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Time taken to the maximum increase in the oxygenated hemoglobin level in calf muscle as a predictor of mild and moderate post-thrombotic syndrome

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ABSTRACT

Background: Near-infrared spectroscopy (NIRS) allows continuous noninvasive monitoring of changes in the tissue levels of oxygenated hemoglobin (O_2Hb) and deoxygenated hemoglobin (HHb) and can identify the severity of chronic venous diseases. Here we investigated the predictors of post-thrombotic syndrome (PTS) using NIRS in patients with a first episode of deep venous thrombosis (DVT).

Methods: The study enrolled 129 patients with DVT. Risk factors in each patient were assessed at presentation. Venous abnormalities confirmed by ultrasound and parameters derived from NIRS were evaluated at 6 months after DVT. On standing, increases in O_2Hb and HHb (ΔO_2Hb_{st} and ΔHHb_{st}) and the times taken for each concentration to become maximal ($_TO_2Hb_{st}$, and $_THHb_{st}$) were measured. During 10 tiptoe movements, O_2Hb showed a continuous decrease (ΔO_2Hb_{ex}), whereas venous expulsion ($\Delta HHbE_{ex}$) and subsequent retention ($\Delta HHbR_{ex}$) were observed. The oxygenation index (HbD; HbD = O_2Hb − HHb) was also calculated at the end of standing and at the end of 10 tiptoe movements (ΔHbD_{st} and ΔHbD_{ex}). Final clinical manifestations were evaluated at 6 years, and PTS was considered to be present if the Villalta score was ≥5.

Results: Thirteen patients were excluded and 116 patients were finally included. Of these, 19 (16%) developed PTS. Among various NIRS-derived parameters, $_TO_2Hb_{st}$ had the highest area under the curve (0.88; 95% confidence interval [CI], 0.80-0.93; $P < .01$) with the best cutoff value ($_TO_2Hb_{st}$ ≤48 seconds). On univariate analysis, variables associated with greater risk for development of PTS were stroke (odds ratio [OR], 5.59; 95% CI, 0.74-42.41; $P = .06$), idiopathic DVT (OR, 4.13; 95% CI, 1.36-12.55; $P < .01$) and iliofemoral DVT (OR, 4.31; 95% CI, 1.48-12.60; $P < .01$) at initial presentation, venous occlusion combined with reflux (OR, 4.24; 95% CI, 1.50-12.00; $P < .01$), and NIRS-derived $_TO_2Hb_{st}$ ≤48 seconds (OR, 43.03; 95% CI, 9.04-204.81; $P < .01$) at 6 months. Multivariate logistic regression analysis finally revealed venous occlusion combined with reflux (OR, 4.80; 95% CI, 1.03-22.36; $P < .05$) and NIRS-derived $_TO_2Hb_{st}$ ≤48 seconds (OR, 53.73; 95% CI, 8.43-342.41; $P < .01$) to be independently associated with PTS progression.

Conclusions: NIRS-derived $_TO_2Hbst$ ≤48 seconds is a promising time-course predictor of PTS progression. (J Vasc Surg: Venous and Lym Dis 2016;4:446-54.)

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It has been reported that post-thrombotic syndrome (PTS) is present in 20% to 40% of patients with deep venous thrombosis (DVT) and severe in 5% to 10% of them.[1,2] Recent studies have shown that risk factors for PTS include ipsilateral recurrent DVT, obesity, mild contralateral venous insufficiency, and residual venous obstruction demonstrated by baseline ultrasound.[3,4] However, severe symptoms of PTS may also develop over time in patients with no apparent history of recurrent DVT. Therefore, reliable identification of patients who are likely to develop PTS in the acute phase of DVT is difficult.

Near-infrared spectroscopy (NIRS) allows continuous noninvasive monitoring of changes in tissue levels of oxygenated hemoglobin (O_2Hb) and deoxygenated hemoglobin (HHb) and can clarify the severity of chronic venous diseases. Previously, we reported that increased

calf muscle deoxygenation measured by NIRS was highly associated with clinical deterioration of post-thrombotic symptoms and found that increased calf muscle deoxygenation was an important follow-up variable for predicting the development of PTS.[5] More recently, we found significant differences in NIRS-derived parameters between patients with and without PTS using quantitative measurements of calf muscle O_2Hb and HHb levels by NIRS.[6] Therefore, the primary purpose of this study was to evaluate predictors of PTS, including characteristics evident at initial presentation and follow-up parameters determined by NIRS at a relatively early stage of DVT, to determine their prognostic impact on PTS development.

METHODS

Patients. Subjects potentially eligible for this study were 129 consecutive patients with a first episode of unilateral acute DVT who received anticoagulation therapy between April 2006 and March 2009. The baseline risk factors for venous thromboembolism investigated included age, gender, body mass index (BMI), presence of active cancer, congestive heart disease, hormone replacement therapy, immobilization, renal failure, surgery, and stroke. Idiopathic (unprovoked) DVT was defined as DVT occurring in the absence of the triggering factors listed before. Patients with an ankle-brachial pressure index of <0.9 and those with thrombophilia, recurrent DVT, fixed joints, muscle atrophy or weakness, or limb swelling due to systemic diseases or lymphedema were excluded, as were patients with inadequate follow-up or data acquisition.

