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Retrospective Study

Risk Factors Affecting the Outcomes of CT-Guided Radiofrequency Thermocoagulation of Thoracic Sympathetic Nerve in the Treatment of Primary Palm Hyperhidrosis

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Background: Primary palm hyperhidrosis (PPH) is a chronic disease characterized by uncontrolled palm-sweating exceeding physiological needs. It negatively impacts the quality of life of the patients and can lead to different degrees of psychological problems. Currently, there are a variety of treatment options for PPH, of which thoracotomy is a first-line treatment that has shown good efficacy. However, since it is an invasive procedure requiring general anesthesia and is often associated with high costs and serious complications, better alternatives should be explored. Computed tomography (CT)-guided percutaneous puncture of radiofrequency thermocoagulation (RF-TC) of the thoracic sympathetic nerve is a promising alternative treatment. It is a minimally invasive procedure that can be performed under local anesthesia and is associated with rapid recovery. However, the factors affecting the duration of the surgery-related benefits and outcomes of CT-guided percutaneous RF-TC of the thoracic sympathetic nerve are unclear.

Objectives: To investigate the factors influencing the outcomes of CT-guided percutaneous RF-TC of the thoracic sympathetic nerve in patients with PPH.

Study Design: A retrospective study.

Setting: This study was conducted at the Pain Department of Jiaxing University Affiliated Hospital (Jiaxing, China).

Methods: After approval by the Ethics Committee of the Affiliated Hospital of Jiaxing College, the data of 232 corresponding patients were assessed. The Kaplan-Meier method was used for survival analysis. Univariate and multivariate analyses were performed to identify factors associated with PPH and to construct a nomogram for predicting postoperative recurrence. Time-independent receiver operating characteristic (ROC) curve analyses were performed to assess the nomogram's predictive capacity.

Results: In the one-year survival analysis model, gender (HR = 1.573, 95%CI: 0.844 to 2.934), age (HR = 0.965, 95%CI: 0.915 to 1.018), disease course (HR = 0.960, 95%CI: 0.908 to 1.015), palm temperature difference (HR = 0.377, 95%CI: 0.287 to 0.495), perfusion index difference (HR = 0.590, 95%CI: 0.513 to 0.680) and hyperhidrosis disease severity scale (HR = 1.963, 95%CI: 0.769 to 5.011) were identified as statistically significant factors in univariate analysis, while palm temperature difference (HR = 0.589, 95%CI: 0.369 to 0.941) and perfusion index difference (HR = 0.357, 95%CI: 0.588 to 0.968) were the independent factors in the multivariate Cox proportional hazards risk model. In the 2-year survival analysis model, palm temperature difference (HR = 0.353, 95%CI: 0.261 to 0.478), perfusion index difference (HR = 0.589, 95%CI: 0.510 to 0.680) and hyperhidrosis disease severity scale (HR = 1.964, 95%CI: 0.771 to 5.006) were the statistically significant factors while palm temperature difference (HR = 0.507, 95%CI: 0.321 to 0.799) and perfusion index difference (HR = 0.789, 95%CI: 0.625 to 0.995) were the independent factors.

Limitations: This single-center retrospective study was limited by its small sample size, short follow-up time, and the possibility of bias resulting from the non-random patient selection.

Conclusion: Palm temperature difference and perfusion index difference were independent risk factors associated with prolonging the surgical benefits and reducing postoperative recurrence of CT-guided RF-TC of the sympathetic nerves in patients with PPH.

Key words: Primary palmar hyperhidrosis, radiofrequency thermocoagulation, COX regression, hyperhidrosis disease severity scale

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Primary palm hyperhidrosis (PPH) is a somatic disorder characterized by excessive sweating of the palms that largely exceeds physiological needs (1,2). Although it usually begins in childhood or adolescence, it can occur at any age and can be a lifelong problem, except for a small number of patients in whom the symptoms could improve naturally over time (3). In Western countries, PPH is thought to affect approximately 0.6% to 1.0% of the population. In 2010, we conducted a national survey and found that the prevalence of PPH among adolescents in mainland China was 2.1% (4).

The treatments of PPH can be non-surgical (i.e., local antiperspirant, oral anticholinergic medicines, ion import method, and more) or surgical (i.e., thoracoscopic sympathectomy) (2). Although thoracoscopic sympathectomy is the most common surgical procedure used to treat PPH (5), it is often accompanied by serious complications. Sympathetic neurotomy can be performed not only through surgery but also by radiofrequency thermocoagulation (6). Radiofrequency thermocoagulation is a minimally invasive procedure that produces heat through denervation at high temperatures (7). Currently, radiofrequency thermocoagulation is used for the treatment of refractory ischemic upper limb pain (8), diabetic foot (9), refractory erythema pain (10), trigeminal neuralgia (11), and others. The use of radiofrequency thermocoagulation for hyperhidrosis was first described by Wilkinson in 1984 (12). This led some authors to investigate its applicability and safety for treating palm hyperhidrosis (7,13). They found that radiofrequency surgery had fewer complications than thoracoscopic sympathectomy and could be an alternative method for treating hyperhidrosis of the palms (14).

Scholars have now reached a consensus on the sympathetic ganglia that innervate the corresponding sweat glands, especially those controlling head and hand sweat. They found that T2 and T3 sympathetic ganglia mostly innervate the head, axillary, and palm sweat glands, while T4 sympathetic ganglia innervate the axillary and palm sweat glands (1,2,15,16). Thus, the T4 sympathetic ganglion was chosen as the target site for the radiofrequency thermocoagulation procedure in this study. Our aim was to identify factors influencing the outcomes of computed tomography (CT)-guided percutaneous radiofrequency thermocoagulation of the thoracic sympathetic nerve, especially those that could prolong the duration of surgery-related benefits and delay postoperative recurrence, to guide clinical practice.

