ORIGINAL ARTICLE

Effects of Air Embolism Size and Location on Porcine Hepatic Microcirculation in Machine Perfusion

Maria-Louisa Izamis,[1] Andreas Efstathiades,[2] Christina Keravnou,[1] Soteria Georgiadou,[3] Paulo N. Martins,[4] and Michalakis A. Averkiou[1]

[1]Department of Mechanical and Manufacturing Engineering, University of Cyprus, Nicosia, Cyprus; [2]Department of Plastic Surgery, Galway University Hospital, Galway, Ireland; [3]Department of Pathology, Cyprus Veterinary Services, Nicosia, Cyprus; and [4]Department of Surgery, University of Massachusetts Memorial Medical Center, Worcester, MA

The handling of donor organs frequently introduces air into the microvasculature, but little is known about the extent of the damage caused as a function of the embolism size and distribution. Here we introduced embolisms of different sizes into the portal vein, the hepatic artery, or both during the flushing stage of porcine liver procurement. The outcomes were evaluated during 3 hours of machine perfusion and were compared to the outcomes of livers with no embolisms. Dynamic contrast-enhanced ultrasound (DCEUS) was used to assess the perfusion quality, and it demonstrated that embolisms tended to flow mostly into the left lobe, occasionally into the right lobe, and rarely into the caudate lobe. Major embolisms could disrupt the flow entirely, whereas minor embolisms resulted in reduced or heterogeneous flow. Embolisms occasionally migrated to different regions of the same lobe and, regardless of their size, caused a general deterioration in the flow over time. Histological damage resulted primarily when both vessels of the liver were compromised, whereas bile production was diminished in livers that had arterial embolisms. Air embolisms produced a dose-dependent increase in vascular resistance and a decline in oxygen consumption. This is the first article to quantify the impact of air embolisms on microcirculation in an experimental model, and it demonstrates that air embolisms have the capacity to degrade the integrity of donor organs. The extent of organ damage is strongly dependent on the size and distribution of air embolisms. The diagnosis of embolism severity can be safely and easily made with DCEUS. ***Liver Transpl 20:601-611, 2014***. **© 2014 AASLD.**

Received October 12, 2013; accepted January 5, 2014.

Air embolisms in donor organs are a well-known occurrence. Wolf et al.[1] demonstrated that before transplantation, one-third of donor livers contain intrahepatic air, whereas Liu et al.[2] showed that embolisms occur largely as a result of the routine handling of donor organs. Air can be actively perfused into the donor organ's vasculature during the delivery of the cold flush at the time of procurement,[3,4] during a venovenous bypass,[5-7] and, as machine perfusion (MP) gains popularity, at the time of the connection and during dynamic donor organ preservation.[8] The passive introduction of air can occur when subatmospheric pressures are generated in the vasculature, such as when the organ is suspended above the fluid line and drained, the vasculature is perforated, or air is encapsulated during an anastomosis.[2,4,9] Air embolisms are

Abbreviations: ALT, alanine aminotransferase; CL, caudate lobe; DCEUS, dynamic contrast-enhanced ultrasound; HA, hepatic artery; LLL, left lateral lobe; LML, left medial lobe; MP, machine perfusion; PV, portal vein.

This work was supported by the Didaktor/0311/78 Fellowship under the Framework Program for Research, Technological Development and Innovation 2009-2010; DESMI 2009-2010 is co-funded by the Republic of Cyprus and the European Regional Development Fund.

The authors have no conflicts of interest to disclose.

Address reprint requests to Michalakis A. Averkiou, Ph.D., Department of Mechanical and Manufacturing Engineering, University of Cyprus, 75 Kallipoleos Street, 1678 Nicosia, Cyprus. Telephone: (+357) 22-892255; FAX: (+357) 22-895389; E-mail: maverk@ucy.ac.cy

DOI 10.1002/lt.23838
View this article online at wileyonlinelibrary.com.
LIVER TRANSPLANTATION.DOI 10.1002/lt. *Published on behalf of the American Association for the Study of Liver Diseases*

problematic because they disrupt tissue perfusion, both within the recipient upon donor organ reperfusion and within the donor organ itself. The pathophysiological consequences of air embolisms entering the venous and arterial systems have been extensively reviewed, particularly with respect to the lungs, heart, and brain.[10,11] In the absence of a patent microvasculature, ischemia compromises organ viability, circulatory collapse is risked, and patient morbidity is increased. Protocols for clamping and flushing have minimized the incidence of air embolism release from donor organs,[4] so recent case reports of fatalities due to air embolisms introduced into the recipient during transplantation are relatively rare.[12] Data regarding the damage sustained by the donor organs themselves, in contrast, are minimal, and they suggest that the effects are generally negligible or reversible.[4,13] However, the size and distribution of embolisms within the donor organ have yet to be correlated with the extent of perfusion disruption and organ integrity. To better understand and predict the impact of air embolisms on hepatic functionality and potentially suggest diagnostic and preventive techniques for reducing high-risk transplants, we investigated the effects of air embolisms of different sizes in the hepatic artery (HA), the portal vein (PV), and both in a porcine liver model. The extent and evolution of perfusion disruption over time were determined during MP of isolated livers with dynamic contrast-enhanced ultrasound (DCEUS). This article demonstrates for the first time that the location and size of microvascular disruptions correlate with reductions in bile production and oxygen uptake and with increases in hepatic resistance and histological deterioration.