Venous duplex ultrasound. The initial diagnosis of acute DVT was made by venous duplex ultrasound examination using a color duplex ultrasound scanner (LOGIQ S8: PRO; GE Healthcare Japan, Tokyo, Japan) with a 5-10 MHz linear array transducer. Iliofemoral DVT was diagnosed if the proximal veins were completely occluded with venous thrombus extending proximally to the external iliac vein. Femoropopliteal DVT was diagnosed if the proximal veins had thrombi without iliac involvement.

After initial diagnosis, patients with proximal DVT were administered unfractionated heparin intravenously for 5 to 14 days during the acute phase, adjusted to maintain the activated partial thromboplastin time at 1.5 to 2.5 times the control value, followed by oral warfarin for at least 8 weeks at an international normalized ratio level of 2 to 3. Patients with calf vein thrombosis received oral warfarin alone. All patients were encouraged to ambulate and wore graduated thigh-high compression stockings for at least 6 months immediately after diagnosis.

Development of venous reflux at the follow-up visit was also investigated using duplex ultrasound. Reflux times of >0.5 second for superficial and perforating veins and >1.0 second for deep veins were considered to indicate incompetence.

NIRS. Changes in calf muscle O_2Hb and HHb were measured by NIRS (OM-200 or OM-300; Shimadzu Corp, Kyoto, Japan) using a light source emitting four laser beams of different wavelength (690 nm, 780 nm, 805 nm, and 830 nm), employed in order. According to the variations in optical density at each wavelength, O_2Hb, HHb, and total hemoglobin are calculated on the basis of the Lambert-Beer law,[7] whereby the change in the hemoglobin concentration can be calculated from the variation in optical density relative to the initial value. As light travels in a shallow arc to a penetration depth of about half the separation distance into the tissue, the penetration depth was estimated to be 2 cm because the light emitter-detector distance of the device we employed was 4 cm.[8] Changes in the hemoglobin concentration were expressed as absolute values.

Recently, we developed a new method for identifying changes in calf muscle O_2Hb and HHb in patients with chronic venous diseases using NIRS.[6,9] First, using adhesive tape, the sensor was placed firmly on the posterior aspect of the calf, over the medial head of the gastrocnemius muscle. Baseline NIRS recording was performed while the patient remained supine for 5 minutes with the leg elevated on a foam block in a quiet room at a constant temperature of 22°C to 25°C. The patient then adopted a standing position without putting any weight on the studied leg, resulting in increases of both O_2Hb and HHb. The patient was then asked to keep still until plateaus had been reached, and increases in O_2Hb and HHb were calculated by subtracting the baseline value from the maximum value (ΔO_2Hb_{st} and ΔHHb_{st}). The time elapsed until the maximum increases in the O_2Hb and HHb concentrations ($_TO_2Hb_{st}$, and $_THHb_{st}$) was also measured.

The patient was then asked to perform 10 tiptoe movements with weight bearing on both legs, resulting in a continuous decrease of O_2Hb. The relative change in O_2Hb was calculated by subtracting the value measured at the end of exercise from that at the beginning of exercise (ΔO_2Hb_{ex}). On the other hand, these 10 tiptoe movements also resulted in venous expulsion ($\Delta HHbE_{ex}$) and subsequent retention ($\Delta HHbR_{ex}$).[10] The oxygenation index (HbD; $HbD = O_2Hb - HHb$) was also calculated at the end of standing and 10 tiptoe movements (HbD_{st} and HbD_{ex}; Fig 1).[11] It took approximately 4 to 5 minutes to complete the examination, and the 10 tiptoe movements were completed within 10 seconds.

Study outcome. The primary end point of this study was the development of PTS. The follow-up protocol was an outpatient visit at 2 weeks, 1 month, 3 months, 6 months, and every 6 months thereafter. Ultrasound-confirmed venous abnormalities and NIRS-derived parameters were evaluated by an experienced examiner at

