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Evaluation of graft anastomosis using time–intensity curves and quantitative near-infrared fluorescence angiography during peripheral arterial bypass grafting

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Abstract

Near-infrared fluorescence angiography (NIR) visualizes blood perfusion using the fluorescence property of indocyanine green (ICG). This study aimed to retrospectively determine the usefulness of a quantitative analysis using NIR to predict the patency of peripheral arterial bypass grafts by measuring their fluorescence luminance intensities (FLIs).

Thirteen grafts in 11 patients who underwent peripheral arterial bypass grafting were divided into a patent graft group ($n=7$) and a failed graft group ($n=6$). The changes in the FLIs of ICG opacification through the graft and distal host artery were retrospectively analyzed using stored NIR data. The time–intensity curves (TICs) of ICG opacification through the graft (Q_{graft}) and distal host artery (Q_{distal}) were measured. Two parameters, $\Delta(Q_{graft} - Q_{distal})$ and integral$(Q_{graft} - Q_{distal})$, were also analyzed.

Although not significant, decreases in Q_{graft} were observed in the failed graft groups. The Q_{distal} of the failed graft group was significantly attenuated as compared with that of the patent graft group. $\Delta(Q_{graft} - Q_{distal})$ increased only in the failed graft group, which indicates widening of the gap in FLI. Integral$(Q_{graft} - Q_{distal})$ was higher in the failed graft group, as it reflects the accumulation of ICG opacification.

The TICs were influenced by anastomotic stenosis in the distal site of the host arteries. Our results indicate that the comparison of $\Delta(Q_{graft} - Q_{distal})$ and integral $(Q_{graft} - Q_{distal})$ quantitatively analyzed using NIR can potentially predict anastomotic stenosis.

Keywords Peripheral arterial bypass graft · Near-infrared fluorescence angiography · Indocyanine green

Introduction

Patients with peripheral arterial disease (PAD) may require bypass grafting. The graft can be simultaneously revised in case of occasional anastomotic failure, and when problems with graft anastomoses construction are detected during surgery. As a substitute for indocyanine green (ICG) opacification, near-infrared fluorescence angiography (NIR) is used to evaluate bypass graft anastomosis [1, 2]. NIR visualizes blood flow through ICG fluorescence; ICG binds to blood plasma proteins [3]. NIR can detect fluorescence luminance intensity (FLI) through the graft upon intravenous administration of ICG, without the selective catheter technique and with protection from radiation [1]. NIR cannot visualize the anastomotic stenosis itself, but can locate the anastomotic stenosis by detecting a decrease in graft blood flow. The conventional NIR assessment, which evaluates visualized blood flow through the coronary artery bypass graft (CABG), is known as "qualitative NIR" [4–7].

Subsequently, the bypass graft has been assessed in a peripheral arterial bypass and skin perfusion after peripheral intervention using NIR [8, 9]. Sometimes, we obtained false-negative results with qualitative NIR in PAD surgery.

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We suspect that one of the causes for the false-negative results was the large individual differences in the peripheral arterial conditions, residual vascular beds, and muscular masses of the patients [10]. We hypothesized that ICG FLI at the bypass graft and anastomosed distal host artery were affected by anastomotic stenosis. We have reported on "quantitative NIR," which calculates the FLI of ICG opacification in the bypass grafts [11]. This retrospective study aimed to use quantitative NIR assessment to differentiate bypass grafts and the anastomosed distal host arteries at risk of anastomotic stenosis.

Materials and methods

Subjects

We assessed patients with arteriosclerosis obliterans and other arterial occlusive diseases who had undergone peripheral arterial bypass grafting at Kochi Medical School Hospital.

Surgical procedure

All saphenous vein grafts were used as reversed grafts. The grafts were anastomosed in an end-to-side manner. The anastomosis technique was dependent on the surgeon and was performed using a continuous suture with a 6-0 monofilament suture material. The anastomosis was performed after heparin injection (1 mg/kg), and the activated clotting time was maintained at > 200 s. Protamine administration was not performed in this series. The graft was assessed using transit-time flowmetry (TTF) under a systolic blood pressure of 100–120 mmHg. Qualitative NIR was also performed to assess for blood flow during surgery. The images were recorded in Smart Draw (SDR) formats with a digital image processing system.

Intraoperative graft assessment

Near-infrared fluorescence angiography

Qualitative NIR was used for intraoperative graft assessment during surgery to identify the presence or absence of blood flow through the grafts. We used the HyperEye Medical System (HEMS; MNIRC-1000; Mizuho Ikakogyo, Co., Ltd, Tokyo, Japan) for the NIR [1, 4]. Excitation of ICG was induced with a LED. The camera was placed 30–50 cm above the surgical field. ICG (0.05 mg/kg) was injected via a central venous catheter, followed by a 10-ml saline flush [1, 4]. Graft opacification was categorized as "smooth opacification" (smooth opacification of the graft) or "poor opacification" (slow or inadequate opacification of the graft) by the surgeons during surgery.