Methods

Patients

This study was approved by the Medical Ethics Committee of Jiaxing University Hospital (Jiaxing, China). The data of 264 patients who underwent CT-guided transcutaneous percutaneous radiofrequency thermocoagulation for primary hand hyperhidrosis at our hospital from December 2017 to December 2020 were assessed.

Inclusion and Exclusion (17)

The inclusion criteria were: unexplained visible sweating of the hands lasting more than 6 months, with at least 2 of the following characteristics: 1) the excessive sweating initially started before the age of 25; 2) occurred symmetrically on the hands; 3) happened more than once a week; 4) significantly impacted the patient's quality of life; 5) had a family history of unexplained sweating; and 6) absence of night sweats.

The exclusion criteria were: 1) patients who refused follow-up; 2) were pregnant or breastfeeding.

Surgical Procedure

On entering the operating room, intravenous access was established. The patient was placed in a prone position on the CT operating table with a soft pillow under the abdomen, and the patient's vital signs were checked. The manual spine counting method was used to identify the T4 intervertebral space, and then a 3 mm scanning interval was used until the optimal puncture plane was identified. The CT machine software was used to plot the puncture path. The optimal puncture level and puncture point on the skin were selected.

The proposed depth and angle of the puncture point and the distance from the midline of the puncture point were recorded using a CT tool ruler. The angle of the CT bed to the frame and the relative distance were recorded at this level. A red line was positioned, and the puncture entry point on the positioning red line was marked with a marker based on the previously measured distance from the centerline. After local anesthesia with 1.0% lidocaine hydrochloride at the selected puncture site, a needle was inserted under CT guidance at the pre-designed angle and depth. A 7-gauge sympathetic needle was inserted through the T4 paravertebral space to the small cephalic margin of the posterior lateral T4 rib (Fig. 1). The tip of the needle was adjusted to test the resistance of the tissue around the tip of the electrode (set between 150-250 Ω), and the sensory and motor electrical stimulation was tested at 0.1-0.5 V and 50 Hz frequency. The sensory test was considered positive if discomforts such as pain, swelling, numbness, or tingling were induced. The motor test was performed using a low-frequency current with parameters set to 0.1-0.5V and 2Hz frequency. If tremors were observed in the corresponding segments of the trunk muscle fibers, the motor test was considered positive. Before confirming the correct position of the radiofrequency tip, the return draw was ensured to be free of blood, fluid, and gas. The temperature was set to 80-95°C, and the thermal coagulation lasted for 120 seconds, for a total of 1-2 cycles of radiofrequency thermal coagulation. After 5 minutes, the patient's hands were examined if changed from cold and wet to warm and dry and for numbness or movement disorder in the limbs. When all conditions were stable after the operation, the patients were returned to the ward.

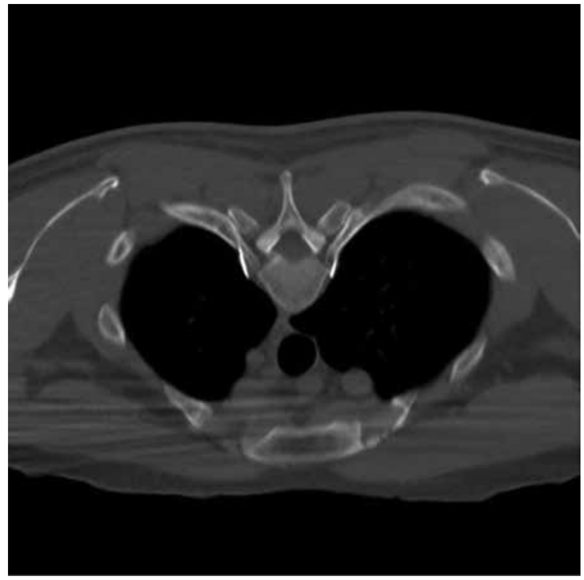

Fig. 1. *Illustration of the CT-guided puncture needle reaching the head of the small margin of the T4 rib.*

Table 1. *Hyperhidrosis disease severity scale (HDSS) is used to evaluate the severity of excessive sweating.*

HDSS score	Questions
1	My sweating is not noticeable and never interferes with my daily activities
2	My sweating is tolerable but sometimes interferes with my daily activities
3	My sweating is barely tolerable and frequently interferes with my daily activities
4	My sweating is intolerable and always interferes with my daily activities

Statistical Analysis

The data were analyzed using the R (version 4.0.2; 2020-06-22) and SPSS version 26.0 (IBM Corporation, Chicago, IL) software. This study screened independent variables following the principle of 10 events per variable (10 EPV). Based on previous studies (18-20) and research experience, independent variables such as age, gender, course of the disease, body mass index (BMI), family history, and hyperhidrosis disease severity scale (HDSS) (Table 1) were included for analysis.

Recurrence was defined as a patient's sweating at follow-up, which was equal to or greater than that observed before surgery (i.e., HDSS ≥ preoperative HDSS at follow-up). A positive result was considered if recurrence was observed. If there was no recurrence or tail amputation at follow-up, the result was considered negative. The time to recurrence was recorded for all patients with recurrence. To further explore the risk factors affecting the prognosis of surgery, one-way Cox regression was used to analyze the association between the independent variables and prognosis. Statistically significant variables were included into a multivariate stepwise Cox proportional risk regression model (Forward: LR), excluding covariance between independent variables and controlling for all confounding factors.

Prognostic plots of T-D and PI-D were constructed. Time-dependent receiver operating characteristic curves (ROCs) were used to evaluate the overall discrimination of the model.