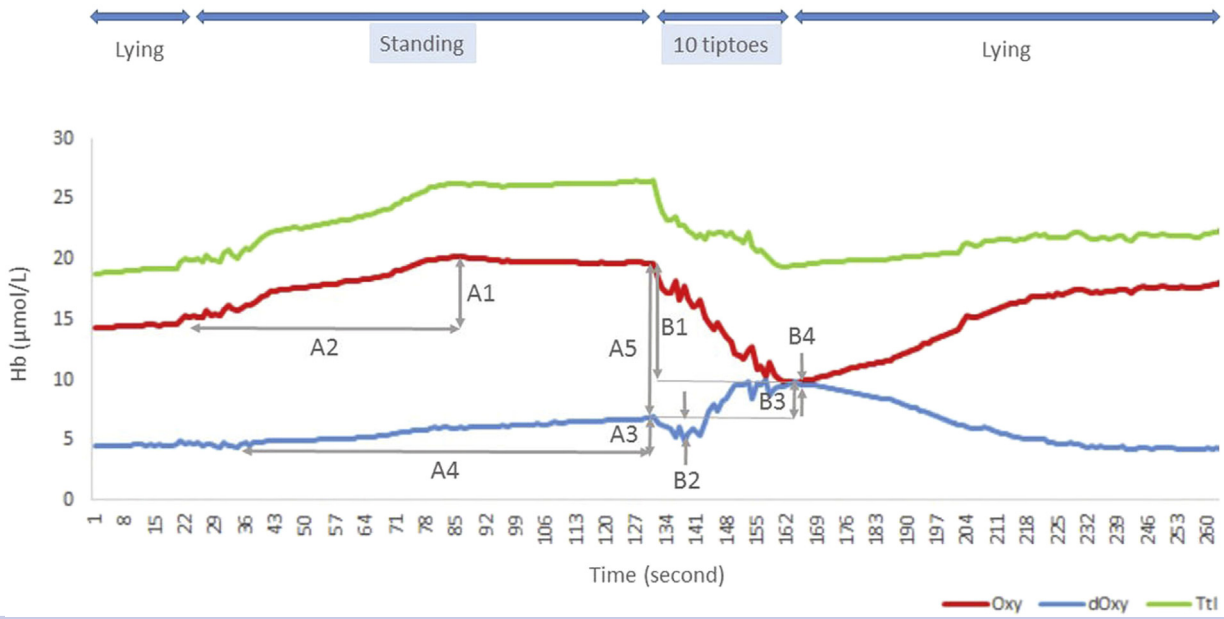

Fig 1. Near-infrared spectroscopy (NIRS) examination. *A1,* Maximum increase in oxygenated hemoglobin (O_2Hb) on standing (ΔO_2Hb_{st}). *A2,* Time elapsed until the maximum increase in O_2Hb ($_TO_2Hb_{st}$). *A3,* Maximum increase in deoxygenated hemoglobin (HHb) on standing (ΔHHb_{st}). *A4,* Time elapsed until the maximum increase in HHb ($_THHb_{st}$). *A5,* Oxygenation index at the end of standing (HbD_{st}). *B1,* Maximum decrease in O_2Hb during exercise (ΔO_2Hb_{ex}). *B2,* Venous expulsion during exercise ($\Delta HHbE_{ex}$). *B3,* Venous retention during exercise ($\Delta HHbR_{ex}$). *B4,* Oxygenation index at the end of exercise (HbD_{ex}). *dOxy,* deoxygenated hemoglobin; *Oxy,* Oxygenated hemoglobin; *TtI,* total hemoglobin.

6 months after DVT. Final clinical manifestations were evaluated at 6 years after diagnosis of DVT, and follow-up of the final patient was completed in March 2015. PTS was considered to be present if the Villalta score was ≥5.[12] A score of 5 to 9 was categorized as mild disease, 10 to 14 as moderate disease, and ≥15 or presence of venous ulcer as severe disease. Moreover, to assess the clinical severity of PTS, the Clinical, Etiologic, Anatomic, and Pathophysiologic (CEAP) classification of the reporting standards for venous diseases was employed.[13] The study protocol was approved by the Institutional Review Board, and all subjects provided informed consent.

Statistical analysis. All data were analyzed using the IBM SPSS Statistics package (version 22; IBM Corp, Armonk, NY). Comparisons of numerical data between patient groups were performed using Student *t*-test, and differences between proportions were evaluated by χ^2 contingency table analysis or Fisher exact test. Continuous data were expressed as mean ± standard deviation. Statistical significance was defined as $P < .05$. The patients' age, gender, BMI, risk factors for venous thromboembolism, and anatomic distributions of DVT were assessed at the baseline, and time-course variables including venous abnormalities and NIRS-derived parameters were evaluated at 6 months after the onset of DVT. Receiver operating characteristic curves were used to evaluate the NIRS-derived parameters. To identify factors that were independent predictors of PTS, potential confounding variables were first chosen using univariate analysis ($P < .10$) and then subjected to multivariate logistic regression analysis.

RESULTS

Patient characteristics. Among the 129 patients evaluated, 13 were excluded from the study because of inadequate follow-up or inadequate data acquisition. Thus, 116 patients were finally included (Fig 2). These comprised 42 men and 74 women with a mean age of 62 years; 13% were aged <40 years, 35% 40 to 65 years, and 52% >65 years. The patients' mean BMI was 24 kg m^{-2}; in terms of BMI classification, 3% were classified as underweight (<18.5 kg m^{-2}), 62% as normal (18.5-25 kg m^{-2}), 31% as overweight (25-30 kg m^{-2}), and 3% as obese (≥30 kg m^{-2}). Thirty-six patients (31%) had unprovoked DVT, and the remaining 80 (69%) had provoked DVT with one or more risk factors.

At initial presentation, DVT was located in the iliofemoral segment in 22 patients (19%), the femoropopliteal segment in 42 (36%), and the calf veins in 52 (45%). Patients were anticoagulated for a mean duration of 18 months. The most common triggering factor for DVT was surgery (45 patients [39%]), followed by hormone replacement therapy (16 patients [14%]) and active cancer (15 patients [13%]).

