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Pharmacokinetics of dexmedetomidine in children. Model validation with TREX study

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Abstract

Introduction: Dexmedetomidine was the sedative agent administered in combination with remifentanil and low dose of sevoflurane in the experimental arm of the TREX trial. The TREX pilot study established infusion rates higher than those initially proposed. This could be attributed to an inappropriate target concentration for sedation or incorrect initial pharmacokinetic parameter estimates.

Methods: The TREX study is a Phase III, randomized, active controlled, parallel group, blinded evaluator, multicentre, superiority trial. The scope is to compare the neurological outcome after standard sevoflurane anaesthesia with dexmedetomidine/remifentanil and low dose sevoflurane anaesthesia in children aged less than 2 years undergoing anaesthesia of at least 2 hours or longer. Dexmedetomidine pharmacokinetics were analysed in the interventional arm of the Italian population.

Results: There were 138 blood samples from 32 infants (22 males and 10 females). The median (IQR) age was 12 (5.2-15.5) months, weight 9.9 (7.3-10.8) kg. None of children were born premature (median postnatal age 39 weeks, IQR 38-40 weeks). Duration of anaesthesia was at least 2 hours, with the longest lasting 6 hours. A three-compartment PK model that incorporated allometric scaling and a maturation function showed observations from the TREX study were consistent with those predicted by a "universal" model using pooled data obtained from neonates to adults.

Conclusions: PK analysis from the experimental arm of the TREX study confirms that plasma concentration of dexmedetomidine is predictable using known covariates such as age and size. The initial target concentration (0.6 mcg.L-1) used to sedate children cared for in the intensive care after cardiac surgery was inadequate for infants in the TREX study. A target concentration 1 mcg.L-1 provided adequate sedation.

Sommario

Introduzione: la Dexmedetomidina è il sedativo somministrato insieme al remifentanil e al sevoflurano, a basse dosi, nel braccio sperimentale dello studio TREX. Lo studio pilota ha determinato la necessità di mantenere dosi di infusione superiori a quelle inizialmente proposte.

Ciò potrebbe essere dovuto ad una concentrazione bersaglio inadeguata per la sedazione o ad errate stime iniziali dei parametri farmacocinetici.

Metodi: lo studio TREX è in Fase III, randomizzato, controllato in modo attivo, multicentrico. Confronta lo sviluppo neurologico in bambini dai 2 anni in giù, sottoposti a procedure chirurgiche dalla durata di 2 h o più. Un gruppo è sottoposto all'anestesia standard con sevoflurano, il braccio sperimentale prevede la somministrazione di dexmedetomidina/remifentanil e basse dosi di sevoflurano.

La farmacocinetica della dexmedetomidina è stata analizzata nel braccio sperimentale.

Risultati: si sono ottenuti 138 campioni di sangue da 32 bambini (22 maschi). L'età media (IQR) era 12 (5.2-15.5) mesi, peso 9.9 (7.3-10.8) kg. Nessuno è nato prematuro (età media postnatale 39 settimane, IQR 38-40 settimane).

La durata dell'anestesia era di almeno 2 h, la più lunga protrattasi per 6 h. Per le analisi si è dimostrato consistente il modello PK a 3 compartimenti. Questo è stato basato sulla scala allometrica che comprende funzioni di maturazione ed ha dimostrato che le osservazioni dello studio TREX erano coerenti con quelle previste dal modello "universale" basato su dati ottenuti da una popolazione che comprende neonati e adulti.

Conclusione: l'analisi PK dal braccio sperimentale dello studio TREX conferma che la concentrazione plasmatica della Dexmedetomidina è prevedibile usando varianti come l'età e la dimensione corporea.

L'iniziale concentrazione bersaglio (0,6 mcg.L-1) usata in terapia intensiva per sedare i bambini dopo cardiochirurgia era inadeguata per i pazienti dello studio TREX. Una concentrazione bersaglio 1 mcg.L-1 ha fornito una sedazione adeguata.

1 Introduction

The debate on the possible risk of anaesthesia on infants' brain dominated the scenes of research and publications of the last two decades. Despite the overwhelming evidence from preclinical studies demonstrating a deleterious effect of anaesthetics on the central nervous system with subsequent cognitive impairment, clinical studies like PANDA (1) and GAS (2) demonstrated the contrary: a relatively short anaesthesia of about one hour does not cause a clinically evident harm to the developing brain and clinical anaesthetic toxicity (1–4).