MATERIALS AND METHODS

Liver Procurement

The studies were approved by the Cyprus National Bioethics Committee and Cyprus National Veterinary Services. All animals were treated humanely in accordance with *Guide for the Care and Use of Laboratory Animals*, which was prepared by the National Academy of Sciences and published by the National Institutes of Health (National Institutes of Health publication 86-23, 1985 revision). Fifteen porcine livers were isolated from 150- to 200-day-old male Landrace pigs and were flushed with 6 to 7 L of ice-cold lactated Ringer's flush solution (Multi-Pharm Co., Nicosia, Cyprus) spiked with heparin (1000 IU/L; LEO Pharmaceutical Products, Ballerup, Denmark) through both the PV and the HA. The warm ischemia time (the time between the start of cardiac arrest and the start of the cold flush) was 30 minutes. The time of static cold storage in lactated Ringer's solution was 2 hours.

Groups and Introduction of Air Into the PV and the HA

Toward the end of the cold flush, the livers were randomly divided into 1 of 5 groups (3 in each group), which were determined by the size and location of the air embolism. We introduced air by allowing a volume of air into tubing to the PV and the HA, and this was followed by 100 to 200 mL of the remaining lactated Ringer's flush solution before the tubing was clamped. Because a lethal volume for adults has been described as 200 to 300 cc[14] or approximately 3 to 5 cc/kg of body weight for a 60-kg adult and our livers weighed 1.2 to 1.5 kg, we chose to define a major air embolism as 4 cc of air (group 1 or major group), which was estimated as approximately 3 cc of air to the PV and approximately 1 cc of air to the HA. Livers that received air in the PV only (group 2 or portal group) or in the HA only (group 3 or arterial group) received approximately 3 cc or approximately 1 cc in the respective tubing. A minor air embolism had half the amount of air at 2 cc or approximately 1.5 cc in the PV and approximately 0.5 cc in the HA (group 4 or minor group). Livers that were unaffected by any air embolisms formed the positive controls (group 5 or none group).

Evaluation of the Impact of Air Embolisms With MP

MP was used as a tool to evaluate the effects of air embolisms over time on the perfusion quality, liver function and structure, and hemodynamic status. The design employed here was scaled up from a system that had been optimized to recover ischemic rat livers[15,16] (Fig. 1). By operating the system at room temperature (20-25°C), we could use an asanguineous perfusate while still ensuring adequate oxygen delivery rates to the liver and a measurable metabolic performance. This was a simpler and more stable system to operate in comparison with normothermic approaches,[17,18] and it was more cost-effective than hypothermic designs based on commercially available organ preservation solutions.[19] The perfusate was a nutritive powder, Williams' medium E (10.8 g/L; W4125, Sigma-Aldrich Corp., St. Louis, MO), which was reconstituted to produce 6 L of perfusate. To this perfusate, we added 2.2 g/L sodium bicarbonate or more as needed to sustain a pH of 7.3 to 7.4 (S5761, Sigma-Aldrich Corp., Medisell Co., Ltd., Nicosia, Cyprus), 1000 IU/L heparin, 2 U/L insulin (Actrapid Penfill, Novo Nordisk, Novo Allé, Denmark), and 0.4 mg/L dexamethasone (Dexamed, Medochemie, Ltd., Limassol, Cyprus). The perfusate reservoir was a 7-L container in which the organ was suspended by a soft, permeable nylon membrane (B004QZ9XKI, Small Parts, Inc., Logansport, IN). The perfusate was circulated by a peristaltic pump (600-rpm Masterflex L/S digital drive, Cole Parmer) out of the organ chamber into an oxygenator (Affinity NT, Medtronic, Minneapolis, MN) supplied with 95% O_2 and 5% CO_2 (Tenaris, Bergamo, Italy); this brought the perfusate oxygen tension to a stable value of 722 mm Hg. The perfusate was then passed into a 500-mL bubble trap (Radnoti, Monrovia, CA), which dampened the pulsatile flow before it was split into 2 flow meters (EW-32461-44 and EW-32460-40, Cole Parmer). The flow was

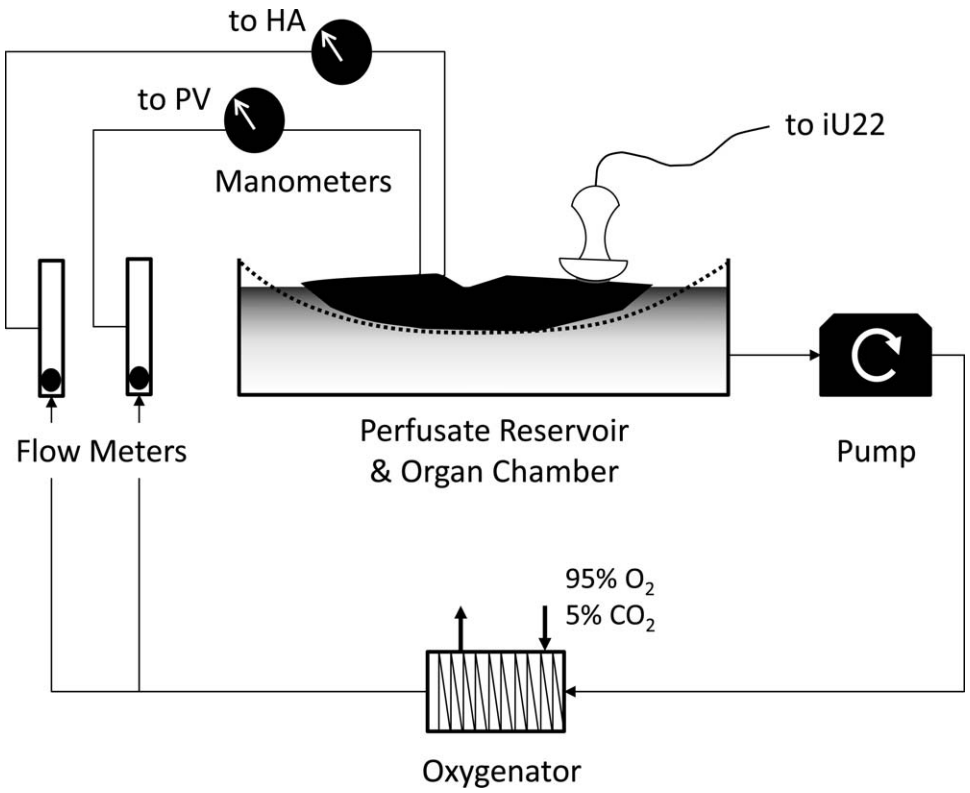

Figure 1. The MP circuit includes a pump that circulates asanguineous perfusate at room temperature (20-25°C) from the organ chamber/perfusate reservoir through an oxygenator and into a bubble trap. The flow is then split into 2 flow regulators that sustain constant pressures across the PV and the HA.

