Background: One goal of wound healing research is to discover agents to accelerate healing. Regulatory agencies have suggested stringent criteria to determine efficacy, that of 100% wound closure. Data analysis at a single point such as 100% closure does not provide detailed information about agent effectiveness over the entire span of healing.

Hypothesis: Wound healing trajectories can provide such information and can be used to demonstrate utility as alternative end points for wound healing trials.

Design: Data from 160 patients in 11 clinical trials of diabetic foot ulcers conducted at 2 centers were evaluated. Wound healing trajectories were constructed for patients whose wounds healed (100% closure) and those whose did not (<100% closure) over a 20-week period. The percentage of patients achieving total healing vs time of treatment was plotted and divided into patients receiving a test agent or placebo.

Results: The healing trajectories were almost identical for patients achieving complete healing at the 2 centers, as were the trajectories for patients with less than 100% closure. However, the trajectories of patients achieving total healing were significantly different from those not achieving 100% closure. Fifty-two percent of all patients achieved 100% healing by 20 weeks; 61% of patients receiving an experimental agent had total healing compared with 39% of placebo-treated patients. Linear regression suggested that all patients would achieve total healing by 37 weeks.

Conclusions: Since wound healing trajectories for diabetic foot ulcers treated at 2 centers so closely mimic one another, trajectories might be useful efficacy end points, and used to compare significant points along a continuum rather than a single static end point. Shifting of the wound healing trajectory from an impaired to a more ideal course may be considered when determining efficacy of new wound treatments.


Since Cohen et al first demonstrated that epidermal growth factor directly stimulated the proliferation of epithelial cells in 1965, effects of this recombinant peptide have been sought. Discovery of other growth factors in the 1970s helped renew scientific interest in wound healing and encouraged clinical optimism that new treatment modalities for chronic wounds would become available. During these past 2 decades, our understanding of the wound healing response, and the roles that growth factors play in regulating this response, has increased substantially.

The first use of topically applied growth factors to accelerate wound healing was reported by Brown et al in 1989. During the past 10 years, many clinical trials involving the application of exogenous recombinant growth factors have been conducted in an attempt to accelerate healing with mixed success. Recently, the reasons for the indeterminate results have been discussed.

The culminating goal of much wound healing research is to discover products and processes that can accelerate wound healing in humans. One problem in the clinical trials to date attempting to discover such therapeutic agents has been trial design, including the methodology and determination of end points. As yet, investigators have not routinely used analytical methods that directly, unambiguously, and unequivocally resolve the bottom-line question: using a statistically robust analysis, does the agent under evaluation actually promote wound healing? Different studies have suggested various outcome measures (eg, absolute wound area remaining, percentage of initial wound area remaining, wound volume remaining, wound perimeter remaining, various definitions of wound healing velocities, and the ease of surgical closure of the remaining wound after therapy). At
PATIENTS AND METHODS

Data were obtained on all patients entered into a total of 11 clinical trials of diabetic neuropathic foot ulcers at the 2 centers utilizing multidisciplinary wound care/research teams. Both centers participated in 3 of the trials, and only the University of Pittsburgh team participated in 6 of the trials and only the University of South Florida/Bay Pines Veterans Affairs Medical Center participated in 2 of the trials.

All patients, regardless of the particular clinical trial, shared 3 basic criteria: (1) all had debridement of all necrotic tissue in and/or surrounding the ulcer prior to study entry and as needed during the trial, (2) all had off-loading of weight to the ulcer area, and (3) all had frequent periodic evaluation by an experienced wound care/research team. Since it was not our intent to focus on the analysis of data from any particular clinical trial, the minimal description of experimental data was sequentially measured diabetic neuropathic foot ulcers in patients treated with various therapeutic wound healing agents, their respective vehicle controls, or standardized care. At all measurement times, the wound perimeters were traced on clear plastic sheets and the wound areas calculated planimetrically. The ulcers were measured weekly for a defined period determined by the parameters of the particular clinical trial, most commonly 20 weeks (140 days).

Since 100% healing (total wound closure) was the efficacy standard used in the majority of these clinical trials, wound healing trajectories were constructed for patients who achieved total healing (100% closure) and those who did not (<100% closure) over a 20-week period at the 2 centers. In addition, the percentage of patients who achieved total healing was plotted vs time of wound treatment (days) for both centers to determine how patients with diabetic neuropathic foot ulcers receiving at least debridement, off-loading, and frequent monitoring heal their ulcers.

