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Fluorometric Prediction of Successful Amputation Level in the Ischemic Limb[a]

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Abstract — The present study was undertaken to compare fluorometric documentation of fluorescein dye delivery with the standard means of determining the level at which an amputation should be performed in the dysvascular extremity. Thirty-nine patients underwent lower-extremity amputation at the level determined by the surgeon based upon physical examination, angiography, segmental pressure indices, and/or pulse volume recordings. In addition, fiberoptic fluorometry was performed preoperatively. After intravenous administration of sodium fluorescein (4-8 mg/kg), fluorometric readings were obtained by placing the fluorometer's light guide on 126 reading sites. Fluorometric findings were evaluated retrospectively, and therefore did not influence the surgeon's decision.

Of the 39 amputations performed overall, only 26 healed. The accuracy of the standard criteria was lowest for the 20 below-ankle amputations, where only 12 cases healed. Alternatively, fluorometric indices separated healing from nonhealing sites in 36 of the 39 cases and in 18 of the 20 below-ankle amputations. Overall, healing sites averaged 94 percent of the fluorescence of the healthy reference area, while nonhealing sites averaged only 29 percent. We conclude that fluorometry should prove to be a valuable adjunct in the assessment of the dysvascular extremity. It uses a low dose of dye, is easy to perform, and is readily repeatable.

INTRODUCTION

The adequacy of nutritive skin blood flow is a major determinant of the healing of an amputation. Poor skin perfusion has been responsible for failure rates as high as 50 percent following lower limb amputations in patients with occlusive arterial disease; accurate determination of such flow would greatly reduce patient morbidity and associated hospital costs (1-8). Unfortunately, no means of assessing skin perfusion has gained widespread clinical acceptance.

Several investigators have employed sodium fluorescein, a nonradioactive tracer dye, to document skin blood flow. Following intravenous injection, the dye distributes to all perfused tissues by diffusing across the capillary walls. When excited by ultraviolet or blue light, fluorescein molecules fluoresce; well-perfused tissues which have acquired the dye appear bright yellow-green. Unstained, nonperfused tissues simply reflect the blue light. While distinct demarcations of extremity skin blood flow are identified readily by

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qualitative visual fluorescence assessment (9-11), the technique is subjective and not very sensitive to gradations in flow, especially in black skin where pigmentation interferes with light transmission.

In 1940, Lange et al. (12, 13) introduced a means of quantitative fluorescence assessment, but their "dermofluorometer" proved to be impractical since it consisted of bulky electrical components that needed to be placed on the patient. In 1980, Silverman et al. (14-16) introduced fiberoptic fluorometry as a means to quantify fluorescence in a more practical, clinically efficient manner. A flexible, sterilizable branched fiberoptic probe transmits excitation blue light to, and emitted fluorescence from, each 1 x 1 cm study site. The data describing the emitted fluorescence registered by a photomultiplier tube are displayed by a digital meter and/or recorded and analyzed by an interfaced computer. Hurford and Silverman (17) reported successful application of fluorometry in 20 patients undergoing amputation at levels selected according to the conventional preoperative criteria at this institution. Preoperative fluorometric assessment discriminated between healing and failing amputation sites with 100 percent accuracy. Their initial application of fluorometry evaluated both the uptake and the elimination of fluorescein in the skin; the test required as long as 4 hours, making it impractical as a diagnostic tool.

The present study was undertaken to determine if documentation of only the delivery (uptake) of dye (a 30-minute procedure) would make the test more efficient and practical, while maintaining its accuracy.