Transit-time flowmetry

The VeriQ system (Medistim ASA, Oslo, Norway) and the mean graft flow (MGF) were used to assess transit-time flow. The MGF was classified as "low flow" when it was < 10 ml/min, which is considered a low-cut-off value [12], as transit-time flow has no cut-off value for peripheral arterial bypass grafting.

Qualitative NIR evaluation and TTF assessment depended on the surgeon. When the graft opacification was categorized as "poor" on qualitative NIR and as "low flow" on transit-time flowmetry, the anastomosis was revised and reassessed during surgery.

Postoperative CT

The grafts were routinely assessed using computed tomography (CT) angiography within 1 week after surgery at our institution. The area was analyzed on the short-axis image of the anastomosis, using SYNAPSE Vincent version 4 (Fuji Film medical CO. Ltd., Tokyo, Japan), and the lengths of the short and long axes of the grafts just above the anastomosis were calculated. The stenosis rate (%) was calculated using the following equation: $1 - [\text{opacification area (mm}^2)] \times 100 / \text{short axis} \times \text{long axis} \times \pi \times 100$.

Quantitative NIR

The change in the FLI of ICG through the grafts and distal artery were retrospectively analyzed using the stored NIR data. This method is referred to as "quantitative NIR" in this study. A region of interest (ROI) of 10×10 pixels was located on the anastomosed area of the graft and the anastomosed distal host artery (Fig. 1a). The mean pixel intensity within the ROI was measured using image analysis software (Image J, NIH Bethesda, Maryland, USA) and the original image intensity acquisition tool [11]. The maximum FLI of the diluted ICG solution was assigned a value of 100%. The FLIs were represented by time–intensity curves (TICs; Fig. 1b).

The FLI, Q, was measured at the graft (Q_{graft}) and distal host artery (Q_{distal}). The FLI of all the grafts and distal host arteries plateaued by 11 s. The difference between the Q_{graft} and Q_{distal} was represented as follows:

$D(Q_{\text{graft}} - Q_{\text{distal}}) = Q_{\text{graft}} - Q_{\text{distal}}$.

The integrated Q values from the start point to 11 s were calculated and represented by: integral/Q_{graft} and

integral/Q_{distal}. The difference between integral/Q_{graft} and integral/Q_{distal} was represented as follows:

integral($Q_{graft} - Q_{distal}$) = integral/Q_{graft} − integral/Q_{distal}.

The quantitative NIR pattern was classified as "excellent" or "insufficient" in this study. The excellent pattern included a value when the result was < 20% of FLI (%) for $\Delta(Q_{graft} - Q_{distal})$ and < 200 for integral ($Q_{graft} - Q_{distal}$).

Study design (Fig. 1c)

Based on postoperative CT findings, an anastomosis was considered a "failed graft" if the stenosis was > 75%. Grafts were categorized as either "patent" or "failed." In some grafts, the anastomosis was revised intraoperatively based on the results of the qualitative NIR or TTF during surgery. Patients with pre-revised graft NIR data were included in the "failed" group. This study examined bypass grafts of the saphenous vein for peripheral artery bypass grafting. Blood flow in vascular prostheses such as Dacron or polytetrafluoroethylene grafts cannot be assessed because of poor ICG penetration; thus, patients with these grafts were excluded.

Statistical analysis

All data were expressed as mean ± standard deviation. The Welch two sample t test and Fisher's exact test was used to compare differences in patient characteristics between the patent and failed graft groups. The Shapiro–Wilk normality test was used as the preliminary normality test for FLI and revealed non-normal distribution of data. A repeated-measures analysis of variance (ANOVA) was applied to compare TICs, Q_{graft}, Q_{distal}, and $\Delta(Q_{graft} - Q_{distal})$. Integral($Q_{graft} - Q_{distal}$) and MGFs from TTF were analyzed using Mann–Whitney U test. Presumption analysis was performed using Mauchly's sphericity test followed by Greenhouse–Geisser and Huynh–Feldt correction tests, if needed. The difference was considered significant when the p value was < 0.05.

