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Learning Curve for Rat Intestinal Transplantation: Milestones From a Single Surgeon's Perspective

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ABSTRACT

Introduction: The rat intestinal transplant (ITx) model is associated with a steep and challenging learning curve (LC) due to the microsurgical skills it requires. Although benchmark values are well-defined in the literature, clear milestones guiding progression along this curve are lacking, which limits its widespread use. Here, we present our methodology for assessing LC progression and achievement.

Methods: We describe the experience of a single surgeon developing the rat ITx model without prior microsurgical training. The LC was divided into 3 stepwise milestones: (1) acquisition of microsurgical skills, (2) development of intestinal graft procurement, and (3) development of intestinal graft implantation. LC progression was assessed using surgical times (milestones 2 and 3) and survival rate (milestone 3), according to the number of procedures performed. Based on anastomosis time, a cumulative sum chart analysis was used to determine the number of procedures required to reach LC proficiency.

Results: After initial microsurgical training, chicken thighs and rat cadavers were used to practice the key steps of the ITx model. Subsequently, intestinal procurement and implantation in living rats were performed to ultimately complete the 3 defined milestones. Analysis of the cumulative sum chart indicated that 34 ITx procedures were required to

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reach anastomosis time targets (linear regression adjusted $R^2 = 0.95$), which was directly associated with an increased 7-day survival rate.

Conclusions: Our methodology, based on stepwise milestones, enabled the development of the rat ITx model. Following the completion of milestones 1 and 2, 34 ITx procedures were required to complete the LC.

Introduction

Acquiring new surgical skills is a standard and continuous challenge in surgery. The learning curve (LC) is often described as the number of attempts needed to achieve these new competencies.[1] Specific outcomes are typically used to assess LC progression, based on benchmark values defined by the literature.[1]

The intestine remains the most challenging organ to transplant. This is due to the graft ischemia sensitivity and the complex donor-recipient interactions.[2] Consequently, the results lag behind those of other solid organ transplants revealing the need for additional preclinical studies, which require strong research model.[2] The rat intestinal transplant (ITx) model is one of the most robust. Since its introduction by Monchik and Russell in 1971,[3-6] numerous technical refinements have been added to expand its applications in various research protocols.

Nevertheless, the model remains challenging due to the microsurgical skills required. Although benchmark values are well-defined in literature, detailed information and clear guidance through the LC are lacking. Therefore, a practical guide outlining its key milestones and their stepwise achievement is needed to facilitate model development, increase its use and ultimately improve ITx research.[7,8] Here, we present the experience of a single surgeon developing the rat ITx model, starting from basic surgical skills.

Materials and Method

Surgeon

The surgeon had no experience with the rat ITx model and no prior microsurgical skills. The LC started from basic surgical knowledges acquired during 3 years of general surgery residency.

Bench, microscope, and instruments

Donor and recipient surgical procedures were conducted using a Zeiss Universal S3 microscope (Fig. 1). The

