Intestinal and multivisceral transplantation: future perspectives

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1. ABSTRACT

The field of intestinal transplantation has experienced a progressive increase in patient and graft survival over the last few years, leading to a parallel increase in the number of programs performing such type of surgical procedures. Indications for intestinal transplant include irreversible intestinal failure, compounded by potential life-threatening complications such as loss of intravenous access, liver failure or multiple episodes of infections. The type of graft that is required is highly individualized according to the patient's original diagnosis and status. Presence of short gut syndrome alone is indication for isolated intestinal transplant; liver failure mandates the use of a liver graft (liver-intestine or multivisceral transplant); intestinal dysmotility disorders with intact liver function require the use of a modified multivisceral graft. Most of the current immunosuppression protocols consist in induction immunosuppression and maintenance doses of tacrolimus. Rejection and infectious complications remain the most common causes of morbidity and mortality; it is therefore essential to closely monitor the intestinal graft to prevent such occurrences. Future developments include: the use of non-invasive markers of rejection; a refinement in surgical techniques; development of advanced immunosuppression protocols; expansion of living related transplant and multivisceral transplantation in selected patients.

2. INTRODUCTION

Intestinal transplantation is now the primary surgical option for patients with irreversible intestinal failure (1-4). Only a few transplant surgeons would have envisioned the dramatic progress that this field has undergone over the last fifteen years. A procedure that was first considered experimental is now performed in over 60 centers in the world (1, 5), is considered cost-effective and currently reimbursed by public and private payors, as well as by Medicaid and Medicare in the United States (6). Despite improvements in patient and graft survival, many are the difficulties that face clinicians involved in this field of transplantation. Rejection still remains a difficult post-operative complication that affects up to two-thirds of the recipients of intestinal transplant; infections, both bacterial and viral, are a leading cause of mortality. Lastly, the monitoring of the intestinal graft after transplant is made more complex by the lack of established, non invasive markers of rejection, coupled with the difficulty in differentiating rejection from infectious enteritis in recipients, unless endoscopy with biopsy of the graft is performed. The constant progress in the field, however, shows promise in disparate areas: surgical techniques such as living related intestinal transplant, innovative immunosuppression protocols, potentially clinically applicable markers of rejection are all coming of age and pushing further the already remarkable achievements that

have been obtained thus far. This review will focus on the current status of intestinal and multivisceral transplantation, with a look at present, upcoming and future innovations.

3.   INDICATIONS FOR INTESTINAL TRANSPLANTATION

As a general rule, intestinal transplantation is indicated when irreversible intestinal failure is present, i.e. when attempts at rehabilitation of the bowel have failed or congenital dysfunction of the intestine precludes its use. Irreversible intestinal failure per se would be adequate to qualify a patient for intestinal transplant; however, the scientific community has recognized as indications to proceed for transplant the development of life-threatening complications (7-9). These complications are: i) loss, or impending loss, of vascular access for total parenteral nutrition (TPN); ii) development of TPN-induced liver failure with cholestatic disease and portal hypertension (which can lead to gastro-intestinal bleeding episodes); iii) episodes of frequent sepsis from central venous line catheters and/or intestinal translocation; iv) recurrent, severe episodes of dehydration. Additional indications for intestinal transplantation are: the presence of functional dysmotility or congenital diseases, such as Hirschsprung's disease, intestinal pseudo-obstruction or microvillous inclusion disease.

For what concerns vascular access, loss of two or more sites for central venous access is considered as an indication for bowel transplant (8-10). Vascular access is not only necessary before transplant, but also for the first few months after transplant when antibiotics, TPN and immunosuppressive medications are still given intravenously.

Liver failure from TPN is the most critical indicator of need for bowel transplant. Liver failure increases dramatically the mortality of patients on the waiting list (11-12). It is possible to replace the intestine when liver failure is not irreversible, with regression of the cholestatic damage to the organ; however, when significant portal hypertension and fibrosis have developed, a liver graft needs to be added to the intestinal graft. One of the late signs of liver failure is the development of gastro-intestinal bleeding episodes, either from esophageal and gastric varices or from enterostomy sites; a rapid progression to sepsis and repeated episodes of bleeding usually causes death in such patients within weeks, necessitating the placement of such patients on the highest priority for transplant (12).

Those patients, who present with congenital or functional diseases of the intestine, should not wait for the development of the abovementioned complications to be listed for transplant. In such cases, the diagnosis of bowel failure is irreversible by the nature of the underlying disease (13, 14). Patients, therefore, should actually be placed on the waiting list for transplant as early as possible, so to avoid loss of access or liver failure and benefit from a better clinical status at the time of transplant.

The original diagnosis that leads patients to the need for intestinal transplant varies in adult and pediatric recipients. In pediatric recipients, the most common diagnoses are: gastroschisis, necrotizing enterocolitis, intestinal atresia, and volvulus (1, 15, 16). In the adult population, most common diagnoses are: mesenteric venous or arterial thrombosis, trauma, Crohn's disease and Gardner's syndrome (1, 2, 17).

4. PRE-TRANSPLANT WORKUP

Once a patient is referred for intestinal transplantation, whether pediatric or adult, he/she will undergo a complex workup, in order to evaluate all other systems and exclude significant additional diseases. In addition, psychological counseling is needed to prepare patients and their families to the post-transplant life.

A complete psychological and social assessment is necessary, including evaluation for substance abuse in adult recipients, since opiates dependency is present in very high rates in pre-transplant candidates.

In all patients, hepatic function is studied to decide whether a liver graft will be necessary: in those patients with perfectly normal liver function tests, no further workup is needed beyond routine laboratory testing and a Doppler ultrasound of the abdomen to evaluate the patency of the portal vein. In those patients where some degree of liver damage is present, a liver biopsy may be indicated. Patients with clear liver failure do not need a biopsy (since it would be dangerous due to from coagulopathy) and are listed for liver transplant.

