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Multimodal postoperative pain management including epidural analgesia for primary bladder exstrophy repair

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Background: Classic bladder exstrophy is a major birth defect requiring complex reconstructive surgery and complex postoperative care and pain management. The hospital recovery is lengthy (four to six weeks) and is accompanied by the challenges of caring for young children and infants needing pain management, strict bed rest, and nutritional care that allows for wound healing.

Methods: In this paper, we review the literature and offer the experiences of a high-volume centre of excellence for bladder exstrophy care. We provide recommendations for epidural catheter placement, catheter tunnelling, postoperative care, and maintenance. We provide guidelines for multimodal medication management strategies and postoperative nutrition requirements.

Conclusions: The goals of care include 1) providing pain management to address incisional pain and bladder spasms and to protect the surgical repair and promote healing by reducing pelvic motion and constipation-associated straining; 2) promoting a successful pulmonary recovery; and 3) optimising nutrition for wound healing. Multimodal pain management using a combination of regional analgesia and oral/intravenous (IV) medications is needed for this challenging patient population. Care is intense and requires an experienced and dedicated interprofessional team.

Keywords: bladder exstrophy, epidural placement, epidural tunnelling, pain management, local anaesthetics, postoperative care, multimodal pain management, nutrition

Introduction

Classic bladder exstrophy is a major birth defect requiring complex reconstructive surgery. The nature of the repair of the exstrophy complex includes the major goals of correction of pelvic diastasis, bladder closure, and abdominal wall closure. The goals of postoperative care focus on the protection of the surgical repair through the recovery period, which balances the need to reduce pelvic motion and disruption of the bony union following osteotomies and to promote abdominal wall healing while attempting to normalise feeding and respiratory care in patients confined to bed rest for four to six weeks. At our institution, the pelvic closure approach is external fixation along with traction for postoperative immobilisation, requiring a lengthy recovery period.

There is abundant evidence that multimodal pain management enhances recovery by improving pain, reducing inflammation, and enhancing respiratory recovery and earlier feeding.[1] Regional anaesthesia or perioperative nerve blocks make it possible to reduce dependence on opioids. Kost-Byerly and colleagues previously reported on the successful use of epidural catheters for bladder exstrophy repair.[2,3] Neuraxial analgesia is just one component of postoperative care recommended for recovery after complex surgeries, and the use of epidurals as a component of anaesthesia in paediatric patients is safe.[4] In classic bladder exstrophy, the use of neuraxial analgesia together with traditional oral and IV opioid analgesia is essential. In this paper, we discuss and review the goals of postoperative pain management in patients having primary closure of classic bladder exstrophy, propose guidelines for multimodal pain management, and discuss epidural placement, its management, and highlight other techniques that lead to best outcomes.

Epidural placement

After induction of general anaesthesia, an epidural catheter is placed. Achieving complete analgesia via epidural can be challenging as many spinal levels need to be covered, extending from the lower thoracic to the sacral.[3,5] Therefore, it is merely used as an adjunct to the multimodal approach when safe and feasible. Catheters can be placed via the caudal region or direct low thoracic/high lumbar insertion. There are currently no catheter tip position studies in bladder exstrophy patients, but our institution's target level is T12. Caudally inserted catheter tips can be threaded to this level, but it is important to confirm correct tip positioning, which can be achieved via ultrasound or radiograph. Radiopaque catheters can be seen on radiographs, thus ideal tip placement can be confirmed without the use of contrast. Correct positioning of catheters that are not radiopaque can be confirmed via radiograph with an injection of OMNIPAQUE™ 180 (GE HealthCare, Inc., Princeton, NJ, USA).[6]

For providers proficient in regional anaesthesia and ultrasound use, ultrasound imaging can be used in young infants to confirm catheter placement. The absence of ossification of the infant's spine allows for excellent acoustic windows to visualise the epidural space and catheter tip.[7] However, by approximately nine months of age, vertebral ossification becomes more robust, impeding the transmission of ultrasound waves, and thus narrowing acoustic windows.[8,9] There are also stimulating catheters that can be used to thread epidurals to a desired location. Providers should utilise the technique that is most readily available and that they are most familiar with to confirm tip placement to maximise accuracy and minimise risks.