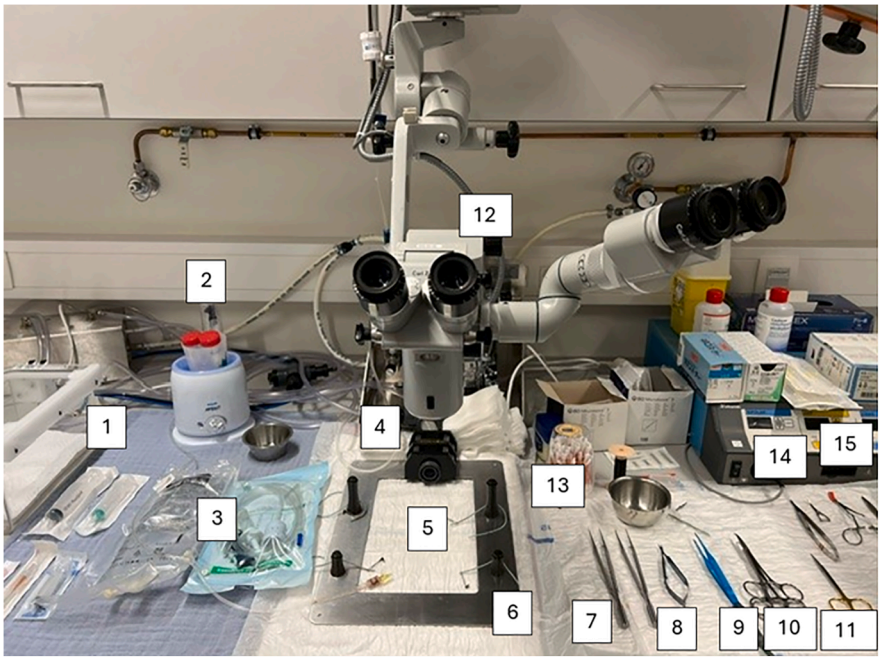

Fig. 1 – Bench table, microscope and instruments. 1, syringe for graft vascular and luminal flush; 2, water heater; 3, i.v. infusion line and NaCl 0.9% perfusion; 4, isoflurane mask; 5, heating pad; 6, abdominal retractor; 7, microsurgery forceps; 8, microsurgery scissors; 9, bipolar forceps; 10, mosquito forceps; 11, macrosurgery scissors; 12, Zeiss Universal S3 microscope; 13, cotton swabs; 14, microsurgery needle holder; 15, vascular clamps.

microsurgical instruments included cotton swabs, microforceps, microscissors, a microneedle holder, vascular clamps (including the Sun Lee clamp),[9] and a rat abdominal wall retractor. An isoflurane ventilator was positioned behind the surgical field. A water heater (to hold warm saline), a heating pad, and a perfusion line were used to maintain donor and recipient homeostasis. A bipolar forceps was employed for coagulating small blood vessels, and 7-0 ligatures were used for larger vessels (7-0 Silk, Fine Science Tool).

Animal model and surgical protocol

General

Six-week-old inbred Lewis rats ($n = 112$) (Janvier Labs, Saint Berthevin Cedex, France) weighing 250-350 g were used as donors and recipients for ITx (Fig. 2). Syngeneic transplantation was chosen to avoid any immunologic effect on LC assessment. Ethical approval was granted by the KU Leuven (P025/2022) and UCLouvain (2022/UCL/MD/13) institutional animal research ethics committees. Sample size was estimated based on previous literature reports.[10] Rats were housed 2-3 per cage in a dedicated facility at KU Leuven and UCLouvain, following European Union guidelines on animal welfare. Standard housing conditions included 14/10-h light/dark cycles and controlled temperature, with *ad libitum* access to water and food. In the postoperative periods, a standardized morbidity score was used to register signs of distress during daily evaluations.

Anesthesia

Rats were not fasted before surgery. Isoflurane (1000 mg/g, Iso-Vet, Dechra, Belgium) inhalation was used for anesthesia induction and maintenance (5% and 1.5% isoflurane, respectively, with 1.5 L/min O2). During surgery, rats were placed on a heating pad, and a 24G tail catheter was inserted for intraoperative fluid perfusion (NaCl 9%, 0.6 mL/min, Baxter).

Donor procedure

A midline laparotomy was performed, and retractors were placed. The graft was carefully manipulated with cotton swabs and kept warm and humid with moist compresses. The procedure began with the derotation of the natural mesenteric torsion and the section of the Treitz ligament. The subdiaphragmatic aorta was dissected, and the superior mesenteric artery (SMA) was freed from surrounding tissues and lymphatic vessels. The ileum, the distal part of the graft, was transected, and the terminal branch of the SMA and superior mesenteric vein were coagulated. The dissection continued along the colon, progressively removing pancreatic tissue and coagulating colonic vessels until the entire colon was mobilized. The jejunal side of the graft was transected, and the portal vein (PV) was dissected free, including the section of the splenic and pyloric veins after ligation or coagulation. For graft flushing, a vascular clamp was placed on the aorta above the SMA origin, the PV was cut at its bifurcation in the liver hilum, and 10 cc of cold saline were slowly perfused through a 24G catheter inserted into the infrarenal aorta. The SMA was cut, and the graft was stored in