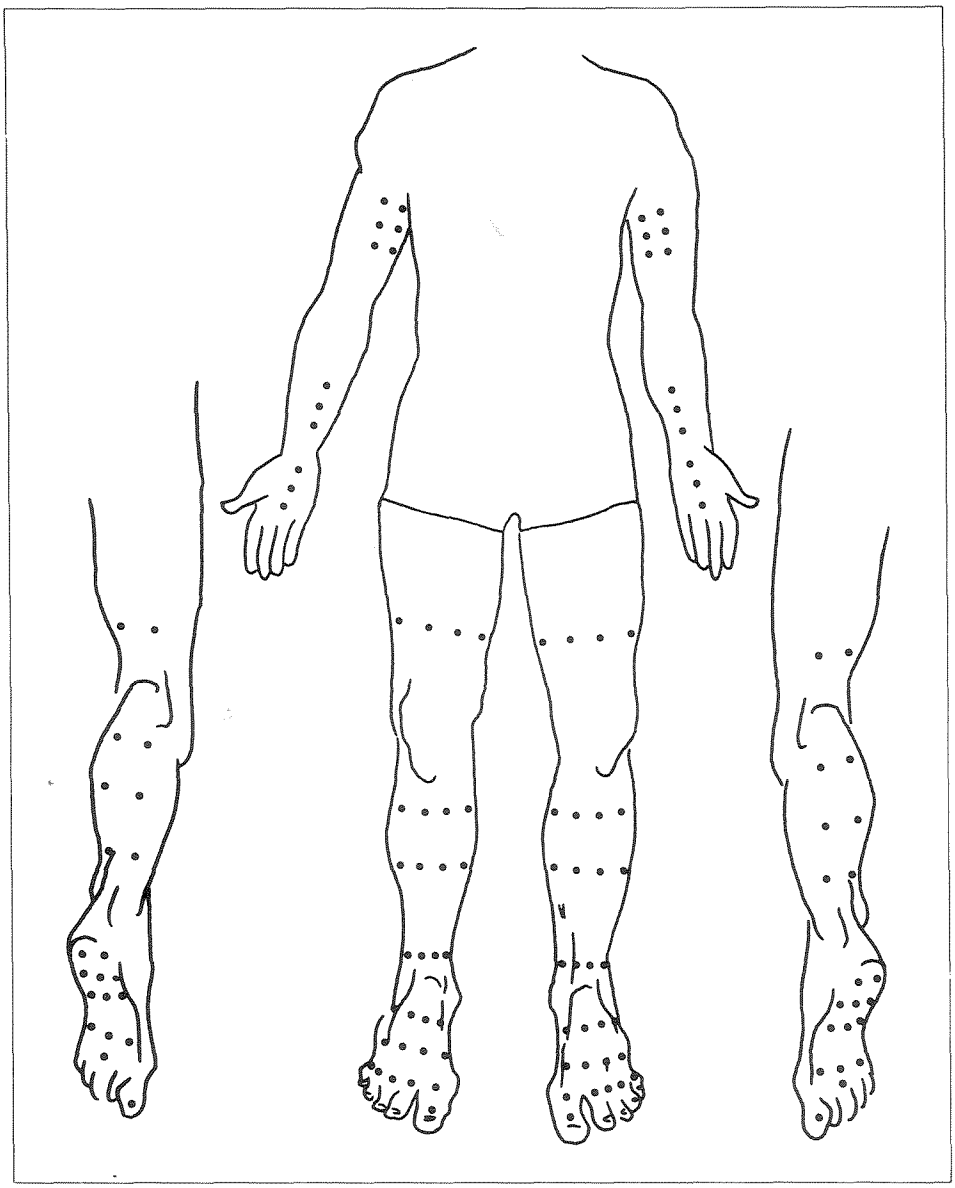

FIGURE 1
Representative grid pattern. Each point represents site of probe placement.

MATERIALS AND METHODS

Study Design

After institutional Human Studies Committee approval had been obtained, patients with nonhealing lower limb ulcers were referred for study (Table 1). Patients who had osteomyelitis within 2 cm of the proposed amputation site were excluded from the study. Thirty-nine amputations (12 digital, 5 transmetatarsal, 3 Symes, 12 below-knee and 7 above-knee) were studied.