Ethical considerations

Patients were made aware of the risk of ICG usage and LED light prior to surgery. All patients and family enrolled in this study gave written informed consent. The technical protocol was performed in accordance with the guidelines of the Ethics Committee for clinical research of the Kochi Medical
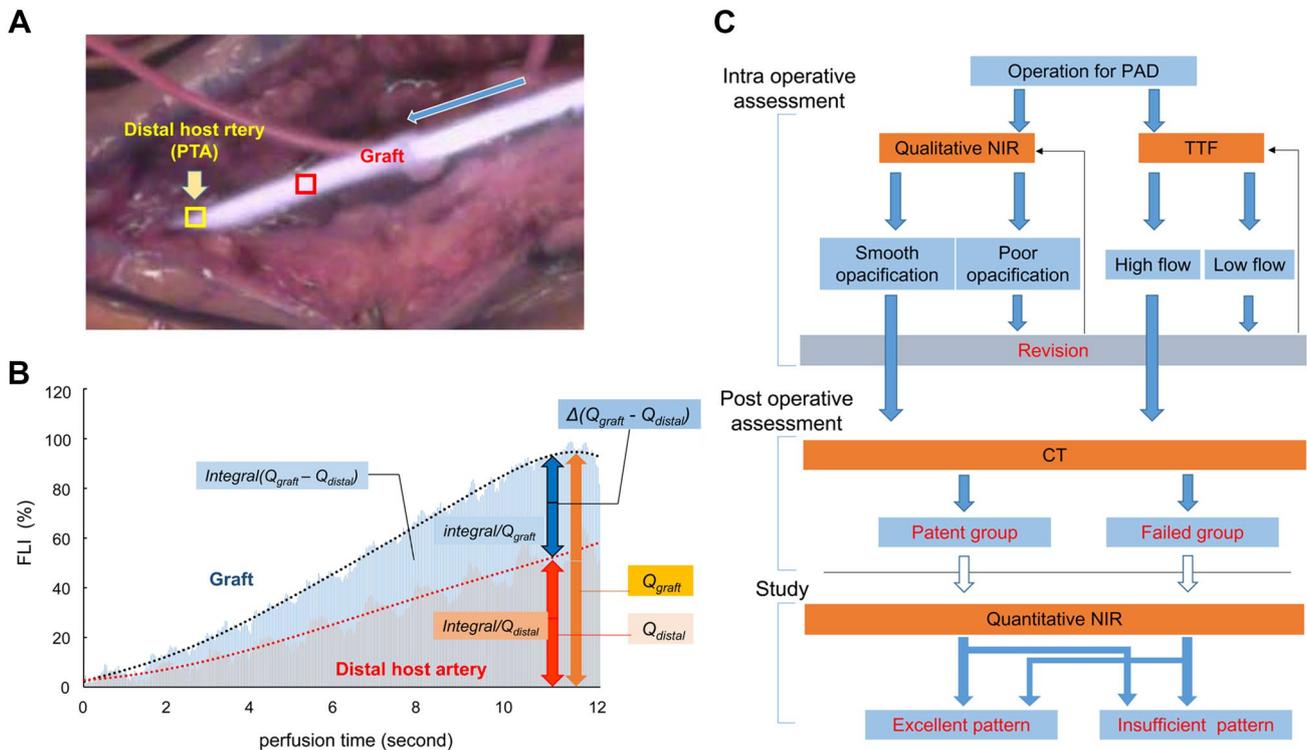

Fig. 1 Near-infrared fluorescence (NIR) angiographic image of femoral tibial artery bypass. **a** The NIR image. The regions of interest (square) were defined in the graft (red) and distal host artery (yellow). **b** The time intensity curve of graft and distal host artery. The luminance intensity is represented by Q_{graft} and Q_{distal}. $\Delta(Q_{graft} - Q_{distal})$ is the difference between Q_{graft} and Q_{distal}. Integral($Q_{graft} - Q_{distal}$) is the difference between integral/Q_{graft} and integral/Q_{distal}. **C** The flow sheet of operation and study. *FLI* fluorescence luminance intensity

School (approval number 30-111) and the Declaration of Helsinki. This was a retrospective study, and additional informed consent was not obtained.

Results

Patient characteristics

In this study, 13 grafts were reviewed. Table 1 shows the characteristics of the patients in the patent and failed graft groups. Seven grafts were categorized as "patent" and 6 as "failed" (Table 2). Three of the 6 graft failures were confirmed using CT. The other 3 graft failures were confirmed using TTF and qualitative NIR, followed by graft revision during surgery. That is, 3 graft failures occurred during the primary surgery.