Results

Baseline Characteristics

Based on the inclusion and exclusion criteria, the data of 264 patients diagnosed with primary palmar hyperhidrosis from December 2017 to December 2020 were assessed. After excluding patients who were pregnant (n = 1), refused follow-up (n = 6) and lost to follow-up (n = 25), a total of 232 patients were included for statistical assessment. The baseline characteristics of all included patients are shown in Table 2.

Follow-up: The longest follow-up period was 48 months, and the shortest follow-up period was 12 months.

Recurrence: By the end of follow-up, December 2021, there were 77 cases of recurrence.

Risk for Recurrence Rate

One-year Follow-up

Of the 232 patients who underwent minimally invasive surgery between December 2017 and December 2020, 103 (44.4%) were men, and 129 (55.6%) were women. For a follow-up period of 12 months, 40 (17.2%) patients experienced an outcome event. The cumulative incidence curve is shown in Fig. 2A. Univariate (UV) and multivariate (MV) Cox proportional risk regression analyses showed that T-D (UV: HR, 0.377 and 95%CI, 0.287 to 0.495; MV: HR, 0.589 and 95%CI, 0.513 to 0.680) and PI-D (UV: HR, 0.590 and 95%CI, 0.513 to 0.680; MV: HR, 0.775 and 95%CI, 0.588 to 0.968) were independent risk factors influencing the recurrence rate of PPH (Table 3A). T-D and PI-D were negatively associated with the primary outcome. The area under the curve (AUC) at 3, 6, and 9 months was 0.865, 0.883, and 0.889, respectively (Fig. 3A). The recurrence rate of PPH after radiofrequency treatment using the nomogram is shown in Fig. 4A. Further, the calibration curve for the one year recurrence-free survival of this model fitted well with the standard curve (Fig. 5A).

Table 2. *Baseline characteristics of patients.*

Variables	
Age, mean ± SD, year	24.89 ± 6.61
Course of PPH, mean ± SD, year	17.87 ± 6.28
Gender, n (%)	
Women	129 (55.6)
Men	103 (44.4)
HDSS score, n (%)	
4	186 (80.2)
3	46 (19.8)
BMI, mean ± SD, kg/m^2	21.1 ± 3.07
Family history, n (%)	
Yes	96 (41.4)
No	136 (58.6)
HR, mean ± SD, bpm	82.21 ± 12.51
NIBP, mean ± SD, mmHg	
SBP	116.85 ± 14.28
DBP	70.69 ± 12.01
Days in hospital, n (%)	
≤ 3	205 (88.4)
> 3	27 (11.6)

HDSS = Hyperhidrosis disease severity scale; BMI = body mass index; HR = heart rate; NIBP = non-invasive blood pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure.

Two-year Follow-up

A total of 139 patients who underwent minimally invasive surgery from December 2017 to December 2019 had 2-year follow-up data. This cohort comprised of 68 (48.9%) men and 71 (51.1%) women, of whom 40 (28.8%) patients had an outcome event. The cumulative incidence curves are shown in Fig. 2B. Univariate (UV) and multivariate (MV) analyses showed that T-D (UV: HR, 0.353 and 95%CI, 0.261 to 0.478; MV: HR, 0.507 and 95%CI, 0.321 to 0.799) and PI-D (UV: HR, 0.589 and 95%CI, 0.510 to 0.680; MV: HR, 0.789 and 95%CI, 0.625 to 0.995) were independent risk factors influencing the recurrence rate of PPH (Table 3B). T-D and PI-D were negatively associated with the primary outcome. The area under the curve (AUCs) at 3, 6, and 9 months were 0.854, 0.871, and 0.888, respectively (Fig. 3B). The recurrence rate of PPH after radiofrequency treatment using the nomogram is shown in Fig. 4B. Similar to the 1-year follow-up cohort, the calibration curve for the 2-year recurrence-free survival of this model fitted well with the standard curve (Fig. 5B).

Adverse Events and Satisfaction

The visual analog scale (VAS) was used to evaluate the pain level of each patient. The majority of the patients (n = 186) reported a VAS of < 4, possibly related to the use of local anesthesia during the surgery. The remaining 46 patients reported an intraoperative VAS of ≥ 4. After surgery, 75 of the 232 patients experienced sinus bradycardia which lasted 2 to 3 hours and eventu-

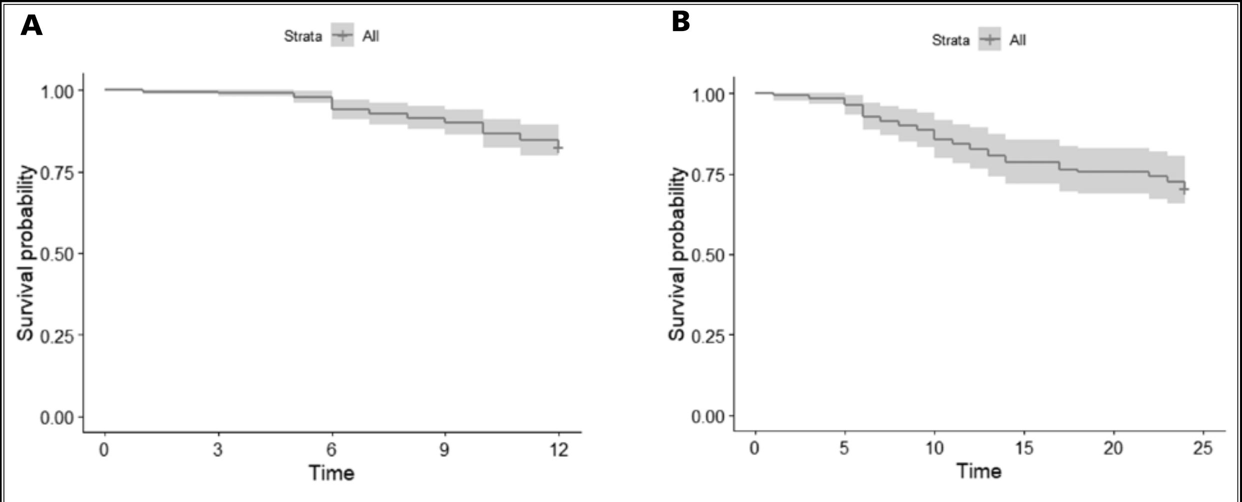

Fig. 2. *Kaplan-Meier analysis demonstrating the cumulative survival rates.*

Table 3A. *Multivariable Cox analyses on potential risk factors for recurrence-free survival.*