Some institutions prefer the caudal approach in young infants, as the risk of spinal cord injury may be less given the more caudate termination of the cord. However, compared to lumbar or thoracic insertion sites, the caudal insertion site is associated with an increased risk of catheter colonisation after three days.[10] Even when placing a catheter via the lumbar or thoracic approach, leaving the catheter in for more than five days is not advised given the risk of infection, unless it is tunnelled. At our institution, all bladder exstrophy epidural catheters are tunnelled, regardless of the insertion site, as they remain in place for 14 days. Tunnelling also decreases the risk of premature catheter dislodgement. Patients can be positioned either in the lateral decubitus or prone position, depending on the approach. The epidurals are initially placed either via a direct thoracic or high lumbar approach and threaded 3–4 cm into the epidural space, or via the caudal approach and advanced to the desired position.

The steps of tunnelling are shown in Figure 1. The catheter is injected with a 0.1 ml/kg test dose of lidocaine 1.5% with epinephrine 1 : 200 000 (max dose of 3 ml) before dosing with bupivacaine or ropivacaine for surgical and postoperative analgesia. Although tunnelling of epidural catheters is ideal, it is not necessary and should not preclude the placement of an epidural catheter; it should just impact the duration of use. Unfortunately, if epidural catheter placement is not possible, there are no other regional anaesthesia techniques that would provide comparable analgesia, both regarding efficacy and duration. A single injection caudal block or bilateral truncal block could provide intraoperative analgesia, but their duration would be limited in the postoperative period.

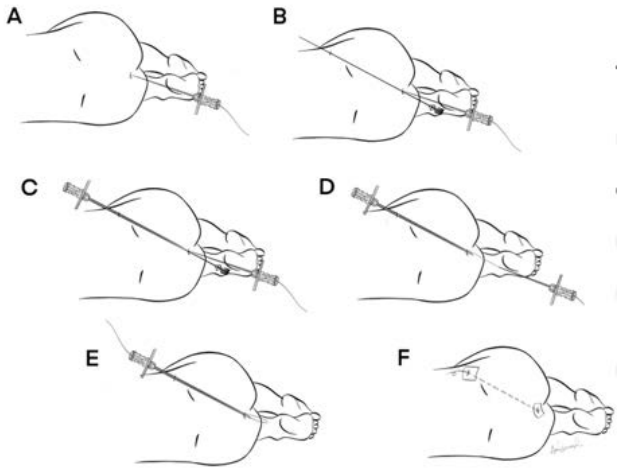

Figure 1: Steps for tunnelling an epidural catheter
A. Standard approaches to caudal, lumbar, or thoracic epidural spaces with an 18-gauge, 2-inch Tuohy (lumbar or thoracic) or Crawford (caudal) needle and catheter advanced to the desired position.
B. A 22 g spinal needle is inserted at the entry site of the Tuohy (or Crawford) needle and advanced under the skin just superior to the iliac crest where it emerges.
C. The spinal needle functions as a guide for a 17 g Touhy needle, without a stylet, until it emerges at the primary needle insertion site.
D. The spinal needle and the original Tuohy needle are then removed, keeping the catheter in place.
E. The catheter is carefully fed through the tunnelling Tuohy needle until the tip emerges at the exit site. Care must be taken to not shear the catheter by the tip of the tunnelling Tuohy needle.
F. The 17 g Touhy needle is then removed, and the catheter is gently pulled to the exit site, being careful not to let the catheter rotate and kink as it is pulled under the skin. The catheter should fall below the skin level and skin glue can then be applied to both the insertion and exit sites. A clear dressing is then applied to the exit site.

Regional anaesthesia safety data

Caudal epidural blockade is one of the most common regional anaesthesia techniques in children. Despite the many benefits of caudal epidural analgesia, it does have some disadvantages. It is time and resource-intensive, requiring a system for regular follow-up, often using a paediatric acute pain service. There are also specific patient contraindications, including infection at the site, coagulopathy, or spinal dysraphism.[11] If there is concern of a spinal/meningeal abnormality, a preoperative anatomic evaluation should be performed by ultrasound or magnetic resonance imaging (MRI). Preoperative laboratory testing is only indicated if the patient or family members have a bleeding history.