Intraoperative qualitative NIR and TTF

Ten grafts (grafts 1–10) showed "smooth opacification" on qualitative NIR and > 10 ml/min of MGF "high flow" on TTF during surgery (Table 2). However, grafts 8 and 9 in the failed graft group showed stenoses on postoperative CT angiography. Three grafts (grafts 11–13) were evaluated as "poor opacification" on qualitative NIR and showed "low flow (< 10 ml/min of MGF)" on TTF during surgery. Patency of graft 11 was reconfirmed using NIR and TTF. Patency of grafts 12 and 13 was confirmed by X-ray angiography after revision. Graft 10 was evaluated and had no anastomosis during surgery, as the MGF was 24 ml/min although the NIR showed low opacification.

Grafts 1 and 8 showed "smooth opacification" and "high flow" on intraoperative NIR and TTF, respectively. Graft 1 was included in the "patent" group (Fig. 2a–d, ESM Video 1), and graft 8 in the "failed" group (Fig. 2e–h, ESM Video 2). An anastomotic cross section of 51.17 mm^2 and 0% stenosis for graft 1, with the corresponding values for graft 8 being 1.18 mm^2 and 99.7%, respectively (Fig. 3a, b), was seen on CT. The CT images of graft 8 showed a slit-shaped stricture in the anastomosis that required anastomosis revision. The redo surgical findings confirmed that an anastomotic suture had hooked onto the opposing inner membrane tissue, causing a stricture. The MGF of the graft improved from 20 to 55 ml/min. The patient's prognosis postoperatively was favorable with the ABPI value rising to 0.81 from 0.6. Graft 9 also had a similar slit-shaped stricture in the graft anastomosis although it was not revised as the patient did not expect revision surgery. For graft 10, the surgeon decided against revision during surgery, because

Table 1 The baseline patient and clinical characteristics

	Patent graft	Failed graft	p value
Patients (grafts)	7	6	
Age	74.4 ± 9.0	65.2 ± 19.0	0.31
Men	4/7 (57.1)	2/6 (33.3)	0.59
BSA	1.5 ± 0.2	1.5 ± 0.2	0.98
Cardiovascular risk factors			
Diabetes mellitus	2 (28.6)	2 (33.3)	1
Hyper tension	4 (57.1)	2 (33.3)	0.59
Dyslipidemia	2 (28.6)	1 (16.7)	1
Smoke (current or previous)	4 (57.1)	2 (33.3)	0.59
Cerebral artery attack	1 (14.3)	0 (0)	1
Coronary artery disease	2 (28.6)	1 (16.7)	1
Chronic kidney disease	1 (14.3)	1 (16.7)	1
Hemodialysis	1 (14.3)	1 (16.7)	1
Previous EVT	3 (42.9)	2 (33.3)	1
Pre-operative variables			
Hemoglobin in g/dL	12.3 ± 0.7	11.0 ± 1.1	0.34
Pre-ABI	0.38 ± 0.27	0.33 ± 0.26	1
Culprit lesion			
SFA	3 (42.9)	2 (33.3)	0.29
PA	2 (28.6)	2 (33.3)	1
PTA	2 (28.6)	2 (33.3)	1
Fontaine classification			
I	0 (0)	0 (0)	-
II	2 (28.6)	2 (33.3)	0.96
III	1 (14.3)	1 (16.7)	1
IV	4 (57.1)	4 (66.7)	1
Operative procedure			
Femoral-popliteal bypass	3 (42.9)	2 (33.3)	1
Femoral-tibial bypass	4 (57.1)	4 (66.7)	1

Bars = mean ± standard deviation, () %

BSA body surface area, *EVT* endovascular therapy, *ABI* ankle brachial index, *SFA* superficial femoral artery, *PA* popliteal artery, *PTA* posterior tibial artery

it showed smooth opacification on qualitative NIR, and TTF showed MGF of 24 ml/min.

Quantitative NIR

The TICs of the graft and distal artery in grafts 1 and 8 were displayed retrospectively by time lapse in quantitative NIR angiography (Fig. 3c, d). Q_{graft} and Q_{distal} increased in graft 1. In contrast, Q_{distal} was attenuated when compared with the Q_{graft} of graft 8. The Q_{distal} was recalculated after revision. Graft 9 showed a TIC similar to that of graft 8. TICs were analyzed and compared among all grafts (Fig. 4). Increased Q_{graft} was observed in the failed group but was not significantly different from that in the patent group ($p = 0.360$;