Factors	Univariable			Multivariable		
	HR	95% CI	P	HR	95% CI	P
Age	0.965	0.915-1.018	0.190			
Gender	1.573	0.844-2.934	0.154			
Family history	0.747	0.390-1.431	0.379			
Course of disease	0.960	0.908-1.015	0.154			
BMI	1.039	0.943-1.145	0.437			
T-D	0.377	0.287-0.495	< 0.001	0.589	0.369-0.941	0.027
PI-D	0.590	0.513-0.680	< 0.001	0.357	0.588-0.968	0.027
HDSS	1.963	0.769-5.011	0.158			

Table 3B. *Multivariable Cox analyses on potential risk factors for recurrence-free survival.*

Factors	Univariable			Multivariable		
	HR	95% CI	P	HR	95% CI	P
Age	0.980	0.937-1.026	0.390			
Gender	1.009	0.547-1.861	0.978			
Family history	1.322	0.715-2.442	0.373			
Course of disease	0.984	0.939-1.030	0.490			
BMI	1.052	0.944-1.171	0.360			
T-D	0.353	0.261-0.478	< 0.001	0.507	0.321-0.799	0.003
PI-D	0.589	0.510-0.680	< 0.001	0.789	0.625-0.995	0.045
HDSS	1.964	0.771-5.006	0.157			

BMI = body mass index; T-D = temperature difference; PI-D = perfusion index difference; HDSS = hyperhidrosis disease severity scale.

ally returned to a normal rhythm. Six patients experienced chest tightness and shortness of breath which were resolved after one day. Three patients had nausea and vomiting, which returned to normal after one day. Compensatory hyperhidrosis after surgery was observed in 156 patients, of whom 3 had severe compensatory hyperhidrosis that interfered with normal life, while the remaining 153 patients had compensatory hyperhidrosis that did not interfere with their normal life. The patients' satisfaction was graded on a score ranging