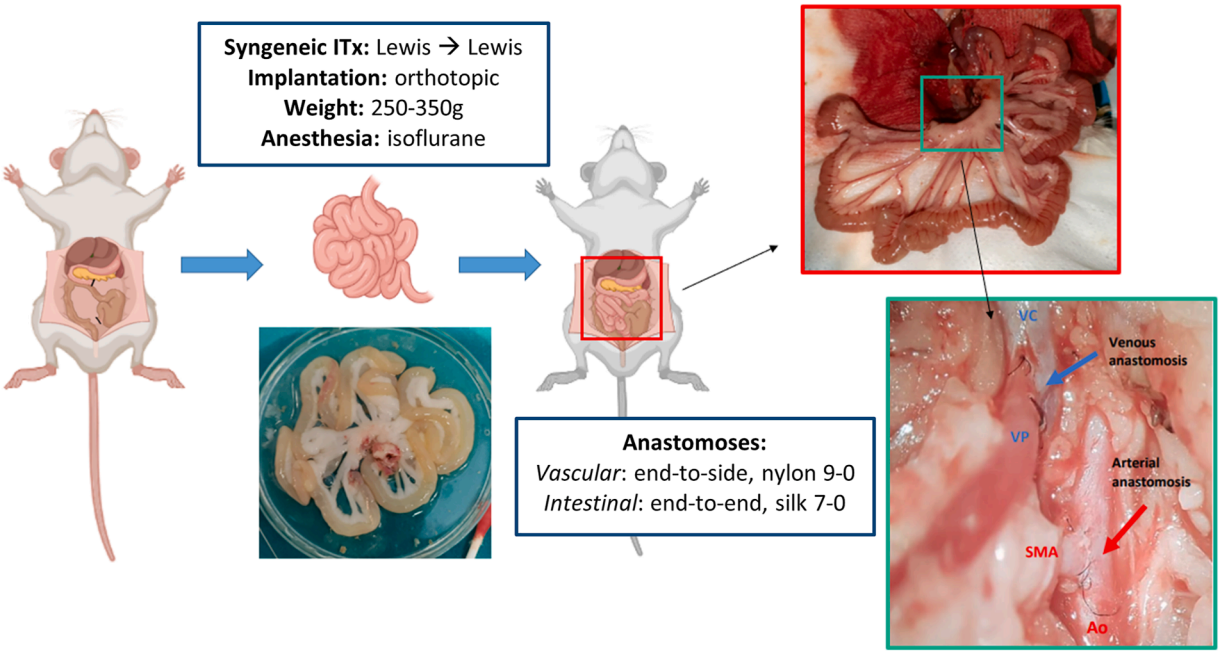

Fig. 2 – Rat intestinal transplant model. In this syngeneic rat intestinal transplant model (lewis to lewis, weight 250-350g), the donor small bowel, from jejunum to ileum, is procured and immediately transplanted into the recipient. It requires two end-to-side vascular anastomoses (Ethilon 9-0), suturing the donor superior mesenteric artery to the recipient aorta and the donor vena porta to the recipient vena cava. Intestinal continuity is restored by one end-to-end jejuno-jejunal and one end-to-end ileo-ileal anastomoses (Nescosuture silk 7-0). Ao = aorta; ITx = intestinal transplantation; SMA = superior mesenteric artery; VC = vena cava; VP = vena porta.

0-4°C saline solution. Finally, the graft lumen was flushed with 20-30 cc of cold saline.

Recipient procedure

Following a midline laparotomy, the infrarenal aorta and vena cava (VC) were freed from surrounding tissues and separated. If necessary, the right gonadal vessels were sacrificed. The graft was placed on the animal's right side, and two end-to-side anastomoses were performed using 9-0 Ethilon (Johnson & Johnson) running sutures: (1) PV to VC and (2) SMA to aorta, with their back side sutured from the inner side. The VC and aorta were sequentially clamped for the anastomoses. After completing the venous anastomosis, the graft PV was clamped, and the VC reopened during the arterial anastomosis. Once the latter was completed, the clamps were removed, and the graft was reperfused. The native small bowel was resected by ligating the mesenteric branches with 7-0 silk, and the intestinal continuity was restored by proximal and distal end-to-end anastomoses using 7-0 silk running sutures. Finally, the abdomen was closed in two layers with 4-0 silk.

Postoperative course

Recipient postoperative analgesia includes ropivacaine (Naropin 2 mg/mL, 3 mg/kg, subcutaneous (SC), administered at abdominal closure), Ceftriaxone (Ceftriaxone Fresenius Kabi 1g, 70 mg/kg, SC, administered once after abdominal closure), and Meloxicam (Metacam 2 mg/mL, 1-2 mg/kg, SC, administered daily during the first 72 h) while rats were kept on a heating pad for 1-2 h after surgery. Animals were checked twice daily during the first 72 h and once daily thereafter.

Euthanasia

All animals were euthanized by exsanguination under general anesthesia at the end of the experiment (7 d post ITx). In case of complicated postoperative course (too high morbidity score, persistent pain despite painkiller, weight loss >25%), rats were prematurely sacrificed by exsanguination under general anesthesia.

