Zinc Deficiency and Clinical Outcome After Infrainguinal Bypass Grafting for Critical Limb Ischemia

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Background: The aim of this study was to identify a relationship between zinc (Zn) deficiency and clinical outcome in patients with critical limb ischemia (CLI).

Methods and Results: Forty-five limbs from 44 patients with CLI who underwent de novo infrainguinal bypass grafting (IBG) were retrospectively reviewed. The patients were divided into a Zn deficiency group (ZD group: Zn <60 μg/dL) and a Zn sufficiency group (ZS group: Zn ≥60 μg/dL). Graft patency, limb salvage (LS), amputation-free survival (AFS), and wound healing were compared between the groups. LS and AFS were examined to identify whether Zn deficiency was an independent predictor. The preoperative factors potentially predictive of Zn deficiency were also analyzed. Twenty-four limbs were categorized into the ZD group. Patients in the ZD group were more likely to have undergone hemodialysis (HD) and have lower serum albumin. The surgical procedures were not significantly different between the groups. Patency, LS, AFS, and complete wound healing rates were significantly lower in the ZD group. Zn deficiency was a negative predictor of LS. Age >75 years and HD were identified as predictors of Zn deficiency.

Conclusions: Zn deficiency was associated with poor clinical outcome. Zn supplementation may improve clinical outcomes during IBG for CLI.

Key Words: Critical limb ischemia; Infrainguinal bypass; Zinc

Critical limb ischemia (CLI) is a limb-threatening condition that requires revascularization to relieve ischemic pain, heal ischemic ulcers, prevent limb loss, and preserve ambulatory status and quality of life. Patients with CLI who do not undergo arterial revascularization have mortality and amputation rates after 12 months as high as 54% and 46%, respectively.[1] In contrast, patients with CLI who undergo successful revascularization survive longer and have a better quality of life than those who receive only medical treatment or undergo primary amputation.[2]

Bypass surgery represents the gold standard for the treatment of CLI with infrainguinal disease. The Bypass versus Angioplasty in Severe Ischaemia of the Leg (BASIL) trial suggested the superiority of the bypass-first strategy in patients with life expectancy >2 years.[3] Additionally, many studies have identified a variety of factors as predictors of the long-term outcome of revascularization for CLI.[4–6]

Zinc (Zn) deficiency causes not only dysgeusia but also a delay in wound healing and decreased immunity.[7–9] Recently, an association has been suggested between Zn deficiency and atherosclerosis.[10,11] However, a relationship between Zn deficiency and CLI has not been clarified. The aim of this study was therefore to identify a relationship between Zn deficiency and clinical outcomes after infrainguinal bypass grafting (IBG) for CLI.

Methods

Study Design
This clinical study was a retrospective study to identify a relationship between Zn deficiency and clinical outcomes after de novo IBG for CLI. This study was conducted in accordance with the mandates of the Declaration of Helsinki. The Nagoya University School of Medicine Institutional Review Board approved the study (approval number: 2019-0332), and all patients provided written informed consent prior to surgery and data collection.

Patients
Consecutive patients with CLI who underwent de novo IBG for CLI (Rutherford categories 4–6) due to arteriosclerosis obliterans at Nagoya University Hospital between May 2012 and March 2017 were retrospectively analyzed.

Table 1. Patient Demographic Data

	ZD group (23 patients/24 limbs)	ZS group (21 patients/21 limbs)	P-value
Patient status			
Age (years)	74±8	70±7	0.091
Female	10 (42)	5 (24)	0.205
BMI (kg/m²)	21.5±5.6	21.4±4.3	0.957
Albumin (g/dL)	3.1±0.6	3.6±0.7	0.010
Hb (g/dL)	10.4±1.9	11.3±2.1	0.162
ALP (IU/mL)	296.7±136.4	303.6±112.5	0.863
CRP (mg/dL)	5.1±6.3	2.0±3.2	0.047
Risk factor			
HT	19 (79)	14 (67)	0.344
DL	9 (38)	14 (67)	0.051
DM	15 (63)	13 (62)	0.967
CAD	14 (58)	14 (67)	0.582
CVD	1 (4)	6 (29)	0.039
HD	14 (58)	5 (21)	0.039
EF <40%	3 (13)	1 (5)	0.611
Medication			
Cilostazol	8 (33)	12 (57)	0.140
Statin	7 (29)	9 (43)	0.369
β-blocker	9 (37)	7 (33)	0.000
Lower limb status (per limb)			
Rutherford classification			0.296
4	0 (0)	2 (10)	
5	17 (71)	14 (67)	
6	7 (29)	5 (24)	
WIfI stage			0.055
1	2 (8)	1 (5)	
2	1 (4)	6 (29)	
3	5 (21)	7 (33)	
4	16 (67)	7 (33)	
Preoperative ABI	0.61±0.34	0.40±0.39	0.056
Preoperative SPP (mmHg)	15±9	15±8	0.963

