cells.[2–10] Angiogenic cytokines include a vascular endothelial growth factor, hepatocyte growth factor, fibroblast growth factor, and granulocyte macrophage colony-stimulating factor.[4,6–10] These cytokines are administered to ischemic tissue as a protein or a gene. However, in patients with peripheral arterial disease classified as Fontaine stage II, the benefit of therapeutic angiogenesis remains controversial.[4,6,8] Nevertheless, several randomized controlled trials have reported reductions in the risk of major amputation in CLI patients who undergo therapeutic angiogenesis.[5,9,10]

Although revascularization is the optimal treatment for CLI patients, therapeutic angiogenesis is expected to become a treatment option for patients who cannot undergo any form of revascularization or for whom past revascularization attempts have failed. Therapeutic angiogenesis can potentially promote unexpected angiogenesis or pathogenic angiogenesis, especially in the presence of a malignant tumor. Although a past or current history of cancer has been an exclusion criterion in past clinical studies, there is still concern over the potential presence of a tumor too small to be detected at the start of the treatment. At least three reported randomized controlled trials using different angiogenic cytokines have evaluated the incidence of cancer during the follow-up period.[6,9,10] In these studies, the incidence of cancer did not differ significantly between the angiogenic cytokine group and the placebo group. However, due to the small sample sizes and the short follow-up periods, the influence of therapeutic angiogenesis on the incidence of cancer remains unclear. We have determined that the cancer incidence in CLI patients should serve as an indispensable natural history for future clinical studies of therapeutic angiogenesis. Few past studies have focused on the cancer incidence in CLI patients. To the best of our knowledge, ours is the first prospective observational study evaluating the cancer risk in CLI patients through intensive examinations to detect asymptomatic cancers.

In this study, we found an increased risk of cancer in CLI patients. It is especially noteworthy that 10% of patients were diagnosed with cancer at enrollment. In the clinical management of CLI patients, the possibility of occult cancer should always be taken into consideration. An extensive screening examination should be considered, especially when a patient plans to undergo therapeutic angiogenesis.

The age and medical history of cerebrovascular disease in these patients were independent risk factors for cancer. However, the significance of a history of cerebrovascular disease remains unknown.

As previously reported, the prognosis of patients with CLI is poor.[11–14] In this study, the prognosis was independent of cancer. It is unclear whether aggressive detection of cancer in CLI patients will contribute to an improved prognosis.

This study has several limitations, including possible bias emerging from differences in the methods of cancer detection, a lack of knowledge of the characteristics of the control population, and our small sample size. Nineteen of 68 patients dropped out before the end of the study. A high number of dropouts could have affected our results. In this study, annual screening examinations were scheduled and all of the cancers detected were asymptomatic. However, only a part of the control population underwent periodic screening examinations. Moreover, data regarding the number of symptomatic cancers in the control population was not available. Additionally, cancer and atherosclerosis share some common risk factors such as smoking. In this study, the characteristics of the control population were not available, so we could not elaborate on why the cancer risk increased in CLI patients.

Conclusion

In conclusion, CLI appears to be associated with an increased risk of cancer. The increased risk of cancer in CLI patients should caution future investigators who are considering using therapeutic angiogenesis on this patient population.

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Author Contributions

Study conception: HS
Data collection: all authors
Analysis: AA
Investigation: all authors
Writing: AA
Funding acquisition: HS
Critical review and revision: all authors
Final approval of the article: all authors
Accountability for all aspects of the work: all authors

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