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Microvascular obstructions in portal bile duct capillaries and hepatic sinusoids during normothermic machine perfusion of marginal human livers

To the Editor:

We read with great interest the recent work of DiRito et al[1] who demonstrated the formation of Rouleaux-like aggregations of red blood cells (RBCs) during normothermic machine perfusion (NMP) of marginal human kidneys. They show that these aggregates cause microvascular obstructions, which can be pharmacologically cleared, improving renal function and decreasing injury.

It is fascinating that aggregates form in a setting thought to be free of clotting factors and platelets. DiRito et al hypothesize that aggregates form because of fibrinogen production by tubular epithelium. This poses the question—whether similar microvascular obstructions form during liver NMP; the organ with the greatest capacity for fibrinogen production.[2]

In this letter we analyze core biopsies taken during NMP of seven human research livers (UK National Research Ethics System; 15/SC/0161) which had been declined for transplant due to steatosis (two DCD, five DBD; six male, one female; mean age 48 years; mean CIT 15 h 27 min). All livers received oxygenated, normothermic, pressure-guided perfusion, either with Medtronic equipment (75 and 5 mm Hg in hepatic artery and portal vein, respectively) or using the OrganOx Metra as per published protocols.[3] The perfusate used is similar to that in DiRito's paper; a RBC-based perfusate, free from platelets and clotting factors (including plasma).

There were no RBC occlusions before NMP in any liver; however, every liver had accumulated RBC occlusions by 1 h. This was true even for livers with CIT of less than 10 h. We display representative images from several livers at a range of time points (Figure 1); all time points shown (5–19 h) are within the accepted duration for NMP in the clinical setting.[3] The liver RBC occlusions display the same morphology on H&E staining (Figure 1) as those shown in the kidneys perfused by DiRito et al.[1] On Martius scarlet blue staining we show that these occlusions are "fibrin rich" as they stain for both RBC (yellow) and fibrin(ogen) in red, again mirroring DiRito's findings.

Occlusions form in liver sinusoids (Figure 1i), often around areas with a heavy burden of steatosis. We also demonstrate occlusion of the portal tract capillaries (Figure 1ii) which supply bile ducts (arrows). This is a key observation as cholangiocytes are known to be extremely sensitive to ischemia,[4] with ischemic-type biliary lesions (ITBL) referred to as the Achilles' heel of liver transplantation.[5] It was hoped that NMP would remove this major hurdle; however, the recent large OrganOx trial showed no benefit of liver NMP on rate of biliary strictures or ischemic cholangiopathy; the portal tract vascular occlusions which we demonstrate (Figure 1ii) could be one potential barrier preventing NMP from ameliorating cholangiocyte ischemia. However, this remains hypothetical, and further research is required.

In conclusion, DiRito et al show RBC aggregates causing microvascular occlusions during renal NMP; here we demonstrate the same phenomenon in liver NMP. These aggregates form within sinusoids and in the portal tract capillaries which supply ischemia-sensitive bile ducts. Future research should investigate the use of plasminogen and tissue plasminogen activator in liver NMP. This combination could clear aggregates, which may improve cholangiocyte oxygenation and could therefore have a role in ameliorating the ischemic bile duct injuries that remain a significant hurdle which NMP is yet to overcome.

KEYWORDS

clinical research/practice, coagulation and hemostasis, disease pathogenesis, ischemia reperfusion injury (IRI), liver allograft function/dysfunction, liver transplantation/hepatology, organ perfusion and preservation, translational research/science