Data given as n (%) or mean±SD. ABI, ankle-brachial index; ALP, alkaline phosphatase; BMI, body mass index; CAD, coronary artery disease; CRP, C-reactive protein; CVD, cerebrovascular disease; DL, dyslipidemia; DM, diabetes mellitus; EF, ejection fraction; Hb, hemoglobin; HD, hemodialysis; HT, hypertension; SPP, skin perfusion pressure; WIfI, Wound, Ischemia, and foot Infection; ZD, zinc deficiency; ZS, zinc sufficiency.

Forty-five patients had preoperative samples collected for Zn analysis. Of these, 1 patient had taken Zn supplements orally and was excluded from this study. Forty-five limbs from 44 patients were enrolled in this study.

Revascularization Procedure and Follow-up
All patients preoperatively underwent contrast arteriography for planning of the appropriate approach and underwent revascularization. The distal anastomotic site was chosen based on the optimal run-off vessel to the diseased toe. The greater saphenous vein was used as a first choice conduit if its diameter was >2 mm, as determined preoperatively on duplex scan in the standing position. If this vein was not accessible, the lesser saphenous vein or an arm vein was used. If the single saphenous vein was too short, a spliced vein graft was created. If all the aforementioned veins were not accessible and the distal anastomotic site was the popliteal artery, a prosthetic graft was used.

Routine follow-up consisted of postoperative visits every month for 3 months, followed by visits at 3-month intervals for 2 years and at 6-month intervals thereafter. Routine surveillance included ankle-brachial index (ABI), skin perfusion pressure (SPP), and duplex ultrasound (US) measurements. Duplex scanning of the graft was the primary method for detecting occult graft lesions. A focal increase in graft velocity (peak systolic velocity >300 cm/s) was considered significant and was followed by arteriography and revision when appropriate.

Cut-Off for Zn Deficiency
The cut-off for Zn deficiency was defined as 60 μg/dL, and was one of the diagnostic items in the guidelines.[12] The patients were divided into a Zn deficiency group (ZD group: < cut-off for Zn deficiency) and a Zn sufficiency group (ZS group: ≥ cut-off for Zn deficiency).

Variables for Analysis
The following data were retrieved as variables for analysis: age, sex, body mass index (BMI), Rutherford classification, WIfI (Wound, Ischemia, and foot Infection) stage, comor-

Table 2. Operative Details			
	ZD group (24 limbs)	ZS group (21 limbs)	P-value
Conduit			0.517
Prosthetic graft	0 (0)	1 (4)	
Non-reversed vein graft	16 (68)	11 (52)	
Reversed vein graft	4 (18)	3 (13)	
Spliced vein graft	4 (14)	6 (31)	
Inflow			0.338
Femoral artery	8 (34)	12 (57)	
Popliteal artery (AK/BK)	8/8 (66)	5/3 (38)	
Tibioperoneal trunk	0 (0)	1 (5)	
Outflow			0.159
Popliteal artery (AK/BK)	1/0 (4)	2/4 (29)	
Tibial artery	11 (45)	9 (42)	
Pedal artery	12 (58)	6 (29)	
Concomitant procedures			
Anatomical bypass	0 (0)	1 (5)	0.280
Extra-anatomical bypass	0 (0)	2 (10)	0.212
Angioplasty of inflow vessel	5 (21)	9 (43)	0.196
Minor amputation	12 (50)	8 (38)	0.550

Data given as n (%). AK, above knee; BK, below knee; ZD, zinc deficiency; ZS, zinc sufficiency.

bidities (hypertension, dyslipidemia, diabetes mellitus [DM], coronary artery disease [CAD], cerebrovascular disease [CVD], hemodialysis [HD], and ejection fraction <40%), medication, preoperative laboratory data (hemoglobin, serum albumin [Alb], alkaline phosphatase and serum C-reactive protein), and surgical procedures.

