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Research Letter

Histopathological Integrity of an Explanted Human Liver with Hepatocellular Carcinoma after Normothermic Perfusion

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Normothermic machine perfusion (NMP) is a validated technology in liver transplantation (LT),[1–3] and is expected to serve as a platform for reconditioning damaged organs.[4] Several groups have envisioned long-term NMP as a potential tool for preclinical research in the drug development pipeline by preserving whole-organ complexity and thereby potentially overcoming some limitations of animal models.[5–7]

However, two main concerns have recently been raised regarding this model. First, the feasibility of perfusing severely damaged livers for several hours to days has not been established. Second, potential histological alterations induced by prolonged perfusion might compromise pathological evaluation and could result in a loss of diagnostic information for the patient.[8]

Our laboratory used a prototype for long-term perfusion (Liver4Life, Apersys, Zurich, Switzerland) capable of preserving a human liver for 3 days.[9,10] The system maintains liver specimens under near-physiological conditions and supports perfusion through both the hepatic artery and portal vein (PV). It incorporates automated glucose control with insulin and glucagon, waste-product removal by dialysis, oxygenation regulation, and simulated liver movement to prevent pressure-related injury, with the aim of enabling long-term *ex vivo* liver perfusion, viability assessment, and functional repair.

Our current objective is to develop an "*in machina*" model based on perfusion of explanted liver tissue from partial hepatectomy specimens for translational oncology research. Although the platform is designed to support perfusion for up to 3 days, this proof-of-concept experiment was interrupted after 18 hours because of technical complications rather than because the platform's intended perfusion duration had been reached.

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Here, we report our first attempt to perfuse a hepatopathic liver with hepatocellular carcinoma (HCC) beyond the Milan criteria,[11] explanted during LT. The indication for LT was alcohol-related hepatopathy complicated by refractory ascites and a 6-cm HCC involving segments VI/VII, along with nontumoral PV thrombosis (Fig. 1). The patient had normal pretransplant serum bilirubin and prothrombin time values. Pretransplant CT showed a patent PV but a narrow intrahepatic portal system.

Written informed consent was obtained from the patient. This study was performed in accordance with French legislation and validated by the French Research Ministry (CODECOH-2023-6114-Explant-Liver-In-Machina). Total hepatectomy was performed with preservation of the vena cava and maintenance of liver inflow until final explantation. The explanted native liver specimen weighed 828 g and measured 24 × 6.5 × 1.5 cm, with segment IV atrophy and segment I hypertrophy.

Our protocol involves hypothermic oxygenated perfusion at a flow rate of 250 mL/min during bench preparation of the surgical specimen before normothermic machine perfusion (NMP). This approach is supported by data suggesting improved tolerance to ischemia-reperfusion injury before NMP.[12] After thrombectomy, we attempted to perfuse the specimen through the PV with the Vitasmart machine (Bridge to Life Ltd., United States), but outflow through the open hepatic veins remained poor. We then created two anastomoses connecting the left and right hepatic arterial branches to a single arterial segment to allow placement of the arterial cannula.

After the perfusion parameters (flow, pressure, and temperature) had stabilized, the explanted liver was placed in the liver chamber, and portal and arterial cannulation was performed. Because the vena cava was preserved during total hepatectomy, caval cannulation was not performed, and outflow was driven by gravity through the perfusion circuit (Fig. 2A, B). The evolution of the flow parameters is shown in Figure 2C. After 18 hours of NMP, perfusion was stopped because of hepatic artery thrombosis and leakage around the PV connection, possibly related to increased intrahepatic resistance. Thus, the planned long-term perfusion was not achieved, and this experiment should be considered a prematurely terminated attempt.

