SURGICAL PROTOCOL ACTIVATED FAT - EPO

The patient is a volunteer who wishes to undergo this operation, which means he or she is fully informed about the procedure of implantation of activated fat into the spinal cord. The patient has requested this treatment in anticipation of the beneficial effects of activated fat on his or her medical status.

1.The patient will sign an informed consent form and will undergo a series of tests including:

- Physical and Neurological examinations, with motor grading by the ASIA scale. This will provide a baseline to compare the safety of the activated fat implantation with the potential neurological benefits. An evaluation based on the Kunming scale will also be done before the surgery.
- An MRI of the injury level: this will allow identification of the lesion and surgical planning.
- Flexion/Extension plain films of the injured spine levels: this will help determine if spinal instability is present and whether or not a spinal stabilization procedure will have to be performed at the time of the operation and implantation of the activated fat.
- Somatosensory evoked potential (SEP) and motor evoked potential (MEP) of the lowers limbs.
- Biological parameters including complete blood count, and a possibly lumbar puncture with Cerebrospinal fluid (CSF) chemistry, protein and cell count. (cytochemical examination of the cerebrofluid liquid)
- Infectious parameters: AIDS, hepatitis B and C (part of the inclusion criteria)

2. Activated Fat Implantation and Surgery

The patient's adipose tissue is obtained from by elective liposuction under local anesthesia (Lidocaïne.) This procedure involves an infiltration step, in which a solution of saline and vasoconstrictor epinephrine (2µg/ml)

is infused into the adipose compartment to minimize blood loss and contamination of the tissue by peripheral blood cells. The final product will be called lipoaspirate adipose tissue (Lipoaspirate).

For mechanical activation, several 10 ml lots of washed lipoaspirated adipose tissue are collected in 50 ml tubes and placed into a sterile element which will bind if they are treated by this machine which exercises mechanotransduction at multiple levels of pressure and orbital force for a set amount of time.

The activated lipoaspirated adipose tissue obtained at this point is referred to as activated fat.

3.Surgery

The patient will be induced by general orotracheal anesthesia using standard neuroanesthesia techniques. Erythropoietin (EPO) (Eprex or similar) is administered prior to the skin incision. 35,000 Units of EPO are administered by slow i.v. infusion within 10-15 minutes (one injection pre and post-

op) This is repeated once every other day for 30 days, at 48 hours intervals. The patient is positioned into the prone position on the operating table; in cases of cervical lesions, the head can be fixed whenever necessary.

The spinal level of the lesion is identified with an X-ray intensifier. A skin incision is made for a multi-level laminectomy, usually on 3 or 4 levels, with the site of the lesion in the middle of the operating field.

Before opening the dura mater, the entire surgical field must be perfectly clean and without any bleeding. The dura mater is then opened all the way, with four stitches, two on each side. The dura mater is then fixed to the lateral muscles. At this point, surgery, continues with a surgical microscope. The first step is the removal of all the arachnoiditis tissue from the posterior face of the spinal cord and dura mater. Once this step has been completed, an entire separation of the arachnoiditis tissue is performed on both sides, down to the roots. The removal of all that this tissue off the dura mater and the spinal cord is achieved, without causing any vascular damage.

The spinal cord and roots can be mobilized laterally to make sure the same amount of tissue is removed on both sides. The ventral approach to the dura mater and the spinal cord is crucial since there are always strong adhesions between the pia and the dura mater. Whenever possible, the cord should be lifted to look for any bone angulation or rule out the presence of any bone fragment that could intrude into the canal and compress the spinal cord.

At the site of the lesion, the anterior dura mater has been opened, on one or both sides, to remove the adhesions on the posterior longitudinal ligament and to correct the bone canal. After complete closure of the anterior dura mater, the next step is to make a complete release between the pia and the dura mater, on all the exposed levels.

The injured spinal cord is in the middle of the operating field and accessible by a small posterior middle line or a line medial to the dorsal root entry zone, depending on whether the injury appears to be central or lateral to one side ; otherwise, a posterior lateral myelotomy is realized made to show the entire contusion area.

Adequate exposure of the gliotic cavity is made with minimal mechanical manipulation.

The gliotic tissue is removed as well as the walls of the cavity, without touching the white matter.

- The cavity is irrigated with artificial CSF and cleaned of debris.
- A biopsy of the borders of the operating site is done, using a micro-Codman for neuropathological analysis; this will allow the release of the accumulated neurotrophic factors at the border of the lesion.
- The activated fat implant is brought into the field and trimmed to fill the intramedullary cavity at the time of implantation.
- If possible, wrap the activated fat with the surrounding intact white matter.
- Make sure the vascular and neural structures are protected; Monitor homeostasis.
- The pia-mater is sutured with an 8-0 prolene suture on the operating field which allows the activated fat to remain in the cavity.