Definitions
CLI was defined as tissue loss/gangrene and resting pain lasting >2 weeks, in accordance with the TASC guidelines.[2] CLI was associated with ankle pressure (<50 mmHg for resting pain; <70 mmHg for tissue loss) or toe pressure (<30 mmHg for resting pain; <50 mmHg for tissue loss). When these measurements could not be obtained because of intractable resting pain or a non-compressible artery secondary to severe calcification, SPP was measured; SPP <40 mmHg was defined as indicative of a critically ischemic limb.[13]

CAD was defined as a history of any revascularization of the coronary arteries. CVD was defined as a history of stroke, cerebral hemorrhage, and/or any revascularization of the carotid arteries. DM was diagnosed in patients who were taking hypoglycemic drugs or who self-injected insulin. Complete wound healing was defined as complete epithelialization of all wounds on the affected limbs. Duration from the initial bypass surgery to complete epithelialization was defined as the healing time.[14]

Clinical Endpoints
The endpoints of this study were graft patency, limb salvage (LS), amputation-free survival (AFS), and complete wound healing. The predictors of Zn deficiency were also assessed.

Statistical Analysis
Data are expressed as mean±SD. Patient baseline characteristics were compared using a t-test or Mann-Whitney test where appropriate for continuous data, and the chi-squared test or Fisher's exact test for categorical data. A logistic regression model was used for the multivariate analysis to determine the factors predictive of Zn deficiency. Additionally, cumulative event rates were estimated using Kaplan-Meier survival curves, and Cox multivariate regression analysis was used to determine the predictors of clinical outcomes (LS and AFS). Any covariates with P<0.05 on univariate analysis were assessed in multivariate analysis. P<0.05 was considered significant. Statistical analyses were performed using SPSS version 23 (IBM, Armonk, NY, USA).

Results

Patient Characteristics
Twenty-four limbs in 23 patients were categorized into the ZD group (Zn <60 μg/dL), and 21 limbs in 21 patients into the ZS group (Zn ≥60 μg/dL).

