

## Role of Anesthetists in Human Embryonic Stem Cells Transplantation in Patients with Spinal Cord Injury

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### Abstract

Spinal cord injury (SCI) is a devastating disorder affecting millions across the world. Still, there is no standard approach for the treatment of SCI. The concept of regenerative medicine using the stem cells to repair tissues has become a reality of new era. Human embryonic stem cells (hESCs) have boosted as a new therapeutic strategy to treat SCI. The implantation of hESCs with reduced pain is the major concern for physicians. Our institute has been using specialized procedures to implant hESCs in a SCI patient via anesthesia techniques. The implantation of hESCs as well as the induction of anesthesia requires an employment of a skilled anesthetist. The authors focus on the novel approach, i.e., use of epidural and caudal routes for introduction of hESCs, as well as the role of an anesthetist in implantation of hESCs in the patients of SCI. An epidural injects hESCs into the region outside the duramater of the meninges whereas; hESCs implantation through the sacral membrane which is approximately three centimeters above the tip of the coccyx and is in continuum with the epidural space is achieved by caudal route. An anesthetist has an edge over the others due to his skills and knowledge regarding anesthetic agents, routes of administration as well as pain controlling strategy. He plays a significant role in hESC transplantation by evaluating a patient's condition, protocol development along with a suitable health care management. Anesthetists might contribute to comprehensive patient care by encouraging the optimal use of multimodal regimens, as well as in implementing novel techniques, ensuring improvement in pain control and minimize adverse events.

**Keywords:** Transplantation; Epidural; Caudal; Anesthetist; Stem cell therapy; Lignocaine; Implantation

### Introduction

Spinal cord injury (SCI) is a devastating ailment affecting millions across the world [1,2]. It results in severe sensory and motor deficits due to the poor regenerative capacity of the adult spinal cord. It is generally a sports, motor vehicle crashes/accidents or fall related injury. It leads to paralysis, loss of sensory and motor functions, accompanied with other multiple health problems such as urinary, cardiac and respiratory dysfunction which deteriorate the quality of life of the patient [3,4]. A series of cellular and biochemical events occurring during the injury are followed by a cascade of reactive changes such as inflammation, hemorrhagic necrosis, edema and demyelination. They, together, result in loss of neurons and myelinating oligodendrocytes, vascular destruction, scarring and axonopathy accompanied with de-nerivation below the central lesion site and cell death [5,6]. The loss of oligodendrocytes and demyelination also leads to the progressive and delayed degeneration of residual axonal tracts [3].

While targeting towards treating or reducing the reactive changes, cell therapy has been investigated as a promising tool to treat SCI. Neural stem cells (NSCs), adult stem cells such as mesenchymal stem cells (MSCs), etc. are the commonly used cells for the treatment of SCI [7]. Characterized by the potential of self-renewal, pluripotency and unlimited propagation, human embryonic stem cells (hESCs) might be a reliable option for cellular replacement therapy. They possess the capability to differentiate and proliferate, as well as produce

undifferentiated hESCs in large number which have the tendency to differentiate under controlled conditions is another important factor to consider them potent [8-10].

The procedure of hESCs implantation is not an easy task to perform where determination of the anatomic location of implantation site is one of the major concerns during the use of hESCs. High cell concentration within the region of interest is the main target for the physician. However, the physicians also prevent the dwelling of cells into other undesirable sites/organs. Intracoronary, transendocardial, transpericardial, intraventricular, intravenous, intramyocardial, etc. routes of catheterization/administration have been previously reported [3,11]. Further developments of catheterization systems for various clinical studies involve various other routes for administering hESCs such as intramuscular, intravenous, intrathecal, epidural, caudal, brachial plexus, popliteal and/or deep spinal muscle injection, etc.

At our institute, the epidural as well as caudal routes have been regularly used for implantation of hESCs during the treatment of SCI. Both the routes involve a specialized procedure requiring anesthetists for the transplantation of hESCs. Anesthesia, being a vital part of transplantation, enables a patient to undergo painless and distress free treatment/operation. Role as well as effect of anesthesia in the implantation of hESCs through various routes is of major concern [12,13]. Of the major three types of anesthesia, viz., general, regional and local, the former two require anesthetists [14]. The present study explains the caudal and epidural method of implantation of hESCs for the treatment of SCI, emphasizing on the role of anesthetist in introduction of hESCs in SCI patients.