Despite this reassuring evidence, another research question remains unanswered: "Does longer anaesthesia cause some long-term neurocognitive effects?"

The attempt to find an answer to this question is dealt within TREX study.

The TREX is a randomized controlled study aimed at demonstrating if an anaesthetic regime based on regional blockade with dexmedetomidine-remifentanil sedation (with low inspired sevoflurane concentration) has equal or better long term neurological effects compared to children undergoing anaesthesia for two hours or longer given sevoflurane alone with regional blockade (ClinicalTrials.gov Identifier: NCT03089905). TREX protocol follows a previously performed and published pilot study aimed testing feasibility and safety of dexmedetomidine/remifentanil-based anaesthesia (5).

Dexmedetomidine was chosen as principle sedative medication because of preclinical evidence of neuroprotective effects and a known non-toxic profile of this drug (6).

One of the secondary outcomes of the TREX study is to examine pharmacokinetics (PK) of dexmedetomidine and its complex pharmacodynamic (PD) interactions with remifentanil and sevoflurane in children. The TREX study is currently ongoing.

The scope of this chapter is to provide a literature review on:

- Administrated drugs used in experimental arm of TREX study:

    o Dexmedetomidine

    o Remifentanil

    o Sevoflurane

- Pharmacokinetic and Pharmacodynamic, with particular attention to the elements to be considered in the pediatric field and the pharmacokinetics of dexmedetomidine

This chapter is structured in eight sections, the first one is focused on some elements of pediatric anaesthesia. In section 2, 3 and 4 characteristics of the medication administrated are illustrated. Section 5 contains considerations on how to choose the better doses. Section 6 illustrates concepts of PK and PD. The last one, section 7, closes the review illustrating the TREX outcomes and how the thesis contribution fits in the current state of the art.

1.1 Pediatric anaesthesia: important aspect to be evaluated

Children deserve the best choice in terms of anaesthesia, they are not mini-adults and this involves differences from the point of view of the effect that anaesthesia has on organ systems (7).

There is some evidence that children who have prolonged procedures are at greater risk, and preclinical data suggest that general anaesthetics might be the mechanism involved. Consequently, it is reasonable to investigate whether there is a superior outcome if the anaesthetic agents which cause the greatest changes are avoided or minimized.

Children undergoing anaesthesia inevitably have surgery or some other procedure being performed. Such surgical procedures may themselves place the child at risk. For example, peri-operative hypotension and inflammation have been identified as potential risk factors for adverse neurodevelopment. Children having surgery are also more likely to have syndromes or conditions that are associated with increased risk for poor neurodevelopment.

1.1.1 Neurological impact

General anaesthesia has been shown to enhance inhibitory transmission through gamma-amino-butyric-acid type A (GABA A) receptors and to reduce excitatory transmission through N-methyl-D-aspartic acid (NMDA) glutamate receptors. This could cause neurodegeneration through apoptosis at the peak of synaptogenesis, which is also related with significant learning and memory deficit in the following years (8).

The impact of anaesthesia may imply processes such as accelerated neural apoptosis or changes in synaptic density and function. Preclinical studies have demonstrated, in younger animals, a deleterious effect of anaesthetics on the central nervous system with subsequent cognitive impairment and this is greater with longer durations of

exposure. Changes have been demonstrated in many species ranging from the nematode to the non-human primate. Some studies have also demonstrated long term behavioural and neurodevelopmental functional changes in animals exposed to prolonged anaesthesia in infancy. There is great controversy over whether or not these animal data are relevant to human clinical scenarios (9,10).

The changes seen in preclinical studies interest Gamma-Aminobutyric acid A (GABA) agonists and non-depolarising neuro muscular blocker (NMDA antagonists) such as volatile anaesthetics (e.g., sevoflurane), propofol, midazolam, ketamine, and nitrous oxide. There is less evidence for an effect with opioid (such as remifentanil) or with alpha 2 (α_2) agonists (such as dexmedetomidine). Furthermore, these preclinical studies have shown a clear dose-response relation: higher dose of anaesthesia (i.e., longer anaesthesia) are associated with more morphologic and functional changes (9,10).

Despite the overwhelming evidence from preclinical studies, it is worth noting that two very important studies PANDA and GAS (1,11) have shown that animal models rarely predict human outcomes and a relatively short anaesthesia of one hour does not cause a clinically evident harm to the developing brain and in neurocognitive competence.