Regional anaesthesia can be safely performed in paediatric patients. Data from a United States large multicentre consortium, the Pediatric Regional Anesthesia Network (PRAN), as well as the European multicentre audit, the French-Language Society of Paediatric Anaesthesia (ADARPEF), report a very low overall complication rate.[12-14] Complications and adverse events are generally defined as neurologic (paraesthesia or neurologic deficit), local anaesthetic systemic toxicity (LAST), infection (generally localised superficial or deep tissue/abscess, rather than widespread infection), vascular (haematoma or puncture), respiratory, catheter malfunction, and dural puncture.[13]

In a study of over 100 000 blocks from the PRAN database, no difference in the risk of neurologic problems comparing caudal to lumbar or thoracic approaches was observed. Most neurologic complications were sensory in nature, resolving over weeks to months with only two cases persisting beyond three months. There were also no cases of permanent motor deficit noted. Ecoffey et al.[4] reported only eight complications related to caudal blocks out of a total of 31 132 regional blocks: six dural taps without postdural puncture headache, one nerve injury, and one local anaesthetic toxicity. Polaner and colleagues analysed data from 18 650 caudal blocks and reported an overall complication rate of 1.9%.[12] The most common complication was block failure with no cases of long-term sequelae.

The incidence of LAST reported by PRAN is overall extremely low at 0.76/10 000.[13] However, children are at a higher risk of toxicity due to two age-dependent factors. The first contributing factor to this increased LAST risk is the immature metabolism of the cytochrome P450 system. This system is immature in young infants and can lead to higher amide local anaesthetic concentrations. The second contributing factor to the increased LAST risk is the altered plasma concentrations of alpha-1-acid glycoprotein (AGP). The low concentration of AGP results in less protein binding to the local anaesthetic, resulting in a high free fraction in circulation.[11] The greater volume of distribution mitigates some of this risk for a single injection, but continuous infusions can result in drug accumulation.

LAST can present with neuro or cardiac manifestations. Haemodynamic instability and cardiovascular collapse should be

treated with Intralipid® 20% along with reduced doses of epinephrine (1 μg/kg) for cardiopulmonary resuscitation. The Intralipid® should be administered as an initial bolus of 1.5 ml/kg IV over one minute, which can be repeated every 3–5 minutes up to a max dose of 10 ml/kg, followed by an infusion at 0.25 ml/kg/min. Neurotoxic seizures should be treated with midazolam 0.05–0.1 mg/kg IV. Propofol, vasopressin, calcium-channel blockers and beta blockers should all be avoided.[14]

Haemodynamic effects of epidurals in infants

It is traditionally taught that infants receiving neuraxial anaesthesia suffer fewer haemodynamic alterations than adult patients. Although the haemodynamic response in infants and children is not completely understood, several studies show that neuraxial blocks in combination with general anaesthesia are well tolerated by neonates and young children.

In one study of children under 10 kg, authors used transoesophageal Doppler monitoring to better understand the haemodynamic changes with epidural plus general anaesthesia. Children anaesthetised with sevoflurane plus epidural bupivacaine containing epinephrine experienced significant decreases in heart rate and systolic and diastolic blood pressures, accompanied by increased stroke volume resulting in an unchanged cardiac output.[15]

Another study compared general anaesthesia either to general anaesthesia plus plain caudal bupivacaine or to general anaesthesia plus caudal bupivacaine containing epinephrine in a study of neonates. Haemodynamics were measured by transthoracic echocardiography. The authors concluded that although there was a significant decrease in systolic blood pressure in the epidural groups, vasodilation related to sympathetic blockade is minimal in neonates.[16]

In another study of children ranging from six months to seven years, study participants were randomised into caudal blockade plus general anaesthesia or balanced general anaesthesia. Haemodynamic responses were measured by standard monitors plus transoesophageal Doppler. No haemodynamic differences could be detected between groups and both had good haemodynamic stability.[17]

