IDENTIFICATION OF A MULTI-GENE SIGNATURE OF DECOMPRESSION STRESS

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Introduction

Decompression stress (DS) can be defined by the probability of decompression sickness (DCS) as predicted by the U.S. Navy linear exponential multi-gas (LEM) model (Gerth 2002). At present, there is no method for testing the adequacy of such a predictor except to observe DCS (a rare event) or by detecting venous bubbles using ultrasound (which correlates poorly with DCS).

A method by which dives could be monitored for DS could lead to safer and more time effective decompression profiles. Determination of a biochemical marker or a panel of markers for DS would elucidate molecular interactions with gas emboli, which could be a starting point for finding adjunctive or non-recompressive therapies for DCS.

Previous studies have shown that creatine phosphokinase, an indicator of tissue damage, has been shown to elevate significantly in subjects diagnosed with DCS (Martin 1972, Shank 2001). Increased clotting activity and evidence of intravascular coagulation has been demonstrated in DCS cases and in asymptomatic divers (Lee 1998, Radziwon 2007). Inflammatory markers are also present at increased levels in cases of DCS (Montcalm-Smith 2003), and following asymptomatic dives with detectable bubbles (Ersson 2003). However, there are currently no validated biomarkers of DS. Additionally, there are no data available describing interactions of physiological pathways in the pathology of DS or DCS.

Genome-wide expression profiling of peripheral blood mononuclear cells has been used for comprehensive exploration of gene patterns or aberrant gene expression associated with cardiovascular disease states and processes (Aziz 2007). We hypothesize that gene expression microarray technology could be used to identify and validate global gene expression patterns characteristic of DS.

Methods

- DUHS, NSMRL and NEDU IRB approval
- N = 54 (US Navy-trained divers) immersed, mixed-gas dives
- N = 9 (US Navy-trained divers) dry, normoxic dives for validation: Three dives/subject: 60, 70 and 80 min @ 47 FSW
- Surface exercise (N = 9), hyperbaric oxygen exposure (N = 9) and diurnal control samples (N = 11) collected
- Paxgene blood tube collection 1hr pre and 2hr post-dive
- RNA extraction and betaglobin reduction prior to microarray processing
- Affymetrix GeneChip U133A for expression data

Figure 1: Data Collection Timeline

- Blood draw (PaxGene tubes), Echocardiogram
- Depth/time/physical activity recorded
- Echocardiogram (Normoxic exposures only)
- Study completion

Microarray Data Analysis

- 24,500 probes RMA normalized (Bioconductor/R script)
- Principal Components Analysis to investigate potential presence of batch effect (Bioconductor/R script)
- Step-down permutation t-test (10,000 permutations) to determine differentially expressed genes between pre and post-dive, and correct for False Discovery Rate and Family-Wise Error Rate (Bioconductor/R script)

Results

Figure 2: 362 covariant genes differentially expressed between pre and post-dive. Rows represent samples, columns represent genes. Red is overexpressed relative to mean; green is underexpressed relative to mean. For the sample key along the right side of the heatmap, red denotes pre-dive samples and blue denotes post-dive samples.

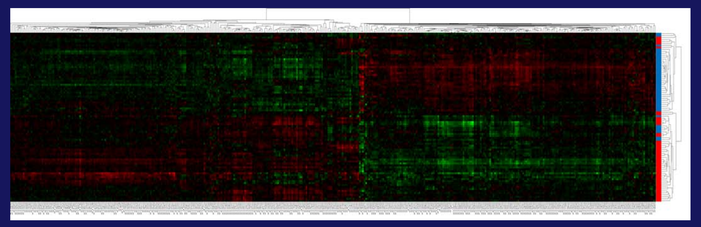
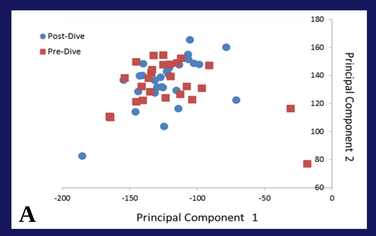


Figure 3: A) Principal component analysis of the independent, normoxic nitrox test dataset, based on all genes on the Affymetrix U133A Genechip. Principal component 1 (PC1) captures the majority of the covariance in the dataset, and principal component 2 (PC2) captures the next largest amount of covariance in the dataset. Overlapping of PC1 and PC2 for the pre and post-dive samples indicates similarity in expression among samples based on all genes. **B)** PCA of the independent, normoxic nitrox test dataset, based on the 362 DS 'signature' derived from the mixed-gas dataset. The majority of covariance captured by PC1 (based on the DS signature alone) is based on pre vs post-dive status. For a PC1 score cutoff of -64, Fisher's Exact test results demonstrate significant differential expression (p=0.0058) between pre and post-dive samples.