Table 2 Patient and graft data during surgery

Group	Graft	Procedure	MGF/TTF (ml/min)	NIR		ABI	
				Qualitative	Quantitative	Before	After
Patent	1	FT	34	Smooth	Excellent	0[c]	0.95
	2	FT	35	Smooth	Excellent	0.60	1.10
	3	FT	84	Smooth	Excellent	0.55	1.04
	4	FT	13	Smooth	Excellent	0.40	0.85
	5	FP	56	Smooth	Excellent	0.44	0.81
	6	FP	207	Smooth	Excellent	0[c]	0.93
	7	FP	57	Smooth	Excellent	0.65	0.83
Failed	8[a]	FP	20/57	Smooth/smooth	Insufficient/excellent[b]	0.48	0.60
	9	FP	37	Smooth	Insufficient	0[c]	0.45
	10	FT	24	Smooth	Insufficient	0.57	1.07
	11[a]	FT	7/15[b]	Poor/smooth[b]	Insufficient/excellent[b]	0.46	0.78
	12[a]	FT	0/22[b]	Poor/–	Insufficient/–	0[c]	0.40
	13[a]	FT	5/29[b]	Poor/–	Insufficient/–	0.45	0.69

NIR near-infrared fluorescence angiography, *CT* computed tomography, *PT* popliteal tibial artery bypass, *FP* femoral popliteal artery bypass

[a]The anastomosis was revised according to the result of intraoperative graft assessment

[b]The value and assessment result after revision during surgery

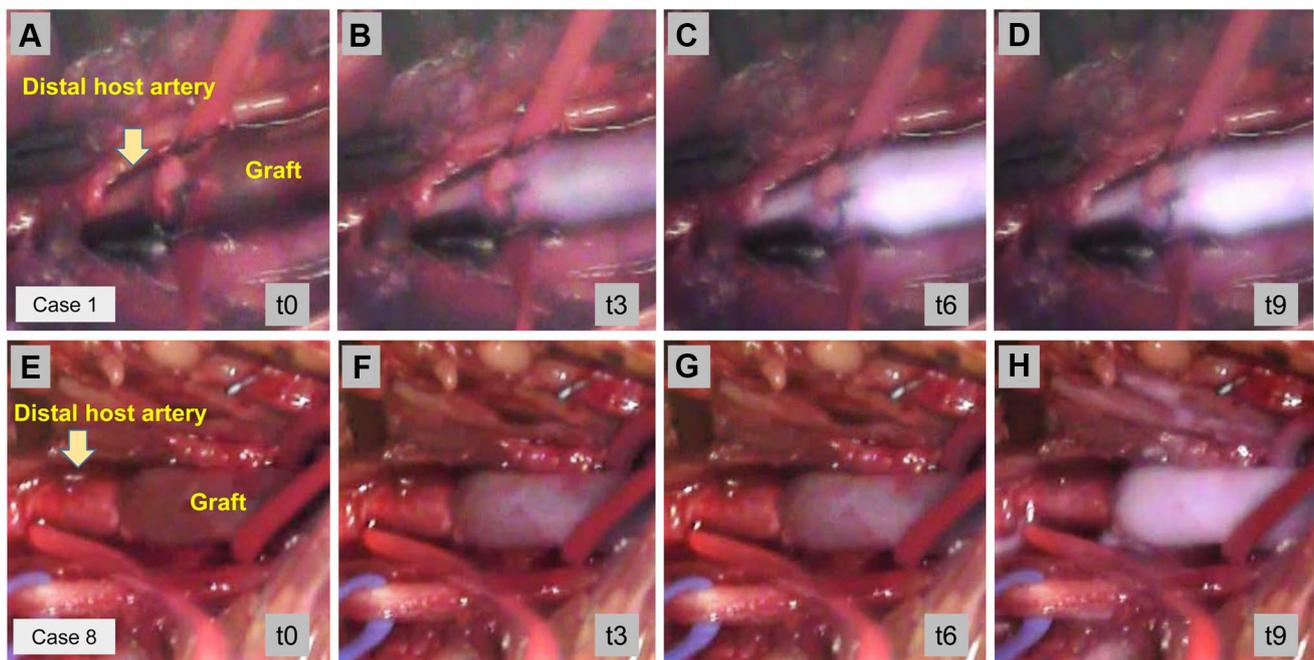[c]Zero include the grafts that has unevaluable blood pressure

Fig. 2 Near-infrared fluorescence (NIR) angiographic image. The change of ICG opacification with time. The ICG opacification of the graft and distal host artery are shown in **a–d** (graft no. 1 of Table 2) and **e–h** (graft no. 8 of Table 2). The "*t*" means elapsed time (second). *ICG* indocyanine green