## Ethics statement

Patients with SCI presented in the institute for hESC therapy. A clear and comprehensive appreciation accompanied with an understanding of the facts, implications, and consequences of the hESC therapy was provided to the patients/guardians. A video graphed and signed informed consent was obtained from each of them, prior to the treatment. The study protocol is approved by an independent ethics committee and is overseen by an institutional review board. The study is conducted according to the declaration of Helsinki [15].

## Cell culture and differentiation

Institute possess a certified laboratory following good manufacturing practice (GMP), good laboratory practice (GLP) and good tissue practice (GTP) using proprietary in-house technology (United States Granted Patent No. US 8592, 208, 52). All the SCI patients are treated as per our standardized protocol that has been validated after establishing safety and efficacy of hESC in SCI [16].

Non-neuronal and neuronal cell lines are obtained from a single, spare, expendable, pre-implantation stage fertilized ovum taken during natural *in-vitro* fertilization (IVF) process with due consent. The cells are cultured, maintained and stored in syringes for further use. The pre-filled, frozen syringes are thawed when required. The cells undergo quality analysis for determination of integrity, viability and microbial contamination [17].

## Cases

The cases diagnosed with SCI were admitted in the institution. On assessment, they were diagnosed as either paraplegic or quadriplegic. The paraplegics receiving caudal, epidural and both were 66, 20 and 43, respectively. Similarly, the quadriplegics receiving caudal, epidural and both were 27, 12 and 23, respectively. The patients were handled by skilled and experienced anesthetists and physicians.

## Prior to transplantation procedure

Before the transplantation procedure, the physician of our institute keeps a record of allergic reactions, medications used by the patients. The patients are asked to avoid drinking and eating anything after midnight, the night before the transplantation. The administration of medicines is also done with a small amount of water, *i.e.*, a sip. The consumption of alcohol and smoking is also restricted before, during and after the procedure.

## Transplantation procedure

The transplantation/implantation of hESCs takes place *via* surgery. Patients are asked to change into a hospital gown, which allows an access to clean the injection area as well as to allow the physician to easily visualize the injection site. After shifting the SCI patients to operation theatre, the hESCs are introduced to the patients using a special procedure involving trained anaesthetists.

**Epidural procedure:** An "epidural" injection or catheter infusion involves the administration of hESCs into the region outside the duramater of the meninges.

The patient is positioned in knee abdominal position in left lateral posture. The lumbo-sacral or lumbo-thoracic area (as per requirement) is thoroughly cleaned using antiseptic agents followed by draping with a cut sheet. As the epidural procedure might lead to

discomfort, the area is locally anaesthetized with 2% lignocaine using 26 gauge (G) needle.

Following the method of loss of resistance (LOR), the epidural catheter is introduced and is fixed depending on the level of injury. The patency of the epidural catheter is assessed by injecting normal saline. After ascertaining the strength, approximately 0.5-0.7 ml of hESCs are transplanted per vertebral space. This is followed by multiple dosing of hESCs using same catheter *in situ* through an infusion pump at the rate of 60 ml/hour. As the hESCs possess a shelf life of half an hour, they are introduced in 5-10 minutes. In case of higher level of injury, *i.e.*, high thoracic or cervical injury, the lumbar catheter is replaced by a single shot cervical epidural injection.

The patients may feel tenderness at the site of needle insertion for a few hours after the implantation which can be treated by applying an ice pack for 10 to 15 minutes once or twice a day. The patients are asked to rest for the remainder of the day whereas; normal activities may typically be resumed the following day. A temporary increase in the pain can occur for several days after the injection due to the pressure of the fluid injected or due to local inflammatory response.

In addition to understanding the general protocol and time involved in the procedure, the patients are advised to discuss with their clinician and physician whether pain medications (or certain other medications) can be taken on the day of the injection.

The patient may receive fluids intravenously. A constant check on pulse, blood pressure and oxygen levels is kept during the entire procedure. After the transplantation procedure, the catheter is removed from the back and the patient is asked to lie on bed for some time till he/she feels fine to move.

The epidural procedure is contraindicated if the patient is suffering from fever/local sepsis/local infection or is on anticoagulant therapy (which may lead to coagulopathy) [18].

**Caudal procedure:** Administration of hESCs through the sacral membrane which is approximately three centimeters above the tip of the coccyx and is in continuum with the epidural space can be achieved by a "caudal" injection.