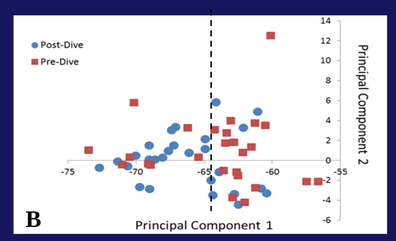
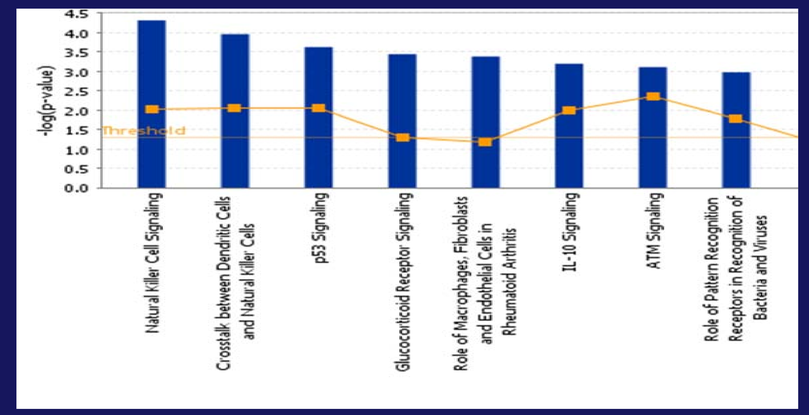


Figure 4: Ingenuity pathway analysis (IPA) identifies canonical pathways significantly differentially expressed following mixed-gas decompression exposures. The Y-axis indicates the negative $\log_{10}$ (p-value) of pathway significance by IPA with a significance threshold of 1.25. The yellow line gives a relative ratio of the number of genes expressed in the pathway divided by the total number of genes in the pathway.



Discussion

In this study, we investigated the use of genome-wide microarray to identify global gene expression patterns associated with DS. Future studies will further investigate expression of these pathways following high DS dives. After filtering genes differentially expressed in the hyperbaric oxygen, exercise and time of day control groups, 726 genes were identified as differentially expressed following mixed-gas decompression exposure. Pathway analysis demonstrated a significant portion of genes were associated with innate immune response. A 362 multi-gene signature of significant, covariant genes was then applied to the independent, normoxic decompression dataset and demonstrated differentiation between pre and post-dive samples. Future studies will focus on validation of individual genes and pathways identified as being differentially expressed in response in DS.

References

Aziz H, Zaas A, Ginsburg G. Peripheral blood gene expression profiling for cardiovascular disease assessment. *Genomic Med*. 2007; 1(3-4): 105-112.

Ersson A. Inflammatory response to hyperbaric trauma. An experimental and clinical study. Department of Anaesthesiology and Intensive Care, Malmo University Hospital, Sweden. Doctoral dissertation. 2003 Sep 26.

Gerth WA, Johnson TM. Development and validation of 1.3 ATA PO2-in-He decompression tables for the MK 16 MOD 1 UBA. Panama City (FL): Navy Experimental Diving Unit. Technical Report No.: 02-10. 2002.

Lee HC, Lin TC, Maa HC. Changes in coagulation factor and its correlation with decompression sickness. 1998. Undersea and Hyperbaric Medical Society Meeting Abstract.

Martin KJ. Decompression sickness: Observations on haematological and biochemical parameters. *J Clin Pathol*. 1972 Nov; 25(11):1004-5.

Montcalm-Smith E, Joice R, Porter W, Valentine D, Routh M, Meyerhoff J, et al. Chemokine and cytokine levels and decompression sickness. 2003. Undersea and Hyperbaric Medical Society Meeting Abstract.

Radziwon P, Olszanksi R, Tomaszewski R, Lipska A, Dabrowiecki Z, Korzeniewski K, et al. Decreased levels of PAI-1and alpha$_2$-antiplasmin contribute to enhanced fibrinolytic activity in divers. *Thromb Res*. 2007 May.

Shank E, Muth C. Diver with decompression injury, elevation of serum transaminase levels, and rhabdomyolysis. *Ann Emerg Med*. 2001; 37: 533–6.

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