Surgical time

All surgical times were recorded independently of milestone achievement and used for LC progress evaluation. Intestinal procurement time (IPT) was defined as the interval from donor skin incision to graft cold perfusion, with a target benchmark of 60 min.[11] Cold ischemia time (CIT) was measured from graft flush to graft anastomosis, after which warm ischemia time (WIT), also referred to as anastomosis time (AT), started. AT was the most critical step of the procedure, and a maximum of 40 min is recommended to ensure recipient survival.[8]

Milestones

The LC was divided into three stepwise milestones: (1) acquiring microsurgery skills, (2) developing the intestinal procurement procedure, and (3) mastering the graft implantation technique. This final milestone, recognized as the most challenging in literature, was further divided into 2 phases: (a) initial anastomosis skill acquisition on nonliving models (chicken thighs, rat cadavers), followed by (b) application to living animals. Milestones were considered achieved once the target procedures were mastered. For Milestone 1, achievement was defined as the acquisition of sufficient microsurgical skills, which included thread and needle handling, notching, and performing anastomosis on various materials. Milestone 2 required the ability to dissect and procure a suitable graft for transplantation in living rats through an uncomplicated procedure, even if IPT benchmark values were not yet achieved. Milestone 3, corresponding to completion of the LC, was validated once AT benchmark values were consistently met, with no postoperative surgical complications (e.g., bleeding, thrombosis, anastomotic leakage, or stenosis) and a 7-d survival rate >50%.[12] Milestone progression was continuously assessed by experienced microsurgeons familiar with rat transplant models.

Statistical Analysis

A cumulative sum chart (CUSUM) of AT was used for LC analysis. The AT of all procedures performed in milestone 3b were selected chronologically and the difference to the sample mean was calculated for each of them. Then, these differences were cumulatively summed to obtain a CUSUM chart. On this chart, a piecewise linear regression model was applied to determine the CUSUM inflection point, identifying the number of procedures required to keep all subsequent AT values below the mean. Statistical analyses were conducted using R (version 4.3.0; Copyright 2023 The R Foundation for Statistical Computing), while other graphs were made using GraphPad Prism (9.3.1; GraphPad Software, San Diego, California).

Results

Milestones

For milestone 1, private institutional microsurgery course at KU Leuven (totaling 35 h) were followed, focusing on basic microsurgery movements on synthetic membranes and isolated chicken vessels (Fig. 3). Simultaneously, a 1-week experimental microsurgery course, not specific to ITx, was attended at the Scandinavian Microsurgery Academy (University of Gothenburg, Sweden). For milestone 2, 14 rat cadavers were used to familiarize with rat anatomy and initiate the first steps of the intestinal procurement procedure. Afterward, the procedure was performed on living animals until all steps were mastered ($n = 6$). Regarding milestone 3, 33 chicken thighs were used as an initial platform to develop anastomose skills (end-to-side, end-to-end). Training then transitioned to rat cadavers until the 40-min AT target was achieved, allowing progression to milestone 3b. Additional technical refinements (rat tail perfusion, donor procedure, bipolar forceps…) for donor and recipient procedures were acquired during a 6-wk training period in an experienced center (Madrid, Spain; $n = 9$, no survival experiments allowed due to local ethical considerations, times registration included in the study).