Pediatric patients require comprehensive workup to rule out additional congenital diseases and evaluate cardiopulmonary function. Former premature infants can present with lung insufficiency and/or chronic lung disease, as well as pulmonary hypertension or cardiac malformations. In patients with respiratory insufficiency, a decision should be made as to whether there is a possibility of weaning the patient from ventilatory support after transplant; if a patient is deemed unable to be ever off support, this patient should not be considered a candidate for transplantation. In addition, a neurological workup should include complete neurological exam with CT scan of the brain, again to exclude malformations.

Renal function must be assessed as well, since long term use of immunosuppressive medications will impair it over time. Therefore, any patient with moderate renal insufficiency at the time of evaluation for bowel transplant, (even in the pediatric age group) should be potentially evaluated for a renal transplant as well. Renal ultrasound can evaluate the size of the kidneys, while functional studies such as a triple renal scan and a 24-hour creatinine clearance will assess true renal function. A transplant nephrologist will then make the final assessment and recommend for inclusion of a renal graft at the time of intestinal transplant, if it were deemed necessary.

Lastly, immunological considerations have to be taken into consideration. For example, patients with

intestinal atresia might present with associated deficits of the immune system such as common variable immune deficiency (18, 19). Recognizing this small subset of patients with immune deficiency is critical since these patients are at a high risk of developing graft-versus-host disease (GVHD) after transplantation.

In adult patients, a full cardiology workup is necessary, to exclude patients with prohibitive risk of cardiopulmonary complications. In those patients who present with diagnosis of tumors, it is imperative that extra-abdominal disease be ruled out, since immunosuppression post-transplant will cause early and aggressive recurrence.

5. TYPE OF INTESTINAL TRANSPLANT

The original concept of intestinal and multivisceral transplantation was first defined by Starzl, who envisioned the use of disparate combination of required organs from a larger 'cluster' that comprised the liver, stomach, pancreas, duodenum, spleen, small and large intestine, and kidneys (20-22). Selective use of some or all of such organs would be decided based on the individual patient's requirements. The organs transplanted would range from an isolated intestinal graft to all of the organs abovementioned together (multivisceral transplantation). Over the years, different variations to such concept have been proposed and implemented, with four major categories of transplant being currently performed at intestinal transplant centers.

5.1. Isolated intestine

The simplest adaptation to the original concept of intestinal transplantation is the use of an isolated intestinal graft (23). The superior mesenteric artery and vein are its vascular supply, with jejunum and ileum (more rarely a segment of ascending colon), being the transplanted segments of bowel.

Indications for isolated intestinal transplant are usually loss of bowel with still intact liver function and absence of portal hypertensive complications. As an example, a patient after resection of bowel for volvulus, mesenteric vein thrombosis, or trauma.

The arterial inflow to the graft can be provided by the native mesenteric artery, but most often is the infra-renal aorta, with or without the use of an extension graft. The venous outflow can be performed in two ways: portal or systemic. In the portal reconstruction, the mesenteric vein is anastomosed to the native superior mesenteric vein or any of the larger veins that drain into the portal vein (splenic vein, for example), if necessary with the use of a venous jump graft. The systemic drainage, instead, is performed by connecting the mesenteric vein of the graft onto the inferior vena cava or one of its tributaries (renal vein, for example). Studies have compared the two types of drainage to see if any adverse effect would come from the systemic drainage that is not physiological; however, no significant difference was observed between the two ways of reconstruction in terms of graft function and patient survival (24). In the end, it is the anatomy of the

recipient's venous system that dictates which option to choose.

Intestinal reconstruction is performed proximally at the duodenal or jejunal level in the recipient with the proximal jejunum of the graft. Distally, a stoma is constructed at the level of the ileum, and the distal ileum or the donor colon is connected to the native colon.

Indications for inclusion of the donor colon are: loss of native ileo-cecal valve and/or previous surgeries that included subtotal or total colectomy in the recipient. In patients who previously received total procto-colectomy, it is possible to perform a pull-through procedure utilizing the donor colon; this, however, can be performed only if the pelvic anatomy is favorable for such procedure. More commonly, an end-colostomy is performed, and a rectal reconstructive procedure can be planned a few months later if the patient's condition allows.

5.2. Liver-intestine transplant

The development of end-stage liver disease in patients with intestinal failure dictates the addition of a liver graft. Liver-intestine transplantation can be performed in block (composite liver-intestine transplant) with both organs being vascularized through an aortic patch that contains both the superior mesenteric artery and the celiac axis (22, 23). In such case, the venous outflow of the intestinal graft will be through the liver graft via the portal vein, and final outflow of the composite graft will be through the hepatic veins. Because all patients have already developed significant portal hypertension, it is critical that the venous outflow from the native stomach, pancreas and spleen be drained using the recipient's native portal vein. This can be accomplished by performing a porto-caval shunt between the native portal vein and the inferior vena cava, or alternatively by connecting the native portal vein onto the graft portal vein.

In order to preserve the alignment of the graft portal vein, dissection and removal of the donor pancreas can be avoided by preserving the head of the pancreas or the whole pancreas, and including them in the graft together with the duodenum (25-28). This presents multiple advantages: it preserves the native liver hilum structures but allows cut down of the liver graft in cases of size mismatch; there is no need for a biliary reconstruction in the recipients, since the bile is drained into the common bile duct and the duodenum; finally, the portal vein is less skeletonized, with lesser risk of kinking.