At the 6-month follow-up point, venous duplex ultrasound revealed no venous abnormalities in 46% of the patients. On the other hand, 18% of the patients had

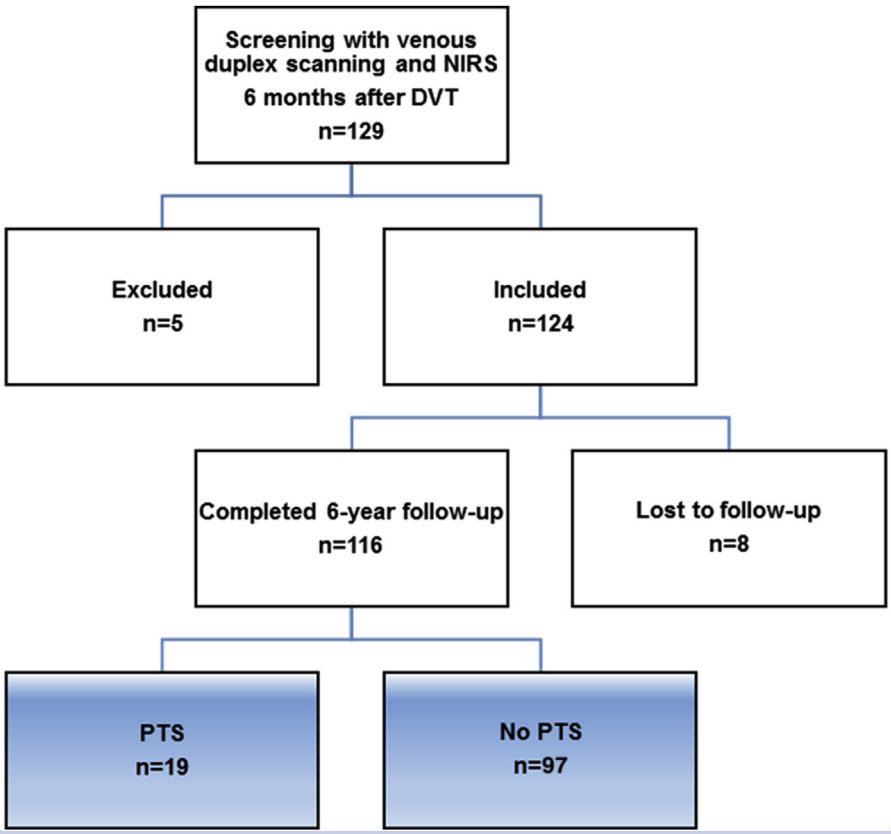

Fig 2. Study flow diagram. In the cohort study, 129 consecutive patients were enrolled; five patients were excluded and eight were lost to follow-up, leaving 116 patients who were eligible for the study. *DVT,* Deep venous thrombosis; *NIRS,* near-infrared spectroscopy; *PTS,* post-thrombotic syndrome.

venous obstruction, 14% had reflux, and the remaining 22% had both obstruction and reflux. No patients had clinical evidence of PTS.

Presenting characteristics associated with PTS. Of the 116 patients evaluated, 19 (16%) finally developed PTS. Table I summarizes and compares the characteristics of the patients with and without PTS. In this cohort, there were 16 patients with mild and 3 with moderate disease, and no patients had severe disease. There were no significant differences in mean age and age category or gender distribution between the two groups. Similarly, there were no significant intergroup differences in BMI or BMI category. The duration of anticoagulation was 19 months in patients with PTS and 18 months in patients without PTS, the intergroup difference being nonsignificant. There were no significant differences in risk factors between patients with and without PTS. On the other hand, patients who had idiopathic DVT had a higher incidence of PTS ($P < .01$). With regard to the anatomic distribution of DVT, the incidence of iliofemoral DVT was significantly higher in patients with PTS than in those without ($P < .01$). On the other hand, femoropopliteal DVT was evenly distributed between the two groups ($P = .10$). By contrast, patients with calf DVT were significantly less likely to develop PTS ($P < .01$).

At 6 months, the proportion of patients with occlusion alone did not differ between the groups. Similarly, there was no significant intergroup difference in the presence of reflux alone. By contrast, the proportion of patients with both reflux and obstruction was significantly higher in the PTS group ($P < .01$).

Table II compares the final CEAP clinical manifestations between patients with and without PTS. Among the 19 patients with PTS, 11 (58%) had severe edema (CEAP C3) and 8 (42%) had CEAP C4. On the other hand, among the 97 patients without PTS, 75 (77%) had no visible venous disease (CEAP C0), 8 (8%) had telangiectasia or reticular veins (CEAP C1), 4 (4%) had varicose veins (CEAP C2), and 10 (11%) had edema. No recurrent DVTs were encountered during the follow-up period of 6 years.

