Nitric Oxide Levels in Wound Fluid May Reflect the Healing Trajectory

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Background

Chronic wounds such as diabetic ulcers, venous leg ulcers and pressure sores are very difficult to heal and can last for months to years in spite of the numerous treatments currently available. In this report, we describe the analysis of nitric oxide metabolites (nitrate and nitrite, NOx) and other biomarkers (MMP-9 and elastase activity) in human wound fluids (various eliogies) and correlate these markers with wound status (progressing or worsening) based on patient’s wound history.

Methodology

Clinical Study: This is a multi-center study to evaluate NOx and protease levels in wound fluid samples in adult patients with either a chronic or acute wound that appears to have enough exudate for sampling (one time point). Following Institutional Review Board approval, written informed consent was obtained from each subject. Subjects could enroll in this study multiple times to allow for collection of wound fluid from the same wound at different time points. All subjects in this study received standard wound care as deemed necessary by their clinician. At enrolment the wound dimensions were recorded and the wound status (progressing, stagnating or worsening) was assigned based on the patient’s medical history.

Analytical Methods: Wound fluid was collected before wound cleansing (pre-cleansing) and after wound cleansing (post-cleansing). Wound fluid for NOx measurement was collected by placing a sterile, nitrate-free pad on the wound to absorb the fluid. Wound fluid for protease measurements were collected utilizing small swabs.

Statistical Methods: Wound area was computed assuming an elliptical area. The correlation coefficients were computed between pre- and post-cleansing samples on a subset of subjects for which data was available. Average of the pre- and post-cleansing metabolites were computed and plotted for each subject based on the wound status (progressing, stagnating and worsening) assigned at enrollment. Preliminary stepwise discriminant analysis and logistic regression analyses were used to determine the analytical or baseline factors significantly associated with wound status.

Results

The study is on-going. This poster describes data obtained at the interim analysis when 100 wound samples had been obtained. A total of 50 wounds provided samples that were suitable for NOx analysis, 33 of which had both pre- and post-cleansing data and the remaining 17 had only either pre- or post-cleansing data available. Of these 50 wounds, 20 were chronic venous ulcers, six were diabetic foot ulcers and the remaining were pressure, arterial, burn, infection, sickle cell or surgical wounds.

Correlation of Pre- and Post-Cleansing samples

Correlation between pre- and post-cleansing swabs was relatively high for NOx (μM) and not as high for elastase and MMP-9 based on the Spearman-rank correlation coefficient. The Spearman-rank correlations and sample size were as follows: ρ (rho)=0.72 for NOx (n=33), ρ =0.33 for MMP-9 (n=33), and ρ =0.41 for elastase (HNE) (n=33). The Pearson correlation for NOx is lowered to 0.50 due to an outlier value in which one subject had a pre-cleansing value of 273μM and a post-cleansing value of 46μM.

Using NOx to Discriminate Progressing from Worsening Wounds

NOx represents the best metabolite to discriminate between progressing and worsening wounds. Stagnating wounds do not separate from the progressing wounds based on NOx analytical results. N=50, 10 progressing wounds, 30 progressing wounds, and 9 stagnating wounds.

NOx as a Diagnostic Tool

The NOx data provides evidence that a two cut-point diagnostic test is better than a single cut-point test at identifying progressing wounds from worsening wounds.

Conclusion

•Correlation of the pre- and post-cleansing swabs is relatively high for NOx and not as high for HNE and MMP-9.
•NOx represents the best metabolite to discriminate between progressing and worsening wounds. Stagnating wounds do not separate from the progressing wounds based on NOx analytical results only.
•Based on the data from this study, MMP-9, HNE and baseline wound area do not significantly help to discriminate between progressing and worsening wounds. NOx is the only parameter that helps.

References


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