The surgeons' determination of amputation sites was based on conventional preoperative assessment. This consisted of clinical evaluations by the surgical team, angiography, and segmental pressure indices determined by Doppler ultrasound and/or pulse volume recordings. Segmental pressures were based on posterior tibial and brachial artery determinations. For amputations below the ankle, readings were obtained with the cuff placed on the lower leg. For below-knee and above-knee amputations, readings were obtained with the cuff on the thigh. Fluorometry (described below) was performed within 10 days prior to surgery, but it did not influence the surgical decision. Amputations were performed by surgical housestaff under attending supervision. An amputation was considered to fail if it evidenced necrosis or if it required reamputation.

Since fluorometric predictive indices were determined retrospectively, a prediction by fluorometry that more of the leg could be salvaged was not tested. Alternatively, a prediction by standard criteria that an amputation would fail was never tested since amputations were performed only at sites determined to heal.

Fiberoptic Fluorometry (Procedures)

As many as 126 fluorometric reading sites were outlined on each subject (Fig. 1), with one to six sites being evaluated at each potential amputation level. Fluorometric assessments were obtained with the perfusion fluorometer* interfaced to a microcomputer with customized software. First, preinjection background readings were obtained by placing the fiberoptic light guide on each reading site (2–3 seconds at each site). Sodium fluorescein (4 mg/kg in whites, 6–8 mg/kg in blacks) was diluted to 20 cc with normal saline and slowly administered via forearm intravenous catheter over a 2-minute interval. At 10 to 20 minutes after injection, fluorometric measurements were obtained; background values were subtracted to obtain corrected dye fluorescence (DF) readings at each reading site. The DF values of the sites at a potential amputation level were averaged, and the relative fluorescence at that level was determined by comparing that average with the average of readings from a common reference area. The comparison, termed the Dye Fluorescence Index (DFI), can be expressed as follows:

$$DFI = (\text{DF of study site average} / \text{DF of reference area site average}) \times (100)$$

In the 39 amputations studied, the forearm served as the reference area.**

The mean of the DFI's of all healing and of all nonhealing amputations were calculated and compared by Student's t-test. Discriminant analysis was performed to determine the optimum fluorometric cutoff distinguishing healing and nonhealing amputations. Retrospectively, the accuracy of fluorometric predictions based on this cutoff was calculated. The false positive rate (incidence of a test falsely predicting ischemia leading to amputation failure) and false negative rate were determined.

Accuracy and false prediction rates also were determined for standard clinical criteria. Analysis of segmental pressure indices were performed in the same manner as for the DFI. In classifications where one or both of the tests previously had been reported to be difficult to interpret (i.e., diabetic patients and below-ankle amputations for segmental pressures; black patients for fluorescence assessment), the individual subgroups were analyzed separately.

TABLE 1
Patient Population

	Diabetic	Nondiabetic
Black	11	16
White	8	4

* Diversatronics, Inc., Broomall, Pennsylvania 19008.

** The balance of the paper contains several comments on the role of reference-area selection in fluorometric assessment.

RESULTS

Surgical Outcome

Table 2 summarizes the results for patient and amputation categories. Of the 39 amputations performed, 26 healed (a 67 percent success rate). The 13 false predictions of healing constituted a 33 percent false negative rate for conventional preoperative criteria. Twelve of the failures were below-ankle amputations (digital, transmetatarsal, and Symes), where there was a 40 percent accuracy and a 60 percent false negative rate for conventional preoperative criteria.

TABLE 2
Amputation results

	Healed (n = 26)	Failed (n = 13)
Patient Category		
Diabetic	13	6
Nondiabetic	13	7
Amputation Site		
Toe	6	6
Transmetatarsal	0	5
Symes	2	1
Below-knee	11	1
Above-knee	7	0

TABLE 3
Predictive indices

	% Fluorescence (DFI)*	Segmental Pressure Index (%)
All amputations		
Healing	93.6 ± 41.9	0.74 ± 0.23
Failing	29.1 ± 12.1	0.68 ± 0.23
Below-ankle amputations		
Healing	91.1 ± 35.8	0.64 ± 0.22
Failing	29.1 ± 12.7	0.66 ± 0.24
Amputations in diabetics		
Healing Amputations	102.2 ± 40.6	0.80 ± 0.18
Failing Amputations	25.4 ± 15.8	0.75 ± 0.23

*$p<0.05$ by Student's t-test for difference between mean DFI (Dye Fluorescence Index) values of healing and failing amputations, but not between mean segmental pressure indices.