- The dura mater is closed using a 4-0 prolene suture, sealed without duraplasty (otherwise, use an autologous material such as fascia cover)
- Hemostasis of the extradural space using Horsley wax for the bony elements and bipolar coagulation for the epidural vein.
- Drainage of the extradural space (mild)
- An assessment of spinal stability is made, and stabilization of the spine using autologous bone graft if necessary.
- Wound is closed in layers of fascia cover and skin

Intra-operative ultrasound (IOU) would allow visualization of the spinal scar tissue, and of the post-traumatic cystic cavity prior to the myelotomy, to define and assess the extent of the myelotomy.

The duration of the the surgery is estimated between 3-5 hours. During the surgical procedure, photos and films of the spinal cord preparation and implantation of activated fat are made. In post-operative care, after the wake-up room, patients are nursed overnight in the Intensice care unit (ICU). They are transferred to the neurosurgical ward the next day and begin physiotherapy exercises as soon as possible.

Neurological assessments, urinalysis and blood tests are done after surgery, at one month, three months, 6 months, and one year post-op.

4. Post-op stage

This 12 month-period will focus on the subject‘s physical rehabilitation at the Tongren Hospital in Kunming, following a specialized program tailored to the patient’s needs, or at the Step by Step Center, in Barcelona, in accordance with the requirements of the Kunming Center.

The patient must demonstrate he or she is physically and psychologically capable and ready to participate in all aspects of the program.

The ultimate goal is to help patients achieve the maximum level of autonomy.

A specific training program will be tailored for each subject to meet his needs and physical abilities, but all training programs will follow the guidelines described below:

The patient should have demonstrated he or she is physically and psychologically capable and willing to participate in all aspects of the program.

The ultimate goal is to help patients become as independent as possible so they can get back to life in their community.

A specific training program will be designed for each subject to fit his needs and capacity but all training programs will follow the guidelines described below:

Body weight-supported treadmill training (BWSTT). Treadmill training, and/or over-ground training with partial body weight support have been shown to accelerate locomotor recovery.

This type of training, with a treadmill or exoskeleton, is currently used in rehabilitation programs after SCI.

Studies have demonstrated that coordinated stepping movements can be induced in patients with complete para/quadriplegia, trained to walk with their body weight partially unloaded, and with external assistance.

5. Complete outline of the rehabilitation program:

- Daily locomotor training on treadmill (speed at the beginning of the training goes from 0.2 m/s up to 0.6m/s, then with variable increase according to the patient's performance. Normal subject: l.5 -m/s.)
- Partial unloading of body weight (up to 80% of the weight may be unloaded); unloading the patient is achieved by suspending him or her from a parachute harness which is connected to an overhead crane at the pelvis level. The degree of unloading during one training session is provided by a scale on the crane.
- The physical therapists or physiotherapists monitor the movements of the legs on both sides, especially knee extension and the transition from stance to swing and vice-versa on each leg.
- The patient is trained to walk by correcting his or her gait and minimize compensation. Sensory stimulation will be encouraged to improve control of weight-bearing and gait swing phases by increasing speed, and decreasing weight support.
- Acupuncture and electrical stimulations at low frequency treatment.

6. Parameters to monitor during program are:

- Balance
- Walking speed (m/s)
- Walking endurance (min)
- Quality-of gait
- Muscle strength
- Spasticity
- Gait symmetry

7. Strength and endurance training.

The training will involve as many muscles available as possible including upper back, posterior shoulders, arms, available abdominal muscle, strained anterior muscles ; the program will be specific and will be performed on a regular basis 36 hours a week.

During training sessions, larger muscle groups (legs) will be trained first followed by the trunk, torso, and arms, and the smaller muscle groups (neck).

Opposing muscles will be exercised in pairs.

Isotonic exercises will be done using a variety of weights including barbells, cuff weights and weight machines.

All exercises must be done in a controlled manner and through a full range of motion.

8. Exercise progression.

Subjects will undergo a double progressive training program: alternating muscle, stress and repetition increase. Muscle stress increase will be achieved through a systematic program of heavier load.

The progression will be gradual (increased intensity and duration of the exercise). The options for progressive resistance exercises are:

1. Increase weight/keep repetitions the same;
2. Keep weight the same/increase repetitions; and,
3. Keep weight and repetitions the same/increase velocity.

The number of repetitions will be increased to reach 12-14 repetitions, then weight will be increased by 5% and so on.

9. Stretching.

Stretching session: at the beginning and at the end of each session.