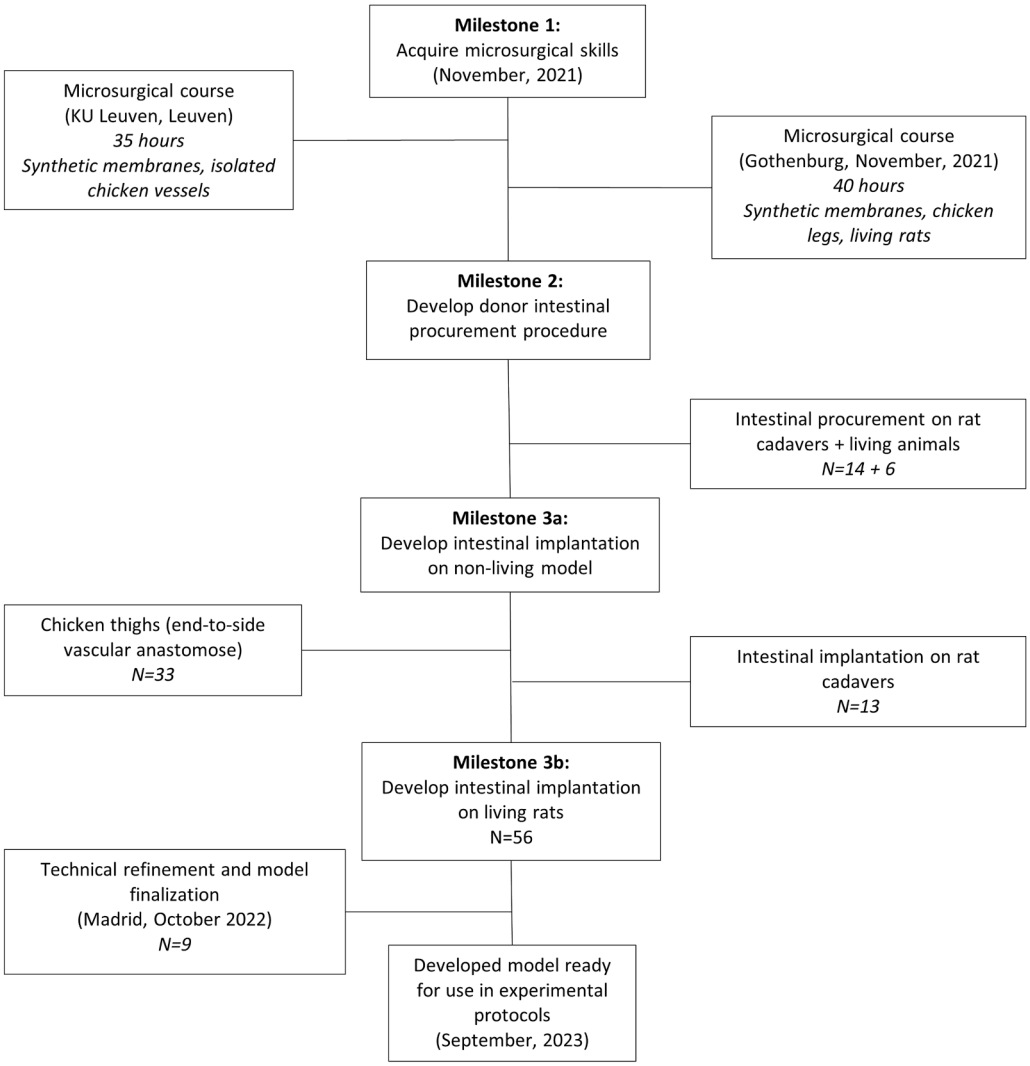

Fig. 3 – Milestones of the learning curve.

LC progression

Across milestone 2 and 3, the 60-min benchmark for IPT was reached by the 40th procedure in living animals (Fig. 4). CIT had a median (range) of 71 (40-117) minutes. Improvement in AT is shown in Figure 5. CUSUM chart analysis with piecewise linear regression identified a breakpoint at procedure 34 ± 0.81 (adjusted $R^2 = 0.95$), indicating that 34 procedures were required to achieve the benchmark AT reported in the literature. As a result, an increased 7-d survival rate was observed afterward (Fig. 6). Survival rates reached 70% in the last 10 ITx procedures (excluding per-operative failures, 2/12, 17%). All deaths were due to distal intestinal anastomose complications (leakage or adhesion-related occlusion), despite perfectly vascularized grafts.

Discussion

In this study, we present our stepwise approach to learn the rat ITx model. The LC was divided in 3 milestones, whose sequential completion ultimately led to model development. Once intestinal procurement was mastered, 34 procedures were required to reach the AT benchmark, the most challenging step of the procedure.

Teaching methodology in microsurgery

Acquiring stereoscopic vision and visuospatial skills is essential for developing the dexterity needed for microscopic work. However, the high costs and ethical approvals required

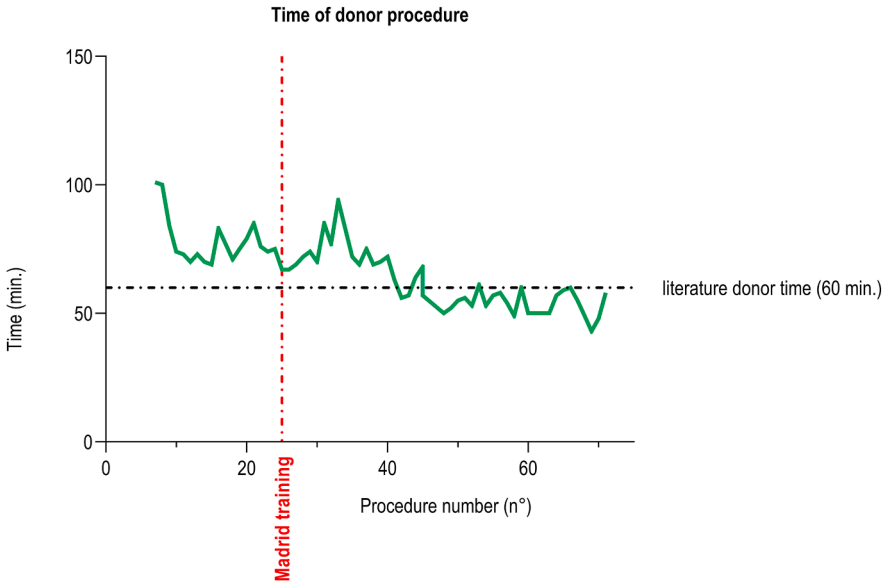

Fig. 4 – Evolution of the donor intestinal procurement time according to the procedure number. Reduction of the donor small bowel procurement time through the learning curve, calculated for living animal procedures ($n = 65 + 6$ unrecorded time). The upper limit of 60 min (horizontal dashed line) refers to the standard of literature to maintain graft quality.

for microscopes and living animals limit practice opportunities. To address these challenges, alternatives using smartphones, iPads, and surgical loupes have been developed.[13,14] For example, Jensen *et al.*[15] trained students using smartphones for magnification and prism glasses for stereoscopic vision, achieving good results at a lower cost.