Using the technique of LOR, hESCs are also transplanted through the caudal route in the caudal epidural space (one of the supplemental routes). The patient is asked to lie in a fetal position. The underlying sacral hiatus are located using the skin folds of the buttocks. With the tip of the coccyx in the natal cleft, the thumb of the same hand is used to palpate the sacral cornua. The sacral area of the patient is cleaned thoroughly using an antiseptic solution and is properly draped. The area is anaesthetized using a local anaesthetic (lignocaine 2%) which is performed by an anesthetist. A special needle of 26 G (2-inch) is introduced through sacro- coccygeal membrane at an angle of 45°. A distinct sound "pop" is felt due to loss of resistance which indicates that the positioning is accurate. The needle is further penetrated parallel to the sacrum. Thereafter, 5 ml of air is injected with hands over the side of the needle tip. On feeling no air and tissue resistance to injection under the skin, hESCs are introduced using a 26 G needle. A number of complications arise while following a caudal procedure. The physician should take care of the site of insertion of needle. If the needle has been inserted correctly, it will swing easily from side to side at the hub while the shaft is held like a fulcrum at the sacro- coccygeal membrane and the tip moves freely in the sacral canal. An early resistance of insertion indicates its incorrect placement.

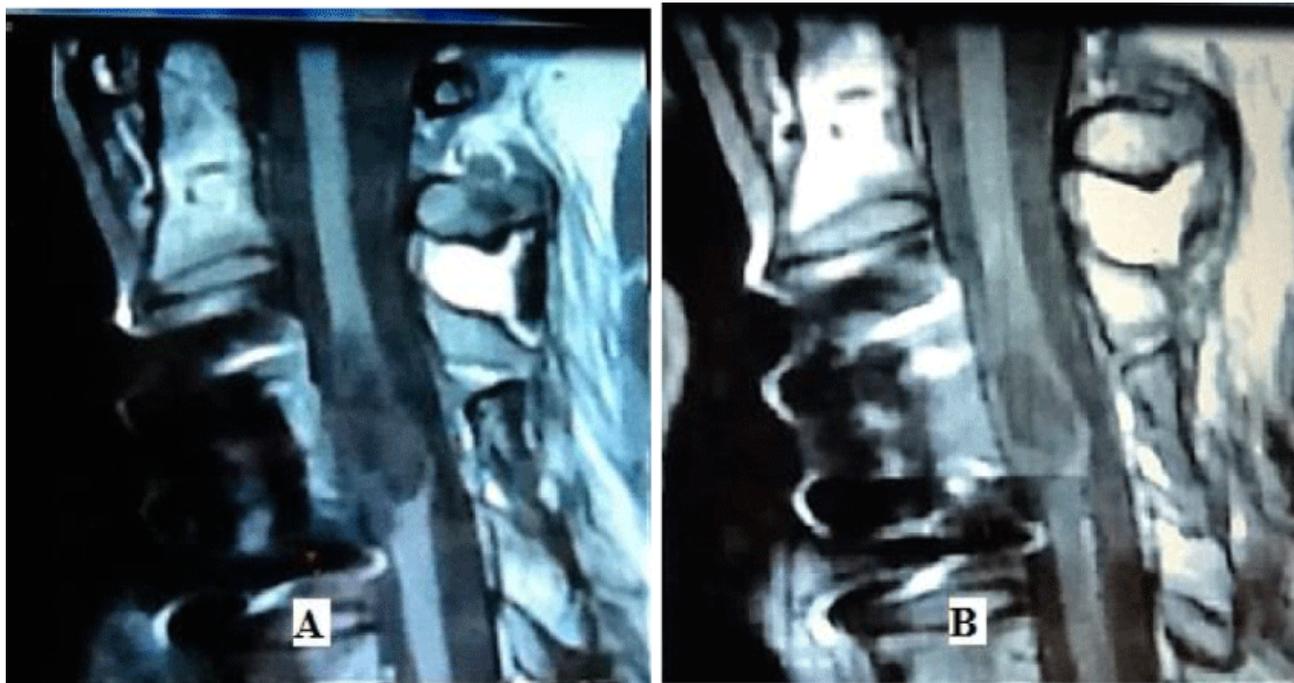
However, a caudal injection is not advised in case of patient refusal, infection at the site, hypovolemic shock, coagulopathies and pre existing neurologic disease.

### Improvement in spinal cord

The regeneration of spinal cord was observed in all the patients. There was a clinical as well as radiological improvement observed in

all the patients, followed by regeneration of spinal cord. Figure 1 represents the tractographic images (before and after hESC therapy) of a patient with SCI using magnetic resonance imaging (MRI).