More haemodynamic studies are needed to further elucidate haemodynamics in infants anaesthetised with epidural plus general techniques. However, three concepts are postulated for the differences in haemodynamic responses to epidural dosing in children versus adults: 1) lower basal sympathetic tone in children; 2) smaller blood volume in children's legs (resulting in less pooling in lower extremities subjected to sympathectomy); and 3) the lower concentration of local anaesthetic used in paediatric epidurals.[16]

Local anaesthetic metabolism and dosing

Bupivacaine and ropivacaine are amide local anaesthetics commonly used for epidural analgesia and anaesthesia in children. Because anaesthetic techniques in children also typically include general anaesthesia, the intraoperative concentrations of bupivacaine and ropivacaine used often fall into analgesic concentrations of 0.25% or lower. Although these are subanaesthetic doses, such concentrations help to reduce requirements for opiates and hypnotic agents and typically allow for doses to remain under toxic limits. Even though bupivacaine has been used for many years, ropivacaine, the S enantiomer of bupivacaine, is less toxic. When plasma concentrations of bupivacaine and ropivacaine were compared, it was found that peak plasma concentrations of the two medications were similar, but ropivacaine plasma levels remained higher for longer.[18] Ropivacaine is also reported to have a longer duration of action. This may be explained by a faster rate of absorption of bupivacaine from the epidural space as well as a higher volume of distribution, as it is more likely to accumulate in fat and neural tissues due to its higher lipid solubility.[18]

Some anaesthesiologists advocate for the use of chloroprocaine in the infant population. This is because of the increased risk of local anaesthetic toxicity in this age group. Amide local anaesthetics (lidocaine, bupivacaine, ropivacaine) are metabolised by the liver, whereas ester local anaesthetics (chloroprocaine) are metabolised by plasma cholinesterase. The increased risk of LAST with amide local anaesthetics demands strict attention to dosing, especially when continuous infusions are used for a long period. Plasma bupivacaine levels have been observed to rise at 48 hours of continuous infusion despite limiting the infusion rate to less than 0.2 mg/kg/h.[19] In contrast, ropivacaine was not found to accumulate in neonates with infusions of 0.2 mg/kg/h, yet its clearance is still age-dependent.[20] Studies of chloroprocaine safety and efficacy are needed, especially related to continuous infusions. However, the existing literature suggests that despite the possibility of lower plasma cholinesterase levels in neonates, the half-life of chloroprocaine in neonates is as short as in adults.[21] Additionally, case reports of inadvertent vascular injection of chloroprocaine producing cardiovascular or neurological toxicity were self-resolved within seconds.[21,22] It is important to note that there is a higher risk of allergic reaction to the ester anaesthetics. Guidelines for dosing epidural local anaesthetics are provided in Table I.

The goal of epidural analgesia is to maximise local analgesia while lessening the need for systemic opioid use.[23] This technique allows for the patient to be more alert and interactive while facilitating early feeding. It is important to educate healthcare teams so they understand that epidurals administering dilute anaesthetics do not cause motor blockade and the lower extremities may be weak but should still be able to move. Therefore, for the patient to remain immobilised, sedation may also be required despite adequate analgesia.

Although there are no formalised recommendations on duration of use, current practice is to remove catheters by day 14 to reduce the risk of bacterial infection. Tunnelled epidural catheter sites are closely monitored to ensure catheter patency and for signs and symptoms of infection or bleeding.[24] Physical exams should occur at least daily and include an assessment of both motor function and sensory levels in addition to visible inspection of the tunnelled catheter exit site. The most common signs and symptoms of infection include erythema or fluctuance around the insertion or exit site, back/hip pain, and fever.[24] If infection is suspected or if a