The ultrasound Doppler segmental pressure index, the conventional preoperative technique most commonly relied upon by the surgeons, did not consistently discriminate between healing and failing amputations (Table 3). The mean ankle/brachial index for all healing amputations was 0.74, while that for all failing amputations was 0.68.

The poor discrimination was especially evident in patients undergoing amputations below the ankle and in patients with diabetes. For patients receiving below-ankle amputations, the average Doppler pressure index was 0.64 for those that healed; this was not significantly different from the mean value for those that failed, 0.66. In diabetics, the pressure index averaged 0.80 at amputation sites that eventually healed and 0.75 at those that failed to heal.

Fiberoptic Fluorometry

Fluorometry proved to be a more reliable predictor of healing (Tables 3 and 4). The mean DFI, an indication of relative fluorescence, clearly distinguished between viable and nonviable amputation sites. Amputation sites that went on to heal had a mean DFI of 94. Those sites that failed to heal had a mean DFI of 29.

Discriminant analysis provided an optimum cutoff between healing and nonhealing indices of 44. This separated healing from nonhealing sites with 93 percent accuracy; only one site with a DFI greater than 44 failed to heal. (This false negative was in a patient with significant atherosclerotic changes of his upper extremity; the reference forearm had low DF readings and thus leg points had spuriously high DFI's.) The two false positive predictions had values of 36 and 38.

In patients undergoing below-ankle amputations and in diabetics, fluorometry clearly distinguished healing and nonhealing sites. It also proved to be highly accurate in black patients, who are traditionally difficult to evaluate with the fluorescein test. In black patients, healing amputation sites had an average

TABLE 4
Comparison of predictions

	Standard criteria*			Fluorescence assessment**		
	Accuracy	False Pos.	False Neg.	Accuracy	False Pos.	False Neg.
Below-ankle	8/20	0/0	12/20	18/20	1/12	1/8
Below-knee	11/12	0/0	1/12**	12/12	0/1	0/11
Above-knee	7/7	0/0	0/7	6/7	1/1	0/6
Overall	26/39	0/0	13/39	36/39	2/14	1/25

*A prediction by standard criteria that amputation would fail was not tested; false positive rate = 0.
**A prediction by fluorescein that more of the leg could be salvaged was not tested.

DFI of 85, while nonhealing sites averaged 30.

There were two minor reactions to fluorescein (one patient had transient nausea, one patient had mild urticaria).

DISCUSSION

Although skin perfusion is a major determinant of stump viability, it is not documented by conventional preoperative techniques such as plethysmography, waveform analysis, and Doppler ultrasound segmental limb pressures. Using such methods as the basis for preoperative evaluation in the present patient population, a 33 percent failure rate was noted, with a 60 percent failure rate for below-ankle amputations. This was not totally unexpected; other investigators also have noted no statistically significant difference in Doppler values in patients with healing and nonhealing below-ankle amputations (18). In many cases, inaccurate predictions may be attributed to the fact that conventional preoperative techniques are not sensitive to gradations in microcirculatory flow and local perfusion. Limb perfusion in diabetics can be particularly difficult to evaluate with these techniques because pressure determinations may be spuriously high in patients with calcified arteries. In diabetic patients, we noted no significant difference between segmental pressure indices at amputation sites destined to healing and nonhealing fates.

To date, no means of measuring skin perfusion has proved to be reliable, inexpensive, and practical clinically. In animal studies, the distribution of radiolabeled microspheres provides a reliable means of assessing capillary blood flow, but this is not clinically useful. Measurements of skin temperature may not discriminate between capillary and nonnutritive shunt flow. Washout of intradermally administered radioisotopes (4,5,7) has been employed successfully in the clinical setting, but it requires expensive imaging equipment and involves needle sticks in hypoperfused tissues. Recent reports have suggested that transcutaneous measurement of tissue oxygen level reflects the extent of skin perfusion (19,20). However, since each site must be warmed for 20 minutes prior to obtaining a measurement, examination at multiple sites is impractical unless multiple measuring probes are used.