During the treatment, no adverse effects or complications were observed.



**Figure 1:** Tractographic images of spinal cord of a patient with spinal cord injury undergoing human stem cell therapy. **A:** Before treatment; **B:** One year after treatment.

### Discussion

The SCI impairs the motor and sensory functioning of the body by damaging the white and grey matter and myelinated fiber tracts and causing myelopathy [19,20]. Recent advances in cell replacement therapy as well as the progression of neuro-protective and regenerative interventions have raised the ray of hope to treat SCI. Several therapeutic interventions such as surgery, physiotherapy, and other pharmacological interventions have been reported to improve the neurological recovery. However, no golden approach is effective in the replacement of damaged neural tissues by enhancing endogenous neural regeneration and anti-inflammatory effect [15].

hESC therapy is gaining attention in the world of cell replacement as these cells possess the potential to heal the damage caused by SCI such as replacement of damaged neurons, re-establishment of lost axonal connections, neuro-protection, etc. [21-23]. Many studies on embryonic stem cells derived from rats report the use of ESCs in treatment of injuries related to brain and spinal cord [24,25]. The effect of hESC therapy in patients with cerebral palsy, cortico-visual impairment, Friedrich ataxia and spino-cerebral ataxia has also been previously reported by the authors [17,26-28].

The institute has been using some specialized procedures for implantation of hESCs during the course of the therapy for the

treatment of SCI. It involves the use of caudal as well as epidural route of administration of hESCs. The epidural as well as the caudal injection procedure takes place by a specialized anesthesiologist [29,30].

An anesthesia focuses a rapid recovery, ensuring reduced pain, few complications, and minimal systematic changes during the entire transplantation period. The use of anesthesia during stem cell transplantation in the human body has also been reported in many studies [31,32]. Lignocaine is a popularly used local anaesthetic for spinal anesthesia. It is mainly used for short surgical procedures due to its predictable onset and dense sensory and motor blocking capacity for moderate duration. Though, many reports suggest the neuro-toxic effects of lignocaine arresting the use of lignocaine for spinal anaesthesia [33-35] However, some studies favor the use of lignocaine as an excellent and safe modality for patients undergoing surgery [36]. As lignocaine was used as an anesthetic agent prior to the procedure, no neuro-toxic effects were observed.

The anesthesia related transplantation surgeries focus on the evaluation of clinically significant outcomes such as quality of recovery, heartbeat, blood pressure, blood glucose level and oxygen level throughout [14]. The role of the anesthetists has an edge over other physicians in concern with providing pre and post-operative

medical care [37]. By optimizing pre-operative anesthesia care and avoiding postoperative adverse events (AEs) and complications, anesthetists are gaining importance for both out-patients and in-patients undergoing transplantation.

The anesthetists play an important role in evaluating patients during the entire procedure of transplantation, i.e., from pre-operative period to post discharge period. They prepare suitable anesthetic plans (requirement of blood for transfusion, technical support factors, etc.) after analyzing patients' medical history. He also keeps a fresh eye on any unexpected and life-threatening events (such

as blood loss and allergic reaction) occurring during the operation [14].

The transplant anesthesiologist is the one who plays traditional and non-traditional roles ensuring a successful outcome of the transplant procedure and program as a whole [38]. The benefits of the anesthesiologist can only be fully realized when they are incorporated into a comprehensive patient care plan involving induction of anesthesia as well implantation of hESCs [39]. Figure 2 represents the responsibilities undertaken by an anesthetist during the entire course of implantation of hESCs.