adjusted to sustain a constant pressure of 5 mm Hg across the PV (0.5 ± 0.2 mL/minute/g of liver) and a pressure of 80 to 100 mm Hg across the HA (0.16 ± 0.06 mL/minute/g of liver). The effluent was then allowed to flow freely from the vena cava back into the reservoir, and this closed the circuit.

Every half-hour for 3 hours, the pH was measured with a pH probe (Amarell Electronic GmbH, Kreuzwertheim, Germany) positioned in the perfusate, and it was maintained at 7.3 ± 0.08. Oxygen tension was measured with an oxygen probe (Microelectrodes, Inc., Bedford, NH) placed in the bubble trap for influx oxygen levels to both the PV and the HA and in the inferior vena cava for efflux measurements. Vascular pressures, flow rates, and bile production were also recorded. The liver gross morphology was photographed hourly with a Canon EOS 450D digital single-lens reflex camera. Histological examinations were conducted with biopsy samples procured at the end of perfusion from the center of each lobe, stored in 10% formaldehyde, processed, and stained with hematoxylin and eosin. Images were captured with a BX51 microscope (Olympus, Tokyo, Japan) and Olympus DP2-Twain–compatible software (ImageReady 8, Adobe, San Jose, CA). Perfusate samples, collected every half-hour and snap-frozen in liquid nitrogen, were evaluated after perfusion for alanine aminotransferase (ALT) release with a standard assay kit (Infinity TR71121, Thermo Electron Corp., Pittsburgh, PA). Livers were weighed at the time of procurement, after static cold storage, and at the end of MP.

DCEUS Assessment of Perfusion Quality

The extent of hepatic perfusion was evaluated with DCEUS, which enabled individual evaluations of the portal and arterial vascular flows because contrast could be introduced into each vessel separately. The contrast comprised microbubbles, which were 0.7- to 10-μm-diameter particles made up of a lipid polyethylene glycol shell encapsulating the inert gas sulfur hexafluoride (SonoVue, Bracco SpA, Milan, Italy).[20] Because the microbubbles were ultrasonic scatterers, they acted as bright reflectors when they were injected in a saline emulsion into the vasculature.[21] Their passage into the PV or HA, through the parenchyma, and into the hepatic veins enabled visualization of the extent of perfusion throughout the microvasculature. As the microbubbles flowed into the capillary bed, trace amounts were left behind because they were captured by endothelial receptors, primarily on Kupffer cells and leukocytes; they temporarily highlighted the perfused liver vasculature without affecting the flow or causing air embolisms themselves.[22] Two groups of perfusion images were subsequently captured with a Philips iU22 ultrasound scanner (Philips Healthcare, Bothell, WA). The first images were taken with an L9-3 linear array probe, which was held in a fixed position

on overlapping left lateral lobes (LLLs) and left medial lobes (LMLs) with a Fisso mechanical articulated arm (Baitella AG, Zurich, Switzerland). An image was captured 20 seconds after a 0.2-mL bolus was administered. Ultrasound imaging was performed at a transmission frequency of 3.1 MHz in the contrast-specific mode and at a transmission frequency of 6.3 MHz in the tissue mode (because contrast side-by-side imaging allowed both routine B-mode images and contrast images to be represented simultaneously). The compression was set at the maximum that was available on the scanner (50 dB) to best accommodate a large range of signals and avoid saturation. The 2-dimensional gain was set at 77% to optimize the signal while minimizing the noise. The mechanical index was 0.05 for the contrast side and 0.04 for the tissue side with the focus at 3.5 cm. The images were extracted with commercial quantification software (QLab 8.1, Philips Medical System, Bothell, WA). A second set of images was captured with an L12-5 linear array probe set at a transmission center frequency of 3.9 MHz in the contrast-specific mode and at a transmission center frequency of 8.2 MHz in the tissue mode. As with the L9-3 probe, the 2-dimensional gain was set at 77%, and the mechanical index was 0.05 for the contrast side and 0.04 for the tissue side with the focus at 3.5 cm. By placing the probe directly in contact with the liver (no lubrication required) and manually scanning the organ's exposed surface, we acquired a series of images that collectively reflected the organ's state of perfusion in 3 dimensions. The nonperfused areas and those with patchy perfusion were indicated on a schematic of the liver. The mechanical index was then increased to 1 to destroy any remaining microbubbles with a C5-1 probe in preparation for the next bolus injection.

Statistical Analyses

All analyses were performed with MATLAB 2010b (version 7.11.0584, 64-bit). For the quantitative group results, differences were analyzed via an N-way analysis of variance. Multiple-group comparisons were performed with the multicompare function in MATLAB with Tukey-Kramer correction for multiple comparisons. Data are expressed as averages and standard deviations. The statistical significance limit was set at $P < 0.05$.

RESULTS

Gross Morphology

Livers across groups could not be distinguished from one another on the basis of color, consistency, stiffness, or sharpness of lobe edges throughout the duration of MP.

