Title: Structural Analysis Of Neonatal Clots Through Intensity-Varied Image Processing

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Introduction: Bleeding is a serious complication in neonates undergoing major surgery with cardiopulmonary bypass and can lead to morbidity and mortality. Currently, this issue is addressed with the transfusion of adult blood products. However, this method is not always sufficient in restoring hemostasis in neonates. This inconstant efficacy may be due to substantial functional and structural differences between neonatal and adult fibrin clots[1]. Currently, there are limited ways to quantify structural differences in fibrin networks. Recent studies evaluating fibrin clot properties have focused on several major methods quantifications such as fibrin branch alignment, fractal dimensionality, and fibrin fiber branching. While many of these quantifications have been computerized through a programmed algorithm, there is a lack of image processing that works consistently with neonatal clots, due issues with not being able to consistently binarize an image with the same threshold. This study looks to validate an image processing algorithm, using a novel binarization method, to better quantify clot structure.

Materials and Methods: Clots were formed from either neonatal or adult purified fibrinogen at a concentration of 2.5mg/mL with the addition of thrombin at three different concentrations (0.25, 0.5, and 1 U/mL). After polymerization, image stacks of the clots were taken by a scanning confocal microscope at 63X at three random locations per clot. 3D stacks were made in ImageJ. Stacked images gave different perspectives of the clots at different angles. Afterwards, image stacks were processed in MATLAB, using a binarization process that varied with the intensity distribution of the image. Each image of each 3-dimensional stack was analyzed individually. Branching points were counted from this binarized image by quantifying intersections of fibers. Fibrin fiber overlap was quantified by calculating the area of a branching point. Fractal dimensionality considered how fibers connected in the third dimension (depth). Lastly, porosity of the clot was also measured as a comparison of a controlled clot quantity (**Figure 1**).

Results and Discussion: The quantities identified above were quantified for adults and neonatal clots with varying concentrations of thrombin. Because the current sample size is relatively low to compare adults and neonates with different thrombin concentrations, pooled results from all thrombin concentrates tested are shown. In adults, this image analysis method showed significantly higher branching points, fibrin fiber overlap, and fractal dimensionality, and a significantly lower porosity. Significance was determined using heteroscedastic two-tailed t-test (p-value = 0.0003 for branching points/μm^2, 0.0009 for fibrin fiber overlap, 0.0365 for Fractal Dimensionality, and 0.0006 for Porosity).

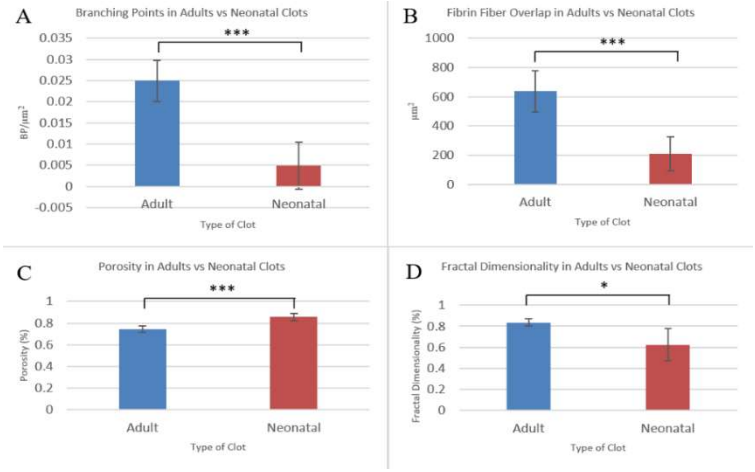

Figure 1: Quantitative differences between adult and neonatal clots from MATLAB Image Analysis. A) Branching Points per square micron B) Fibrin Fiber Overlap (uM^2) C) Porosity (%) D) Fractal Dimensionality (%) p*: < 0.05, p: < 0.01, p***: < 0.001.**

Conclusions: This new method of image analysis to quantify clot structure has consistently shown differences in clot structure between adult and neonatal clots. This technique could allow for better characterization of clots for future hematological studies. Future steps include increasing sample size and increasing runtime of the algorithm.

References:

1. Brown, A. C., Hannan, R. T., Timmins, L. H., Fernandez, J. D., Barker, T. H., & Guzzetta, N. A. (2016). Fibrin Network Changes in Neonates after Cardiopulmonary Bypass. Anesthesiology, 124(5), 1021–1031. doi:10.1097/ALN.0000000000001058