It is not yet known what kind of impact could have a longer exposure. Scientific community is still trying to understand if a longer anaesthesia causes some long-term neurocognitive effects and if there are anesthesia regimens with better neurodevelopment outcomes.

Precisely for the neurological impacts that anesthesia can have, Dexmedetomidine is chosen as the main drug of the anesthesia regimen in investigation arm of TREX study. This is because in preclinical studies it has been proven non-toxic and protective from the neurological point of view (12).

1.2 The main characteristics of Dexmedetomidine

Dexmedetomidine is a drug becoming increasingly important in pediatric anaesthesia. It is an α2receptor agonist, analogue of clonidine but much more selective (α2:α1 ratio of 1620:1) (4,5). It has various effects:

- Sedative

- Anxiolytic

- Sympatholytic

- Sparing of analgesics (13).

It has a central action on the locus coeruleus through pre- and post-synaptic receptors that produce a state of unconsciousness similar to natural sleep in the 2-3 states of non-REM (4,5).

1.2.1 Haemodynamic effect

Dexmedetomidine, being an α2 agonist, is a direct peripheral vasoconstrictor which achieves this effect by activating vascular smooth muscle α2B adrenoceptors. The impact of this response during dexmedetomidine infusion remains poorly quantified (14)

In the study of Anderson et al., it is consistently observed an initial short-term peak in α2 agonist-induced vasoconstriction, followed by pseudo steady state of vasoconstriction (4,8,15,16).

These vasoconstrictive effects were associated with concomitant increases in intra-arterial blood pressure (BP) and decreases in heart rate (HR). α2 agonist-induced vasoconstriction might have been rapidly attenuated either by α2 agonist mediated

release of nitric oxide from endothelial cells or by the release of other compensatory vasodilators.

Dexmedetomidine biphasic haemodynamic alteration is characterized by: vasoconstriction, baroreflex-mediated bradycardia and vasodilatation.

This involves a first phase of hypertension with relative bradycardia followed by relative hypotension for normalization of the rate (17,18).

However, the inhibition of sympathetic activity at cardiac level maintains more stable hemodynamics in response to stress, decreasing (according to various studies) death from myocardial ischemia in the postoperative period (19,20).

1.2.2 Better respiratory control

Another positive aspect of dexmedetomidine is that it does not produce excessive respiratory depression because, during sedation, it does not suppress the breath center and does not alter the morphology and dynamics of the upper respiratory tract (4,21).

1.2.3 Analgesia

The analgesic effect of dexmedetomidine is mediated by α-receptors, inhibiting pain transmission in locus coeruleus. In the spinal cord it also binds to posterior horn neurons at presynaptic level and at postsynaptic levels in intermediate neurons, thus inhibiting pain transmission signals to the brain by regulating the flow of potassium and calcium ions. At peripheral level dexmedetomidine blocks nociceptive neurons activation and the release of nociceptive peptides.

The efficacy of analgesia is controversial because in some studies this effect has been shown to be insufficient in the pediatric field (22), while in others (16,23–26) excellent results have been found. There is a variability of inter-individual response to the

perception of pain following the administration of dexmedetomidine, due to genes decoding for α2A and α2C receptors (27).

The most interesting aspect of this drug is the possibility of reducing the dose of opioids, such as morphine, in postoperative period. The use of intraoperative dexmedetomidine infusion allows an improvement control pain in the immediate with lower opioid requirement, also associated with a reduction of nausea and vomiting (16,23–26).

1.2.4   Possible solution for emergency agitation

A problem occurring with sedation or general anesthesia is delirium, whose incidence is particularly high with inhalation anesthesia. In this case the use of dexmedetomidine has produced interesting results. Emergency agitation (AE) or delirium (ED) presents as a sudden complex of psychomotor disorders, such as perceptual disturbances, delusions and disorientation (manifested as hallucinations, convulsions, crying, swaying, and kicking in bed). This depends on the nervous system immaturity, surgery type, anaesthesia type and duration, anxiety in the preoperative period linked to an unfamiliar environment and also on postoperative pain.

The action on α2receptors, through a sedation and anxiolytic process, strikingly decreases the incidence of this phenomenon and improves its course (22,28). Dexmedetomidine has a positive effect on delirium compared to placebo, midazolam and opioids and the optimal dose (ED95) is 0.30 mcg/kg. However, there is no evidence of superiority over other drugs such as propofol, ketamine, clonidine, melatonin, chloral hydrate and ketofol (28–30).