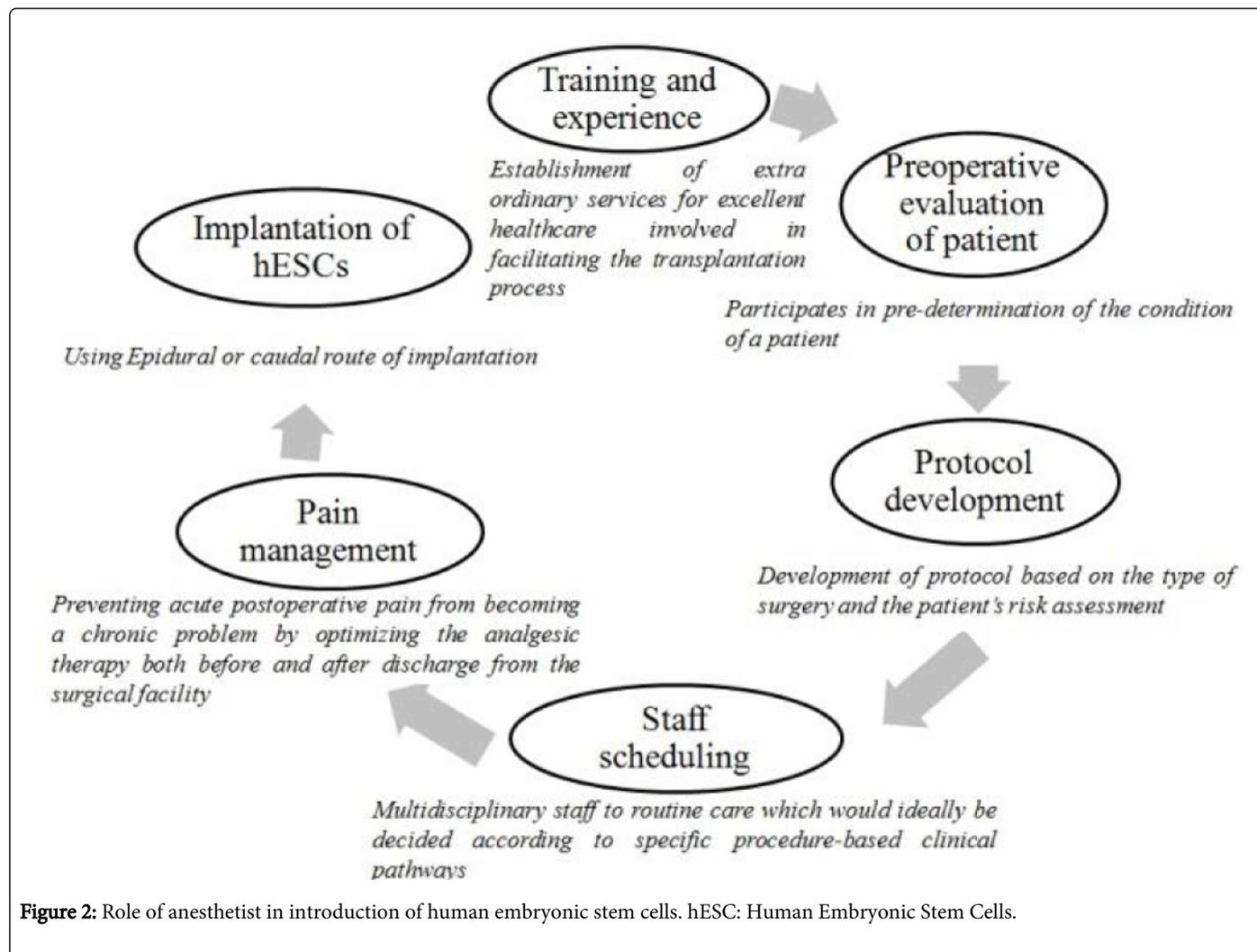
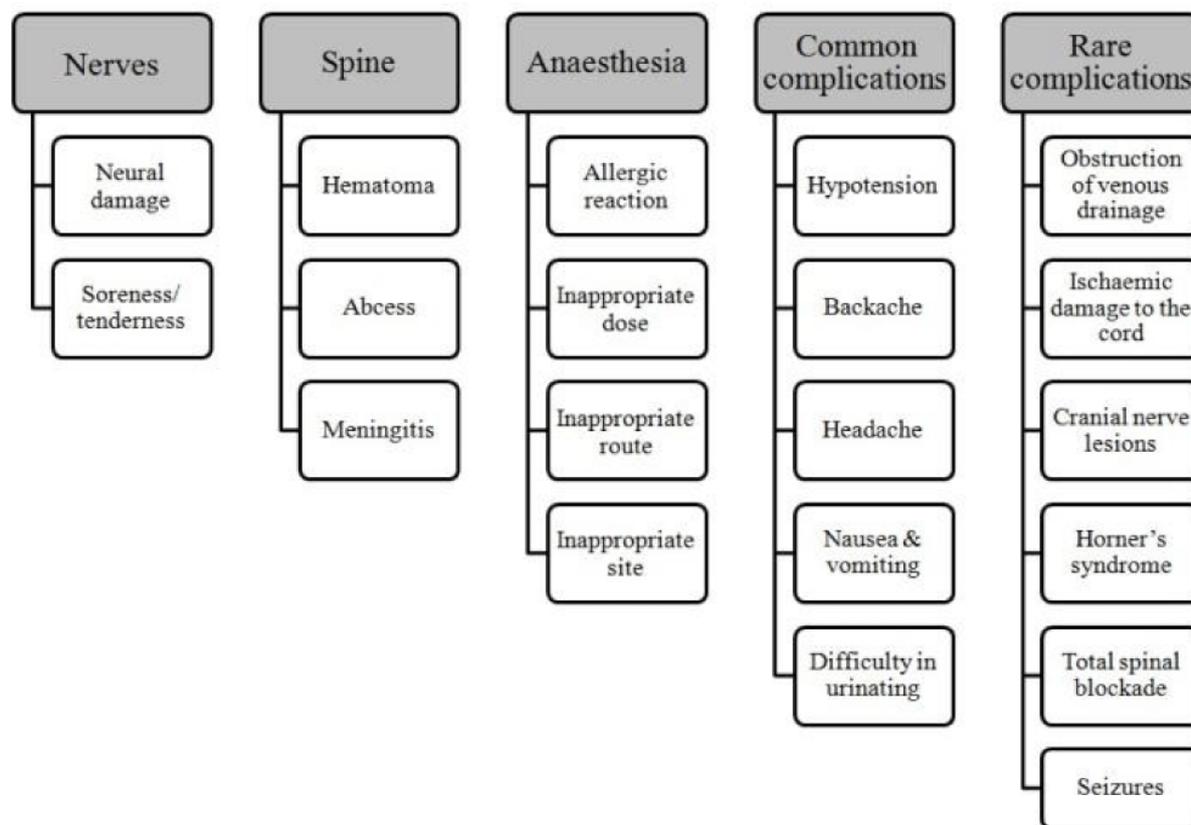


