Research paper

Intraoperative neurophysiology monitoring in scoliosis surgery in children

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A B S T R A C T

Objective: Intraoperative neurophysiology monitoring (INM) is thought to reduce the risk of postoperative neurological deficits in children undergoing scoliosis and spine deformity surgery. INM is being used increasingly despite conflicting opinions, varied results, non-standard alarm criteria and concern regarding cost effectiveness. In this paper we present our experience with INM in scoliosis and spine deformity surgery in children, propose alert criteria and preferred anaesthetics in clinical practice.

Methods: We retrospectively analysed our experience with INM in 56 children who had 61 scoliosis and spine deformity surgeries.

Results: INM was successfully undertaken with transcranial electrical motor evoked potentials (TcMEP) and somatosensory evoked potentials. There were no injuries due to INM. Four children had 5 alerts during 4 surgeries. A postoperative deficit was seen in one child only. No new postoperative deficits were seen in any child who did not have an alert during INM. Total intravenous anaesthesia was better for INM compared to inhalational anaesthetics.

Conclusions: INM is useful in scoliosis surgery; it is likely to mitigate the risk of new deficits following surgery. We recommend alert criteria for TcMEPs that include multiple facets – amplitude, stimulus paradigm, morphology. We recommend propofol and remifentanil, in preference to sevoflurane and remifentanil for anaesthesia during INM.

Significance: Our study adds to the literature supporting the role of INM in scoliosis surgery in children. We provide guidelines for alert criteria in clinical practice and recommend the use of total intravenous anaesthesia as the preferred anaesthetic option.

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1. Introduction

Paediatric neurosurgical and orthopaedic spinal procedures, as well as other surgeries in children with “unstable spine” are at risk of adverse neurological sequelae. The risk appears to be higher for those with pre-existing deficits, especially neurological, and those with multisystem involvement. Tailored intraoperative neuromonitoring (INM) with transcranial electrical motor evoked potentials (TcMEP), somatosensory evoked potentials (SSEP), free running electromyograms (EMG) and stimulus triggered EMG (Stim EMG) may mitigate the risk of permanent injury and adverse neurological outcomes during certain surgeries (Cheng et al., 2014; Lieberman et al., 2008; Senkoylu et al., 2017; Novais et al., 2017; Neira et al., 2016; Piasecki et al., 2018; Purger et al., 2015; Samdani et al., 2016; Galloway and Zamel, 2011; Pastorelli et al., 2015; Jea, 2014; Sala et al., 2010; Nuwer et al., 2012b; Fehlings et al., 2010; Langeloo et al., 2003; McIntyre et al., 2016).

An evidence-based guideline update on intraoperative spinal monitoring with somatosensory and transcranial electrical motor evoked potentials (Nuwer et al., 2012b) concluded that INM is effective to predict an increased risk of paraparesis, paraplegia and quadriplegia in spinal surgery. A systematic review (Thirumala et al., 2017) of the diagnostic accuracy of motor evoked potentials to detect neurological deficit during idiopathic scoliosis...
correction, estimated the probability of a postoperative neurological deficit with a positive TcMEP change as 26.3%. In clinical practice when an alert is raised based on INM, the clinical team intervenes to try and reduce the risk of adverse events. While there is animal data that shows that action taken in response to an alert during INM can improve neurological outcome, it is difficult to ascertain this with certainty in humans (Thirumala et al., 2017; Ney and van der Goes, 2014; Nuwer, 2016). INM in spinal surgeries is shown to be cost saving in cost effectiveness models and clinical practise (Ney et al., 2013; Ney and van der Goes, 2014; Nuwer, 2016).

In this paper we present our experience and assess the role of INM in scoliosis surgery in childhood. We also make recommendations regarding the parameters that should constitute an alert in INM for scoliosis in children, as well as the optimal anaesthetic protocol during the INM.

2. Methodology

Ethics approval was obtained, as required by our institution, for this retrospective analysis of INM in children who had surgical procedures for scoliosis. The data was used in real time for clinical decision making.

The study included all children in whom we undertook INM for scoliosis and orthopaedic spinal surgery between 2012 and 2018. TcMEPs and SSEPs were monitored during INM.