Liver Perfusion Quality

The disruption of perfusion appeared as black areas devoid of the ultrasound contrast agent (Fig. 7). Both

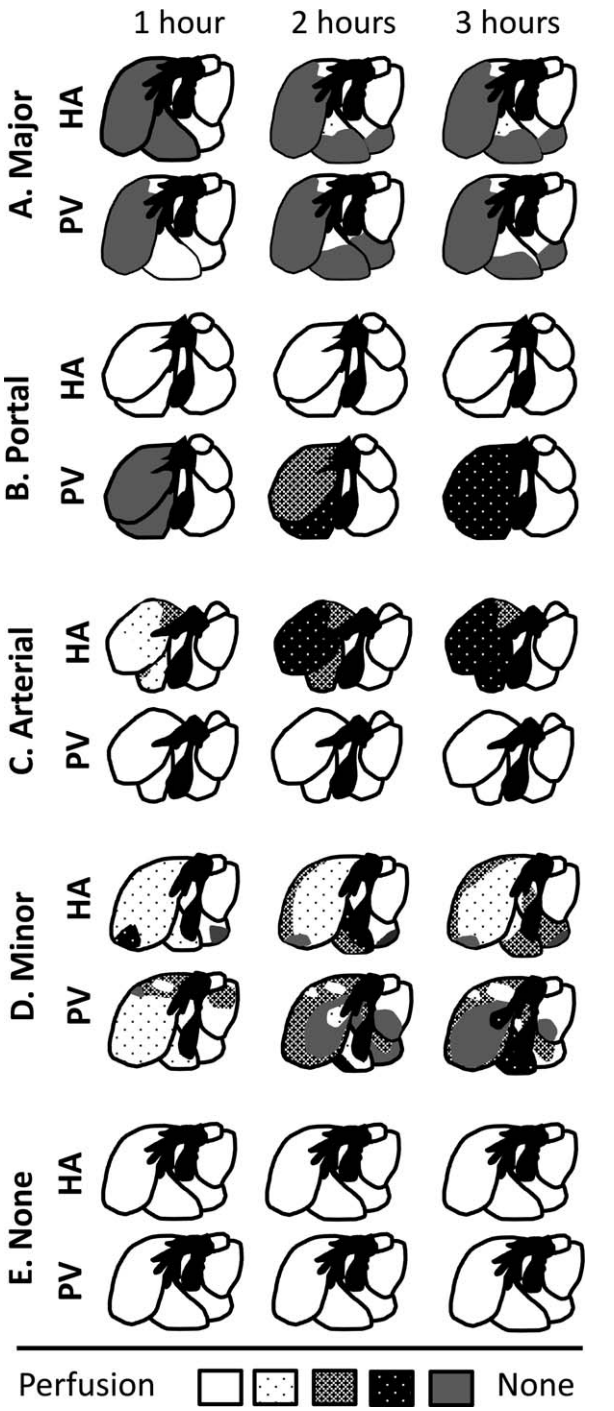

Figure 2. After the injection of either the PV or the HA with contrast, free-hand scanning of the livers with a Philips L12-5 probe revealed the extent of perfusion disruption by air embolisms throughout the entire liver. (A) A major embolism in both the PV and the HA caused complete disruption of the flow in the LML and LLL as well as segment V of the right medial lobe. (B) A major portal embolism disrupted the flow in the LLL and LML. (C) A major arterial embolism resulted in deteriorating flow over time in the LLL and LML. (D) A minor embolism in both the PV and the HA produced generally deteriorating flow that affected regions throughout the liver. (E) No embolisms resulted in stable perfusion in both the HA and the PV over time.

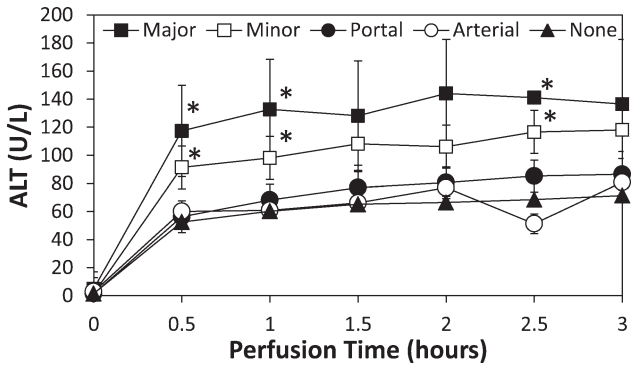

Figure 3. Livers with minor and major embolisms affecting both the PV and the HA showed dose-dependent increases in the release of ALT. *Significantly different from groups with arterial, portal, or no embolisms ($P < 0.05$).

major and minor embolisms produced disruptions in perfusion that failed to improve and often further deteriorated over time. Although major embolisms generally completely blocked flow to a region (Figs. 2A-C and 7A-C), minor air embolisms produced either patchy perfusion with diffuse regions showing no presence of contrast or homogeneously perfused regions at very low contrast intensities (Figs. 2D and 7D). Because air frequently migrated through the lobes, the extent of perfusion in these organs changed continuously. Livers without air embolisms were homogeneously perfused with contrast throughout both the portal vasculature and the arterial vasculature (Figs. 2E and 7E). Routine B-mode imaging