The present study strongly suggests that preoperative fluorometric assessment of fluorescein delivery can accurately predict the outcome of an amputation, and that addition of this technique would significantly improve preoperative evaluation. Assessment of the distribution of fluorescein can provide a direct indication of perfusion, since the dye is transported by the functional circulation and diffuses across the capillary membrane. Considering that factors other than perfusion can influence ultimate viability, the 93 percent accuracy noted in the present study suggests great potential for the technique.

Fluorometry offers several advantages over the traditional fluorescein test (qualitative assessment under ultraviolet illumination). It is more sensitive to gradations of perfusion (15), even in black skin (21). In addition, it provides reliable assessments of perfusion after several injections of dye — by subtracting as background the fluorescence remaining from a previous injection, one can document the distribution of a repeat injection. Silverman et al. (16) have reliably employed four injections within a 60-minute period. Thus, fluorometric results can be confirmed and the effectiveness of a therapeutic intervention, such as vascular reconstruction, may be monitored. These features overcome many of the limitations of the traditional fluorescein test in the assessment of the dysvascular extremity (9–11).

Moreover, fluorometry permits a lower dose of dye to be employed (4–8 mg/kg for fluorometry versus 10–40 mg/kg for visual assessment). Not only does this prevent noticeable staining of the skin, but it also reduces the potential systemic effects associated with fluorescein. These include nausea and vomiting, urticaria, hypotension, and respiratory compromise (22,23). (In light of the potential risks associated with dye injection, a physican should be available.) Recent evidence suggests that fluorescein causes a release of histamine in certain individuals (24). If fluorescein behaves like other potentially direct histamine-releasing agents, then the amount of release is proportional to peak plasma levels. Slow injection of a low-dose dilute solution, as employed in this study, minimizes the possibility of a reaction and permits discontinuation of dye administration if signs or symptoms arise. In more than 200 slow injections of a low dose of dye, we have noted no major reactions and less than a 2 percent incidence of minor (nausea, urticaria) reactions.

By evaluating delivery of dye only, we successfully shortened the fluorometric test to less than 30 minutes compared to the 4-hour test that was previously employed (17). Assessment of dye delivery is best accomplished by comparison to a reference area. In the present study and in previous studies (17,21,25), this has proved to be effective even in black skin. By administering a higher dose, we maintained a high signal to background (noise, reflectance, background fluorescence) ratio in black patients.

In the present study, we used the forearm as a reference; however, in a patient with significantly compromised forearm flow, this resulted in a low reference value and therefore spuriously high DFI determinations (resulting in the one false negative

prediction). Presently, the shoulder and chest are being evaluated as reference areas, as they may be less prone to such atherosclerotic changes.

Since there are local variations in flow, more than one reading site should be studied at each potential amputation level (especially at the anticipated level). Averaging the DFI's at each level allows the data to be more representative of the average flow in each area. It should be noted that the palm and sole exhibit unique fluorescence patterns. Fluorescence may peak more quickly and at higher values because these areas are richly perfused. In black patients, these areas also peak quickly, but at much higher values relative to other areas, since the palm and sole are less pigmented and thus transmit more fluorescence.

CONCLUSIONS

This study confirms the importance of skin perfusion in the selection of amputation level in the dysvascular extremity. By documenting skin perfusion, fluorometric assessment of fluorescein dye delivery appears to have overcome the major limitations of segmental pressure assessment. This is most evident in diabetic patients, where vessel calcification distorts pressure assessment by conventional techniques, and in patients undergoing below-ankle amputations. The increased precision (in black as well as white patients), repeatability, and safety of fluorometry make it preferable to qualitative (visual) assessment of fluorescein staining ■

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