An alternative technique in liver-intestine transplantation involves the use of separate grafting of the two organs (non composite liver-intestine transplant) (29, 30). This technique is accomplished by utilizing separate arterial and venous anastomoses for each of the transplanted organs. The liver is implanted through standard techniques, and the intestine is connected as if it was an isolated intestinal graft. The major advantage in such technique lies in the fact that each organ is functionally separate, so that if severe intestinal rejection occurs and requires removal of the intestinal graft, this can

be accomplished without sacrificing the vascular supply of the liver graft (29, 30).

Reconstruction of the enteric continuity is performed in a similar fashion as in isolated intestinal transplantation, most commonly connecting the graft jejunum to the most distal segment of the remaining native proximal intestine. If the donor duodenum is not included in the graft, then biliary reconstruction needs to be performed as well with a hepato-jejunostomy. Distal intestinal reconstruction is performed as in the isolated intestinal transplant technique.

5.3. Multivisceral transplantation

The centerpiece of abdominal multi-organ transplantation, multivisceral transplantation is defined as simultaneous transplantation of the following organs as a single block, based on the vascular supply of the aorta and inferior vena cava: liver, stomach, duodenum, pancreas, small intestine, with optional transplantation of the spleen, large intestine and kidneys (20-22, 31). The vascular inflow is from the aorta with its celiac, superior mesenteric and renal arteries; the venous outflow is the inferior vena cava, where the hepatic veins and the renal veins drain into.

The advantages of multivisceral transplantation are multiple: first, this type of transplant replaces all dysfunctional units in the digestive tract, keeping the anatomical and tri-dimensional alignment of its parts. By the same concept, removal of the native stomach, pancreas and spleen eliminates all residual portal hypertension, therefore obviating the need for a porto-caval shunt in the native organs. In pediatric patients, where abdominal volume is of utmost importance, much space can be gained by splenectomy in the recipient.

Most importantly, however, multivisceral transplantation might confer a higher degree of protection from severe rejection, as compared to recipients of liver-intestinal grafts. This was observed in one series of over 100 multivisceral transplants, although additional data to confirm such observation is still missing (31, 32). Additionally, transplantation of the stomach and pancreas does not add significant risks in terms of rejection or infection susceptibility (31).

There are multiple indications for performing multivisceral transplantation, primarily based on the need for liver and intestinal grafts, combined with the need for replacement of additional dysfunctional units within the abdominal domain. Neoplasms of the digestive system (pancreas tumors, neuro-endocrine tumors of the intestinal tract, and desmoid tumors in the mesentery) or pre-cancerous syndromes (familial adenomatous polyposis) are a primary indication for multivisceral transplantation (33, 34). As long as there is no extra-abdominal spread of the original tumor, the procedure that will guarantee the most complete oncological resection is the exenteration of the involved organs. Another indication is in patients with multiple prior surgeries in whom there are multiple adhesions, complicated at times by entero-cutaneous fistulas. In this group of patients we can include those

patients with previous radiation enteritis, in whom not only the intestine is dysfunctional but also the stomach and pancreas are chronically damaged. In the pediatric population, where smaller and smaller candidates are being evaluated, multivisceral transplantation can be offered as primary option, since it decreases the number of anastomoses that need to be performed, with less risks of technical complications (biliary leaks, arterial or portal thrombosis and so on) (31).

5.4. Modified multivisceral transplantation

Modified multivisceral transplantation is defined as multivisceral transplantation without the liver (23, 31). It is a surgical option reserved for those patients in whom the liver function is still well preserved but there is a generalized intestinal dysfunction. Candidates for modified multivisceral transplantation are primarily patients with dysmotility disorders (Hirschsprung's disease, intestinal pseudo-obstruction, megacystis microcolon intestinal hypo-peristalsis syndrome), congenital mucosal diseases (microvillous inclusion disease), or in whom there is a high risk of cancer (familial adenomatous polyposis). The organs that are transplanted are: stomach, duodenum, pancreas, small bowel (and, optionally, spleen and large bowel). The arterial inflow is once again given by the aorta and its celiac and mesenteric branches; the outflow is through the portal vein, which is connected to the recipient portal vein just outside the preserved native liver (31). The most important advantage in this type of operation is the removal and substitution of all segments of the gastro-intestinal tract which are either dysfunctional or at potential risk for cancer, while preserving the native liver and connecting in an anatomical and physiological way the venous outflow of the organ cluster.

Interestingly, recipients of modified multivisceral transplants, even though they do not have a liver graft, do seem to be protected as well from severe rejection just like multivisceral patients, as compared to recipients of isolated intestine or liver-intestine grafts (31). Again, this has been observed by the authors of the study in a large cohort of patients, but still needs to be confirmed by other centers (31).

6. SURGICAL TECHNIQUES

6.1. Donor operation

Most of the surgical techniques for retrieval of the different types of intestinal grafts have been previously described in detail (23, 34, 35). There are, however, some points that are worth considering.

In the retrieval of isolated intestine, most of the dissection of the vascular pedicle at the base of the mesentery can be performed in the donor before cross-clamp. The inferior pancreatic-duodenal artery need to be preserved with the pancreas graft, if this is utilized for transplant. The line of division for superior mesenteric artery will therefore need to be at the lower edge of the pancreas below the takeoff of the inferior pancreatic-duodenal artery if this organ is retrieved for transplant; alternatively, the superior mesenteric artery will be divided

at the level of the aorta if the pancreas is not utilized for transplant (35). The superior mesenteric vein is divided near the inferior edge of the pancreas below the corresponding pancreatic-duodenal vein (if present); otherwise, the superior mesenteric vein is divided within the pancreas, right at the level of the takeoff of the splenic vein if the pancreas is not used for transplant.