Alb was significantly lower in the ZD group than in the ZS group, whereas HD was significantly higher in the ZD group than in the ZS group. Lower limb status and other clinical characteristics were similar between the 2 groups (**Table 1**).
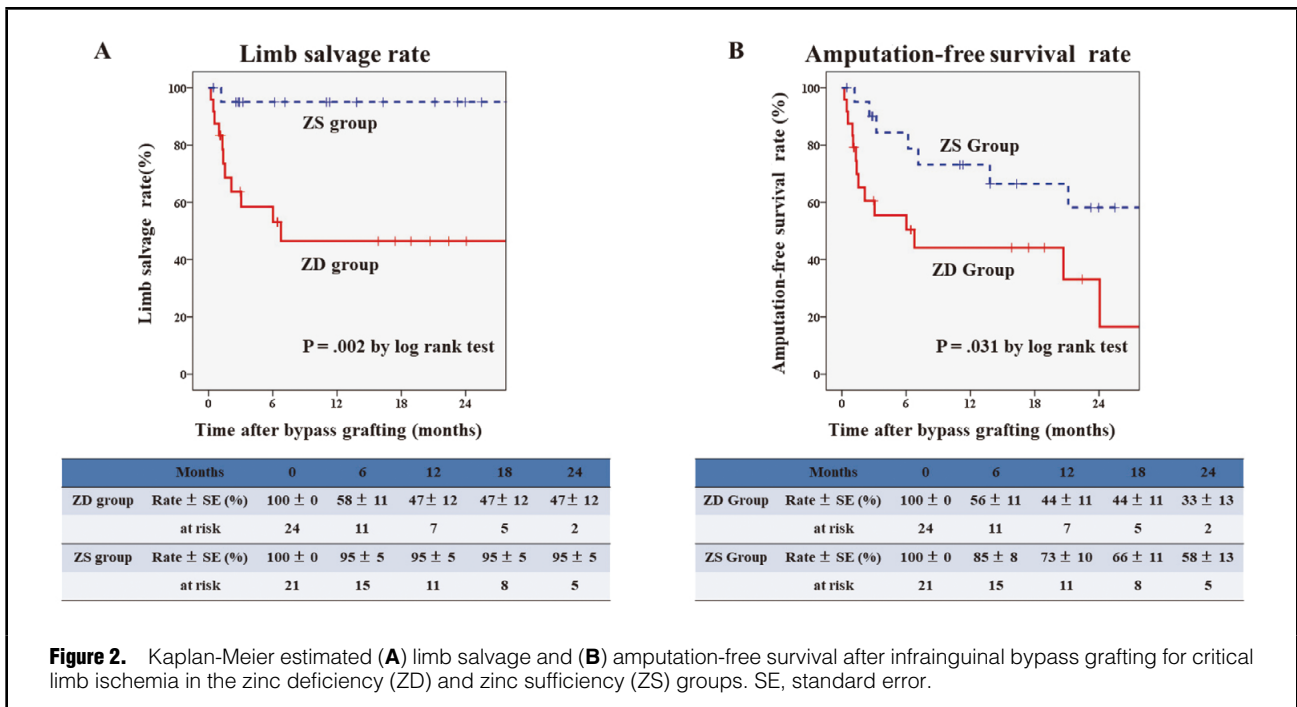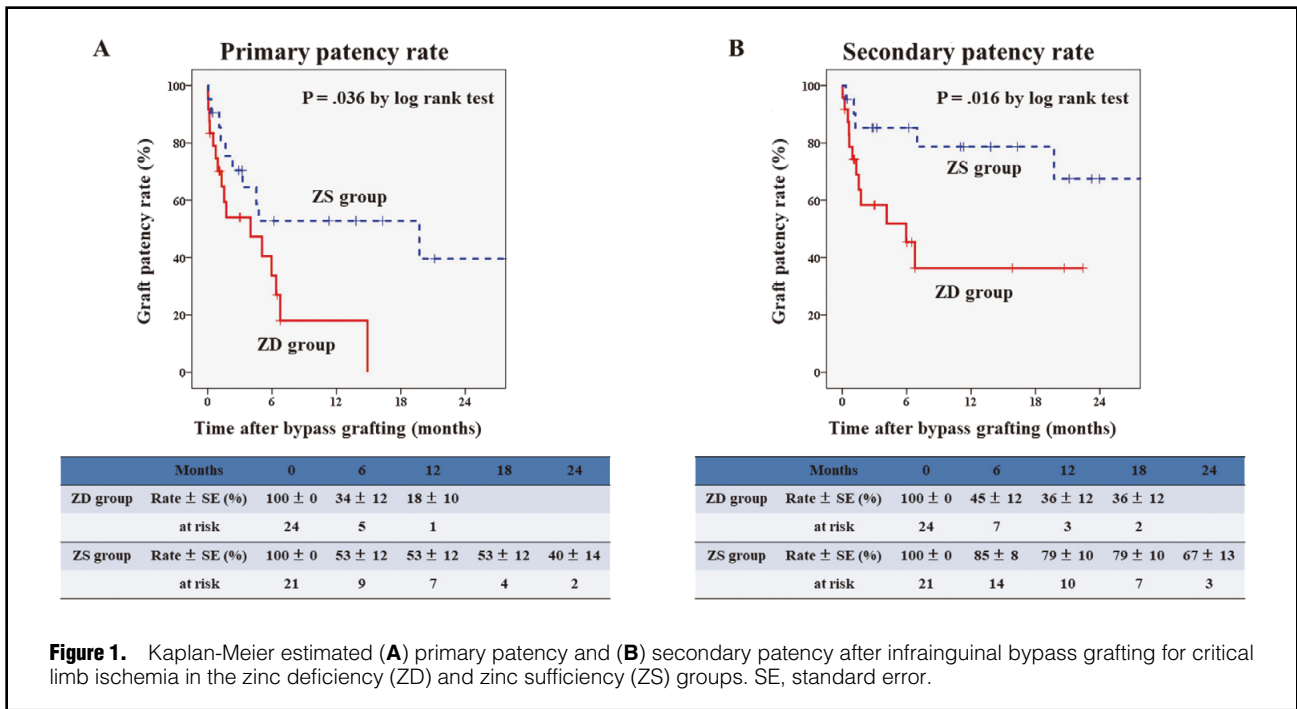
Surgical Procedures
The conduit, the proportion of proximal inflow arteries and distal outflow arteries, and the concomitant procedures were similar between the 2 groups (**Table 2**).