Children in the study were reviewed by the neurologist prior to surgery, clinical profile (including a detailed neurological evaluation including history and examination) noted and the INM planned. Any neurological impairment including sphincter disturbances were documented. Pre-operative SSEP studies were undertaken, if the patient was cooperative. The planned INM was discussed with the child (when appropriate) and family.

Anaesthesiatics used during the INM were propofol and remifentanil (Total Intravenous Anaesthesia, TIVA) or sevoflurane and remifentanil (Inhalation Anaesthesia, IA). In addition, some children received ketamine boluses or infusions during the INM and some received morphine. No neuromuscular paralytic agent was administered during the surgery and INM; a single dose of atracurium was often used at induction. Some children on propofol and remifentanil had also received sevoflurane briefly for induction. The anaesthesiatics team was requested to notify the neurophysiologist team when there was a change to the anaesthetic regime. Bispectral index (BIS) was monitored by the anaesthesiast to aid in the evaluation of depth of anaesthesia (Sigl and Chou, 1994).

INM was undertaken using the Medtronic NIM-ECLIPSE machine. TcMEPs and SSEPs were obtained after anaesthetic induction, prior to commencement of surgery for baseline values. TcMEPs and SSEPs were recorded on exposure of the spine. They were monitored during the surgery and at completion of the surgery (before recovery from anaesthesia).

TcMEPs were evoked using trains of 5–9 stimuli (usually 5 stimuli), pulse width of 200–400 μs, and stimulus intensity (SI) 62–200 V. When using trains of 9 pulse width was 200–300 μs. The recording bandwidth for the TcMEPs was 30–1000 Hz and the screen display was 100ms/cycle long. Screw electrodes over the scalp were used for transcranial electrical stimulation and SSEP recordings. Scalp electrodes were placed at C3, C4, C1, C2, CZ, and FZ of the international 10–20 system (‘Guideline thirteen: guidelines for standard electrode position nomenclature. American Electroencephalographic Society’, 1994)). The best bipolar combination of scalp electrodes for TcMEPs (based on stimulus parameters needed to evoke MEPS with minimal movement artefact) were determined at the onset and used for the rest of the INM. Motor evoked potentials were recorded, using needle electrodes from the foot muscles (abductor digitii minimi and abductor hallucis), leg muscles (gastrocnemius and tibialis anterior) and thigh muscles (hamstrings or quadriceps femoris). At least one upper limb muscle from each side was sampled for control and comparison in children with thoraco-lumbar surgeries. In those with INM for cervical spine surgery, hand muscles (abductor pollicis brevis, abductor digitii minimi), forearm muscles (flexor digitorum superficialis or extensor digitorum communis) and the deltoid were also monitored for TcMEPs. Insertions of intravenous or intraarterial cannulas in the forearm or hand also determined choice of muscles on each side.

SSEPs were evoked with stimulus intensities of 8–25 mA, stimulus frequency of 4–5 Hz, and stimulus duration of 300 μs. SSEPs were recorded with bandpass of 30–1000 Hz. Display duration was 100 ms for the lower limb SSEPs and 50 ms for the upper limb SSEPs. The posterior tibial nerve (PTN) at the ankle was stimulated on each side to evoke lower limb SSEPs. Evoked responses were monitored over the popliteal fossa, scalp electrodes and mastoid electrodes. In most children one recording was made over the cervical spine or suboccipital region, unless the surgical incision was high. SSEPs were acquired from at least one upper limb (using median nerve stimulation) for thoracolumbar surgeries. For cervical spine interventions we monitored SSEPs from stimulation of the median nerves bilaterally or median nerve on one side and ulnar on the other (because of ease of access). Multiple montages were evaluated in each INM and the best selected for monitoring (Hanson et al., 2016).

An alert was called if there was a significant change in the INM, as determined by the neurophysiologist. With regard to TcMEPs an alert was raised if one or more of the following changes were observed on at least two consecutive trials without an obvious explanation (e.g. anaesthetic bolus): There was a sudden loss of MEPs, a decrease in the amplitude of the MEPS of >50–75% (with minimal change in the upper limb TcMEP in thoracolumbar surgeries, or a unilateral change), an increase in the latency of the MEPS of >50% or significant change in the morphology (reduction in number of phases and complexity) and duration of the MEPS from one or more muscles, or there was an increase in the stimulus intensity required by >25%, or an increase in the number of stimulus trains required. A combination of the above parameters was often used in the clinical decision making to raise the alert. With regard to SSEPs the protocol was for an alert if there was a significant drop in amplitude (>50%) or increase in latency (>10%) of the SSEPs. An alert resulted in all three teams – surgery, anaesthesiology and neurophysiology discussing and exploring possible reasons for the alert. This then led to appropriate action being taken. We did not undertake a ‘wake up test’, thought by some to be complementary (Chen et al., 2015) to INM, when an alert was called.