When a composite liver-intestine graft is retrieved, careful attention has to be paid at the level of the aorta, so to encompass both the celiac axis and the superior mesenteric artery in the common aortic patch or conduit that will be utilized for arterial inflow in the recipient operation. The portal vein is not divided; the duodenum and the head of (or all) the pancreas can be included in the graft.

Same attention to the aortic conduit needs to be made when retrieving a multivisceral graft. It is always better to include as long a segment of the thoracic aorta as possible in the arterial conduit, since this could be used in the recipient as an interposition graft. The organ cluster from pediatric donors, especially those weighing less than 8 kilograms, needs to be handled with extreme carefulness. It is very easy to twist or stretch the small vascular pedicles in such small donors, and this can cause discoloration and ischemia in the liver and intestine, which poses the graft at risk for primary non function. Lastly, the space between the takeoff of the mesenteric artery and the takeoff of the renal arteries is often very narrow in small pediatric donors; if the kidneys are not part of the multivisceral graft, the division line on the aorta must be exactly in between the two structures, so as not to compromise vascular supply in either of them.

The esophagus is divided distally, near the diaphragm. We include donor spleen and colon in the retrieval operation for multivisceral and modified multivisceral transplants (31, 32, 36). The donor colon is divided at the level of the descending colon, with blood supply from the middle colic vessels, thus assuring a good length of large bowel and the presence of an ileo-cecal valve in the graft. The spleen can be removed at the back table if size matching is uneven, if the blood group is compatible but not identical, and in cases of immune deficiency in the recipients (to minimize the risk for graft-versus-host disease).

In recipients of modified multivisceral transplant, the retrieval technique is similar to a regular multivisceral, except for the vascular supply to the liver (36). In fact, the liver will be utilized for a recipient other than the recipient of the intestinal graft, so it needs to be harvested separately. The common bile duct is divided and left long on the liver side and tied on the intestinal graft side, since it will not be used. The lines of division for the hepatic vessels will be: for the portal vein, the superior edge of the pancreas, for the hepatic artery right below the gastro-duodenal artery (which is double ligated and divided) and above the splenic artery, which must go with the modified multivisceral graft (together with the left gastric artery). The open stump of the proper hepatic artery is over sown at the back table.

Flushing of the organ with preservation solution is accomplished through retrograde perfusion via the infra-renal aorta, and University of Wisconsin solution is most commonly utilized. Alternatively, histidine-tryptophan-ketoglutarate (HTK) solution can be utilized as a perfusate flushing solution (37). The graft is then packed and taken to the back table.

6.2. Recipient operation

The most challenging aspect in the recipient operation comes from the previous history of surgeries in the patients. Patients have often previously undergone extensive resections, resulting in loss of abdominal domain, significant adhesions, entero-cutaneous fistulas, and, when the liver is in failure, portal hypertension with collateral vessels and varices.

In isolated intestinal transplantation, removal of part of the native intestine might be necessary; if possible, it is preferable to maintain the native ileo-cecal valve. The native mesenteric artery and, especially, the superior mesenteric vein, if still present and patent, should be dissected and utilized for the vascular anastomoses. The aorta can be accessed in the retroperitoneum and a segment of the infra-renal aorta is chosen if the native mesenteric artery is not suitable for anastomosis. Similarly, the inferior vena cava can be exposed for the venous anastomosis if the graft is to be drained in a systemic fashion.

In liver-intestine transplantation, the hepatectomy is usually performed with preservation of the native inferior vena cava (piggy-back technique). The venous anastomosis for outflow is at the level of the hepatic veins, while the arterial inflow will be through an aortic graft in composite liver-intestine transplant, which will include both the celiac axis and the mesenteric artery. Once again, in liver-intestine transplant it is necessary to drain the native portal vein that serves the stomach, pancreas and spleen, via a porto-caval or porto-portal shunt.

In multivisceral transplant, the most important technical step is rapid devascularization of the native abdominal organs (31). This can be accomplished in a caudad or cephalad approach to the superior mesenteric and celiac vessels, which are mass clamped at the beginning of the organ resection. In the cephalad approach, the native stomach is divided just below the gastro-esophageal junction, and then lifted downwards. This will expose the supraceliac aorta, allowing the surgeon to slip two fingers on both sides of the aorta. A vascular clamp is then applied over the celiac axis and superior mesenteric artery, without dissecting such vessels separately. In the caudad approach, the intestine is mobilized and lifted upwards, until the left renal vein is identified, then a clamp is applied from the bottom up, again encompassing the superior mesenteric and celiac arteries. Once the vascular inflow has been cut off, the splanchnic organs can be removed with marked decrease in blood loss. The liver is usually removed retaining the native inferior vena cava. The arterial inflow is provided through an aortic conduit, placed at the level of

the infra-renal aorta; the venous outflow is at the level of the hepatic veins. Gastro-intestinal reconstruction, from proximal to distal, is performed as follows: a gastro-gastrostomy is created between native and donor stomach; a pyloroplasty is performed; a colo-colostomy (or ileo-colostomy if the large bowel is not included in the graft) is created onto the native colon; an ileostomy is created to monitor the graft as a terminal, loop, or Bishop-Koop type.