NIRS measurements. Table III compares the NIRS-derived parameters in patients with and without PTS measured at 6 months after DVT. Adoption of a standing position caused increases in the level of both O_2Hb and HHb. While patients were standing, there was no significant difference in ΔO_2Hb_{st} between those with and those without PTS (8 ± 6 μmol/L, 11 ± 10 μmol/L; $P = .44$). Similarly, there was no significant difference in ΔHHb_{st} between patients with and those without PTS (11 ± 10 μmol/L, 14 ± 7 μmol/L; $P = .18$). The values of HbD_{st} in the

Table I. Comparison of characteristics between patients with and without post-thrombotic syndrome (*PTS*)

	PTS (n = 19)	No PTS (n = 97)	*P* value
Characteristics at initial visit			
Age, years	57.2 ± 15.5	61.3 ± 18.3	.27[a]
Age category			
<40 years	3 (15.8)	12 (12.4)	.69[b]
40-65 years	9 (47.4)	32 (33.0)	.23[b]
≥65 years	7 (36.8)	53 (54.6)	.16[b]
Male gender	10 (52.6)	32 (33.0)	.10[b]
BMI, kg m^{-2}	25.2 ± 2.3	22.6 ± 3.1	.34[a]
BMI category			
<18.5 kg m^{-2}	1 (5.3)	3 (3.1)	.52[b]
18.5-25 kg m^{-2}	10 (52.6)	62 (63.9)	.36[b]
25-30 kg m^{-2}	8 (42.1)	28 (28.9)	.26[b]
≥30 kg m^{-2}	0 (0)	4 (4.1)	.37[b]
Duration of oral anticoagulation, months	18.9 ± 14.7	18.1 ± 28.2	.90[a]
Risk factors			
Active cancer	1 (5.3)	14 (14.4)	.28[b]
Congestive heart failure	0 (0)	1 (1.0)	.66[b]
Hormone replacement therapy	4 (21.1)	12 (12.4)	.32[b]
Immobilization	0 (0)	12 (12.4)	.11[b]
Renal failure	2 (10.5)	5 (5.2)	.37[b]
Surgery	5 (26.3)	40 (41.2)	.22[b]
Stroke	2 (10.5)	2 (2.1)	.06[b]
Idiopathic DVT	7 (36.8)	12 (12.4)	<.01[b]
Distribution of DVT			
Iliofemoral DVT	8 (42.1)	14 (14.4)	<.01[b]
Femoropopliteal DVT	10 (52.6)	32 (33.0)	.10[b]
Calf DVT	1 (5.3)	51 (52.6)	<.01[b]
Venous abnormality at 6 months			
No abnormalities	1 (5.2)	52 (53.6)	<.01[b]
Obstruction	5 (26.3)	16 (16.5)	.31[b]
Reflux	4 (21.1)	12 (12.4)	.32[b]
Obstruction and reflux	9 (47.4)	17 (17.5)	<.01[b]

BMI, Body mass index; *DVT*, deep venous thrombosis.
Categorical variables are presented as number (%). Continuous variables are presented as mean ± standard deviation.
[a]Student *t*-test.
[b]Pearson χ^2 test or Fisher exact test.

two groups were also similar (13 ± 14 μmol/L, 10 ± 15 μmol/L; $P = .61$). In contrast, the time taken for the increase in O_2Hb concentration to become maximal ($_TO_2Hb_{st}$) was significantly shorter in patients with PTS than in those without (38 ± 33 seconds, 63 ± 35 seconds; $P = .04$). There was no significant difference in the value of $_THHb_{st}$ between patients with and those without PTS (190 ± 73 seconds, 210 ± 59 seconds; $P = .38$). In patients who were not at risk for development of PTS, O_2Hb and HHb gradually increased simultaneously until plateaus had been reached. In contrast, patients who were at risk for development of PTS had reduced $_TO_2Hb_{st}$. The $_TO_2Hb_{st}$ curve reached a plateau within a short time and then began to decrease while HHb continued to increase.

Table II. Numbers of patients with each Clinical, Etiologic, Anatomic, and Pathophysiologic (CEAP) clinical class at 60 months

Class	Signs and symptoms	PTS (n = 19)	No PTS (n = 97)
C0	No visible or palpable signs of venous disease	0 (0)	75 (77)
C1	Telangiectasia or reticular veins	0 (0)	8 (8)
C2	Varicose veins; distinguished from reticular veins by a diameter of 3 mm or more	0 (0)	4 (4)
C3	Edema	11 (58)	10 (11)
C4	Pigmentation, eczema, lipodermatosclerosis, or atrophie blanche	8 (42)	0 (0)
C5	Healed venous ulcer	0 (0)	0 (0)
C6	Active venous ulcer	0 (0)	0 (0)

Values are expressed as No. (%).