Similarly, various cost-effective model alternatives that do not require ethical approvals have been reported.[14,15] These include nonorganic models (surgical gloves, gauzes, silicone tubes, three-dimensional–printed structures…) and nonliving organic models (Japanese noodles, flower petals, chicken eggs, human placentas…).[13-15] In addition, cadavers from humans, pigs, rabbits, chickens, turkeys, and rats are frequently used for microsurgical training. In our experience, chicken thighs proved to be a convenient model, offering a wide range of microsurgery exercises (dissection, ligature, anastomose) while being easily accessible in regular food shops.[16-18] However, these nonliving models cannot fully replicate the "beating heart" conditions. Tissue dissection and vascular anastomoses require arteries and venous blood flow for training, which are also linked to decision-making skills and appropriate reaction when facing urgent per-operative complications.[13]

Finally, LC evaluation requires specific microsurgery assessment tools.[15] "Objective Structured Assessments of Technical Skills" are training programs where trainees perform increasingly complex structured tasks, validated by a supervisor based on specific endpoints.[19] The stepwise approach we propose is built as such, where milestone achievement targets are checkpoint to assess LC progression. Accordingly, 34 procedures were needed in the last milestone to achieve the 40-min AT target. Subsequently, we observed a 7-d survival of more than 50%, corresponding to LC achievement according to literature.[8,10,12,20]

The rat ITx model

The steep LC of rat ITx limits its widespread use.[20] Numerous procedural details beyond microsurgical skills can impact the outcome. Male rats weighing 200-300g are typically used for ITx.[21] A heating pad and intraoperative intravenous fluid perfusion are crucial for donor and recipient hemodynamics, which subsequently reduce graft injury.[8,22] The donor/recipient strain combination induces different immunological reactions (e.g., rejection, graft-versus-host disease) that must be anticipated.[23] In our study, we chose syngeneic Lewis-to-Lewis ITx to exclude immunological influences.

During procurement, constant graft humidification and careful manipulation with cotton swabs are crucial to avoid graft lesions.[21] In addition, a reduced IPT is associated with increased recipient survival rates and should remain below 60 min. The use of bipolar coagulation forceps significantly reduced this time.[11,12,20,21] Donor bleeding is the most common complication, often occurring during PV dissection, especially at the section of the pyloric or splenic veins, the two main PV branches.[11] Such bleeding can compromise graft quality, so ligature may be preferred over coagulation. *In situ* vascular flushing through the infrarenal aorta appears superior to direct SMA flushing on the back table, likely due to better controlled flush pressure, which can cause microcirculatory injury.[21,24] The volume of perfusion varies, typically between 2 and 5 mL, and the choice of preservation solution depends on the team's preference, ranging from standard saline to specific preservation solutions.[21] While heparin is