The specimen was then disconnected and sent for histopathological analysis. The assessment was performed according to the routine protocol in a blinded manner by pa-

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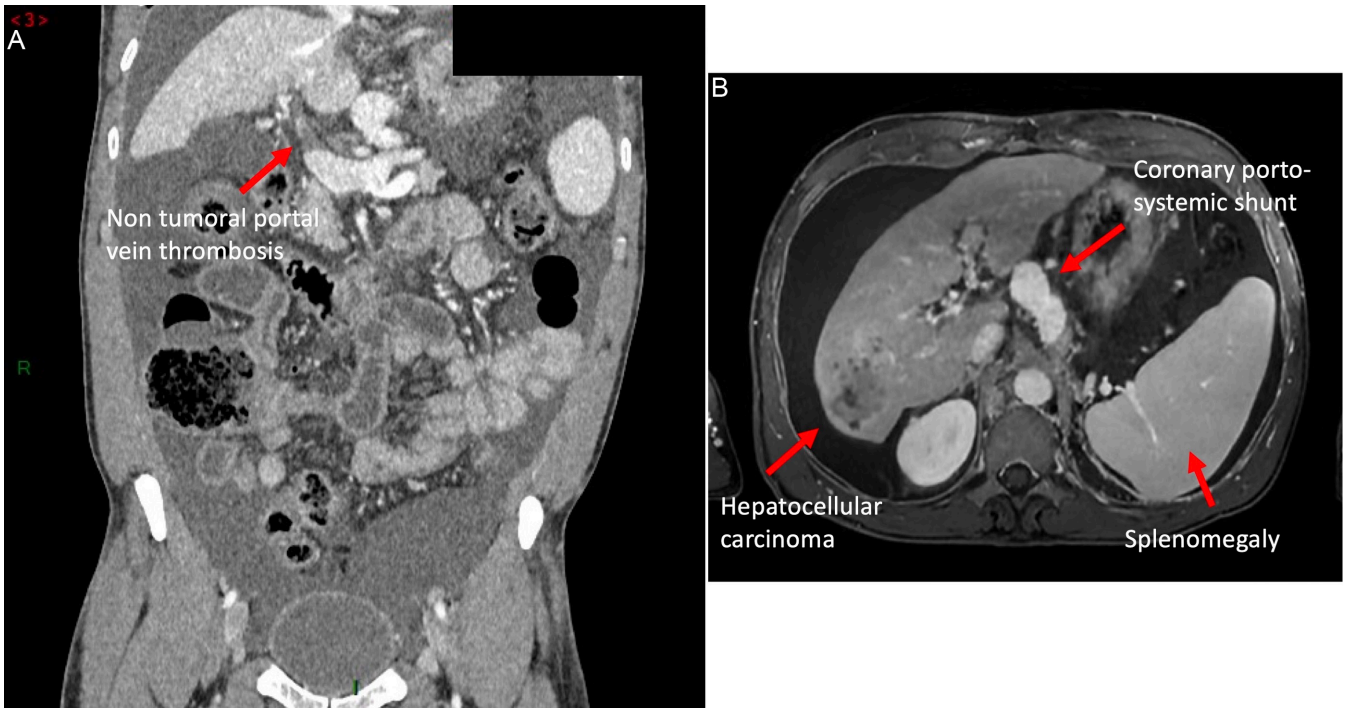


Fig. 1. Pretransplant CT scan of the transplant recipient. CT, computed tomography.

thologists AS and MS, who were unaware that the specimen had undergone perfusion.

Histologically, the tumor was a moderately differentiated HCC (WHO grade II), predominantly microtrabecular (60–70%) with a macrotrabecular component (30–40%) and focal pseudoglandular areas (<10%). Vessels encapsulating tumor clusters were present in a minor proportion (approximately 30%).[13] Peritumoral microscopic vascular invasion was identified. Immunohistochemistry demonstrated diffuse glutamine synthetase expression, consistent with a phenotype associated with *CTNNB1* mutation (Fig. 3).

The nontumoral liver parenchyma showed architectural remodeling without cirrhosis, including stenosis and muscularization of portal venules, hyperarterialized portal tracts, sinusoidal dilatation, and irregular periportal regenerative changes, consistent with porto-sinusoidal vascular disorder (Fig. 3).[14] Moreover, partially recanalized PV thrombosis was present without evidence of tumor invasion of the portal vein.