A clinical assessment of the child (including a neurological evaluation) was undertaken and documented in the post-operative period (after extubation and within the next 3 days). Any new deficits were specifically documented. Long term follow-up was also documented.

3. Results

Fifty-six children had 61 occasions of intraoperative monitoring. They ranged in age from 4 to 17 years at the time of the INM; median age was 12. INMs were undertaken for surgery related to thoracolumbar scoliosis in 52 and for cervical spine fixation or correction in 4 children. Nineteen patients were syndromic or had specific other diagnosis: Duchenne muscular dystrophy-3,
cerebral palsy-3 (one child also had VACTERL anomalies), Prader Willi syndrome-2, achondroplasia-2, and 1 each with Escobar syndrome, Ehlers-Danlos syndrome, connective tissue disorder (unspecified), Mobius syndrome, Morquio syndrome, Klippel Feil syndrome, valproate embryopathy, hyper-IgE syndrome and neurofibromatosis.

There were no adverse events reported or seen as a result of the monitoring. All children were intubated at the time of INM. None of the children had any oro-lingual trauma as a result of TcMEPs.

Twenty-one children had abnormal neurological findings prior to surgery. Two of the 4 children who had alerts during surgery had pre-operative neurological deficits. Pre-operative SSEPs were undertaken in 55 children. They were suboptimal in 3 and abnormal in 4 children. Pre-operative SSEPs were normal in the 4 children who had alerts during surgery.

Propofol (range 2–14 mg/kg/h) and remifentanil (range 0.1–0.5 μg/kg/min) were anaesthetics used for the INM in forty-three patients (TIVA arm). Nineteen had sevoflurane (<0.9 MAC) and remifentanil (range 0.1–0.5 μg/kg/min) (IA arm). Anaesthetists used the Bispectral Index to monitor the depth of anaesthesia (Sigl and Chamoun, 1994). Twenty-six received ketamine either as boluses or infusions. Ketamine infusions appeared to have additional sedational and analgesic effects, without impacting on the INM.

Two patients in the inhalational anaesthetic (IA) arm had an anaesthetic change from sevoflurane to propofol because of difficulty obtaining TcMEPs. One child with probable anaesthetic/haemodynamic related cardiorespiratory compromise was changed from propofol to sevoflurane, but subsequently also received propofol.

C1–C2 was the most frequently used combination for delivering TcMEP stimulus, with C3–C4 being next. Trains of 5 were most frequently used for TcMEP monitoring (range 5–9). The SI required for the TcMEPs varied from 62 to 200 V (most frequently used SI was 89 V). The duration of the TcMEP stimulus was mostly 400 μs (range 200–400). TcMEPs were monitored prior to the start of surgery, during surgery and at the completion of surgery. During crucial portions of the surgery they were monitored more frequently. The most frequently used stimulus paradigm for each INM was recorded. There was a statistically significant difference (p = 0.000027) in the average most frequently used SI for the propofol/TIVA group (100.1 V), compared to the sevoflurane/IA group (146.3 V).

Prior to start of surgical manipulation or correction, TcMEPs were obtained in all INMs; in one they were obtained inconsistently at the start. In this child while SSEPs were present and remained stable during and after the surgery, TcMEPs in the lower limbs were inconsistently obtained initially, they were less consistent during the surgery and were present at the end of surgery; there were no new deficits following tightening of the rod for scoliosis. In one child with a previous lower limb disarticulation, TcMEPs and SSEPs were monitored to and from the single normal lower limb.

TcMEPs often varied slightly from one trial to another (in the muscles MEPs were evoked from, the latency, morphology, amplitude). Often as the anaesthetic progressed, during the main part of the surgery, there was a need to gradually increase the stimulus parameters, especially the SI. TcMEPs remained stable with no clinically significant change during or after the surgery in 57 INMs (in 52 children).