Fig. 4a). The Q_{distal} in the failed group was significantly decreased as compared with the patent group ($p = 0.0083$; Fig. 4b). The difference in value between Q_{graft} and Q_{distal} is expressed as "$\Delta(Q_{graft} - Q_{distal})$". ANOVA revealed a significant difference between the $\Delta(Q_{graft} - Q_{distal})$ in the patent and failed groups ($p = 0.017$; Fig. 4c). $\Delta(Q_{graft} - Q_{distal})$ was increased in the failed group only, indicating that a widening of the gap in the TICs. Integral(Q_{graft}
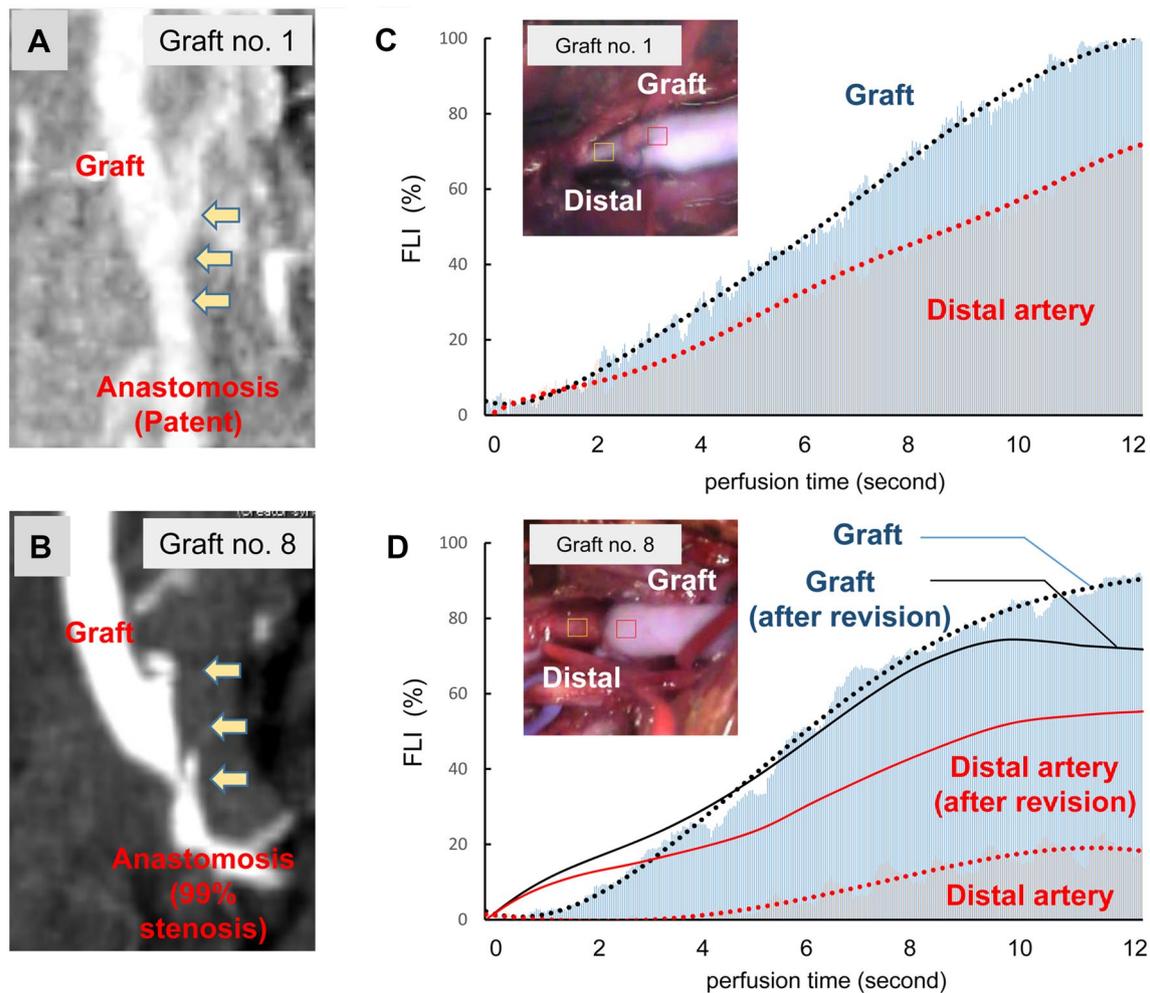
Fig. 3 The computed tomographic (CT) images and the time–intensity curves (TICs) from near-infrared fluorescence angiography. **a** The patency of anastomosis corresponding to graft no. 1. **b** Severe anastomotic stenosis at the heal position, corresponding to graft no. 8. **c** TICs described from graft no. 1. The TICs were measured from the graft and distal host artery. **d** TICs described from graft no. 8. The dotted line depicts pre-revision data. The continuous lines depict data after revision. *FLI* fluorescence luminance intensity

$-Q_{distal}$) was significantly different between the two groups ($p = 0.019$; Fig. 4d).