Ten tiptoe movements produced a continuous decrease of calf muscle O_2Hb in both groups, and there was no significant intergroup difference in ΔO_2Hb_{ex} (−15 ± 10 μmol/L, −11 ± 7 μmol/L; $P = .22$). On the other hand, this maneuver resulted in venous expulsion ($\Delta HHbE_{ex}$) and subsequent retention ($\Delta HHbR_{ex}$). The $\Delta HHbE_{ex}$ value was significantly decreased in patients with PTS relative to those without PTS (−3 ± 4 μmol/L, −6 ± 3 μmol/L; $P = .04$). On the other hand, there was no significant difference in $\Delta HHbR_{ex}$ between the groups (8 ± 9 μmol/L, 6 ± 3 μmol/L; $P = .28$). Similarly, there were

Table III. Near-infrared spectroscopy (NIRS)-derived parameters compared between patients with and without post-thrombotic syndrome (*PTS*)

	PTS (n = 19)	No PTS (n = 97)	*P* value[a]
On standing			
ΔO_2Hb_{st}, μmol/L	8 ± 6	11 ± 10	.44
ΔHHb_{st}, μmol/L	11 ± 10	14 ± 7	.18
HbD_{st}, μmol/L	13 ± 14	10 ± 15	.61
$_TO_2Hb_{st}$, seconds	38 ± 33	63 ± 35	.04
$_THHb_{st}$, seconds	190 ± 73	210 ± 59	.38
During exercise			
ΔO_2Hb_{ex}, μmol/L	−15 ± 10	−11 ± 7	.22
$\Delta HHbE_{ex}$, μmol/L	−3 ± 4	−6 ± 3	.04
$\Delta HHbR_{ex}$, μmol/L	8 ± 9	6 ± 3	.28
HbD_{ex}, μmol/L	−6 ± 25	−5 ± 14	.91

Values are expressed as mean ± standard deviation.
[a]Student *t*-test.

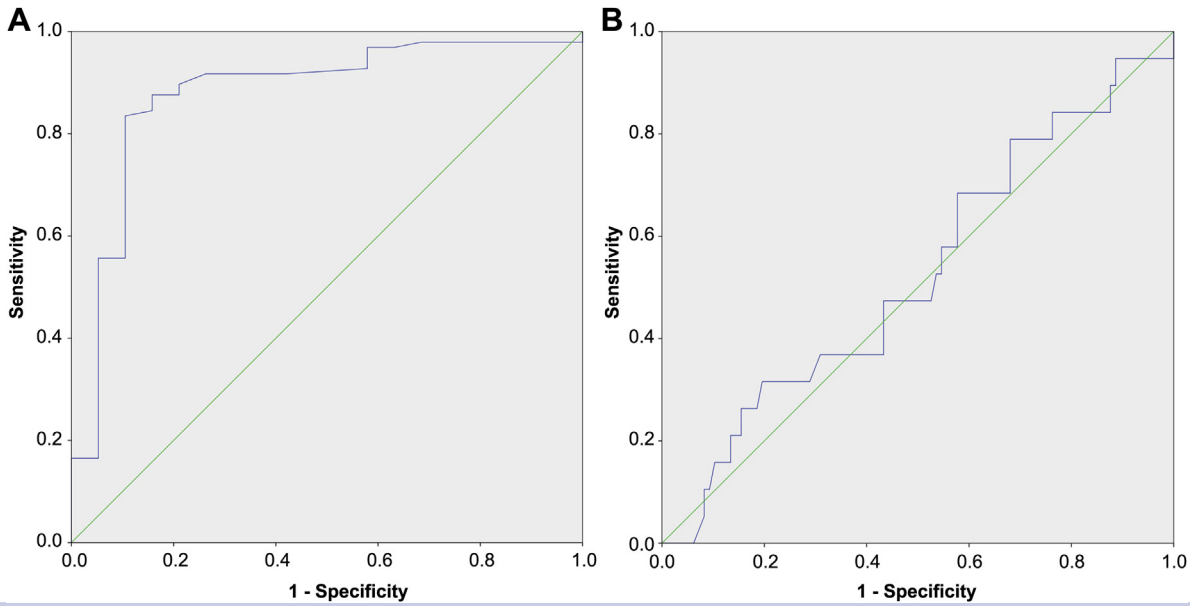

Fig 3. Ability of near-infrared spectroscopy (NIRS)-derived confounding parameters to predict post-thrombotic syndrome (PTS). **A,** NIRS-derived $_{T}O_2Hbst$ ≤48 seconds had the highest area under the curve (0.88; 95% confidence interval [CI], 0.80-0.93; $P < .01$) with a sensitivity of 90% and a specificity of 83%. **B,** When a ΔHHbEex cutoff point of −0.87 μmol/L was used, the clinical discrimination performance was not significant with a sensitivity of 32% and a specificity of 80% (area under the curve, 0.53; 95% CI, 0.43-0.62; $P = .73$).

no significant intergroup differences in HbD_{ex} reduction (−6 ± 25 μmol/L, −5 ± 14 μmol/L; $P = .91$).