1.2.5   Clinical Use

Dexmedetomidine is approved by EMA for:

- Sedation in Intensive Care Unit (ICU) in adults who require a non-profound level of sedation (that allows awakening in response to verbal stimuli corresponding to the value from 0 to - 3 of the Richmond Agitation-Sedation Scale, RASS)

- Sedation of non-intubated adult patients prior to and/or during diagnostic or surgical procedures requiring sedation (i.e. procedural/awake sedation).

In the pediatric field, a profile similar to adults has been demonstrated in children over one month, who are mainly in the postoperative phase. These children were evaluated for treatment up to 24 hours in the ICU. Data for newborns (28–44 weeks of gestation) are limited and only for maintenance doses ≤ 0.2 mcg/kg/h (31).

Clinical uses in pediatric field for which a useful application has being highlighted: pediatric intensive and critical care, premedication, emergence agitation, airway procedure, invasive procedure, neurosurgery procedure, regional anaesthesia, radiological studies, treatment of cyclic vomiting syndrome.

1.2.5.1 *Pediatric intensive and critical care*

Infusion lasted 24 hours and cardiovascular and haemodynamic effects and quality of sedation were monitored. The quality of sedation was controlled through various scores such as Ramsay Sedation Scale, Pediatric Intensive Care Unit (PICU) sedation score, tracheal suctioning score and Bispectral Index (BIS). In critically ill children there are case reports in which dexmedetomidine has been used for sedation and has anxiolytic effect during mechanical ventilation. A particular area of interest is the intensive care for pediatric burns, where it is often difficult to adequately sedate these patients. The addition of dexmedetomidine to opioids or benzodiazepines made it possible without significant adverse events. Even in cardiothoracic critical care Dexmedetomidine has been used in children breathing spontaneous (32)

1.2.5.2 *Premedication*

Perioperative anxiety is a common phenomenon in children and α2receptor agonist such as dexmedetomidine have been shown to be a good choice for premedication. In children with extreme anxiety there are successful cases of Dexmedetomidine administered before non invasive procedure. Possible ways of administration for this indication are oral or intranasal.

The oral administration has a bioavailability of 15%, while the transmucosal bioavailability is 80% (23,33–36).

1.2.5.3 *Emergence agitation*

As previously explained, dexmedetomidine allows to reduce the incidence and duration of delirium, which particularly occurs with the use of some anesthetics such as sevoflurane (23,32).

1.2.5.4 *Airway procedure*

Airway procedures may require spontaneous ventilation due to the risk of aspiration in children who have breathing problems. Dexmedetomidine allows to maintain spontaneous ventilation avoiding respiratory depression, maintains airway tone, reduces airway reflexes, making the examination more feasible and keeps respiratory and hemodynamic profiles stable (36–38).

Example of airway procedures in which patients need to be awake or only mildly sedated are:

- Rigid bronchoscopy
- Thyroplasty with vocal cord medialization, treatment of dysphonia
- Laryngoplasty (39).

1.2.5.5 Invasive procedure

For invasive procedure, Dexmedetomidine is not sufficient to obtain an adequate depth of anaesthesia. Therefore, dexmedetomidine is administered with other anesthetics. The goal is to obtain adequate anesthesia, exploiting the positive effects of dexmedetomidine.

These are:

- Opioid sparing

- Less incidence of emergence agitation

- Reduced dose of other drugs such as remifentanil and sevoflurane resulting in fewer adverse effects with optimal sedation.

Moreover it has a good margin of safety in children who have a cardiovascular and respiratory critical status (23,32).

1.2.5.6 Neurosurgery procedure

Some neurosurgical interventions, i.e. the resection of a brain tumor or of an epileptogenic focus, require special anaesthetic settings. During craniotomy the patient must be sedated, but neurological feedback for resection is subsequently required. For this reason, patient must be awake and cooperating in an adequate anesthetic regimen to avoid episodes of respiratory depression or hypotension etc. Dexmedetomidine is helpful in this kind of procedures, especially because it maintains respiratory drive allowing, in case of epilepsy, to localize the focus while maintaining the epileptic activity (36).

1.2.5.7 Regional anaesthesia

Adding Dexmedetomidine to a caudal anesthetic (as bupivacaine) allows pain relief and prolongation of postoperative analgesia in pediatric patients. The addition of

Dexmedetomidine to local anesthetic did not cause statistically significant effect on hemodynamics and adverse events (32,36).