Clinical Outcomes
The primary graft patency rates at 6 months and 1 year tended to be lower in the ZD group (34% and 18%, respectively) than in the ZS group (53% and 53%, respectively; P=0.036; **Figure 1A**). The secondary graft patency rates at 6 months and at 1 year were also significantly lower in the ZD group (45% and 36%, respectively) than in the ZS group (85% and 79%, respectively; P=0.016; **Figure 1B**).

The LS rates at 1 and 2 years were significantly lower in the ZD group (47% and 47%, respectively) than in the ZS group (95% and 95%, respectively; P=0.002; **Figure 2A**).

Figure 1. Kaplan-Meier estimated (**A**) primary patency and (**B**) secondary patency after infrainguinal bypass grafting for critical limb ischemia in the zinc deficiency (ZD) and zinc sufficiency (ZS) groups. SE, standard error.

Figure 2. Kaplan-Meier estimated (**A**) limb salvage and (**B**) amputation-free survival after infrainguinal bypass grafting for critical limb ischemia in the zinc deficiency (ZD) and zinc sufficiency (ZS) groups. SE, standard error.

Eleven legs were amputated in the ZD group, 4 of which were amputated due to widespread infection despite patent bypass grafts. In contrast, only 1 leg was amputated in the ZS group due to graft occlusion.

The AFS rates at 1 and 2 years were significantly lower in the ZD group (44% and 33%, respectively) than in the ZS group (73% and 58%, respectively; P=0.031; **Figure 2B**).

Cumulative complete wound healing was compared between 24 limbs in the ZD group (Rutherford 5, 17 limbs;

and 6, 7 limbs) and 19 limbs in the ZS group (Rutherford 5, 14 limbs; and 6, 5 limbs). The patient demographic data and operative details were similar between the 2 groups except for Alb and HD (**Table 3**). The cumulative complete wound healing rates at 6 months and 1 year were significantly lower in the ZD group (48% and 48%, respectively) than in the ZS group (86% and 86%, respectively; P=0.019; **Figure 3**).

Table 3. Patients With Tissue Loss: Demographic Data			
	ZD group (23 patients/24 limbs)	ZS group (19 patients/19 limbs)	P-value
Patient status			
Age (years)	74±8	70±6	0.187
Female	10 (42)	5 (26)	0.349
BMI (kg/m²)	21.5±5.6	21.5±4.5	0.959
Albumin (g/dL)	3.1±0.6	3.6±0.7	0.017
Hb (g/dL)	10.4±1.9	11.3±2.2	0.154
CRP (mg/dL)	5.1±6.3	2.2±3.3	0.074
Risk factor			
HT	19 (79)	12 (63)	0.314
DL	9 (38)	12 (63)	0.129
DM	15 (63)	13 (68)	0.000
CAD	14 (58)	13 (68)	0.542
CVD	1 (4)	5 (26)	0.072
HD	14 (58)	4 (21)	0.028
EF <40%	3 (13)	1 (5)	0.618
Medication			
Cilostazol	8 (33)	10 (53)	0.230
Statin	7 (29)	8 (42)	0.521
β-blocker	9 (37)	6 (32)	0.755
Lower limb status (per limb)			
Rutherford classification			0.892
5	17 (71)	14 (74)	
6	7 (29)	5 (26)	
WIfl stage			0.140
1	2 (8)	1 (5)	
2	1 (4)	4 (21)	
3	5 (21)	7 (37)	
4	16 (67)	7 (37)	
Preoperative ABI	0.61±0.34	0.44±0.39	0.128
Preoperative SPP (mmHg)	15±9	16±8	0.685

Data given as n (%) or mean±SD. Abbreviations as in Table 1.