The recipient operation in a modified multivisceral transplant can be performed in different ways, according to the number of native organs that are preserved. The common baseline concept is to leave the unaffected native leave in situ, so that the donor liver can be utilized for another recipient. Arterial inflow is performed in all cases through a graft from the infra-renal aorta. The most conservative technique spares the native duodenum, pancreas and spleen, which are left in place (17). A variation to this technique leaves the native liver and spleen intact, with their tributary vessels (17). In these instances, the portal vein of the graft (taken at the superior margin of the donor pancreas) is anastomosed to the native superior mesenteric vein, below the splenic vein takeoff. Biliary and pancreatic drainage is accomplished by a side-to-side duodeno-duodenostomy in the first variant, and with a duct-to-duct anastomosis in the second variant. The original technique of modified multivisceral transplant leaves only the native liver in place. In such case, the most important technical point is in the preservation of the native hepatic artery, requiring careful dissection of each of the branches of the celiac axis, with preservation of any aberrant right or left hepatic artery (22). The portal vein in the recipient is divided as far from the liver as possible, usually within the pancreas, so that it can be later connected to the portal vein of the modified multivisceral graft. Gastro-intestinal reconstruction is similar to a multivisceral transplant, save for the need for biliary reconstruction, which is performed via a Roux-en-Y hepato-jejunostomy, created with a loop of the intestinal graft.

Abdominal closure after intestinal transplant can be difficult for multiple reasons: previous loss of domain of the abdominal cavity in the recipient, organ edema after reperfusion, scars from previous surgeries, need for partial resection of the abdominal wall if desmoid tumors or entero-cutaneous fistulas are present, and so forth. Forced primary closure will cause organ dysfunction and abdominal compartment syndrome. The options available to the surgeons range from the use of absorbable or non absorbable mesh for smaller fascial defects, to the use of temporary placement of silastic mesh and serial closure operations. An alternative approach in cases of large wound defects is transplantation of the abdominal wall, a vascularized, composite graft encompassing the anterior abdominal wall with its skin, subcutaneous tissues and rectus abdominis muscles, whose blood supply is based on the inferior epigastric vessels (38).

7. LIVING-RELATED INTESTINAL TRANSPLANTATION

The utilization of a live donor for intestinal transplant has been achievable only in the last few years, when clinical results in intestinal transplantation started improving to the point of making the surgery on the donor worth its risks for the survival of the intestinal graft in the recipient (39-41). Currently, over 30 live donor small bowel transplants have been performed in the United States, with several additional case reports from Europe and Asia. Same considerations that apply for any other type of live donor need to be applied in intestinal segmental graft donors: the potential donors need to have same ABO blood group, excellent health, imaging studies that show anatomy of the intestine compatible for donation, and psychological clearance.

The donor graft is retrieved from the ileum, with a segment of 150-200 centimeters in length (42). In the donor, the last 20 cm of distal ileum are preserved, as well as the ileo-cecal valve; care must be given to measure the remainder of the jejunum-ileum in the recipient to make sure that at least 60% of the length of the native intestine is preserved. The vascular supply to the graft is through the last branch of the superior mesenteric artery that takes off from the ileo-colic artery, and its correspondent mesenteric vein branch. In the donor, the operation is completed by reconstitution of intestinal continuity and over sowing of the vascular stumps.

The organ is flushed with preservation solution at the back table until effluent form the vein is clear. Timing of donor and recipient operation must be controlled so to minimize cold ischemia time.

The recipient operation is similar to an isolated intestine transplant (40); most of the time, the inferior vena cava is utilized for venous drainage (systemic drainage). Care must be paid in the technical aspect of the anastomoses, since the small size of the artery and vein makes twisting and kinking of the vessels easy to occur. One technical point is to create the opening in the receiving vessels (aorta and vena cava) wider than the diameter of the implanted mesenteric vessel, so to minimize the possibility of thrombosis.

Combined live donor liver-intestine transplantation has recently been described as well, mostly in pediatric recipients (43); in such cases, the graft is by necessity non composite, with individual anastomoses for each of the two components (liver and intestine).

The field of live donor intestinal transplantation is still in its early stages. Important concerns for the status of the donor have to take priority every time live donor bowel transplant is considered; in addition, long term follow-up studies are required to evaluate the growth and development of recipients of a segmental graft as compared to those who receive full length intestinal grafts from deceased donors (44).

8. IMMUNOSUPPRESSION

The great improvements in the field of intestinal transplantation would have not been possible without the availability and then refinement of potent

immunosuppressive protocols. The history of bowel transplant immunosuppression can be roughly divided in eras: the first era when induction drugs for immunosuppression were not available or not utilized early after transplant, resulting in almost invariable rejection of the graft (1, 17, 31). In the second era, induction immunosuppression became standard, which resulted marked improvements in the capacity of controlling rejection, but a high rate of infections and post-transplant immunosuppression-related complications (17, 31, 45). In the third, and last era, which we are currently in, induction immunosuppression has been adjusted so to limit the amount of immunosuppression that is required after the immediate post-transplant period (for instance, protocols that avoid steroids), and keeping with the improvements in patient and graft survival (45-48).

Almost all centers currently use induction immunosuppression therapy for intestinal transplant: it can consist in monoclonal, anti interleukin-2 receptor antibodies (such as daclizumab or basiliximab), or anti-lymphocyte antibodies such as thymoglobulin or alemtuzumab (Campath 1-H). Most anti-lymphocyte antibodies are given for a short course of therapy during the immediate peri-operative period; anti interleukin-2 antibodies are administered intermittently over the first 6 months post-transplant. In our experience, pediatric recipients do not tolerate well Campath 1-H induction therapy, therefore alternative anti-lymphocyte agents or daclizumab are preferred (32).

The most commonly utilized maintenance immunosuppression drug is the calcineurin inhibitor tacrolimus. Sirolimus is rarely utilized as primary maintenance drug in addition to tacrolimus (49); however, sirolimus alone or in combination with tacrolimus is often used in patients with worsening renal function or repeated episodes of rejection. Mofetil mycophenolate is seldom utilized, again usually in patients after multiple episodes of rejection or with significant nephrotoxicity. Maintenance steroid therapy (usually 3-9 months post-transplant) is necessary for patients with interleukin-2 inhibitors induction therapy, while steroids can be avoided in patients who undergo induction therapy with anti-lymphocyte antibodies. Additionally, patients with anti-lymphocyte induction therapy can be maintained at a lower baseline level of tacrolimus than patients who did not get anti-lymphocyte agents (45-48).