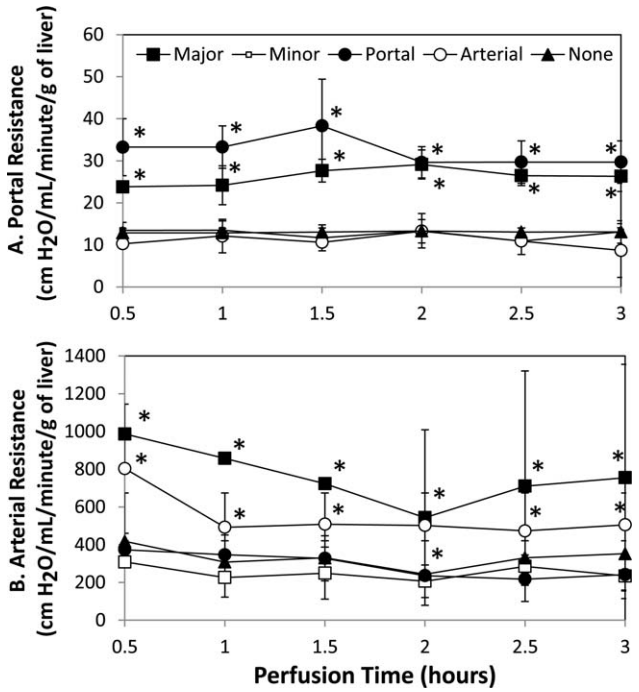

Figure 4. (A) Major air embolisms increased portal resistance in comparison with livers with minor or no air embolisms. (B) Major air embolisms increased arterial resistance in comparison with livers with minor or no air embolisms. *Significantly different from other groups ($P < 0.05$).

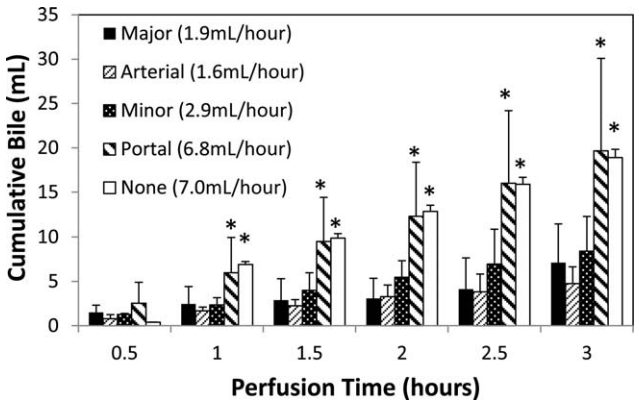

Figure 5. Bile production declined only in the presence of an arterial embolism. *Significantly different from other groups ($P < 0.05$).

revealed that all livers were otherwise structurally healthy, although occasionally air embolisms were observed at the start of MP in the hepatic veins of livers across groups (data not shown), and this appeared to be a function of the procurement process. These embolisms did not appear to affect perfusion, as demonstrated by the livers of group 5, and they disappeared over the course of perfusion.

Histology and ALT Release

At the end of 3 hours of perfusion, the architectural integrity and the presence of necrosis were evaluated via hematoxylin and eosin staining of biopsy samples taken from the center of the LLL, LML, and caudate lobe (CL) of each liver. Possibly because the vascular access to the CL is smaller than that to the other lobes and because of the obtuse angle to hepatic inflow, air embolisms were never found here, and histologically, the CL generally appeared healthy and intact across groups and served as a useful comparison for lobes within a particular liver. Major air embolisms produced clear signs of cellular damage. In agreement with the migration of air within livers, the hepatic architecture varied tremendously within the affected lobes. Here, the LLL had large focal regions of

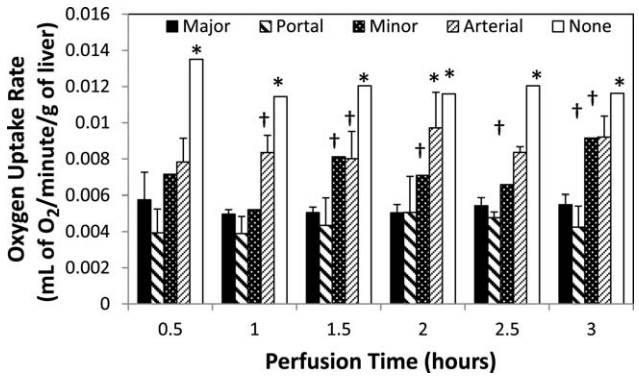

Figure 6. The oxygen uptake rate declined with increasing volumes of air in the liver. *†Significantly different from other groups ($P < 0.05$).

hypoperfused cells adjacent to otherwise healthy-appearing tissue, whereas damage to the LML was diffuse throughout (Fig. 8A). Interestingly, significantly less damage was seen in livers with only 1 affected vascular supply (Figs. 8B,C). Livers with minor embolisms in both the portal and arterial vasculatures had a similar but less severe response in comparison with livers with major embolisms (Fig. 8D). Livers without air embolisms had normal-appearing architecture (Fig. 8E).

ALT, released by damaged cells into the perfusate, correlated with the observed histological damage, and a dose-dependent increase was demonstrated in livers with minor and major air embolisms (Fig. 3). Livers with arterial or portal air embolisms, in contrast, did not differ significantly with respect to ALT release from livers without embolisms. The majority of the measured ALT was released during the first half-hour of perfusion, after which values generally plateaued or showed insignificantly inclining trends. Because livers were not flushed of lactated Ringer's solution before the initiation of perfusion, any ALT produced during cold ischemia was released during the early stages of perfusion and was included in these measurements.