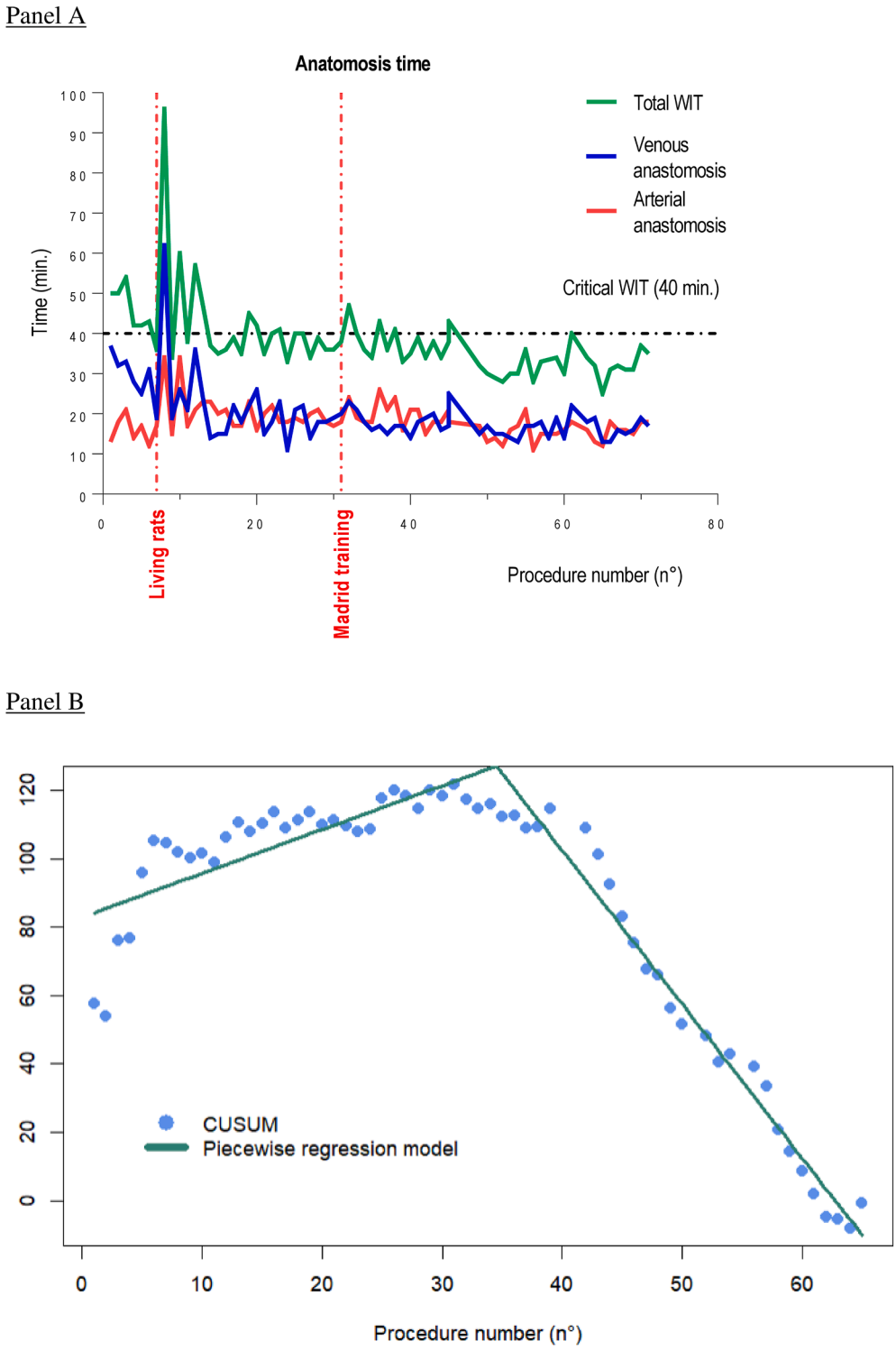

Fig. 5 – *Improvement of anastomose time according to the procedure number Panel* (A) Evolution of the arterial and venous anastomose time during the learning curve ($n = 71 + 7$ unrecorded time). Forty minutes of total anastomose time is proposed in the literature as survival limit and was set as learning target (horizontal dashed line). *Panel* (B) Cumulative sum chart of the total anastomose time (living rats procedures, $n = 61 + 4$ unrecorded time). In a cumulative sum chart, data are chronologically organized and the difference to the mean is calculated for each of them. Subsequently, these differences are cumulatively summed and represented on the chart. Finally, a piecewise regression model is applied on the dataset to calculate the inflexion point. In this dataset, the inflection point was procedure number 34. CUSUM = cumulative sum of the difference to the mean; WIT = warm ischemia time.

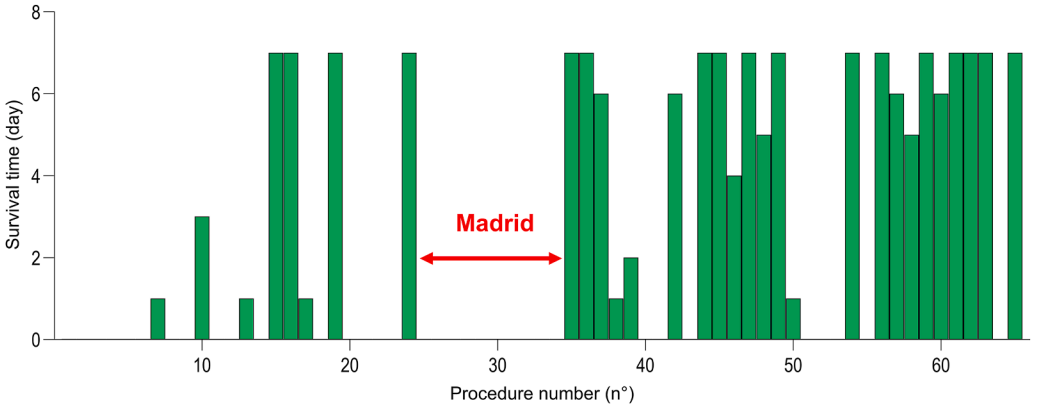

Fig. 6 – Rat 7-d survival according to the procedure number. Rat 7-day survival following intestinal transplantation ($n = 65$). Rats unable to recover postsurgery due to technical issues (primarily anastomose failures) are also depicted on the graph with a survival duration of 0 days. No survival experiments were conducted during this period (procedures numbered 25 to 33) due to local ethical considerations.