Predictors of Clinical Outcome

On multivariate analysis, Zn deficiency (hazard ratio [HR], 8.607; 95% CI: 1.036–71.532, P=0.046) was identified as a predictor of LS, regardless of HD and low serum Alb (**Table 4A**). In contrast, low serum Alb (HR, 4.385; 95% CI: 1.557–12.345, P=0.005) and DM (HR, 2.036; 95% CI: 1.074–4.504, P=0.029) were identified as predictors of AFS. Zn deficiency (HR, 2.036; 95% CI: 0.191–1.261, P=0.140) was not identified as a predictor of AFS (**Table 4B**).
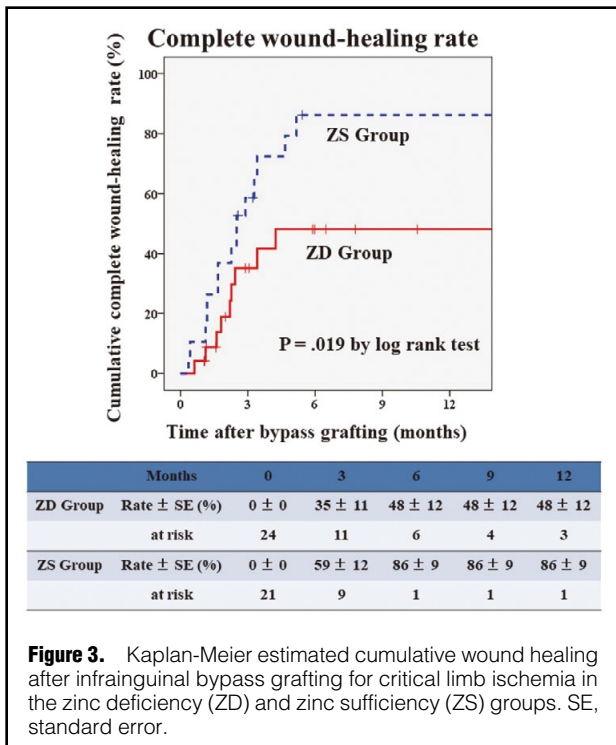
On multivariate analysis, age >75 years (HR, 6.211; 95% CI: 1.358–28.571, P=0.018) and HD (HR, 7.633; 95% CI: 1.628–35.714, P=0.019) were identified as predictors of Zn deficiency (**Table 5**).

Discussion

CLI is the final stage of peripheral artery disease, and revascularization is essential for treating CLI. When deciding on the revascularization method, it is important to obtain information about the patient's risk factors. Many studies have identified a variety of factors (e.g., age, DM, HD, serum Alb) as predictors of long-term outcome after revascularization for CLI.[14–16] Additionally, several scoring systems with risk stratification and confirmed validity regarding the prediction of outcomes have been introduced.[3,4,17] To our knowledge, however, no study has identified a relationship between trace elements and CLI. This is the first detailed description of the relationship between trace elements and the clinical outcomes of patients with CLI.

Several studies have clarified the relationship between Zn and cardiovascular disease, and thus, we focused on Zn out of the various trace elements.[18–20] Zn is an indispensable trace element in the body, and the daily dietary Zn requirement is generally estimated to be 10–15mg/day.[8] Zn is primarily absorbed from the duodenum, the jejunum and, to a lesser extent, the ileum.[7,21] The absorption rate is generally 30–40% of the daily intake of Zn. Zn is secreted in the small intestinal lumen in pancreatic and small intestinal juices. More than 90% of the dietary Zn ingested is ultimately excreted in feces, and <10% is excreted in urine.[7] In diabetic patients, Zn excretion through urine is significantly increased by osmotic diuresis.[22] Zn deficiency in patients with chronic kidney disease is mainly due to a decrease in intestinal absorption.[23] Furthermore, Zn is lost via the dialysis membrane, especially in HD patients,[24] which might be why DM was, interestingly, not a predictor of Zn deficiency in the present study.[7] Furthermore, in the

Complete wound-healing rate

Months	0	3	6	9	12
ZD Group Rate ± SE (%)	0 ± 0	35 ± 11	48 ± 12	48 ± 12	48 ± 12
at risk	24	11	6	4	3
ZS Group Rate ± SE (%)	0 ± 0	59 ± 12	86 ± 9	86 ± 9	86 ± 9
at risk	21	9	1	1	1