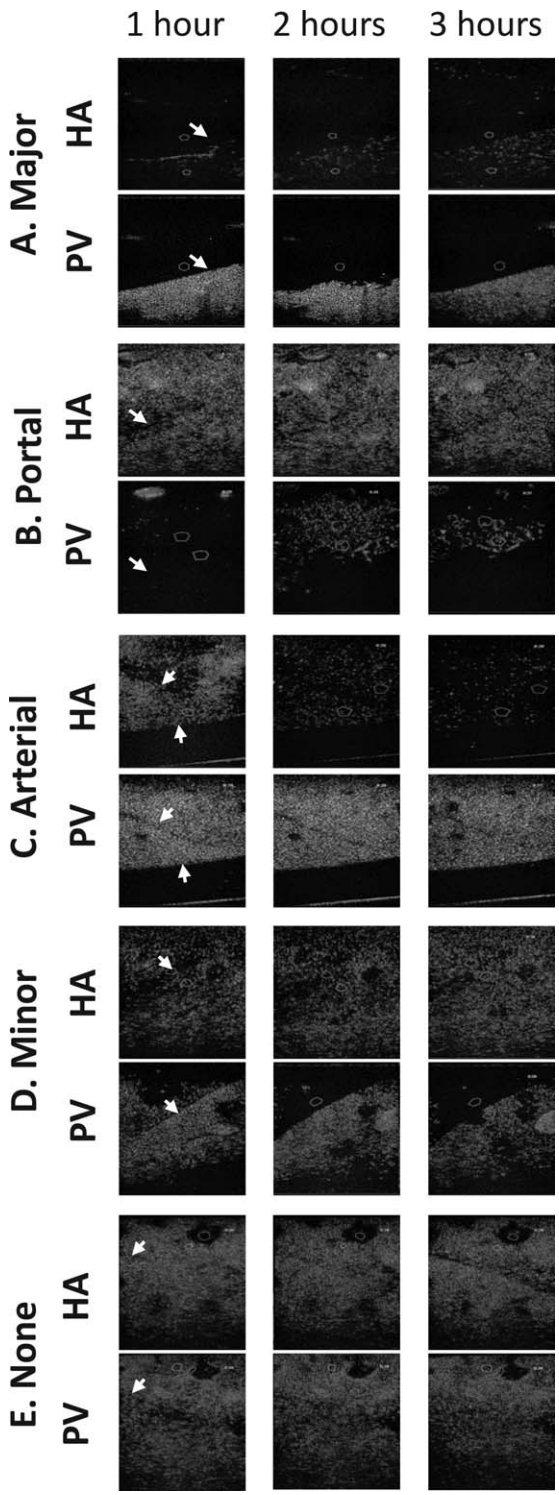

Figure 7. A Philips L9-3 ultrasound probe was fixed in position over the LLL and LML of the liver, and an hourly screenshot was taken on the Philips iU22 ultrasound machine 20 seconds after the administration of a contrast bolus into either the PV or the HA. The contrast and hence perfusion appear as gray regions; otherwise, the images are black. White arrows demarcate liver edges (ie, overlapping LLL and LML and superior surface of the liver). (A) A major air embolism caused the complete disruption of perfusion in the LLL of both the HA and the PV and in the HA branches of the LML. (B) A major embolism in the PV caused complete disruption of perfusion in the LLL and the LML, and there was partial recovery of the flow in the former over time; the flow through the HA was unaffected. (C) A major embolism in the HA caused deterioration in the flow to the LLL and the LML over time, whereas the PV flow was unaffected. (D) A minor embolism in both the PV and the HA reduced flow homogeneity and volume (intensity) over time. (E) No air embolisms resulted in the stable, homogeneous presence of contrast in both the HA and the PV.

Hepatic Resistance, Bile Production, Oxygen Consumption, and Liver Weight Gain

The presence of air embolisms was expected to affect the dynamic MP measures of vascular resistance, bile production, and oxygen consumption. Edema, manifested as hepatic weight gain, was measured as a possible indicator of the extent of ischemia/reperfusion damage caused by embolism-induced hypoperfusion.

Throughout the 3 hours of perfusion, vascular resistance was significantly increased from control values in those livers with major embolisms; portal resistance was significantly increased in livers with major portal embolisms (Fig. 4A), and a similar trend was observed in livers with major arterial embolisms (Fig. 4B). Minor embolisms had little impact on vascular resistance, and there was no significant difference from the control group.

Bile flow, shown to be the most powerful indicator of biliary function,[23] was significantly reduced by the presence of arterial embolisms, both major and minor (Fig. 5), in accordance with the fact that the bile duct blood supply is provided exclusively by the HA.[24] Major portal embolisms did not affect bile production,

