Supplementary material

Skorek P, Skubera M, Natorska J, et al. Role of red blood cells in hemostasis and whole blood clot contraction in adults after the Fontan procedure. Pol Arch Intern Med. 2023; 133: 16412. doi:10.20452/pamw.16412

Whole blood clot contraction

Clotting was initiated by addition of 40 μL of activation mixture (CaCl2 and human thrombin at final concentrations of 0.01 M and 1 U/ml, respectively) to 960 μL of prewarmed for 5 minutes at 37°C citrated whole blood. Samples were incubated at 37°C for 1 hour and the clot size was measured as a difference between the initial sample volume and the fluid volume around the clot after its retraction and was expressed as a percentage. To provide new insights into the in vitro clot contraction we performed analysis of erythrocyte compression inside the whole blood clot. Clotting was initiated using the similar model as for the clot size measurement in a final volume of 50 μL. Polyhderocytes formation was analyzed using scanning electron microscopy (SEM). For that purpose whole blood clots were washed in 0.1 M NaCl and fixed in 2.5% glutaraldehyde, dehydrated by a graded series of ethanol concentrations and frozen in tert-Butyl alcohol for 2 hours. Then, clots were dried in a vacuum and coated with gold. Microphotographs were acquired using a scanning electron microscope (JEOL JCM-6000, Japan). We analyzed 40 images for each clot and then assessed, using ImageJ (US National Institutes of Health), the areas covered by polyhedrocytes (defined as areas with polyhedrocytes content of at least 50%). Results were presented as the percentage of polyhedrocyte-positive areas.

To the best our knowledge there are no reference ranges for clot contraction of polyhedrocytes formation. Therefore in-house controls are required to interpret the data.