Survival analyses were done using the Kaplan-Meier method, with both log-rank and Wilcoxon statistics to test for differences between groups. Percentage healing refers to the diminishment of wound area relative to day zero as follows:

\[ \frac{\text{Area (Day 0)} - \text{Area (Day x)}}{\text{Area (Day 0)}} \times 100. \]

Calculations and graphs were done using JMP software (SAS Institute Inc, Cary, NC) and Sigma Plot software (SPSS Inc, Chicago, Ill).

a meeting sponsored by the National Institutes of Health and Food and Drug Administration (FDA) in 1993 titled, “Clinical Trial Issues in Topical Wound Healing Biologies,” end points for wound healing clinical trials were discussed. Wound closure rates, operative wound closure severity, and quality-of-life end points were suggested, in addition to using the Wound Healing Society’s definitions of “ideal” or “acceptable” wound healing and complete wound closure.20

The FDA and a committee of the Wound Healing Society have suggested the most stringent criteria to determine efficacy for a new wound healing agent, that of total (100%) wound closure.19 Stromberg et al of the FDA have stated, “With respect to outcome measurements, complete rather than partial closure of wounds provides the best objective evidence of clinical benefit and therefore is the preferred primary endpoint.”21 They did indicate that in certain indications end points of a clinical trial other than complete (100%) closure may be considered as long as they are validated measurements of patient benefit.

Healing of open wounds follows an exponential course, with the rate of change of wound area progressively decreasing as the residual wound area approaches total closure. DeNouy10 was able to express this phenomenon by developing an equation and designing a curve that represents the theoretical evaluation of the healing of a wound.10 This curve is similar to the growth curve originally described by Gompertz.22 Because inclinations of this exponential curve diminish with time, attempts have been made to develop a linear rate of healing. Gilman14 introduced the concept of using the wound perimeter to neutralize the effect of varying size wounds in calculating the rate of healing. This concept appeared to work satisfactorily for both diabetic15 and venous status ulcers.16 However, researchers using this manipulation to determine a rate of healing found a different initial healing rate and overall healing rate.16 This may be due to the various processes of healing necessary to close these wounds as suggested by Snowden.14 Tallman et al17 further manipulated the raw data measurement to attempt to get a single healing rate by calculating a rate for each time interval measured and averaging the various intervals. This they called a mean-adjusted healing rate.

Hokanson et al18 proposed an analytical strategy that combined a mathematical model to approximate the actual wound closure measurements and an end point analysis similar to that used in failure-time studies. This approach leaves the raw data for wound area free of mathematical transformation. That group has shown that this analysis worked when applied to large groups of animal wound healing studies.22-24 To date, no large studies of human wounds have been evaluated to determine whether such a strategy could be used as an end point to determine efficacy of therapeutic agents in wound healing.

Using a dynamic healing trajectory or healing time curve may allow the prediction of healing of an individual wound. To determine a population time to healing, survival study methods as described by Kaplan and Meir25 can be used. These have been reported for wound healing studies and are useful when there are a significant number of patients who do not complete the entire time course of the study or do not reach the end point under investigation (eg, complete healing).26,27

Over the past 10 years, 2 multidisciplinary wound research teams, one based at the University of Pittsburgh, Pittsburgh, Pa, and one based at the University of South Florida/Bay Pines Department of Veterans Affairs Medical Center, Bay Pines, Fla, have participated in 30 prospectively randomized clinical trials enrolling 610 patients. These included 160 patients with diabetic neuropathic foot ulcers.

The purpose of this report is to demonstrate the usefulness of the wound healing trajectory (plot of percent
age of wound closure vs time of wound treatment) in helping to determine efficacy of novel therapeutic agents for wound healing.

### RESULTS

A total of 160 patients with diabetic neuropathic foot ulcers met inclusion/exclusion criteria and were entered into institutional review board–approved prospectively randomized clinical trials. Of all the patients entered into these trials at the 2 centers, 73 achieved total healing (100% closure) within 20 weeks.

The wound healing trajectories were almost identical for patients achieving complete healing at the 2 centers (Figure 1). This was true whether one evaluated the mean or the median percentage healing vs time of treatment. The trajectories for patients with less than 100% closure over the period of the trials were also remarkably similar. However, the trajectories of the patients achieving total healing were markedly different from those not achieving 100% closure.