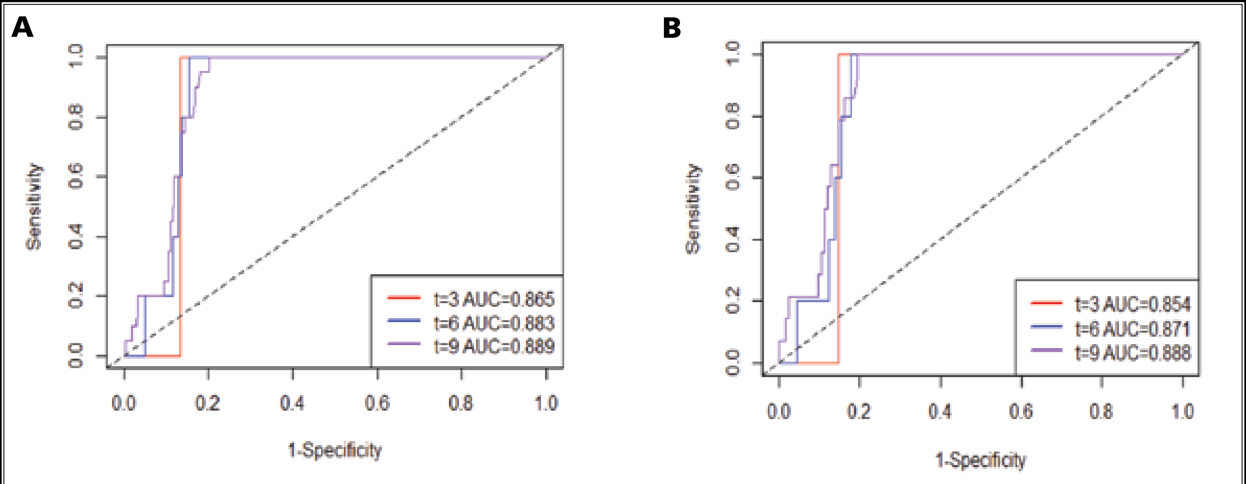

Fig. 3. *Time-dependent ROC.*

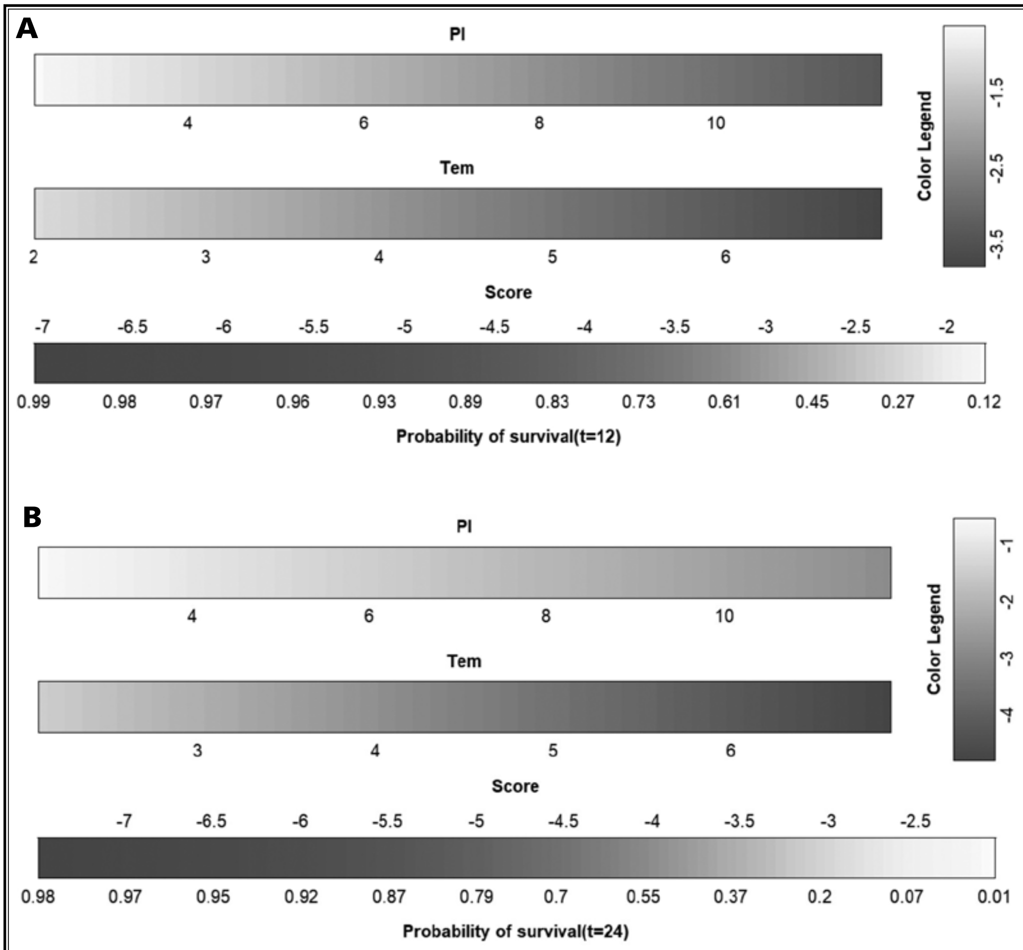

Fig. 4A. *Nomogram for predicting the recurrence rate of PPH after radiofrequency. The score was obtained by the addition of every risk factor corresponding to the predicted recurrence rate.*

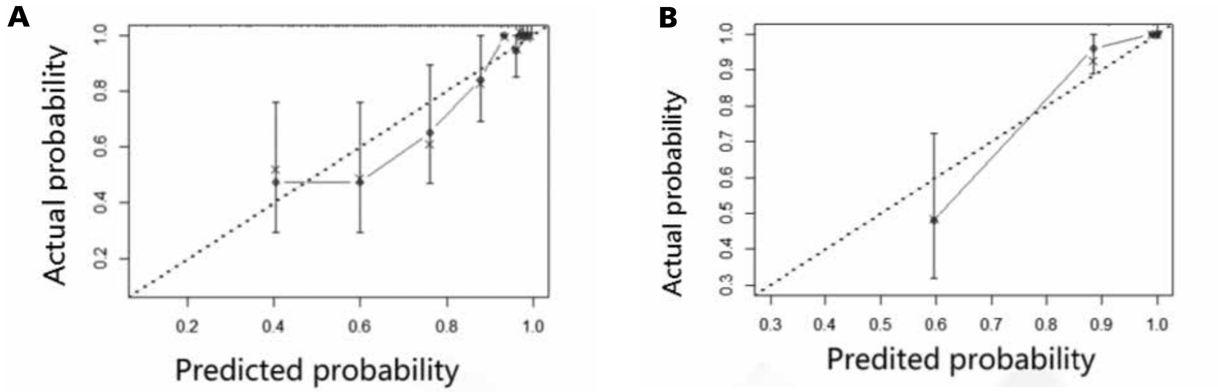

Fig. 5. *Calibration curves.*

from 0 to 10, with 0 indicating very dissatisfied and 10 indicating very satisfied (0-2 indicating dissatisfied, 3-5 indicating slightly satisfied, 6-8 indicating satisfied, and 9-10 indicating very satisfied). The results showed that the overall patient satisfaction was 6.34.

Discussion

Primary hyperhidrosis is one of the oldest skin diseases and can often be an embarrassing condition (21). Uncontrolled and continuous sweating symptoms can have serious implications on the affected individual's social interaction and daily activities, leading to significant physical and mental problems. Some previous studies found that PPH had a genetic predisposition, and inheritance appears to be autosomal dominant or recessive (22). In this current study, 96 of 232 patients (41.4%) had a family history, which was concordant with previous findings.

There are a variety of treatments available for primary hyperhidrosis, ranging from conservative treatment with psychological therapy, oral medication, topical medication, radiation ionization, and local injections to surgical treatment with percutaneous open-chest or thoracoscopic sympathectomy (2). Most patients opt for thoracoscopic sympathectomy as medication therapy is often either ineffective or only provides a temporary antiperspirant effect (23). The success rate of surgical intervention in the literature ranges between 90% to 97% (24). The most commonly used surgical procedure is thoracoscopic sympathectomy. However, it has a risk of serious complications such as Horner's syndrome, hemopneumothorax, intraoperative arrest, or brachial plexus injury. The most unacceptable complication is compensatory hyperhidrosis. About 80% of patients develop compensatory hyperhidrosis after thoracoscopic sympathectomy, and more than half of them report that compensatory hyperhidrosis affects their daily lives (25). Thus, a less invasive, more economical, and safer alternative is required. At present, patients with primary hyperhidrosis have been treated with minimally invasive treatments such as percutaneous radiofrequency thermocoagulation or chemical (ethanol, phenol, etc.) blockade of the sympathetic nerve chain with the aid of a CT machine and have achieved good results (26).