Lower limb SSEPs were undertaken prior to, during and at the end of surgery with stimulation of the PTN on each side. SSEPs from the upper limb were undertaken for control and for monitoring in appropriate cases. Stimulation at PTN, median or ulnar was delivered above twitch threshold and the SI required was between 8 and 25 mA. They were obtained in 60 of the 61 INMs. Far field potentials over the mastoids, or cervical/suboccipital region were often (not always) more robust and stable, compared to the scalp potentials. No alerts were called on the basis of the SSEPs. They remained clinically stable in all children in whom they were monitored.

SSEPs were monitored less frequently than TcMEPs during the surgery. Averages of 200–500 were required to get reproducible traces and this took time. SSEPs were more difficult to obtain – as they were more affected by the electromagnetic milieu of the operating theatre (for example there was more interference with use of cautery). SSEPs were acquired at opportune moments. At times the surgeon was requested to briefly pause to get a readable SSEP. SSEPs from the upper limb were more easily obtained than from the lower limbs. SSEPs were repeated following an alert, and after discussion between the three teams a plan of action formulated and executed.

Alerts were called during the INM on 5 occasions: during 4 INMs (for thoracolumbar scoliosis surgery) in 4 children (there were two alerts during one INM in one child, one of which was a miscommunication alert, the other a true alert). All the alerts were called based on TcMEP changes. Details of these alerts and the actions taken are shown in Table 1. Only one child had a new deficit postoperatively. This was initially quite significant. There was almost complete recovery over 8 weeks. At one-year follow-up power was at least 4/5 in the lower limbs, similar to the preoperative examination. Fig. 1 illustrates a few recordings of TcMEPs prior to, during an alert and after recovery of TcMEPs in Case 4. Fig. 2 shows SSEPs from the lower limbs prior to and after an alert in this child. There were no new postoperative deficits without an INM alert.

Of the 61 occasions of monitoring there were alerts during 4 surgeries. In 57 surgeries there were no alerts and no new postoperative neurological deficits. In 3 of 4 surgeries with alerts there was a recovery of MEPs after corrective action. In 1 patient with alert there was no recovery of MEPs and a new postoperative neurological deficit was noted. Fisher Exact Test was significant (two tailed p = 0.032) for predicting a new postoperative deficit if there is an alert and no recovery of MEPs after corrective action.

4. Discussion

Intraoperative neuromonitoring is now considered “standard of care” during paediatric spine surgery (Nuwer et al., 2012a; Ney et al., 2015; Vadivelu et al., 2014; Ferguson et al., 2014). It is not a perfect test and its effectiveness is still being debated. Sensitivity and specificity of INM in predicting and preventing postoperative deficits are difficult to ascertain because of many reasons including the low rate of new neurological deficits, different INM protocols, non-standardised alarm criteria, heterogeneous groups of patients (some with pre-existing neurological deficits), and variable levels of experience and expertise of the clinical team. There is inherent limitation in assessing the specificity of INM changes when those changes result in clinical interventions by anaesthetists or surgeons (Nuwer et al., 2012b). Therefore, it is difficult to calculate Receiver Operating Characteristics curves. Prospective trials and registries with thoughtful and innovative design may help confirm the essential role and efficacy of INM in clinical practice (Eccher et al., 2014; Jea, 2014; Nuwer, 2016; Nuwer et al., 2012a). In this paper we report on our clinical experience and propose some practical recommendations for INM in scoliosis surgery.

We undertook 61 INMs with TcMEPs and SSEPs in 56 children having scoliosis and spine deformity surgery, with no adverse effects from the INM. TcMEP is a highly sensitive and specific tool for detecting spinal cord motor impairment or injury during spine surgery (Kim et al., 2013; Legatt et al., 2016; Thirumala et al., 2016;
Children with alerts during INM. (Legatt et al., 2016; Kim et al., 2013). Our experience is in keeping early siren of impending spinal cord injury.