Treatment of rejection episodes consists in an increase of baseline immunosuppression, combined with steroid boluses and weaning cycle for episodes of mild rejection; if no response is noted within 24-48 hours, then anti-lymphocyte agents should be utilized (50). Moderate and severe rejection episodes require use of anti-lymphocyte agents in the greatest majority of cases. It is important to treat rejection early and aggressively, because even a mild episode of rejection can rapidly progress into severe, exfoliative-type of rejection if not treated properly (51, 52). It is, therefore, mandatory that close monitoring of the graft be performed during treatment of rejection episodes.

9. MONITORING OF THE GRAFT

A great challenge to the field of intestinal transplantation is the fact that no serological marker for the diagnosis of acute rejection exists, such as creatinine or liver function tests in the diagnosis of rejection for recipients of renal and liver grafts, respectively.

Since clinical intestinal transplantation became widespread, there has been a necessity of creating a stoma to monitor the graft visually and with endoscopies. The stoma is usually constructed in the distal ileum, which is the segment of bowel most susceptible to rejection. Visual inspection of the stoma can only detect gross alterations in the intestine, such as changes in the blood supply or severe mucosal sloughing. Endoscopic examination, coupled with histological analysis of the mucosal biopsies obtained during the endoscopy, remains the gold standard for evaluation of an intestinal graft. Even endoscopic exams, per se, are not always accurate in the analysis of early changes in the graft mucosa. Histological evaluation of the mucosal biopsies remains the most reliable indicator of rejection. The community of transplant pathologists has been using standardized criteria for the diagnosis of acute cellular rejection (53). It is important that dedicated transplant pathologists be in charge of all samples' readings.

A step forward in the monitoring of intestinal grafts has been the implementation of frequent protocol endoscopic monitoring and zoom video endoscopy (54, 55). The first calls for twice weekly endoscopies in the first month after transplant and subsequently twice per week monitoring in the following two months. In addition, every time a patient experiences an episode of rejection, endoscopy should be performed very often (even daily or every other day, if necessary). This will allow the clinician to control tightly the evolution of the changes on the intestinal mucosa. The second advancement has been the use of zoom video endoscopy: it allows magnification of the intestinal mucosa up to more than 100 times, with the possibility of examining the intestinal villi and deviation from normal anatomical appearance. The parameters that are examined are: the height, tip and vascular pattern of the villi, the mucosal friability and the background erythema in between villi (55). A score is assigned to each parameter, and the sum of such scores generates a final score, which correlates to histological analysis. Because of size limitations, zoom video endoscopy can only be utilized in recipients of intestinal grafts beyond the early pediatric age, that is when the donor graft is older than 2 years of age.

The clinical implementation of non invasive markers of rejection is still in its infancy. Multiple substances have been evaluated, and the ones that offer the greatest promise to become effective tools for the clinician are still utilized in pilot studies. Citrulline has been evaluated as a blood marker of intestinal function (56). The test can be performed in blood, serum or even dried-blood-spot assay (56, 57). A decrease in the levels of citrulline has been associated with moderate and severe rejection, but not as significantly with mild rejection (58).

The advantage of such monitoring is that patients can easily send in a test, even without going to the laboratory in the case of the dried blood spot; whenever the citrulline levels decrease, even if the patient is asymptomatic, this will prompt an endoscopy to rule out rejection. A second marker of rejection, calprotectin, has been evaluated in the stool of intestinal transplant recipients (59). The level of stool calprotectin will increase when a patient is experiencing rejection, but remain normal when other intestinal pathologies (e.g., viral enteritis) are present. Additional markers such as perforin and granzyme B have been studied in the peripheral blood of intestinal transplant recipients; these markers, however, are not intestine-specific (60).

10. COMPLICATIONS

10.1. Rejection

Rejection still remains the hardest management issue in this field of transplantation. Multiple factors play a role: the susceptibility of the intestinal graft, which is comprised of many different tissues and cell types; the presence of a large amount of donor lymphatic tissue, with its potential for immune interaction with the recipient; the colonization of the intestine with bacteria and viruses, which can easily translocate in to the blood stream and trigger septicemia once the integrity barrier of the mucosa is disrupted (61).

Acute cellular rejection can develop rapidly, over the course of a few days, and left untreated progresses into exfoliative-type of rejection, with complete loss of the mucosal surface, bleeding and septicemia (51, 52). Unlike hepatic and renal grafts, intestinal transplant rejection rarely occurs without signs or symptoms. The most common manifestations are: diarrhea (or increased stoma output), which at first is clear then bloody; fever; dehydration; nausea and vomiting. Early diagnosis by means of endoscopy and biopsy is critical. Timely treatment is critical. Empiric treatment with a bolus of steroids can be given even if unable to perform endoscopy or histological analysis within 24 hours. Treatment of rejection, as previously described in the section dedicated to immunosuppression, should be with steroids in cases of mild rejection; in moderate or severe rejection, it will be necessary to utilize anti-lymphocyte agents to arrest the progression of the damage to the intestine. Supportive measures need to be instituted as well, such as placing the patients on intravenous nutrition, giving prophylactic antibiotic therapy for gut decontamination and systemically if sepsis occurs, and instituting frequent endoscopies to monitor the resolution of the episode.