The key finding of this experiment was that tumor architecture and grading, assessment of vascular invasion, and interpretation of the background liver pathology remained assessable. In addition, we did not observe artifactual changes that would prevent accurate evaluation of the main histological features of tumor and nontumor tissues. Notably, despite difficulties with arterial cannulation, no misleading ischemic, necrotic, or artifactual changes interfered with characterization of the tumor or underlying liver disease. No histological signs of ischemia-reperfusion injury were identified. In this single specimen, 18 hours of NMP did not appear to compromise histopathological assessment, although additional studies are needed to determine whether this finding is generalizable.

This experiment also illustrates that long-term NMP of explanted liver specimens remains technically challenging and that adjustments to NMP systems originally designed for liver grafts may be required when perfusing diseased livers. Chronic liver disease can alter liver architecture and thereby increase vascular resistance. Because of the resulting hemodynamic impairment, a standardized, one-size-fits-all approach to perfusion parameters may be inappropriate. Models have been developed to simulate pathological conditions such as fibrosis and portal hypertension and to describe intrahepatic circulation, and these models could be used to help define perfusion parameters.[15,16] Another strategy could be to directly measure portal and arterial flow using vascular flow probes in the transplant recipient before division of the hepatic pedicle to replicate similar perfusion conditions.

We emphasize that partial obstruction of the portal vascular bed can alter portal inflow and contribute to perfusion failure. This case highlights the importance of PV patency and careful case selection. Further experiments should evaluate specimens without severe portal hypertension to determine whether perfusion outcomes can be improved and technical complications reduced.

Although we did not achieve the planned long-term perfusion of the surgical liver specimen, this single experiment provides preliminary evidence that 18 hours of NMP did not compromise histopathological assessment.

This preliminary experience also highlights the technical difficulty of perfusing explanted livers with tumors and the need to individualize the perfusion protocol for each case. Further studies are needed to define the potential role of explanted human liver models in translational oncohepatology.

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Conflict of interest

The authors have no conflict of interests related to this publication.