Survival analysis of patients entering these clinical trials at the 2 centers who achieved total healing each week is shown in Figure 2. This was broken down further for each individual center, and these analyses showed that 52% of all patients at the 2 centers entered into a prospectively randomized clinical trial and receiving the standard care of debridement, off-loading, and regular evaluations healed their wounds 100% by 20 weeks. There was no statistical difference between the 2 centers when the survival analyses were compared (log-rank \( P = .36 \); Wilcoxon \( P = .27 \)). Almost no total healing occurred until 28 days (4 weeks). After 28 days, a linear relationship occurred, with 3% more patients achieving 100% closure per week (12% per month).

Although no trials of patients with diabetic foot ulcers conducted at the 2 centers had greater than 20-week duration, a linear regression analysis was performed to predict when all patients would achieve 100% closure. Based on the linear relationship demonstrated in Figure 2, this would occur at 37 weeks.

When the survival analysis was separately determined for patients receiving any experimental agent vs control patients not receiving such treatments, the patients receiving experimental agents had a statistically significant higher incidence of 100% closure (Figure 3). Sixty-one percent of patients receiving one of the experimental agents had total healing (100% closure) at 20 weeks vs 39% of control patients (log-rank \( P = .02 \); Wilcoxon \( P = .04 \)). The linear regressions of the 2 groups suggested that the experimental agents group would all reach 100% wound closure by 32.7 weeks and the controls by 46.1 weeks.

### COMMENT

Improvement in wound healing is a goal that has rapidly gained the interest of clinicians, researchers, and in-
The percentage healing to reach an arbitrary fraction of 100% closure can be determined. As Hokanson et al have described, the distributions of these fractional closure times and statistical differences in these distributions can then be determined using statistical methods similar to those used for failure-time or survival analyses. For each group the time required to reach an arbitrary percentage of healing, such as 50%, 75%, and 90%, can be determined. These distributions of times to an event (eg, 75% wound closure) can then undergo standard statistical failure-time analysis. These techniques can be used to compare 2 experimental groups or generalized for comparing multiple groups.

Many of the problems of comparing healing in multiple groups are averted with this method. The wounds are normalized by using a fractional decrease in wound size or percentage closure to be consistent with the analysis of chronic wounds that vary greatly in size. This allows use of raw data and does not require manipulation by introducing the perimeter or a second step of using the perimeter and multiple small time intervals. These latter techniques may not be necessary in diabetic neuropathic foot ulcers that are of limited size, as McGrath and Simon have suggested that the relative rate of closure is remarkably independent of initial wound size.

Healing trajectories provide more information about the entire continuum of the wound healing processes. Hokanson et al have illustrated that a test performed on data from a single point (eg, 100% closure) may not provide accurate guidance about the actual effectiveness of novel therapeutic agents over the entire span of the healing process. All wounds normalized to initial size begin at 0% healing and all wounds that heal during the defined period of the trial have 100% healing. Statistical comparisons at these extremes may be noninformative and stress the need for statistical techniques that allow dynamic comparisons at biologically meaningful interim values. This point is in agreement with Polansky and van Rijswijk, who state that healing time curves (wound healing trajectories) are a “moving picture” of healing that provide more detail than the “snapshot approach” in which only the proportion of patients healed (100% closed) at the end of the study is assessed.

Wright et al have suggested that “normal” and “impaired” healing exist on a continuum and are a compromise from “ideal” healing. In attempting to accelerate acute incisional wound healing depicted by a breaking strength or tensile strength vs time curve, they discuss the concept of moving the wound healing process in the direction of the “ideal.” This essentially shifts the breaking strength vs time curve to the left. Conceptually, the wound healing trajectories used to depict the data of the chronic diabetic neuropathic foot ulcer wounds are similar to the gain in wound strength curves used to depict data from healing acute surgical incisions. Therefore, shifting the trajectory of the patients with less than 100% closure in a defined time period in Figure 1 to the trajectory of patients attaining total closure could be used to determine new therapeutic agent efficacy. If the predictive value of such wound healing trajectories can be determined for the various other types of human chronic wounds, such as pressure ulcers and venous stasis ulcers, possibly shorter
clinical trials can be constructed relying on specific shifts of the wound healing trajectories from impaired healing toward an ideal end point.

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