CT-guided injection of anhydrous ethanol is easily accepted by patients due to its minimal invasiveness and beneficial short-term results. However, one disadvantage of this technique is that anhydrous ethanol is fluid and has a risk of upward diffusion, which could lead to Horner's syndrome. If injected into a blood vessel, it can cause thrombosis, leading to pulmonary embolism and spinal cord or even brain infarction (27). To this end, we have explored the use of radiofrequency technology for thermocoagulation to affect the thoracic sympathetic chain, achieve longer efficacy and decrease the risk of complications.

Radiofrequency thermocoagulation of the sympathetic nerves for the treatment of hyperhidrosis has been shown to be safe and effective in numerous studies (6,7). During the procedure, although the thoracic sympathetic nerve chain is located deep in the anterior aspect of the thoracic spine and partially outside and in front of the pleura, the puncture needle can still reach the puncture location under CT guidance, which improves the accuracy of the puncture and reduces pain and ray exposure time to the patient. CT helps to select the best puncture path and reduces the risk of complications such as hemopneumothorax and pulmonary atelectasis. However, the location of the sympathetic nerve is indeed determined by stimulation rather than CT. We reach the target point and perform radiofrequency thermocoagulation and then observe whether the increase in palm temperature is ≥ 3°C or a 5-fold increase in the end-finger perfusion index or the patient's hands change from moist and cold to dry and warm, which is considered a successful procedure and sent back to the ward (28). In this present study, 205 (88.4%) patients had a hospital stay of ≤ 3 days. Thus, the proposed radiofrequency thermocoagulation procedure can shorten the length of stay, reduce treatment costs and save medical resources. However, the risk of recurrence is a major cause of reduced patients' satisfaction after the procedure and should be thoroughly investigated for wider acceptance of radiofrequency thermocoagulation. In a study by Purtuloglu et al (7), recurrence of hyperhidrosis after thoracoscopic sympathectomy was reported to be 5%, while recurrence after radiofrequency was 25% . Concordantly to previous study findings, we also observed that patients who underwent radiofrequency thermocoagulation had a high recurrence rate. In this study, 77 of 232 patients recurred by December 2021. However, only 3 of 232 patients had compensatory hyperhidrosis that impacted their daily lives, compared to other serious complications associated with thoracoscopy. Further, although 156 of 232 patients who underwent radiofrequency thermocoagulation also had compensatory hyperhidrosis; this did not affect their daily lives. The mechanisms underlying the occurrence of compensatory hyperhidrosis after ablation is currently debatable. Some authors suggest that the development of compensatory hyperhidrosis may be a "reflex sweating" secondary to a reflex in the hypothalamic sweating center following ablation of the sympathetic chain (29), while others suggest that the level of sympathetic neurolysis is directly related to the development of compensatory hyperhidrosis (30).

Another complication of radiofrequency ablation that reduces patient satisfaction is intraoperative pain. It was reported in 46 of 232 patients (intraoperative pain score of ≥ 4) at follow-up despite the use of intraoperative local anesthesia. In response to the intraoperative hyperthermic radiofrequency injury nerve pain experienced by patients, a small amount of fentanyl was administered during the follow-up procedure. However, it was not only ineffective but also accompanied by side effects such as nausea and vomiting (n = 3) and necessitated better analgesic modality to improve the patient's comfort. In addition, a short period of sinus bradycardia was experienced postoperatively in 75 patients, which could be related to sympathetic inhibition.

Previous studies have shown that the palm temperature can increase after radiofrequency thermocoagulation. Thus, palm temperature measurements were used as objective data to evaluate the effectiveness of the procedure (effectiveness of nerve damage) (18,19). The current study finding was also consistent with previous studies. Our analysis showed that the palm temperature difference and perfusion index difference were significantly associated with the duration of the surgical outcome. Higher elevated palm temperature and perfusion index were associated with a lesser risk of recurrence and prolonged the duration of the surgical benefits. In the one-year survival model, the risk of recurrence was reduced by 0.411 for every 1°C increase in T-D and by 0.643 for every one unit increase in PI-D. In the 2-year survival model, the risk of recurrence was reduced by 0.493 for every 1°C increase in T-D and by 0.211 for every one unit increase in PI-D. We speculate that greater T-D and PI-D could be associated with more complete damage to the sympathetic nerves and a longer time to recurrence. Thus, intraoperative palm temperature difference and the end-finger perfusion index difference could be promising indicators for deciding the duration of radiofrequency thermocoagulation to obtain better surgical results and delay recurrence.

As a whole, the radiofrequency thermocoagulation procedure is a minimally invasive procedure that was associated with quick recovery, lesser financial stress, shorter hospital stays, and fewer complications. However, its medium- to long-term outcomes should be improved. During follow-up, approximately one year after the procedure, more than half of the patients reported that their palms had started to slowly become moist again. Although this was not as severe as before the procedure to the point of interfering with daily life, the effects of the procedure could slowly fade with time, and the sweaty palms could aggravate even interfere with the patient's life again. We speculate that the high recurrence rate of radiofrequency thermocoagulation could be related to the incomplete severance of the sympathetic nerve and the slow repair of the residual sympathetic nerve over time (4).

Based on the results of this study, surgeons can preoperatively inform patients about the risks and benefits of radiofrequency thermocoagulation. Here, we report that the 1- and 2-year recurrence rate was approximately 17.2% and 28.8%, and the probability of developing life-altering compensatory hyperhidrosis after surgery was approximately 1.3%. From a patient's perspective, these data could guide them to make a better decision on whether or not to opt for this treatment. From a surgeon's perspective, the findings of this study showed that the surgeons could predict a patient's risk of recurrence based on the intraoperative palm temperature difference and perfusion index difference.