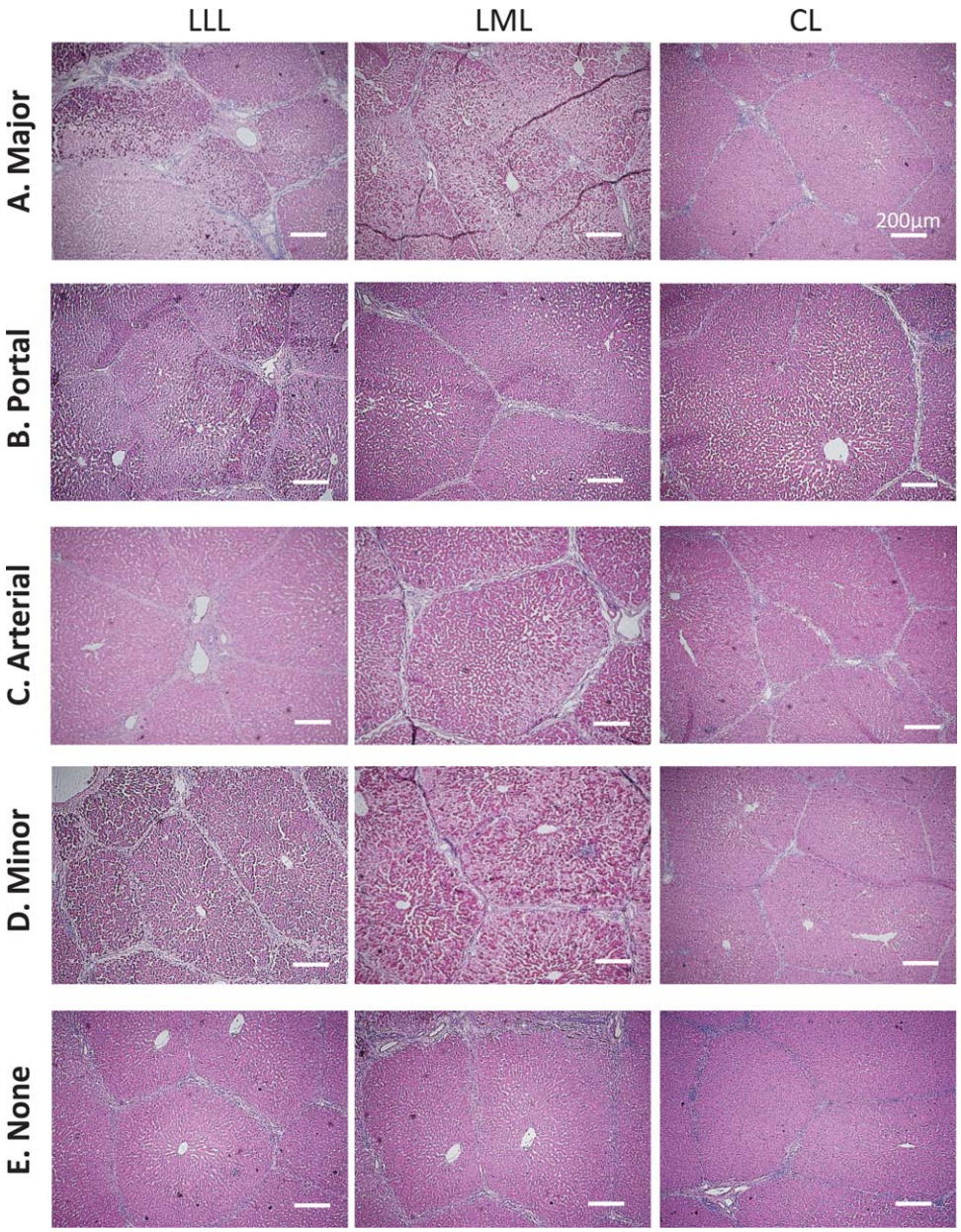

Figure 8. Biopsy samples taken from the centers of the LLL, LML, and CL after 3 hours of MP were stained with hematoxylin and eosin. The staining depicted a dose-dependent impact of the air embolism size on the overall architectural and cellular integrity. (A) Major air embolisms caused large areas of cell damage in regions where liver perfusion by both the HA and PV had been disrupted. Disruption of just the PV (B) or HA (C) however, resulted in significantly less cell damage. (D) Minor air embolisms in both the PV and HA caused similar but less severe damage than major embolisms. (E) Livers with no air embolisms had normal-appearing histology.

and there was no significant difference from the control group ($P > 0.05$). Bile production was steady throughout perfusion in all groups.

Hepatic oxygen consumption served as a general indicator of the presence of incomplete perfusion in livers with embolisms because those livers consumed significantly less oxygen than the control group (Fig. 6). Certain trends could be discerned among livers with air embolisms; major embolisms to both the HA and the PV and to the PV alone resulted in the lowest oxygen consumption across the groups. Minor and arterial embolisms, reflecting the smaller volume of liver affected by the air embolisms (particularly because the artery supplies a third of the liver's blood volume), had improved oxygen consumption, although it was generally less than that of livers with no embolisms.

Weight gain proved to be a highly nonspecific and inconsistent indicator of the presence of air embolisms. An average weight gain of 32% ± 15% was observed in all livers after cold ischemia. Two-thirds of the livers that had major and minor embolisms in both the HA and the PV gained an additional 17% ± 9.1% and 8.0% ± 3.9%, respectively, at the end of

perfusion, whereas the remaining third showed no change in or loss of weight. All livers in the other groups had an average weight loss of 17% ± 11% with no significant differences between groups.

DISCUSSION

Although air embolisms are a rare source of major complications for patients, they are nevertheless a common occurrence in donor organs. It is important, therefore, to understand when and to what extent they are likely to be injurious. In this study, we evaluated the impact of air embolisms on the liver, an organ that appears to be less vulnerable to the ischemic effects of air embolisms because of its dual blood supply. Major histological damage and evidence of intracellular enzyme leakage (ALT) occurred only when embolisms were present in both the HA and the PV and disrupted perfusion in the same zones. Moreover, the majority of the cellular damage appeared to occur during static cold storage because a substantial release of ALT occurred in the first half-hour of perfusion when lactated Ringer's solution was flushed from the liver into the perfusate; very little was released during the remainder of perfusion. In this model, air was introduced at the start of static cold storage and during flushing of the PV and the HA, and because of the orientation of the porcine hepatic vasculature, there was an increased propensity for air to enter the LLL and the LML, which increased the likelihood of a common region being deprived of perfusion. This led to the observation that when air became entrained in both the PV and the HA, the magnitude of the perfusion disruption and subsequent cellular damage increased with the size of the embolism. Additionally, because air embolisms could occasionally be seen migrating from one region to another, it is possible that multiple regions could be affected over the course of reperfusion.

A major embolism in the PV alone caused detectable changes in dynamic MP metrics, such as increases in the hepatic resistance to flow and decreases in oxygen consumption, and this suggests that some cells were potentially compromised by ischemia. However, there appeared to be no significant changes in histology or increases in ALT within 3 hours of MP, and this suggests that there was substantial compensation by the collateral arterial vasculature.

Embolisms in the HA also did not produce any obvious histological changes according to simple hematoxylin and eosin staining or significant releases of ALT, yet a reduction in the production of bile was observed when the arterial flow was compromised, even with a minor air embolism. Because the HA is the exclusive source of the biliary blood supply,[24] this finding is an important consideration for the handling of livers from donors after cardiac death, which are already predisposed to a high incidence of biliary complications after transplantation,[25,26] and supports the argument that efforts to ensure a patent arterial macro- and microvasculature may be key to minimizing poor long-term outcomes.