Table I: Local anaesthetic dosing for epidural

Drug name	Dosing	Administration notes
Ropivacaine 0.1–0.375%	0.2–0.4 mg/kg/h Max dose 0.5 mg/kg/h	Ropivacaine and bupivacaine can be given by bolus every 90–120 minutes, or by infusion. The volume and dose of a bolus depend on the position of the catheter tip. Subsequent bolus doses are reduced by 30–50% of the original bolus. Subsequent chloroprocaine dosing is best administered by infusion due to its rapid hydrolysis by pseudocholinesterase.
Bupivacaine 0.125–0.25%	0.2–0.4 mg/kg/h Max dose 0.5 mg/kg/h	
Chloroprocaine 1–3%	5–7.5 mg/kg/h Max dose 9 mg/kg/h	
Lidocaine	0.6–0.8 mg/kg/h Max dose 1.4 mg/kg/h	

Table II: Medications, dosing, indication, and potential side effects

Drug name	Dosing/route of administration	Indication	Potential side effects
Opioids			
Morphine	IV 0.05–0.1 mg/kg/dose q2–4h PRN Also used as PCA with basal/bolus options	Acute pain	CNS and respiratory depression, nausea, vomiting, constipation, hypotension, and urinary retention; may cause histamine release causing itching and bronchospasm
Hydromorphone	IV 0.01–0.02 mg/kg/dose q2–4h PRN Also used as PCA with basal/bolus options	Acute pain	CNS and respiratory depression, nausea, vomiting, constipation hypotension, and urinary retention Note: May cause less pruritus than morphine
Fentanyl	IV 0.5–1 μg/kg/dose q1h PRN Also used as PCA with basal/bolus options	Acute pain	Bradycardia, pruritus, respiratory depression, chest wall rigidity, and hypotension
Oxycodone	PO 0.5–1 mg/kg/dose q4h PRN	Acute pain	CNS and respiratory depression, nausea, vomiting, constipation, and histamine release
Methadone	IV or PO 0.05–0.1 mg/kg/dose q6–12h	Acute pain, potential withdrawal from opioids	CNS and respiratory depression, hypotension, and bradycardia Note: Long half-life of 19 h in children, accumulation may occur and require dose readjustment
Butorphanol	IV 0.03–0.5 mg/kg/dose q4h PRN Max dose 2 mg	Sedation, pruritus, acute pain	Drowsiness, dizziness, insomnia, nausea, and vomiting
Non-opioid adjuncts			
Acetaminophen	IV, PO or PR 10–15 mg/kg/dose q4h PRN	Acute pain	May increase the toxicity of barbiturates
Diphenhydramine	IV or PO 0.75–1.5 mg/kg/dose q6h	Sedation, pruritus, nausea, or vomiting	Sedation, nausea, vomiting, and blurred vision; may cause paradoxical reactions in young children
Phenobarbital	IV or PO loading dose 15–20 mg/kg divided q6h, followed by 3–5 mg/kg/24 h divided BID	Sedation	Drowsiness, hypotension, and cognitive impairment; use with caution in renal/hepatic impairment Note: Will need trough 10–14 days after continuous dosing
Clonidine	PO 0.5–2 μg/kg/dose Transdermal patch also available Max dose 25 μg/kg/24 h	Sedation, acute pain	Bradycardia, hypotension, dizziness, fatigue, and constipation Note: Cannot stop abruptly; taper dose when stopping
Dexmedetomidine	IV starting dose 0.3 μg/kg/h Max dose 2 μg/kg/h	Sedation	Bradycardia and hypotension Note: Can only be used in ICU/OR and cannot stop abruptly; taper dose when stopping
Bladder spasms			
Diazepam	0.05–0.2 mg/kg/dose q3–4h Max dose 0.6 mg/kg within 8 h period	Bladder spasms, sedation, anxiolysis	Hypotension and respiratory depression
Lorazepam	IV or PO starting dose 0.05–0.1 mg/kg/dose q4–6h PRN Max dose 2 mg/dose	Bladder spasms, sedation, anxiolysis	Respiratory depression, dizziness, and mood changes; may cause paradoxical reactions in young children (10–30% in < 8 years)
Oxybutynin	PO 0.1–0.2 mg/kg/dose q6h Max dose 15 mg/day Transdermal patch: ½ patch ages < 2 years, full patch ages > 2 years	Bladder spasms	Anticholinergic effects: drowsiness, confusion, constipation, blurred vision, dizziness, and dry mucous membranes Transdermal patches bypass first-pass metabolism by the gut and thus reduce constipation