Chronic rejection is not as frequent as acute rejection (62). It is now being observed more often, since results of short term survival have improved and more patients are still alive with their graft years after the transplant. It mostly consists in changes in the macro- and micro- vasculature of the graft, with resulting arterial intimal hyperplasia and strictures, sub-mucosal fibrosis, glandular drop-out and clinical inability of the bowel to perform its absorptive functions (63). A less common form of chronic rejection has been described as encapsulating peritoneal sclerosis, which usually is clinically manifest by intestinal obstruction (64). There is no real treatment for chronic rejection, and most patients require graft removal and re-transplantation.

10.2. Infections

If rejection presents the biggest clinical challenge to the clinicians, infections still are the leading cause of death in patients after intestinal transplant (1, 16, 17, 31). Bacterial, viral and fungal infections are a constant threat, especially in the early post-operative period. In pediatric patients, there is an inverse relationship between the development of viral infections (especially respiratory) and age (32). Patients younger than one year are particularly vulnerable to such type of infections.

Immunosuppression plays an important role in the pathogenesis of infections, since all intestinal transplant recipients need, on average, higher level of baseline immunosuppression as compared to recipients of other solid organ transplants. In addition, frequent episodes of rejection mandate the use of sharp increases in baseline immunosuppression with additional courses of anti-lymphocyte or steroid treatments. This will, in turn, cause high susceptibility in the recipients towards all types of infection, including opportunistic infections.

The graft itself is at the same time a source and a target of infections: on one side, the physiological colonization with bacteria can make it easy for translocation into the bloodstream if the integrity of the mucosa is disrupted, as it happens during rejection episodes (65-66); on the other side the transplant intestine itself is often infected with pathogens such as *Clostridium difficile, Rotavirus* or *Enterovirus* strains, which can mimic signs of rejection by causing profuse diarrhea (67).

It is important to pursue tight infection control practices in such group of patients, in order to avoid cross-contamination. The emergence of multi-drug resistant strains of bacteria presents a clear danger to bowel transplant recipients; many of the patients with prolonged intensive care unit stays become colonized with vancomycin-resistant *Enterococcus* strains, methicillin-resistant *Staphylococcus* or similar. In the pediatric population, respiratory viruses like *Adenovirus, Influenza virus,* and *Respiratory Syncytial virus* can rapidly spread and be a cause of major morbidity and mortality.

Opportunistic infections are seen as well. For instance, invasive tissue fungal infections with *Aspergillus* strains, *Rhizopus* or *Mucor* species can be observed in the nasal sinuses, soft tissues of the extremities and/or trunk. Similarly, *Nocardia* or *Pneumocystis carinii* pneumonia has been described as well as infection with *Histoplasma* and *Coccidiomycosis.*

Viral infection or reactivation by *Cytomegalovirus* has become less significant as in the past, where mortality was much higher. Most programs implement strict prophylaxis protocols with the utilization

of gancyclovir or its derivatives, with additional use of specific hyper-immune globulins (Cytogam) during the peri-operative period (67, 68). Such prophylaxis is even more necessary in patients of CMV-positive grafts who had negative pre-transplant serology, since this category of patients is at highest risk for primary infection post-transplant.

10.3. Post-transplant lymphoproliferative disease

There is an increased incidence in the development of post-transplant lymphoproliferative disease (PTLD) in recipients of intestinal grafts (17, 31, 36, 69). Epstein Barr virus (EBV) has been linked as causative agent of PTLD, although not all forms of PTLD are EBV-derived. It is of utmost importance to monitor patients' EBV levels during the post-operative course by means of quantitative polymerase chain reaction (PCR), so that a positive test in a previously negative recipient or a log increase in the number of viral copies in an already positive patient triggers investigations to rule out PTLD. Pediatric recipients are at higher risk of developing PTLD than adult patients (17, 70).

PTLD can involve the transplant intestine, manifesting with chronic diarrhea, as well as all other organs and systems. In the mild forms of the disease, patients will present with weight loss, hypo-albuminemia, nausea and low grade fevers. More advanced forms present with lymphoadenopathy, palpable abdominal masses, and rapid weight loss, fevers. Endoscopy can reveal lymphatic hyperplastic lesions, nodules or ulcerations in the transplant and/or native intestinal mucosa (71). Histologically, there is a dense lymphoplasmic cellular infiltrate in the intestinal mucosa, with disruption of the glandular architecture and crypts' distortion. Immunohistochemistry can reveal a preponderance of CD20 positive cells (B lymphocyte lineage); special stains for EBV virus can be positive in the areas of lymphatic infiltrates. Confirmatory diagnosis of PTLD is obtained by gene rearrangement studies from the tissue biopsies, looking for T or B lymphocyte clonal populations.

The first principle for PTLD treatment is reduction of immunosuppression; because of the high requirements for the bowel graft, it might not be possible to stop immunosuppression completely, unless dictated by the patient's clinical scenario (72). Second, aggressive antiviral therapy is warranted in cases where EBV is positive; this is accomplished by the use of gancyclovir and Cytogam. If there is preponderance of CD20 positive cells, the depleting, anti-CD20 monoclonal antibody rituximab has been successfully utilized (73). While less aggressive forms of PTLD can be treated with a short course of rituximab (4 weekly injections), more aggressive forms require prolonged treatment, at times even lasting a few months (74). When PTLD is not EBV positive, or when the cell type is not predominantly of CD20 positive, then chemotherapy is required.

10.4. Graft-versus-host disease

Graft-versus-host disease (GVHD) is not very common in recipients of intestinal transplants; however, its management is fairly complex (75). Pathogenesis of GVHD can be traced to the presence of a large load of immunologically competent cells within the intestinal graft at the time of transplant. Such cells migrate out of the intestine and engage disparate tissues in the host, causing specific organ damage (76).