In the TTF evaluation, the MGF values of the grafts in the patent and failed graft groups were not significantly different (69.4 ± 64.7 and 15.5 ± 14.0 ml/min, $p = 0.071$, Fig. 4e).

Discussion

This study retrospectively assessed the TICs of grafts and distal host arteries during peripheral arterial bypass grafting. The Q_{distal} in the failed group was significantly decreased, although increases in Q_{graft} were observed in the patent and failed groups. The reason for reduced bypass flow in the peripheral arterial bypass could not be determined, unlike in a coronary artery bypass. The influence of anastomotic stenosis on ICG opacification through

the graft or post-stenotic artery in an ex vivo model has been described [13]. We have shown that a change in graft FLI value might be a predictor for future graft failure in CABG [11, 14]. The qualitative NIR evaluating blood flow through grafts may produce a false-negative result during peripheral artery bypass grafting. The FLI of the distal host artery was not evaluated. The quantitative NIR detected anastomosis trouble, although qualitative NIR and TTF could not detect slit-like stenosis in grafts 8 and 9; therefore, the quantitative NIR has the potential to detect severe anastomotic stenosis. However, this study could not show a statistical difference due to small sample size. Grafts 11, 12, and 13 showed anastomotic stenosis on TTF and both NIR. Three grafts were simultaneously revised. The retrospective comparison of the ICG opacification of graft 11 between primary and revised graft noticed us the flow insufficiency of the primary graft. Graft 10 was
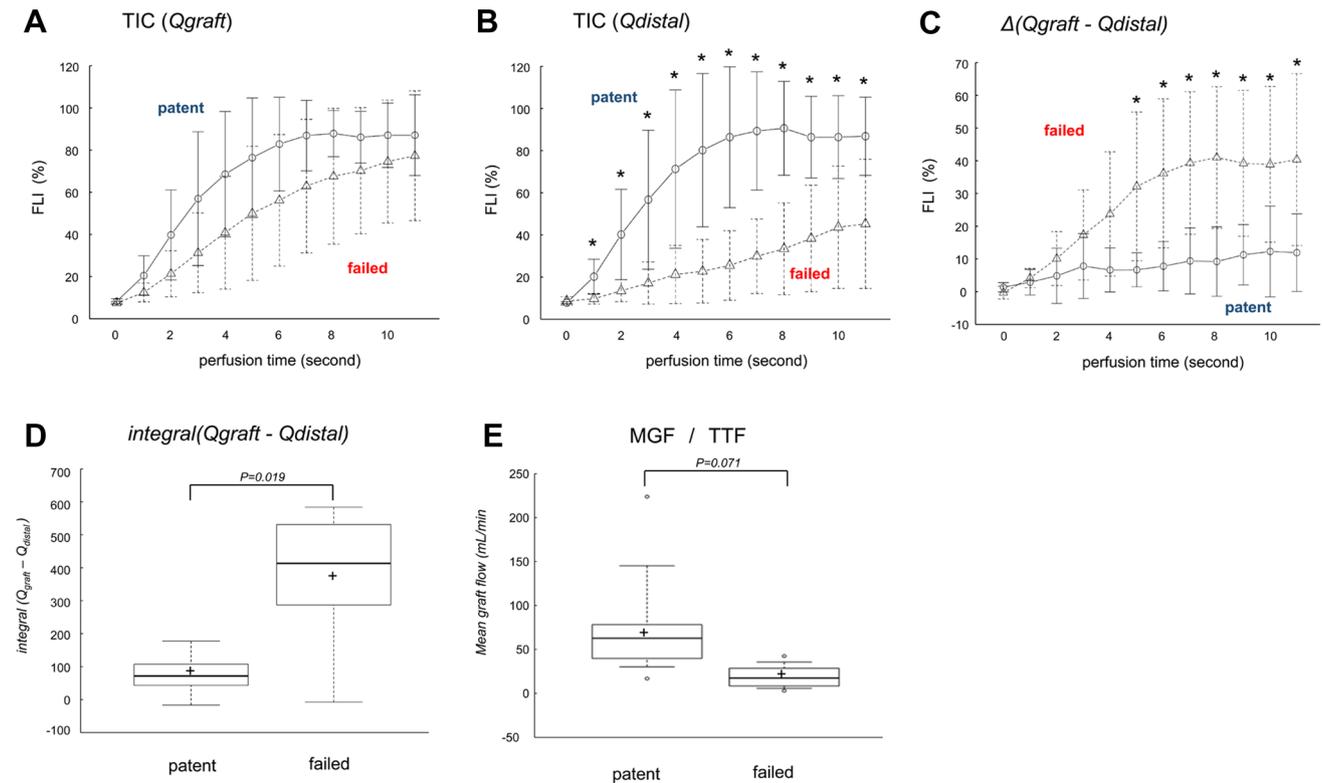
Fig. 4 Time intensity curves (TICs) on the near-infrared fluorescence angiographic image and mean graft flow (MGF) of Transit time flowmetry (TTF). **a** The TICs of the graft (Q_{graft}) between the patent and failed grafts with no significant difference. **b** The TICs of distal artery (Q_{distal}) between the patent and failed grafts. The Q_{distal} in the failed graft had significantly decreased compared to the patent graft (asterisk). **c** The $\Delta(Q_{graft} - Q_{distal})$ increased gradually in the failed graft. The $\Delta(Q_{graft} - Q_{distal})$ of the patent group showed no increase by time (asterisk). **d** Box-and-whisker plots demonstrating the distribution of integral($Q_{graft} - Q_{distal}$) of the graft between the patent and failed grafts were significantly different. Plus: means average value. **e** Box-and-whisker plots demonstrating the distribution of MGF of the TTF between the patent and failed grafts were significantly different. Plus: means average value. *FLI* fluorescence luminance intensity