Limitations

The limitations of this study were as follows: first, the nonrandomized, single-center, and retrospective design of this study could not exclude outliers. Second, the small sample size could not allow further segmentation or validation of the findings.

Conclusion

In summary, CT-guided percutaneous radiofrequency thermocoagulation of the thoracic sympathetic nerve was found to be a safe and effective option for treating PPH. However, it was also associated with a high risk of recurrence. The differences in palm temperature and perfusion index were determining factors affecting the outcomes of radiofrequency thermocoagulation and could be used intra-operatively as guidance to reduce the risk of recurrence and improve patient's treatment satisfaction.

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Supplementary material available at www.painphysicianjournal.com

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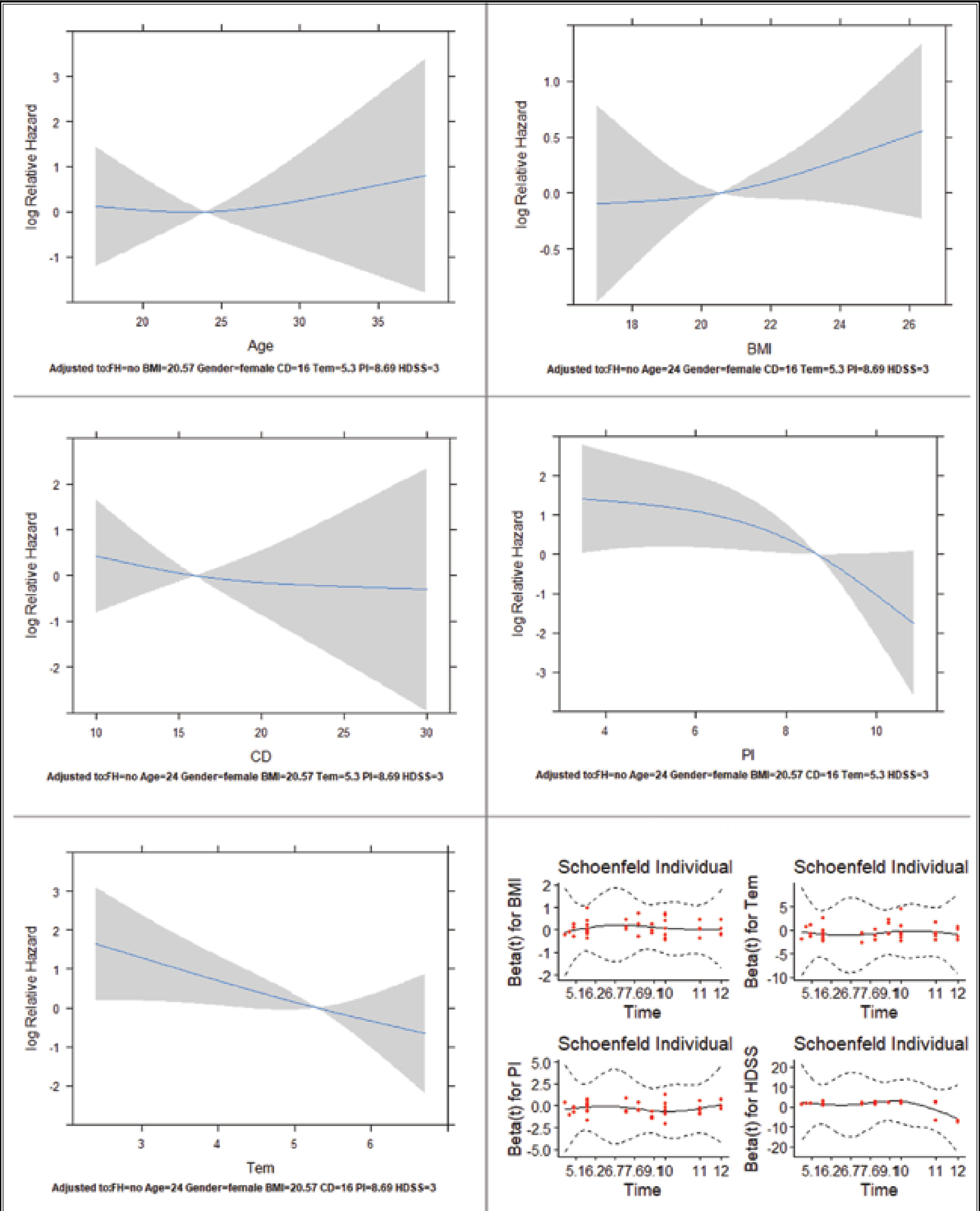
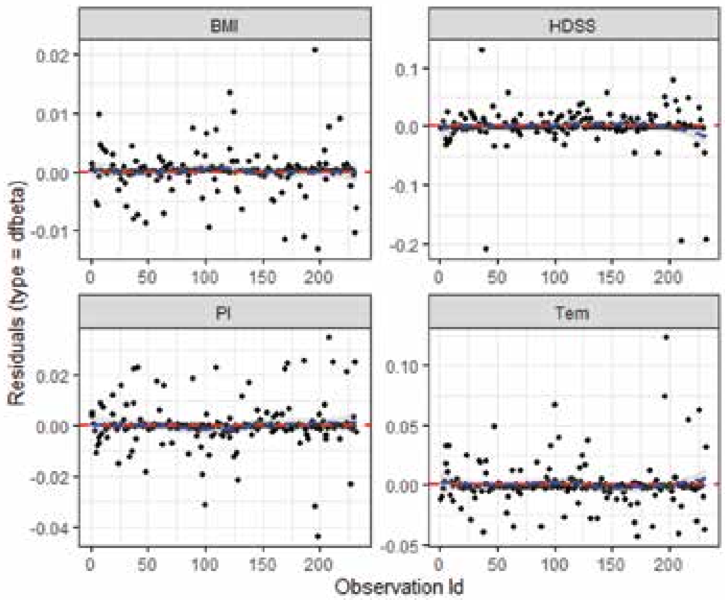
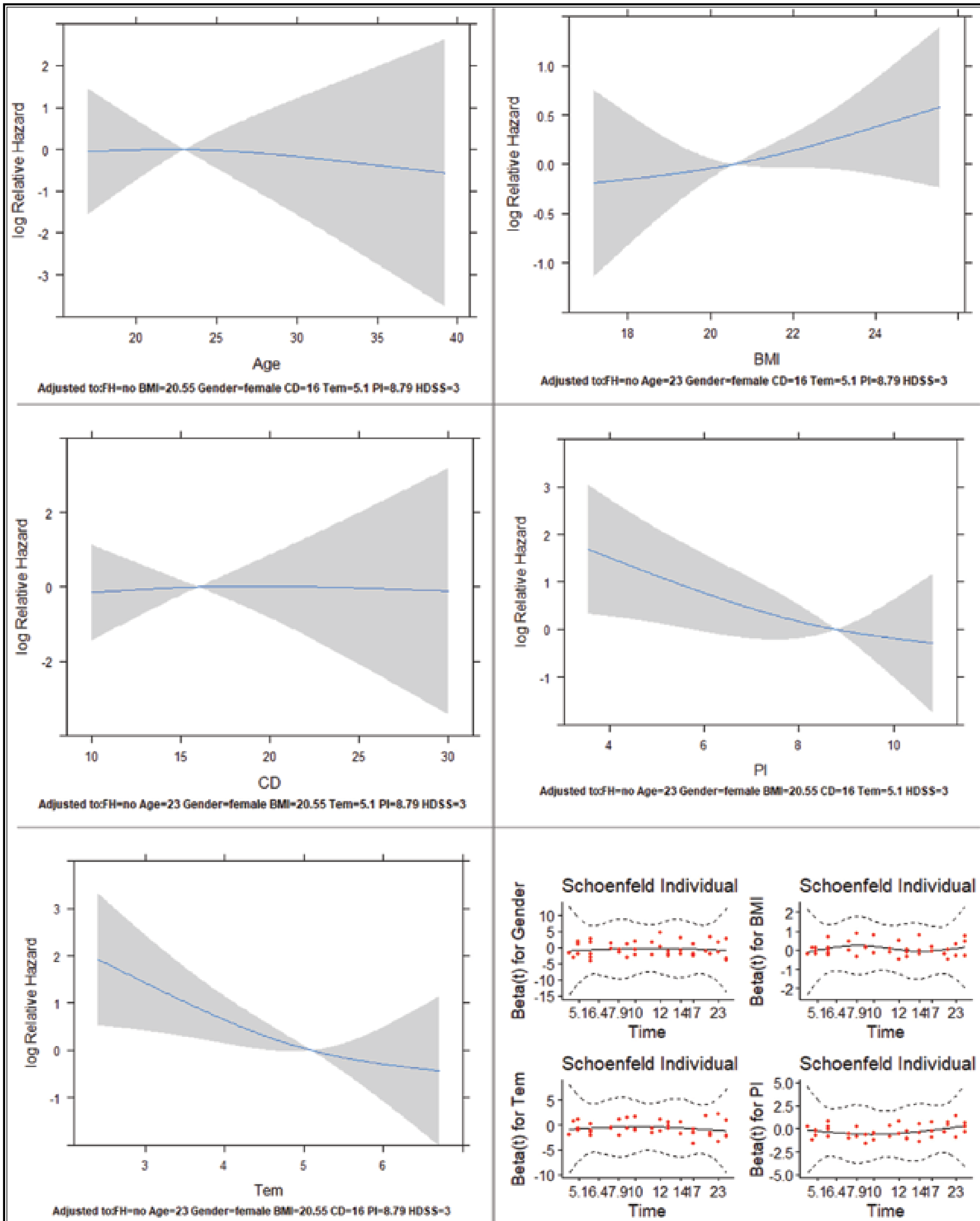