The intestinal graft is not involved by the immunological process, but the native intestine can, and diagnosis can be made by tissue biopsy. Symptoms, therefore, are rarely seen in the gastro-intestinal system; rather, the skin is most commonly involved, with subsequent target organs being the liver, lung and bone marrow. Particular attention needs to be given to those patients whose primary diagnosis is intestinal atresia, because there is a higher incidence of immunological disorders in such population, with a well-known increase in the risk for GVHD (18).

Skin GVHD manifests with a papulo-macular rash, rapidly desquamating, on the hands, feet, trunk and extremities; liver GVHD causes cholestatic hepatitis, with increasing bilirubin levels and organ dysfunction; lung GVHD results in respiratory distress, oxygen requirement and diffuse infiltrative lung disease; bone marrow involvement is manifested by thrombocytopenia and, in serious cases, aplastic anemia (75).

Treatment of mild GVHD is usually successful; an increase in immunosuppression with boluses of steroids and/or topical treatment with tacrolimus ointment usually resolves the skin lesions. However, if GVHD progresses to multi-organ involvement, escalation of immunosuppression is often necessary but can lead to significant infectious complications. If at all possible, it is suggested to use the least amount of immunosuppression necessary to control the disease, or alternative therapies, such as the use of monoclonal antibodies such as daclizumab or infliximab.

11. FUTURE DEVELOPMENTS

The progress in bowel transplantation over the last few years is only bound to continue; it is realistic to expect that graft and patient survival rates will continue to parallel the ones of liver transplant recipients (1). More and more national health systems and private insurance companies will hopefully continue to fund such type of transplant in a move that will parallel kidney and liver transplant provisions. The real challenges for the clinician are on multiple levels.

Education is a key factor to increase the number of referrals and their timings. Gone should be the days when a patient presents for evaluation either late (with liver overt failure or after complete loss of venous access), or worse, after having done personal research, since their own physician was not aware of or did not consider intestinal transplant a real therapeutic option. Transplant surgeons and physicians should dedicate part of their practice to informing the local communities and hospitals, taking time to explain how much this field of transplant has advanced over the last few years and how critical is early referral. A close relationship with pediatric and adult gastroenterology

groups is also essential, not only for issues of bowel rehabilitation and patient referral, but also for long-term post-operative follow-up once patients return to their homes.

From a referral standpoint, there is going to be a trend for earlier evaluation of potential candidates; this will in turn translate to more patients being evaluated before liver failure ensues. On the other side, younger and smaller patients in the pediatric population will be referred. The challenges in small (less than one year of age) pediatric patients come from a perspective of organ donation, since there is not a large pool of deceased donors in this age group. In addition, patients in this group tend to have significant higher risks of developing infections, especially respiratory, and present with an overall higher morbidity and mortality. It is up to the clinicians to come with aggressive protocols of infection monitoring and prevention to overcome such complications.

Multivisceral transplantation might also find more clinical applicability, if the preliminary findings of immunological benefit from rejection were to be confirmed by other groups. In patients with dysmotility problems, modified multivisceral transplantation should, in our opinion, be the procedure of choice.

The inclusion of the spleen in a multivisceral or modified multivisceral graft is a controversial topic of debate. Pre-clinical data (77) and clinical data from one center's experience (16, 31) suggest a potential benefit from a rejection standpoint, without an increase in the risk of developing GVHD. Only long term follow-up and larger patients' groups will be able to clarify the real impact of inclusion of the spleen in a multivisceral graft.

The donor large intestine (including ileo-cecal valve) should also be widely utilized. We have not observed any increase in the incidence of rejection or infectious complications, while preserving an important physiological mechanism for fluid reabsorption, and decreasing the chances for dehydration in the recipients.

The next years will also be an occasion to study patients longitudinally, now that survival rates are higher. Important issues of physical and psychosocial development in children, quality of life and return to work in adults can only be addressed now that we are starting to have larger cohorts of patients with longer times for follow-up.

The most promising field is going to be in the non invasive monitoring of the intestinal grafts. The critical factor is to find a test or a battery of tests that are easy to perform for the patients, even at home. The ideal marker should be a stable substance, whose measurements can be easily produced by the laboratories within hours. Whether it will be citrulline or calprotectin, or any other new molecule real-time utilization will be a key factor. Moreover, a reliable test could avoid performing unnecessary endoscopy in many patients, with significant cost savings and less morbidity. It is likely that a combination of tests in a panel might be a more comprehensive solution. For instance, if a patient starts experiencing diarrhea, he/she could send a paper strip with a few drops of blood and/or a small sample of stool. This specimen could be taken to the laboratory, and tested for multiple substances at the same time. Its results would allow the clinician, by the end of the day, to decide whether it is the case to admit the patient and perform endoscopy or just follow up over the next days without a need for admission or invasive tests.

Another field of future developments is in live donor intestinal transplantation. Aside from ethical issues related to the safety of donors, it will be critical to compare long-term results of such procedure with data from deceased donors. The main reasons for the use of live donors have been the long waiting time, a better immune matching and short ischemia time during surgery; however, if all potential deceased organ donors were actively recruited for intestine donation, the waiting time would be shorter. The major issue lies with the pediatric population; because of the scarcity of pediatric donors, waiting times are long and patients deteriorate while on the list. In such cases, live donor intestinal transplantation combined with techniques for organ size reduction at the time of transplant can become a significant alternative.

Implementation of new immunosuppressive protocols is also a key factor in the future of intestinal transplantation. The major goal is to minimize long-term immunosuppression and its deleterious side effects, while at the same time achieving functional tolerance. One question still open is whether long term minimization of immunosuppression might lead to a higher rate of chronic rejection, with late graft loss.

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