occluded on postoperative CT, despite MGF of 24 ml/min and smooth opacification on qualitative NIR. Quantitative NIR of graft 10 showed an insufficient pattern (supplement data). Graft 10 may have had severe anastomotic stenosis, as in grafts 8 and 9.

We studied the usefulness of measuring Q_{graft} and Q_{distal} and analyzed two parameters, $\Delta(Q_{graft} - Q_{distal})$ and integral($Q_{graft} - Q_{distal}$). The FLI did not significantly differ between the graft and the distal host artery if anastomotic stenosis was absent. Evaluating $\Delta(Q_{graft} - Q_{distal})$ would be reasonable for comparing FLIs between the graft and distal host artery. $\Delta(Q_{graft} - Q_{distal})$ may minimize the influence of individual differences in hemodynamics and peripheral vascular condition. We considered that integral($Q_{graft} - Q_{distal}$) may reflect ICG FLI accumulation. Reports showed that the differences in graft flow velocity vector and isovelocity contour depend on the anastomosis shape [15]. In contrast, some reports showed no significant difference between the flow rates of bypass grafts with and without stenosis or string phenomenon [16], and could not predict graft patency [17]. Our study results showed no significant difference in the

decrease in Q_{graft} in the failed graft group. $\Delta(Q_{graft} - Q_{distal})$ and integral($Q_{graft} - Q_{distal}$) may be useful for the comparison of FLI between the graft and distal host artery.

If a hybrid operating room is available, X-ray angiography can accurately evaluate the anastomosis, although this requires selective catheter technique and protection from radiation. The TTF evaluates only graft flow. This report showed that the increase in FLI and flow through only the graft does not necessarily reflect anastomotic status. Graft evaluation with NIR and TTF has shown usefulness for CABG [4, 11]. In peripheral arterial surgery, there are large individual differences in perfusion area. Thus, the anastomotic stenosis often does not decrease graft flow. Conventional TTF and qualitative NIR could not predict an anastomotic stenosis. Quantitative NIR is useful as it evaluates blood flow in the bypass and distal host arteries.

Limitations

The first limitation of this study is the small sample size. We could not verify the relationship between the changes in the FLI of ICG and the MGF of TTF. The comparison of MGF and integral($Q_{graft} - Q_{distal}$) resulted in a correlation function, r, of -0.31 and a p value of 0.241 (data not shown). Second, patient characteristics, hemodynamics, and graft condition were not matched as this was a clinical study, and graft quality, diameter and length, anastomotic site, inflow, runoff, residual vascular beds, and muscular mass of the patients could not be matched [10]. Evaluating $\Delta(Q_{graft} - Q_{distal})$ and integral($Q_{graft} - Q_{distal}$) would be reasonable in the comparison between the graft and distal artery. Third, there was a time lag between the NIR during surgery to the postoperative CT assessment. Some graft obstructions may be caused by non-technical problems such as graft kinking, reduced graft flow due to hypotension, and inappropriate anticoagulation in the postoperative period. Although grafts 8 and 9 were revealed, a slit-shaped anastomotic stenosis was thought to be a graft failure by technical error.

Conclusion

This study analyzed the dynamics of the FLI of ICG opacification using NIR and compared TICs between the graft and distal host artery during peripheral arterial bypass grafting. We show that increased $\Delta(Q_{graft} - Q_{distal})$ and integral($Q_{graft} - Q_{distal}$) might be useful predictors of a compromised anastomosis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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