Air in the hepatic veins was a somewhat common observation across groups, and it was likely a result of unclamping the hepatic outflow and allowing a passive introduction of air into the vasculature as the flush solution drained out. These embolisms appeared to be of no consequence for the perfusion quality or organ integrity. Because they are likely expelled during reperfusion, however, they represent a greater risk of causing downstream complications.

The diagnosis of the size and distribution of air embolisms is helpful in predicting the magnitude of the injury that donor organs could sustain if the embolisms are not eliminated. Injury could begin with embolism-induced inadequacy of the initial flush of the organ and result in its disqualification.[3,13] DCEUS proved to be extremely useful in this regard. A semiquantitative assessment of the extent of flow disruption in the PV and the HA (major, minor, or none) strongly correlated with the degree of histological and functional damage observed during MP. This was especially impressive in those cases in which there was no macroscopic evidence that as much as 50% of the flow in the organ was disrupted. DCEUS can also be used to assess the severity of flow disruption in the absence of MP simply by the inclusion of contrast in the organ flush solutions. DCEUS is a simple-to-use mobile tool that is also low-cost, safe, continuous, noninvasive, non-ionizing, and perfusion- and parenchyma-specific.[27,28] DCEUS is used to characterize systemic abnormalities such as ischemia,[29] fat,[30] and fibrosis[31,32] as well as focal abnormalities such as microvascular disturbances,[33,34] nodular hyperplasia,[35] and tumors.[36,37] DCEUS is also being developed as a means of providing focal treatment through tissue or clot destruction,[38,39] drug delivery,[40,41] and molecular targeting.[42] DCEUS is typically used in radiology, cardiology, obstetrics, and gynecology, and its adaptation to the field of transplantation may be highly advantageous. If we consider only its application to the work at hand, we find that it can replace the use of magnetic resonance imaging and transesophageal echocardiography in the detection of air embolisms both ex vivo and in vivo[2,3,43] while significantly enhancing the specificity of organ monitoring during MP. A particularly powerful feature of DCEUS is its ability to extract quantitative metrics from the trajectory of contrast through the liver or from image analysis after acquisition.[44,45] Subsequent studies will explore whether a perfusion index can be defined that quantifies the extent of perfusion in the organs examined. This can then be numerically correlated with metabolic outcomes of MP as well as the effects of the severity of ischemia/reperfusion injury on graft function after transplantation.

MP is evolving into a superior dynamic donor organ preservation and organ recovery modality in comparison with the current gold standard of static cold storage. Up to 50% of kidneys in the United States are

currently machine-perfused, and clinical trials for the liver and heart are in progress.[19,46,47] Healthy organs appear to derive microcirculatory benefits from MP,[15] and its ability to continuously monitor the organ over time, provide necessary treatment, and quantitatively describe and predict the time to optimal viability strongly supports its widespread use in recovering suboptimal organs to expand the current organ donor pool. We have recently demonstrated the feasibility of these powerful concepts in a rat model of ischemic damage.[15,16] An index of ischemic severity was quantified, and the time to optimal transplantability was predicted, simply through the use of metabolic measurements made during MP.[48,49] Furthermore, by providing donor organs with oxygen and nutrients and removing cytotoxic metabolic byproducts in a minimally immunogenic environment, MP reduces ischemia/reperfusion injury upon transplantation.[50] It is possible, therefore, that the model used here has underestimated the amount of damage that air embolisms can produce in donor grafts that are transplanted directly without MP treatment. The results of the present study also highlight several additional considerations in the use of MP. The first is that although MP can be used to diagnose the presence of air embolisms with the aid of DCEUS, embolisms disrupt its function and reduce the accuracy of its viability metrics. Second, MP itself can be a source of air embolisms through the introduction of air into the vasculature at the time of the connection to the device or through any leakage or existing air in the device, as has been seen in cardiopulmonary and hemodialysis units.[51,52] An evaluation of the presence of air is not routinely performed during MP, and data on the incidence of its occurrence are limited,[8] but the results here suggest that this perhaps should be given greater consideration. Finally, MP may have a role in both sustaining and eliminating air embolisms. Depending on the oxygenation mechanism used in MP, hyperbaric saturated gas conditions may encourage the formation and prevent the dissolution of air embolisms.[53] By favoring hypobaric conditions and taking advantage of the ability to adjust flow rates and directions, MP may be well equipped to remove air embolisms before transplantation. Future studies with transplant models are warranted to ascertain the consequences of air embolisms and MP's role in reducing them.

In conclusion, air embolisms can significantly disrupt vascular flow. Because the liver has a dual blood supply, the damage caused by air embolisms is dependent on their size and location. Cellular damage is visible when the flow of both the PV and the HA is disrupted in the same region, although the disruption of arterial perfusion, even to a minor extent, also reduces biliary integrity. The occlusion of the portal microvasculature alone appears to cause minimal-to-no structural changes; however, reduced oxygen consumption suggests that mild ischemia may potentially result during the early hours after reperfusion. Embolisms in the hepatic veins do not appear to affect perfusion in the liver, but because they are dispelled during MP, they may present a risk of embolization downstream. DCEUS is a simple and cost-effective approach for detecting and quantifying vascular air embolisms in biological tissue and for assessing microcirculation integrity, and it may have an important role to play in the evaluation of donor organs, particularly during MP preservation. Because disruption of the microcirculation, even to a minor extent, negatively affects tissue integrity, using MP to maximize hepatic flow may have a resoundingly positive effect on donor tissue quality and the recipient rate of recovery.

ACKNOWLEDGMENT

The authors gratefully acknowledge Bracco SpA for the SonoVue used in this study and Kypros Stylianou for his invaluable technical assistance.

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