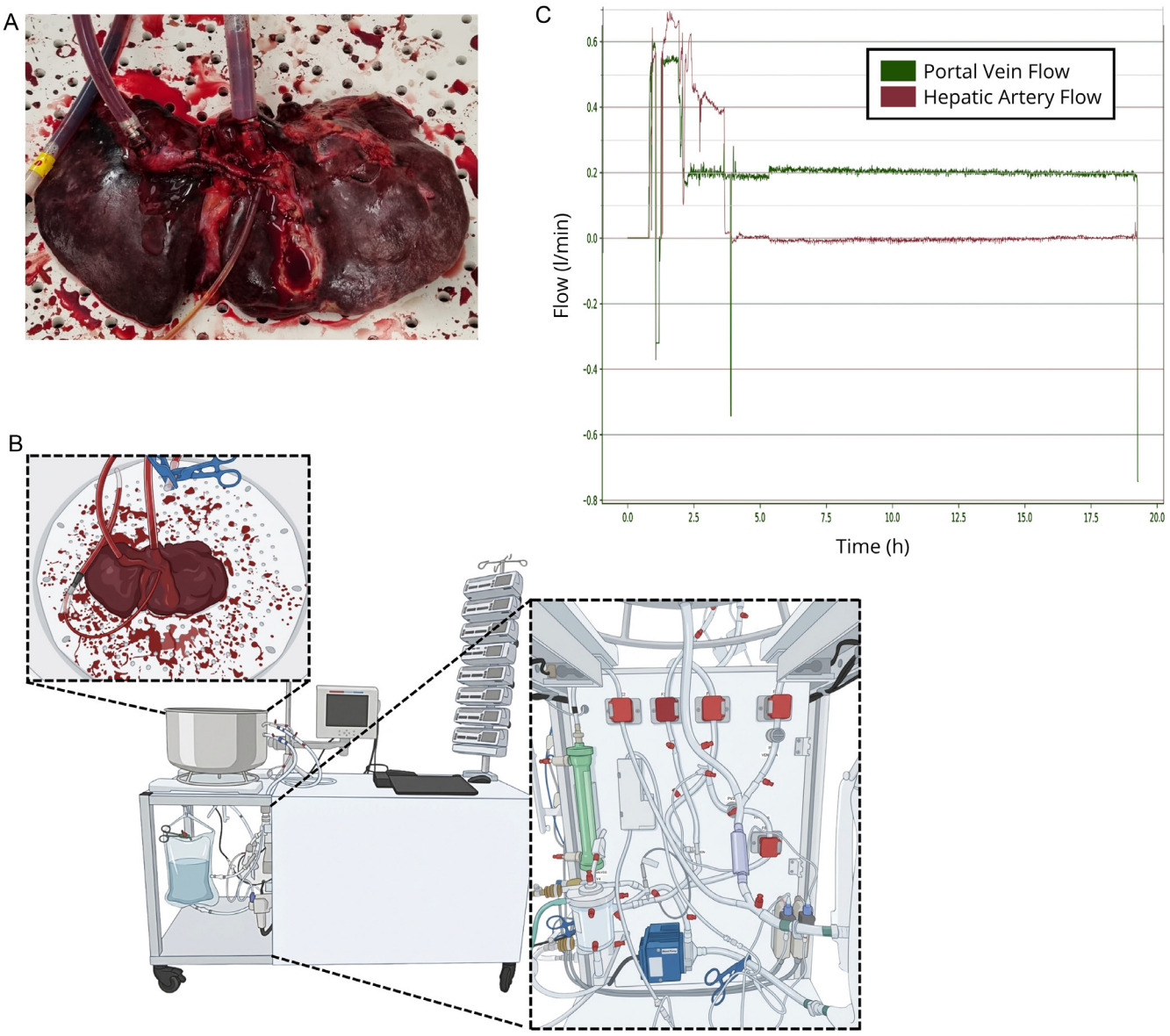


Fig. 2. Normothermic machine perfusion of the explanted liver. (A) Macroscopic appearance of the liver. (B) Schematic of the Liver4Life machine. (C) Hepatic arterial and portal venous flow parameters.

Author contributions

Study concept and design (SB, EV, MAA), acquisition of data (SB, EF, AS, MS, MAA), analysis and interpretation of data (SB, AS, MS, MAA, EV), drafting of the manuscript (SB, AS, MAA), critical revision of the manuscript for important intellectual content (EF, EV, MS), administrative, technical, or material support (EF), and study supervision (MAA). All authors have approved the final version and publication of the manuscript.

Ethical statement

Written informed consent was obtained from the patient. This study was performed in accordance with French legislation and validated by the French Research Ministry (Approval: CODECOH-2023-6114-Explant-Liver-In-Machina). This work was conducted in accordance with the Declaration of Helsinki as revised in 2024.

Data sharing statement

Data can be shared on reasonable request.