Deformity/scoliosis surgery, with a significant change being an value. Our study supports the usefulness of INM in paediatric 2014) have also suggested that INM has a 100% negative predictive value without an alert during INM and other studies (Ferguson et al., 2014) have similar to the 1.38% deficit reported in a systematic review of the diagnostic accuracy of motor evoked potentials to detect neurological deficits during idiopathic scoliosis correction (Thirumala et al., 2017; Thirumala et al., 2016). There were no new neurological deficits postoperatively. In one child there was hemodynamic compromise which resulted in an alert. Stabilizing the hemodynamic status resulted in recovery of TcMEPs. A new postoperative neurological deficit was only seen in one surgery with INM (1.6%), in one child of 56 (1.8%): this is similar to the 1.38% deficit reported in a systematic review of the diagnostic accuracy of motor evoked potentials to detect neurological deficits during idiopathic scoliosis correction (Thirumala et al., 2017; Thirumala et al., 2016).

Currently there are no standard alarm criteria for TcMEPs (Legatt et al., 2016; Kim et al., 2013). Our experience is in keeping with the reported literature. There is intrinsic variability in the TcMEPs. There is universal consensus that loss of TcMEPs should be an alert. However, it may be too late then to avoid neurological sequelae. An alert triggered by a smaller change may enable appropriate intervention and prevent a deficit, even though this may result in more false alerts. Three sets of criteria have been proposed and described in the literature for alerts: threshold of stimulation criteria (Calancie et al., 1998), amplitude criteria (Langeloo et al., 2003; Muramoto et al., 2013; Kobayashi et al., 2014) and morphology criteria (Quinones-Hinojosa et al., 2005). Our paradigm for an alert, as outlined in the methodology, is based on a combination of the reported literature. There is intrinsic variability in the reported literature. There is intrinsic variability in the

<table>
<thead>
<tr>
<th>Patient</th>
<th>Other clinical features</th>
<th>Surgery</th>
<th>Anaesthetic</th>
<th>Alert</th>
<th>Reason for alert</th>
<th>Action taken</th>
<th>INM Outcome</th>
<th>Postoperative outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Scoliosis</td>
<td>IA</td>
<td>IA</td>
<td>1. Amplitude of all MEPs (UL and LL) decreased, latency of MEPs increased, Stimulus Thresholds for TcMEPs increased</td>
<td>Anaesthetic: Muscle relaxant given without notifying neurophysiologist</td>
<td>Established cause</td>
<td>LLL MEPs elicited again 35 min later, gradually returned to normal</td>
<td>No post-operative neurological deficit</td>
</tr>
<tr>
<td>2</td>
<td>Escobar syndrome</td>
<td>Scoliosis</td>
<td>TIVA</td>
<td>Amplitude of all MEPs (UL and LL) decreased, latency of MEPs increased, Stimulus Thresholds for TcMEPs increased</td>
<td>Haemodynamic instability: hypotension, tachycardia, cause – ?cardiac anaesthetic</td>
<td>Likely Surgical</td>
<td>No recovery of LLL MEPs, improved RLL MEPs but did not return to normal</td>
<td>No new neurological deficit</td>
</tr>
<tr>
<td>3</td>
<td>Prader Willi syndrome</td>
<td>Scoliosis</td>
<td>IA</td>
<td>MEPs abruptly lost in LLL, impaired (reduced amplitude, increased latency, change in morphology) in RLL, no change UL MEPs</td>
<td>Likely Surgical</td>
<td>The most recently inserted screw at the apex was removed and the track probed - it seemed intact. A persuasive insult to the cord (after the use of a small mallet with the pedicle finder) was thought to be the most likely cause.</td>
<td>No new neurological deficit</td>
<td></td>
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<tr>
<td>4</td>
<td>Moebius syndrome</td>
<td>Scoliosis</td>
<td>TIVA</td>
<td>MEPs changed then lost in LLL at the end of initial scoliosis correction, smaller change in RLL MEPs, no change in UL MEPs</td>
<td>Likely Surgical</td>
<td>Dealing with a huge curve. In response to the alert, the rod was removed, it was contoured to achieve a lesser correction and repositioned.</td>
<td>LLL MEPs elicited again 11 min later, gradually returned to normal</td>
<td>No new neurological deficit</td>
</tr>
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</table>

Abbreviations: IA Inhalational Anaesthesia; LL Lower Limb, LLL Left Lower Limb; MEP Motor Evoked Potential; RLL Right Lower Limb; TIVA Total Intra Venous Anaesthesia; UL Upper Limb.
posed after a pilot study (Segura et al., 2017). The ease of application of this empirical ratio in clinical practise, its reliability, specificity, sensitivity and usefulness need to be determined in larger trials. With current knowledge, it seems sensible to utilize multiple facets of TcMEPs for alarm criteria.