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

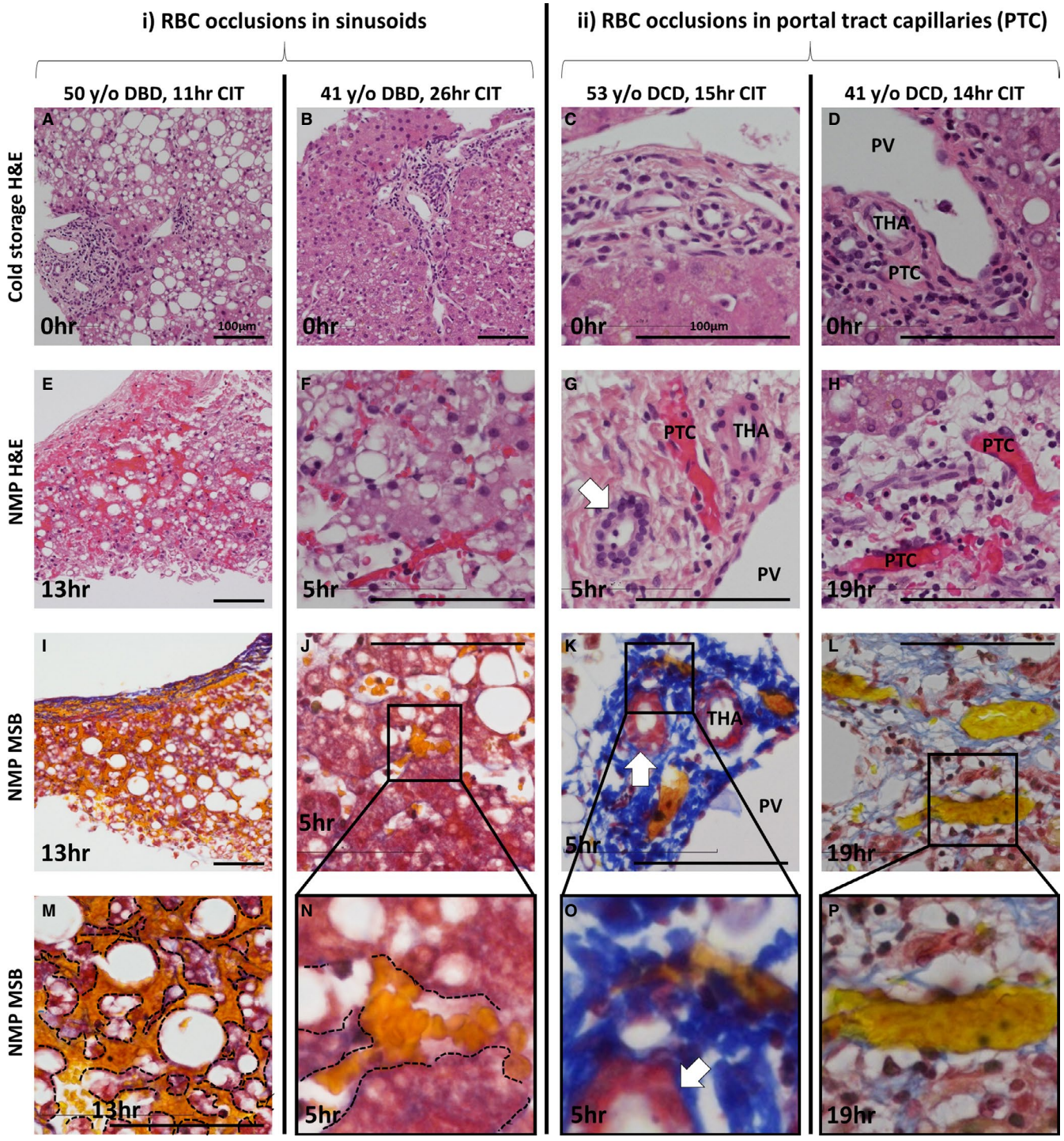

FIGURE 1 Fibrin(ogen)-rich red blood cell (RBC) aggregates cause microvascular occlusions during liver normothermic machine perfusion (NMP). Representative images of seven human livers which were declined for transplantation due to steatosis. A "cold storage" biopsy was taken immediately before NMP, and then at a range of time points after initiation of NMP (details denoted in each image). Hematoxylin & eosin (H&E) stains demonstrate clear vascular structures with no RBC aggregates prior to NMP (A–D), and subsequent increasing accumulation of RBCs obstructing both sinusoids (E, F, I, J, M, N) and the portal tract capillaries (G, H, K, L, O, P) which supply bile ducts (white arrows). Martius scarlet blue (MSB)-stained samples (red = fibrinogen, yellow = RBC) confirmed the presence of aggregates, and demonstrated that these aggregates are fibrin(ogen) rich; RBC within aggregates stained orange (yellow RBCs surrounded by red), in contrast to RBC outside of aggregates, which are stained yellow. All scale bars are 100 microns. White arrows indicate bile ducts. Dashed lines are outlining the hepatic sinusoids between the liver cell plates on the high-power magnification in panels N and M. CIT, cold ischemic time; DBD, deceased following brainstem death; DCD, deceased following circulatory death; PTC, portal tract capillaries; PV, portal vein; THA, terminal hepatic arteriole [Color figure can be viewed at wileyonlinelibrary.com]

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