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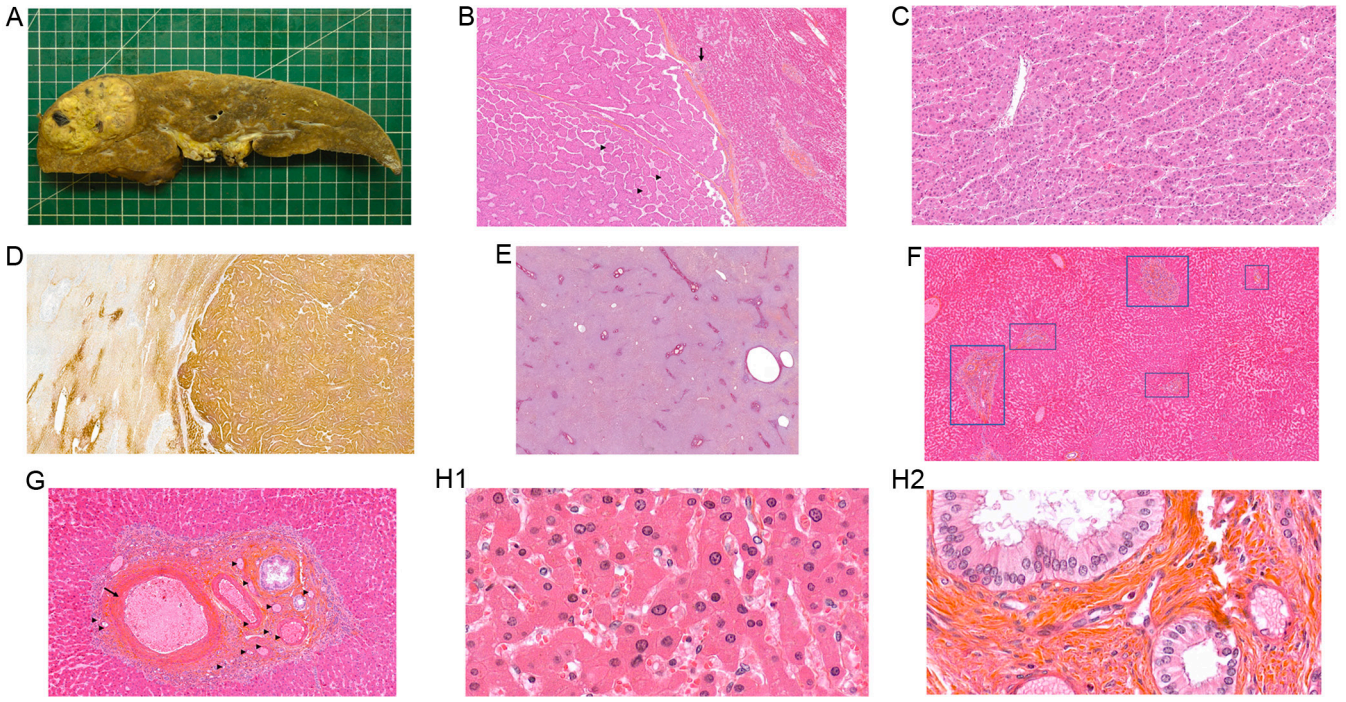


Fig. 3. Histology of the explanted liver after 18-hour normothermic machine perfusion. (A) Gross pathology. Gross examination showed an unencapsulated 60-mm tumor with a 14-mm subcapsular satellite nodule and no macroscopic vascular invasion. The nontumoral liver showed no evident fibrosis or nodularity. (B) Hepatocellular carcinoma, moderately differentiated (left). Arrow: microscopic vascular invasion. Arrowhead: vessels encapsulating tumor clusters (VETC) phenotype with a focal macrotrabecular pattern (HES, ×50). (C) Hepatocellular carcinoma, moderately differentiated, at high magnification (HES, ×200). (D) Glutamine synthetase immunostaining showing strong, diffuse staining of tumor cells, consistent with a phenotype associated with *CTNNB1* mutation (×50). (E) Background liver. Mild bridging fibrosis (F2 according to METAVIR) with recognizable portal tracts and central veins, without cirrhosis. Picro Sirius Hematoxylin staining, ×25. (F) Nontumoral liver (HES, ×100) - Portal tracts (squares) lacking a portal venule profile (complete portal venule stenosis), without septal fibrosis, consistent with porto-sinusoidal vascular disorder. (G) Nontumoral liver (HES, ×200) - Muscularized portal venule (left) with thickening and fibrosis of the portal venule wall and an irregular layer of smooth muscle cells (arrows), associated with numerous profiles of portal arteries (arrowheads; hyperarterialized portal tract). (H) Nontumoral liver at high magnification (HES, ×600), showing preserved cytological integrity of cholangiocytes within portal tracts (H1) and preserved hepatocyte morphology and fine hepatic cord architecture (H2). HES, Hematoxylin-Eosin-Saffron; VETC, vessels encapsulating tumor clusters.

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