Structural Analysis Of Neonatal Clots Through Intensity-Varied Image Processing

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Background

Many neonates are born with congenital heart defects and require corrective surgery with cardiopulmonary bypass (CPB)

Neonates are especially prone to post-operative bleeding, which often results in bleeding complications

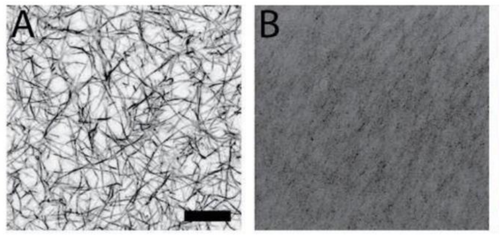
Currently addressed through adult blood transfusion products

This treatment has inconstant efficacy and not always sufficient to restore Hemostasis

Neonatal Fibrinogen

- Quantitative and Qualitative differences in clot structure between neonates and adults
- Possess immature form of fibrinogen
- Normally not an issue for a healthy neonate
- Immature Coagulation System leads to distinct differences in clot properties

Adult Fibrin network **Neonatal Fibrin network**



Confocal Microscopy. Scale bar= 20 um Modified from Brown et al. *Anesthesiology.*(2016)

Objective

This study looks to validate an image processing algorithm, using a novel binarization method, to better quantify clot structure.

- 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.
- Currently, 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.

Materials and Methods

Adult or Neonatal Fibrinogen

+

0.25, 0.5, or 1 U/mL Thrombin

63x Scanning Confocal Microscope

Analysis in ImageJ and MATLAB

MATLAB

- Binarization process that varied with the intensity distribution of the image, using a multi-scaled Hessian filtering method to identify tubular structures
- Structure sensitivity based on the mean, median, and mode of the intensity distribution.

$$Structure\ Sensitivity = \frac{median\ *mode}{mean}$$

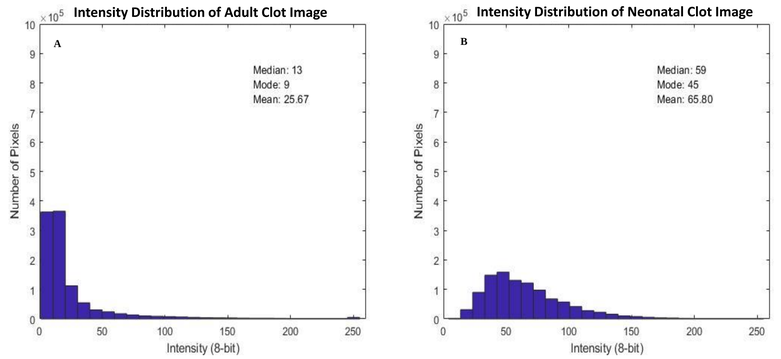


***Figure 3**: Intensity Distribution of an A) Adult Clot and a B) Neonatal Clot. The mean, median, mode of intensity values are also shown for both.*

Results

Each image of the 3-dimensional stack was analyzed individually. Branching points were counted from this binarized image by quantifying the 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 2**).

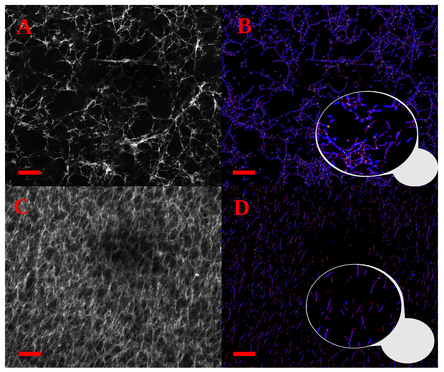


***Figure 2**: A) Grayscale image of an Adult Clot. B) Layered Image of Adult Clot showing binarization (blue), skeletonization (red), fibrin fiber overlap (tan), and branching points (white). C) Grayscale image of a Neonatal Clot. D) Layered Image of Neonatal Clot. Images A and C were taken via confocal microscopy. Scale bar = 25 µm.*

Results

Quantitative differences were found between neonatal and adult clots (**Figure 3**). One-tailed heteroscedastic t-test were done comparing adult versus neonatal clots with equal concentrations across all quantification methods (**Table 1**).

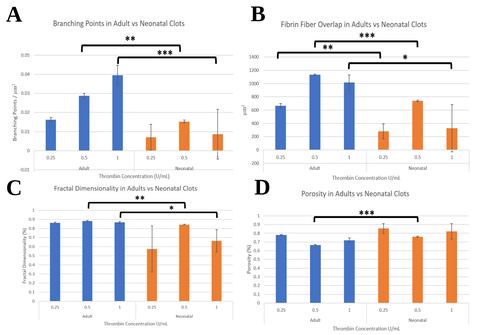


Figure 3**: Quantitative differences between adult and neonatal clots from MATLAB Image Analysis. A) Branching Points per square micron B) Fibrin Fiber Overlap (µm²) C) Fractal Dimensionality (%) D) Porosity (%) p*: < 0.05, p**: < 0.01, p: < 0.001. Sample Sizes: $n_{A,0.25}$ = 2, $n_{A,0.5}$ =3, $n_{A,1}$ =2, $n_{NN,0.25}$ =3, $n_{NN,0.5}$ =3, $n_{NN,1}$ =4.*

***Table 1**: Statistical significance (**bolded**) of quantitative measurements across different concentrations between Adult and Neonatal clots.*

	P-Values			
Concentration (U/mL)	Branching Points	Fibrin Fiber Overlap	Porosity	Fractal Dimensionality
0.25	0.069271471	**0.009527124**	0.071200348	0.093123495
0.5	**0.00036674**	**0.000101832**	**0.000912833**	**0.001897563**
1	**0.007067894**	**0.012950173**	0.053086958	**0.021983738**

Conclusions & Future Work

- Quantification of branching points, fibrin fiber overlap, and fractal dimensionality all seem to be good indicators of an adult clot with significance with 1 U/mL of Thrombin.
- A larger sample size would likely lead to significance of these quantifications across all concentrations.
- Other quantifications of these clots should also be taken into consideration such as alignment of the fibers, especially since there were several clots that had different variations of characteristics than the ones described here.
- Future steps include incorporating these other quantifications like alignment, increasing sample size, and decreasing 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

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