BID – twice a day, CNS – central nervous system, ICU – intensive care unit, IV – intravenous, OR – operating room, PCA – patient-controlled analgesia, PO – by mouth, PR – per rectum, PRN – *pro re neta* (when necessary)

fever of unknown source occurs, the catheter should be removed immediately. MRI should be considered if any neurologic impairment is noted.[24]

Multimodal postoperative pain management

After the closure of the bladder in the operating room, it is imperative to have effective postoperative pain control and medical immobilisation for successful outcomes. To achieve effective immobilisation, the patient will need to receive multimodal pain and sedation management, usually managed by a paediatric pain management specialist. Regardless of the type of postoperative immobilisation strategy, effective immobilisation should strive to minimise or eliminate hip flexion or lateral rocking of the patient's hips. Immobilisation is especially important during the first two weeks, as this stresses both the soft tissue and bony pelvic closures and can lead to failure. During this time, the sedation regimen is titrated to achieve immobility yet allow optimal ventilation. Care in the paediatric intensive care unit (PICU) can be considered if needed for titration of pain/sedation medications when patients are difficult to sedate optimally. The most used medications for this type of immobilisation management include local anaesthetics through tunnelled epidural catheters, opioids, non-opioid adjuncts, alpha-2 agonists, and benzodiazepines. Pain and sedation scores are monitored.[2,23]

The two most common types of surgical pain that are felt in the immediate postoperative period are surgical/incisional pain and bladder spasms. Surgical pain management is most important in the first and second week, which is when a continuous epidural infusion is used. After two weeks, the patient's incisional pain is reduced and the epidural infusion and opioids can be weaned with the hope of requiring only as-needed medications before discharge. Medication management is then focused on immobility and bladder spasms. Some patients are discharged with a medication-weaning schedule.

Intermittent IV opioids are used as first-line pain relief with epidural pain management. If several doses are needed, IV patient-controlled analgesia (PCA) is utilised for pain management. Table II summarises medications used for pain and sedation at our institution, including dosing recommendations, indications, and side effects. Considering that not all hospitals carry the same pharmaceuticals, each individual practice should design a regimen with their stock medications that will lead to the best possible outcomes for their patients. Postoperative bladder exstrophy care requires a high-intensity, well-planned strategy that is most likely to succeed if it is multimodal.

Bladder spasms occur due to the normal postoperative healing process as well as the urinary tubes used to divert urine flow, which are very irritating to the newly closed bladder. Typical signs and symptoms of bladder spasms include arching of the back, sudden irritability, spraying of urine through and around the urinary tubes, sudden awakening from a sound sleep, new bloody drainage from the suprapubic tubes, sudden reaching for the diaper area in pain, vocalisation of sudden scrotal or penile pain, or vocalisation of the severe urge to urinate. Epidural analgesia may decrease pain from bladder spasms but, due to the dual innervation of the bladder (Figure 2), patients often require supplementation with spasmolytics and/or muscle relaxants.[2] Typically, at our centre, patients are ordered around-the-clock oxybutynin to reduce the occurrence and severity of bladder spasms, while diazepam is used more often to treat acute episodes of bladder spasms.

Constipation or straining during bowel movements can be major contributors to postoperative bladder spasms and should be avoided. Patients with bladder exstrophy have an anterior anus, distorted pelvic floor anatomy, and sphincter displacement, which predisposes them to constipation.[3] At our institution, patients are typically started on glycerine suppositories or polyethylene glycol within the first few days after bladder exstrophy closure to avoid this.