Static stretching: gradual stretch without forcing, followed by holding of the final stretch

position for 60 sec.

In addition, clinical and para-clinical investigation will be performed on a regular basis and

will include:

- Evaluation based on the Kunming scale
- ASIA motor score
- Sensory functions evaluation
- Adverse event(s)
- MRI of the activated fat integration site
- CSF analysis if needed
- Biological parameters (blood + urinalysis)
- SEP and MEP of lower limbs

10. RISK ANALYSIS

The predominant risks to the patient in this study are those of any operation, including :including:

- The risk of medical complications of anesthesia, up to death.

o The risk of infection related to the neurosurgical portion of the operation, including meningitis, myelitis and/or wound infection.

o The risk of neurological worsening following spinal cord exploration, debridement and implantation of activated fat. This risk is higher in patients with cervical lesions and/or in patients a pre-op Asia score above A. (with preservation of function below the lesion.)

o The risk of secondary injury due to swelling of the spinal cord in the post-operative period; this would cause a patient, in particular a quadriplegic patient, to eventually become injured at a higher level.

o The risk of late complication, in particular secondary syringomyelia in the cranial segment of the spinal cord

o The risk of post-operative complication such as pneumonia, deep venous thrombosis, eschars and urinary infections.

Risks will be minimized by scrupulous attention to medical, surgical and anesthetic detail in the management of these fragile patients.

The risk of investigation is justified because of the uniformly dismal prognosis of patients with severe spinal cord injury. These patients have virtually no chance of walking again, despite state-of-the-art neurological, surgical and medical care.

The hope of some return of neurological function below the level of injury justifies the risk of the investigation.

11. DATA MANAGEMENT

Detailed report of the surgery and the duration of the surgery for each patient will be provided, as well as a video recording of each of the procedures under the surgical microscope.

The post-surgery period-corresponding to the hospitalization will be recorded, as well as any reaction following the implantation of activated fat, medication given, any complication, and recordings of the usual biological parameters.

Motor Score

Motor and sensory improvement will be evaluated following the Asia motor scale and the Kunming scale (grade 1 to 5.) The patient's recovery will be expressed in percentage for Asia grades.

Asia evaluation of sensory functions will be done with pin prick scores between 0 and 112 for the 28 index levels.

Proprioception:

Change from baseline in proprioception and best improvement will be obtained from a score ranging between 0 to 12 for the thorax and the abdomen, below the level of the spinal lesion, and between 0 to 16 for the lower limbs. Relative change (%) from the baseline will also be calculated. The corresponding formula for two time points Tl and T2 will be : [(T2 -Tl)/Tl] x 100.

Evoked potentials

Improvement in SEP and /or MEP will each be assessed by any change in latency or in amplitude of evoked potential tracings.

Latency will be calculated as the time interval in milliseconds, between the stimulus and a specific point on the evoked potential waveform. Amplitude will be stated in microvolts expressed in reference to the peak. These metrics will be collected from evoked potential tracings off hard copy printouts, or preferably from computer processed measurements. In case of repeated sampling measurements per time point, the median of the latency and amplitude, derived from repeated measurements, will be used.

Analysis of efficacy

Efficacy criteria

Primary criteria:

- Voluntary movement distal to the injury following activated fat implantation

Secondary criteria:

- Improvement in sensory function distal to the initial injury
- Recordable SEP and/or MEP of lower limbs

Analysis of Safety

Incidence of adverse events for the total duration of the study, including the surgical procedure, and changes in any biological parameter including CSF analysis.

12.1 OBLIGATIONS

The protocol and informed consent for this trial can be modified. Every serious or unexpected adverse event during the implantation surgery, that might affect the patient's safety, must be brought to the neurosurgeon’s attention.

Informed Consent

A signed informed consent must be obtained from each volunteer patient prior to the trial. Each participant will be given written information describing the nature and duration of the trial.

This shall take place under conditions where the participant has adequate time to consider the risks associated with his participation in the trial. Two copies of the informed consent will be signed: one will be given to the participant and the other to the Head Neurosurgeon.

12. 2. Data recording

Data resulting from the protocol will be recorded by the Head Neurosurgeon and the staff of the institute responsible for the patient’s care in the report forms used by the Tongren Hospital.

Similarly, parameters of the rehabilitation training program will be recorded by the rehabilitation center. The head neurosurgeon and the staff of the clinic will maintain adequate records for the trial including medical records, laboratory reports, informed consent forms, safety records, and other pertinent data, such as letters and administrative documents. Data resulting from the post-operative

physiotherapy program will be recorded by the hospital staff responsible for the rehabilitation protocol, with the objective of showing the changes in the patient's locomotor performance.