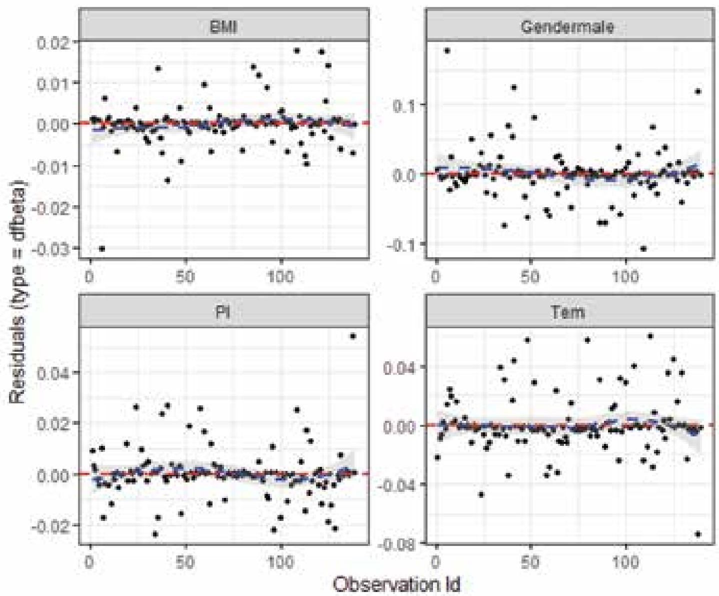
Supplemental Fig. 1. *One-year.*



Supplemental Fig. 1 (cont.). ***One-year***.



Supplemental Fig. 2. *Two-years*.



Supplemental Fig. 2 (cont.). ***Two-years***.