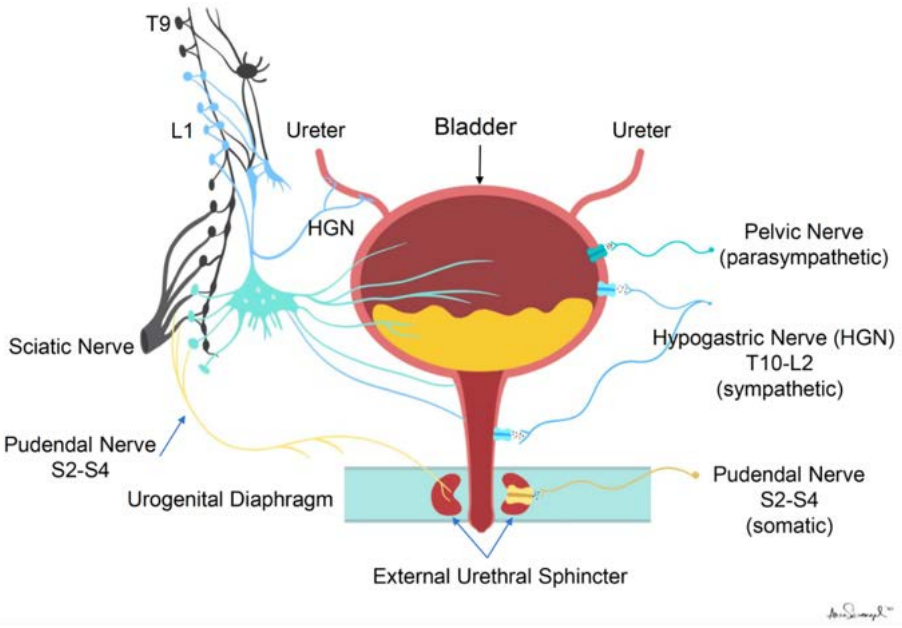

Figure 2: The complexity of bladder innervation; the image shows the range of neural involvement and the implications for the necessity of intentional epidural catheter placement to achieve the best postoperative pain management (adapted from Fowler et al.[5])

Postoperative nutrition

To minimise abdominal distention during the immediate postoperative period, a nasogastric tube is recommended to decompress the stomach. Patients often have an expected, prolonged postoperative ileus. Patients are typically given parenteral nutrition on postoperative day one, if under two years of age or at baseline nutrition risk. Other patients should have parenteral nutrition started if there is no return of bowel function or persistent abdominal distention by postoperative day five. For all patients, once there is a return of bowel function and minimal abdominal distention, feed slowly with trickle enteral feeds via a nasogastric tube, clears by mouth, or breastmilk, and slowly advance to full feeding or a regular diet while watching for abdominal distention that can lead to bladder closure failure.[3] When patients are not meeting their nutritional goals, nasogastric tube feeding can be used supplementally. Because nutrition is so important for postoperative healing, dieticians should be used to optimise and meet caloric goals for all exstrophy patients. Ultimately, optimal nutrition includes developmentally appropriate nutrient-dense food sources such as breast milk, formula, lean meats, dairy, and fruits and vegetables.

Conclusions

Postoperative care for primary bladder exstrophy repair is a complex endeavour requiring a well-informed multidisciplinary team led by protocols of care involving regional and IV medication regimens. Multimodal care is essential. In this paper we have provided a comprehensive review of recommended care options and Table III summarises the key points.

Table III: Key points regarding postoperative pain management in primary bladder exstrophy closure

Good postoperative immobilisation is defined as minimal flexion or lateral rolling of the hips when in external fixation and traction.
Multimodal analgesia is critical after a primary bladder closure with or without osteotomies.
• Adjuncts to epidural analgesia include opioids, benzodiazepines, and bladder antispasmodics
• Daily paediatric pain management requires examination of the epidural site and effectiveness, as well as assessment of pain control, immobilisation, and sedation effectiveness
• Constipation can increase bladder spasms and stress the surgical repair; opioids frequently cause constipation
• A daily bowel regimen should be instituted and optimised
Paediatric regional anaesthesia is safe and effective.
• Tunnelling the epidural catheter extends its use from three days (non-tunnelled) to 14 or more days postoperatively
• LAST is treated with Intralipid® 20% and reduced doses of epinephrine
• Ropivacaine is less cardiotoxic and preferred over bupivacaine in infants > 3 months of age; chloroprocaine is preferred in infants < 3 months of age due to concerns of immature metabolisation and protein binding

Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

The authors declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the Second World Conference on Research Integrity in Singapore, in 2010. This work is exempt from the Institutional Review Board approval because it did not involve human subjects.

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