Ability of NIRS-derived confounding parameters to predict PTS. Using NIRS-derived $_{T}O_2Hb_{st}$ and $\Delta HHbE_{ex}$ as possible discriminative confounding variables, a separate receiver operating characteristic curve analysis was conducted to identify the most suitable cutoff points with the highest accuracy and minimal false negativity and false positivity for discrimination between the PTS and non-PTS groups (Fig 3). Of these, NIRS-derived $_{T}O_2Hb_{st}$ ≤48 seconds had the highest area under the curve (0.88; 95% confidence interval [CI], 0.80-0.93; $P < .01$) with a sensitivity of 90% and a specificity of 83%. In contrast, when a $\Delta HHbE_{ex}$ cutoff point of −0.87 μmol/L was used, the clinical discrimination performance was not significant, with a sensitivity of 32% and a specificity of 80% (area under the curve, 0.53; 95% CI, 0.43-0.62; $P = .73$).

Predictors of PTS. Univariate analysis revealed that variables associated with greater risk for development of PTS ($P < .10$) were stroke (odds ratio [OR], 5.59; 95% CI, 0.74-42.41; $P = .06$), idiopathic DVT (OR, 4.13; 95% CI, 1.36-12.55; $P < .01$) and iliofemoral DVT (OR, 4.31; 95% CI, 1.48-12.60; $P < .01$) at initial presentation, venous occlusion combined with reflux (OR, 4.24; 95% CI, 1.50-12.00; $P < .01$), and NIRS-derived $_{T}O_2Hb_{st}$ ≤48 seconds (OR, 43.03; 95% CI, 9.04-204.81; $P < .01$) at 6 months (Table IV).

The confounding predictors of PTS were then finally tested using multivariate logistic regression analysis (Table V). Although univariate analysis had suggested that thrombus involvement of the iliofemoral venous segment at initial presentation was a predictor of PTS, this factor was not found to be significantly associated with the development of PTS. Similarly, stroke and idiopathic DVT were shown not to be predictors of PTS. At the 6-month point, venous occlusion combined with reflux was predictive of PTS (OR, 4.80; 95% CI, 1.03-22.36; $P < .05$). Furthermore, $_{T}O_2Hb_{st}$ ≤48 seconds detected by NIRS was the strongest predictor of PTS at the 6-month follow-up point (OR, 53.73; 95% CI, 8.43-342.41; $P < .01$).

DISCUSSION

Since the introduction of NIRS in 1977 by Jöbsis as a valid and noninvasive method for measurement of tissue oxygenation,[14] numerous studies have developed and refined the NIRS approach for investigating skeletal muscle hemodynamics and metabolism in vivo.[15-19] In a previous study to investigate the correlation of clinical severity with NIRS-derived HHb in patients with chronic venous diseases, we found that the filling index (FI-HHb), calculated by dividing 90% of the HHb by the time taken to fill 90% of the HHb on standing, and the retention index (RI-HHb), determined as HHbR/HHbE during exercise measured by NIRS, were increased in patients with advanced chronic venous diseases.[20] Moreover, RI-HHb >2.9 measured at 6 months after DVT was shown to have a strong capability for predicting advanced symptoms of PTS.[10] These results appear to reflect the possibility that increased calf muscle deoxygenation during

Table IV. Univariate analysis to evaluate potential predictors of post-thrombotic syndrome (PTS)

Variable	OR	95% CI	*P* value
Variables at initial visit			
Age			
<40 years	1.33	0.34-5.24	.69
40-65 years	1.83	0.68-4.95	.23
≥65 years	4.13	1.36-12.6	.16
Male sex	2.26	0.83-6.11	.10
BMI			
<18.5 kg m^{-2}	1.74	0.17-17.69	.64
18.5-25 kg m^{-2}	0.63	0.23-1.69	.36
25-30 kg m^{-2}	1.80	0.65-4.93	.25
≥30 kg m^{-2}	0.96	0.92-1.00	.36
Risk factors for DVT			
Active cancer	0.33	0.41-2.67	.28
Congestive heart failure	0.99	0.97-1.01	.66
Hormone replacement therapy	1.89	0.54-6.64	.32
Immobilization	0.88	0.81-0.94	.11
Renal failure	2.16	0.39-12.08	.37
Surgery	0.51	0.17-1.53	.22
Stroke	5.59	0.74-42.41	.06
Idiopathic DVT	4.13	1.36-12.55	<.01
Distribution of DVT			
Iliofemoral DVT	4.31	1.48-12.60	<.01
Femoropopliteal DVT	2.26	0.83-6.11	.10
Calf DVT	0.05	0.01-0.39	<.01
Variables at 6 months			
Venous abnormalities			
No abnormality	0.05	0.01-0.38	<.01
Obstruction alone	1.81	0.57-5.73	.31
Reflux alone	1.89	0.54-6.64	.32
Obstruction and reflux	4.24	1.50-12.00	<.01
NIRS-derived parameters			
$_{T}O_2Hb_{st}$ ≤48 seconds	43.03	9.04-204.81	<.01
$\Delta HHbE_{ex}$, μmol/L	2.52	0.83-7.68	.10

BMI, Body mass index; *CI*, confidence interval; *DVT*, deep venous thrombosis; *OR*, odds ratio.