Figure 3. Kaplan-Meier estimated cumulative wound healing after infrainguinal bypass grafting for critical limb ischemia in the zinc deficiency (ZD) and zinc sufficiency (ZS) groups. SE, standard error.

present study, old age (≥75 years old) was a predictor of Zn deficiency. Several studies have determined that Zn deficiency in elderly people is due to a decline in dietary zinc intake with advancing age.[25,26]

Occasionally, legs with tissue loss are amputated due to widespread infection or a delay in wound healing even though the bypass grafts are patent. Several reports have noted decreased immunological function and delayed wound healing as a result of Zn deficiency.[7,8,25] Zn is crucial for the normal development and function of cells that mediate non-specific immunity, such as neutrophils and natural killer cells. Zinc deficiency also affects the development of acquired immunity by preventing both the growth and certain functions of T lymphocytes, such as activation, T-helper 1 cytokine production, and B-lymphocyte help. Similarly, B-lymphocyte development and antibody production, in particular immunoglobulin G production, is compromised. Macrophages are also adversely affected by Zn deficiency.[27] In general, there are 3 major stages of wound healing: inflammation, proliferation, and remodeling.[28] Zn deficiency delays wound healing as a result of decreased nuclear factor-κB activation, reduced expression of pro-inflammatory cytokines (interleukin-1β and tumor necrosis factor [TNF]-α), and decreased neutrophil infiltration during the inflammation stage of wound healing.[29] Additionally, Zn deficiency decreases the function of fibroblasts during the remodeling stage.[30] In the present study, Zn deficiency was associated with a lower rate of graft patency. Several mechanisms explain this association. Zn deficiency exacer-

Table 4. Predictors of Limb Salvage and Amputation-Free Survival

Predictors	Univariate analysis	Multivariate analysis		
	P-value	OR	95% CI	P-value
A. Limb salvage				
Zinc deficiency	0.002	8.607	1.036–71.532	0.046
Albumin <3.0 g/dL	0.001	3.003	0.828–10.869	0.094
HD	0.009	1.834	1.628–35.714	0.428
DM	0.096			
WIfI 3–4	0.168			
B. Amputation-free survival				
Zinc deficiency	0.031	2.036	0.191–1.261	0.140
Albumin <3.0 g/dL	0.000	4.385	1.557–12.345	0.005
HD	0.002	1.680	0.626–4.504	0.303
DM	0.007	2.036	1.074–4.504	0.029
WIfI 3–4	0.098			

Abbreviations as in Table 1.

Table 5. Predictors of ZD

Predictors	Univariate analysis	Multivariate analysis		
	P-value	OR	95% CI	P-value
≥75 years old	0.045	6.211	1.358–28.571	0.018
BMI <20 kg/m²	0.920			
Albumin <3.0 g/dL	0.235			
DM	0.967			
HD	0.038	7.633	1.628–35.714	0.019
Rutherford 6	0.685			

Abbreviations as in Table 1.

bates the detrimental effects of specific fatty acids (e.g., linoleic acid) and inflammatory cytokines, such as TNF, on vascular endothelial function.[10] In addition, it is well known that oxidative stress promotes intimal hyperplasia in vein grafts.[31,32] Zn deficiency has also been reported to increase oxidative stress.[8,33] It is possible that Zn deficiency increases the graft failure caused by intimal hyperplasia. These mechanisms are considered to affect LS in CLI patients with Zn deficiency.

Study Limitations

This study had some limitations. First, this was a single-center, retrospective study. Second, the patient group was small. Third, the detailed mechanism underlying the differences is unclear.

Conclusions

Zn deficiency is associated with lower rates of LS, AFS, and wound healing. Zn supplementation may improve graft patency and LS via its antioxidant and anti-inflammatory effects during IBG for CLI, especially in advanced-age and HD patients, although prospective multicenter studies are needed to confirm this hypothesis.

Data Availability

The de-identified participant data will not be shared.

Disclosure

The authors declare no conflicts of interest.

IRB Information

The Nagoya University School of Medicine Institutional Review Board approved this study (approval number: 2019-0332).

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