recommended to prevent vascular thrombosis (either systemically or within the flush), we did not use it and encountered no related complications.[21] Graft luminal flushing is recommended in the literature but must be done cautiously to avoid ballooning injuries.[21,25,26] CIT should remain below 60-90 min, and recipient surgery should start immediately after graft procurement.[11,21]

The infrarenal implantation of the original model developed by Monchik and Russel remain the gold standard, with the classic end-to-side arterial anastomosis usually performed, with or without an aortic tube.[3,27] As an alternative, the cuff technique was developed, where the recipient renal vessel is inserted into a short plastic tube and everted around it, while the donor SMA is pulled over.[21,28] This method is less challenging and quicker to perform (and to learn), but requires a left nephrectomy and limits graft length due to the smaller caliber of the renal artery.[21,29] A bi-aortic cuff variant addresses this issue by replacing an infrarenal aortic segment with a donor segment that includes the SMA origin.[30] The standard cuff technique can also be used for the vein, independently of the arterial anastomosis type. In addition, a more physiologic end-to-side mesenterico-portal anastomosis has been described, which does not significantly affect the outcome, even for rejection and bacterial translocation, but remains technically challenging with a higher rate of venous thrombosis.[5,20,21,31,32] Regardless of the type of anastomosis, the WIT is more critical for recipient survival, with a maximum of 40 min.[8,11,21,33] Zhang *et al.*[34] demonstrated its validity as an LC assessment marker in their series of 450 consecutive rat ITx procedures.[10] Finally, the restoration of lymphatic circulation is feasible but remains technically challenging without proven benefit.[35]

Orthotopic intestinal transplantation (OITx) and heterotopic intestinal transplantation (HITx) are distinguished by the type of intestinal anastomoses performed.[36,37] OITx involves recipient enterectomy and the restoration of intestinal continuity by graft interposition. This approach makes the recipient immediately dependent on graft function for its nutrition. A minimal graft length of 15 cm (25%) is needed for adequate weight gain, which can be used to monitor graft function.[38] Although OITx is more translational, it is associated with longer surgical times, higher complication rates, and lower survival rates.[21] In HITx, the native intestine is preserved, and the graft is excluded from transit continuity. Although less physiologic, HITx is suitable for studies not dependent on intestinal continuity.[21,39] Ostomies, such as jejunostomy, ileostomy, or both, can be performed in both models, providing benefits for graft inspection and sampling.[21,40]

Technical failure is defined as death within the first 3 postoperative days, while immunological reactions typically appear around day 5 in allogeneic settings.[12,41] In a series of 400 consecutive ITx procedures, Zhong *et al.*[42] reported survival rates of 90% for HITx and 86% for OITx. To reduce complications associated with OITx, a two-step procedure (initial HITx followed by OITx) has been successfully described too.[43]

Conclusions

Rat ITx is crucial for ITx research, enabling various experimental protocols from ischemia-reperfusion injury to immunologic studies. Our method divides the LC into three stepwise milestones, achieving LC proficiency upon completion. According to the CUSUM chart, 34 procedures were needed to meet the WIT objective set by the literature, the most critical step. Guidance from microsurgical experts was essential in achieving these goals, highlighting the value of national and international collaborations. The study also underscores the need for practice in microsurgical training and the importance of alternative training models.

Disclosure

None declared.

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Disclosure of Generative AI

During the preparation of this work, the authors used ChadGPT in order to improve the manuscript language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Ethical Clearance

KU Leuven ethical approval (P025/2022), 2022. UCLouvain ethical approval (2022/UCL/MD/13), 2022.

CRediT authorship contribution statement

Antoine Dubois: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Javier Serradilla:** Writing – review & editing, Validation, Methodology, Investigation. **Ane M. Andres:** Writing – review & editing, Validation, Methodology, Investigation. **Francisco Hernandez:** Writing – review & editing, Validation, Methodology, Investigation. **Jacques Pirenne:** Writing – review & editing, Validation, Methodology. **Laurens J. Ceulemans:** Writing – review & editing, Writing – original draft, Validation, Supervision, Conceptualization. **Eliano Bonaccorsi-Riani:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization.

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