TcMEPs, are thought to be highly vulnerable to inhalational anaesthetics, especially sevoflurane (Kim et al., 2013; Chong et al., 2014) and more easily elicited and monitored with TIVA protocols. Our preference is for TIVA for INM during spine surgery. However, the anaesthetists in our hospital prefer Sevoflurane based anaesthesia in some children. Nineteen children received sevoflurane and remifentanil during the INM. In 17 children TcMEPs were successfully recorded. In 2 children the anaesthetic was changed to propofol/remifentanil due to difficulty in obtaining TcMEPs. The mean most frequently used SI was significantly higher in children who received IA, compared to those who received TIVA. We would recommend the use of propofol + remifentanil TIVA as the preferred anaesthetic option for INM in scoliosis surgery. The addition of ketamine is helpful.

SSEPs monitor the sensory pathways. It is an advantage that the test does not cause any movements. SSEPs have less variability between trials and the alarm criteria (decrease in amplitude of >50% and increase in latency of >10%) are well established and generally accepted. However, the time taken to average 200–500 stimulations and their susceptibility to artefact in the operating theatre, often results in SSEPs being monitored less frequently. They are also thought to be less sensitive in detecting new neurological deficits, especially motor deficits. We did not have any alerts raised from SSEP changes. Use of wireless systems (Farajidavar et al., 2016) may enhance the role and ease of use of SSEPs in INM.

In conclusion TcMEPs may help detect and prevent new neurological deficits during spine surgery for scoliosis and other deformities. TcMEPs are easier to elicit, may detect changes earlier than SSEPs and are thought to be more sensitive in identifying spinal cord injury (Neira et al., 2016; Koht and Sloan, 2016). In our series of 61 INMs in 56 children, we assume that the INM alert and resultant surgical corrective action prevented new deficits in two children. In one child hemodynamic compromise caused the alert. In one child where the TcMEPs continued to remain abnormal the child had a new neurological deficit at the end of surgery. We recommend INM with TcMEP and SSEP for spine deformity surgery in children, with propofol (and remifentanil) being the preferred anaesthetic option for INM.

Fig. 1. TcMEPs recorded in case 4 at time points prior to an alert (A), at alert (B) and after the alert (C–I). Remedial action taken after the alert resulted in gradual return of TcMEPs (F–I). At the alert (B) note significant changes in amplitude, morphology and duration of LLL TcMEPs, with smaller changes in the RLL TcMEPs, with smaller changes in RLL TcMEPs. At C, D and E (3, 4 and 7 min after alert) loss of LLL TcMEPs and smaller changes in RLL TcMEPs. At F (14 min after alert) there is partial recovery of LLL TcMEPs and normalization of RLL TcMEPs. At G, H and I (15, 16 and 42 min later) there is further recovery of LLL TcMEPs. Left UL (hand) TcMEPs remain unchanged. Left UL (forearm) TcMEP channel has been switched off as it was not sampled (due to IV/IA lines). Right UL TcMEPs are of small amplitude in the illustrated figures; they were larger in other montages (not illustrated). TcMEPs were elicited using C4-C3 electrodes (C4 anodal). Stimulus parameters were trains of 5 pulses at 400 Hz, pulse width 400 μs, stimulus intensity 111 V. Calibration bars at bottom right: horizontal 10 ms, vertical 20 μV–100 μV (calibration varies between muscles but is constant for each muscle across A–I). Muscles illustrated from top to bottom in each subfigure were left hand, forearm, hamstring, tibialis anterior, gastrocnemius, foot x2 followed by the right sided muscles in the same order. Abbreviations: LL Lower Limb, LLL Left Lower Limb; TcMEP Transcranial Motor Evoked Potential; RLL Right Lower Limb; UL Upper Limb.
preferred anaesthetic modality. We propose using amplitude, threshold of stimulation and morphology criteria to raise an INM alarm/alert. It is essential (Epstein and Stecker, 2014; Husain et al., 2011) that the INM team is competent: neurologists/neurophysiologists and technologists are well trained. INM should be tailored to the surgery and the child. There should be active communication between the neurophysiology, anaesthetic and surgical teams.

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Conflict of interest

We have no conflict of interest. The paper has not been presented at a meeting or published elsewhere.

References