Table V. Multivariate logistic regression analysis to evaluate final predictors of post-thrombotic syndrome (PTS)

Variable	β	Wald	OR	95% CI	*P* value
Stroke	1.00	0.31	2.73	0.08-92.63	.58
Idiopathic DVT	−0.02	0.01	0.98	0.16-5.99	.98
Iliofemoral DVT	1.40	2.45	4.07	0.02-23.63	.12
Obstruction and reflux	1.57	4.00	4.81	10.3-22.36	<.05
$_{T}O_2Hb_{st}$ ≤48 seconds	3.98	17.78	53.73	8.43-342.41	<.01

CI, Confidence interval; *DVT*, deep venous thrombosis; *OR*, odds ratio.

exercise may predict future venous malfunction in patients with a first episode of DVT.

Another relevant issue related to chronic venous disease is the role of arterial perfusion in relation to clinical symptoms. Several investigators have applied various plethysmographic methods for detection of arterial perfusion and found increased resting arterial perfusion and reduced arterial inflow during the hyperemic response in patients with severe chronic venous disorders.[21-23] These findings lend support to the view that chronic venous insufficiency is a condition that affects both the venous and arterial systems of the lower extremities at the microcirculation level. The disadvantages of plethysmographic examination include the use of different systems for assessment of venous and arterial inflows. Accordingly, little is known about the venous and arterial inter-relationship in patients with chronic venous diseases revealed by various forms of plethysmography. In contrast, NIRS can examine calf muscle O_2Hb and HHb simultaneously in every patient with DVT without the use of occlusion cuffs while the patients are standing and doing exercise, thus better reflecting natural activities during daily life.

In our previous study comparing patients with and without confirmed PTS, we found that in PTS patients, $_{T}O_2Hb_{st}$ was significantly decreased on standing and that during exercise, $\Delta HHbE_{ex}$ was significantly decreased whereas $\Delta HHbR_{ex}$ was significantly increased. Similarly, the falls in HbD_{ex} were more pronounced in PTS patients.[6] In the present study, $_{T}O_2Hb_{st}$ ≤48 seconds was the only NIRS-derived predictor of PTS at the 6-month follow-up point. Therefore, it appeared that NIRS-derived O_2Hb was able to reveal the earliest change in PTS rather than the change in HHb during follow-up of patients with DVT.

The mechanisms underlying the changes in O_2Hb during the development of advanced symptoms of PTS still remain unclear. As we assumed in our previous studies,[6,9] the fact that $_{T}O_2Hb_{st}$ is reduced in patients with advanced symptoms of chronic venous disease may indicate impairment of the venoarteriolar reflex. Long-standing venous hypertension ultimately causes changes in the microcirculation[23] and may result in reflex arteriolar vasoconstriction in response to venous hypertension. Patients who have venous obstruction and reflux at 6 months after DVT are expected to have chronic exposure to venous hypertension, unlike those without venous abnormalities. In our previous study, we found that patients with confirmed PTS had significantly decreased $\Delta HHbE_{ex}$ and increased $\Delta HHbR_{ex}$ relative to those without PTS. This finding reflects the fact that patients with confirmed PTS have more severe impairment of the calf muscle pump than those without.[6] However, in the present study, we did not find any predictive value of changes in both $\Delta HHbE_{ex}$ and $\Delta HHbR_{ex}$ at 6 months, suggesting that impairment of the calf muscle pump may develop in the later phase. In this background, we

were able to clearly identify patients at risk for development of PTS as well as candidates for intervention that would decrease the risk for development of PTS at 6 months. At this point, patients who were found to be at risk for PTS were encouraged to wear compression stockings for more than 6 months. In contrast, patients who were found to have no risk for development of PTS at the 6-month follow-up point were ready to stop wearing compression stockings. Furthermore, patients with a high risk of PTS were potential candidates for subsequent neovalve construction.[24]

There were some limitations to the interpretation of our NIRS findings. We did not find any significant relationships between baseline characteristics and risk factors for PTS. This was mainly due to our relatively small sample size and the high interindividual variability of the data. Only changes in $_{T}O_2Hb_{st}$ suggest that the observed effect is independent of small sample size and reflects a physiologically relevant phenomenon. Therefore, to confirm the relationship between the investigated parameters and the clinical prediction of PTS, a large sample size will be required. Furthermore, in this cohort, we did not find any patients with advanced symptoms of PTS (Villalta sore ≥15, presence of venous ulcer, or CEAP clinical class C5 or C6) at 6 years. Nevertheless, we believe that our findings may have important implications for investigations of the microcirculation in the context of post-thrombotic sequelae.

CONCLUSIONS

We have found that in patients with DVT, venous occlusion combined with reflux was predictive of PTS at the 6-month point. Furthermore, $_{T}O_2Hb_{st}$ ≤48 seconds was the only strong NIRS-derived predictor of PTS. These findings suggest that chronic exposure to venous hypertension resulting from venous obstruction and reflux could affect calf muscle oxygenation at a relatively early stage of DVT. Further studies using a multicentered approach will be needed to confirm the usefulness of NIRS for evaluation of disease severity.

AUTHOR CONTRIBUTIONS

Conception and design: TY
Analysis and interpretation: TY
Data collection: YH, AO, HK, AH, MO
Writing the article: TY
Critical revision of the article: TY, MN, HS
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