Figure 2: Role of anesthetist in introduction of human embryonic stem cells. hESC: Human Embryonic Stem Cells.

Stem cell transplantation involves a highly trained anesthetist to perform the implantation of hESCs. His task begins with training the other integral members participating in the transplantation procedure. His mentoring is followed by the initial evaluation of patients including follow ups which adds depth to the physician – patient relationship. Understanding the complexity of patients, he keeps an oversight on the multiple medical issues associated with the disease. The ultimate decision making meetings involve a protocol formation and review. It is followed by the selection as well as scheduling of staff which involves the selection of candidates to participate during the surgical transplantation. The complexity of the case with the

associated patho-physiology and risks requires 'one-on-one' attention from the trained, experienced, transplant anesthesiologist, and clearly exceeds the level of general physician. During the transplantation, the pain control is more effective due to the anesthetist – patient rapport developed during the consultation [38]. The anesthetists of the institution are special/different from rest as they have introduced hESCs in patients' body, instead of blocks or anesthesia for the first time, which makes the study novel. A focus of the risks associated with the route of administration of hESCs is also performed by the anesthetists (Figure 3).



**Figure 3:** Risks associated with epidural and caudal route of administration.

Taking appropriate steps in minimizing risks associated with transplantation, while understanding the behavior and importance of hESCs, is the major target to be achieved by an anesthetist. Selection of appropriate anesthetic in optimal dose while maintaining normal organ system function might lead to improved patient care at a reduced cost [40]. An absolute management and care should be considered as a multidisciplinary strategy to improve the outcome of patients undergoing surgery, rather than a sub- specialty limited to one medical profession. As a member of the multi- disciplinary team, the decisions of the anesthesiologist should have a direct impact on the ability to achieve a fast-track recovery after transplantation [38]. Reports support the improvement in outcome of a surgery by involvement as well management of anesthetist [38,41]. Furthermore, it also minimizes the common postoperative AEs which might lead to reduced or delayed recovery and prolonged hospital stay, accompanied with a better pain control and patient satisfaction [42,43].

## Conclusion

The role of the anesthesia techniques would ideally expand beyond the time of hospitalization since effective pain control and appropriate hESC implantation is as important as achieving successful convalescence. This is the right time for anesthetists to take a more active role as peri-operative physicians in implantation of hESCs with reduced pain and adverse effects. Future advances in hESC therapy require interdisciplinary collaborations involving anesthetists,

surgeons and nursing care [44]. However, diversity in the role of anesthesiologists' interventions beyond the operating and recovery rooms is also suggested.

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