1.2.5.8 Radiological studies

Imaging procedures can cause anxiety in children due to foreign environment, claustrophobia and separation from parents. As a consequence, it is often necessary to sedate or administer anxiolytics to carry on the procedure. This can cause respiratory depression and excessive sedation. Dexmedetomidine, tested for this purpose, was well tolerated with no significant effects on blood pressure. In addition, all patients maintained spontaneous ventilation without mechanical support. The procedure could be MRI, CT, nuclear medicine and electroencephalogram (EEG) (32).

1.2.5.9 Treatment of cyclic vomiting syndrome

Cyclic vomiting syndrome is a rare disease characterized by repeated episodes of vomiting, nausea, photophobia, phonophobia, and intense prostration bouts during hours to days and ceasing without warning. Its etiology is still not well kwon and an adequate treatment has not been found yet. However, in some case reports, dexmedetomidine has been shown to be effective in resolving acute episodes (32).

1.2.6 Co-administration of Dexmedetomidine and Remifentanil

α2 agonists are not general anaesthetics and neither is Dexmedetomidine, which can be safely combined with sedative or analgesic drugs. Thanks to its characteristics, combination with moderately high doses of remifentanil results in deeper sedation associated with less respiratory depression and better analgesia (40).

1.3 The main characteristics of Remifentanil

Remifentanil is the drug associated with dexmedetomidine that is administered in the experimental arm of TREX study.

Remifentanil is a synthetic opioid widely used in pediatric anesthesia, it has a great affinity for μ opioid receptors, less for κ and δ receptors. (41–43).

Remifentanil is characterized by:

- Greater volume of distribution in children that decreases with age

- Fast onset

- Ultra short acting with rapid clearance

- High potency (41,44).

This opioid can be administered in relatively high doses and allows for profound intraoperative anaesthesia and analgesia, infusion for long durations and in rapid extubation and awakening thanks to its quick elimination (45).

1.3.1 Haemodynamic effect

Despite maintaining a good hemodynamic stability, remifentanil can give rise to bradycardia with possible negative chronotropic effect. In particular, the haemodynamic effect of remifentanil and sevoflurane combination was studied in literature, showing a pressure and cardiac index decrease without significant changes in cardiac output (46,47).

Another aspect that has favored its wide use is the more predictable pharmacokinetics compared to other opioids.

1.3.2 Hyperalgesia

Remifentanil, being an opioid, may cause hyperalgesia resulting in increased pain intensity and postoperative morphine consumption. Systemic α2 agonists, including Dexmedetomidine, have been shown to have an analgesic (also thanks to the release of acetylcholine in the spinal cord) and anti-shivering effect (48,49).

1.3.3 Low dose Sevoflurane added to Remifentanil/Dexmedetomidine: adequate anaesthesia

The TREX group decided that a pure remifentanil/dexmedetomidine regimen would not be universally acceptable in several surgical procedures. However, adding low dose of sevoflurane would be acceptable. Indeed, in the pilot study (3) a very low dose sevoflurane was found to be effective in providing adequate anaesthesia when remifentanil/dexmedetomidine alone was inadequate.

1.4 The main characteristics of Sevoflurane

Sevoflurane is an inhalational anaesthetic, which can be found as a colorless, non-flammable, volatile liquid. Administration is generally done with ease through masks. Sevoflurane has rapid onset and arousal time and it can be used for inhalation induction, associated with bronchodilation, in both children and adults (50,51).

Sevoflurane is a safe, anaesthetic agent whose pharmacokinetic would not seem to be too different between adults and children (49,50).

1.4.1 High incidence of emergence agitation

In children the incidence of emergence agitation is very high in the period following general anaesthesia with sevoflurane (incidence 17-83%). Studies show

Dexmedetomidine efficacy in reducing the incidence of delirium caused by sevoflurane.

In particular, combining the two drugs can bring benefits since it allows to administer lower concentrations of sevoflurane (52,53).

1.5 Choice of doses and considerations

We are now going to focus our attention on a combination of drugs, currently studied to understand if it could bring optimal results in more outcomes.

This anaesthetic regimen based on dexmedetomidine/remifentanil plus low doses of sevoflurane (when needed) was evaluated as safe in the pilot study on which the current TREX project is based (3).

It was complex to identify the correct dose and still there are cases where this needs to be fixed, nonetheless the TREX study defined the doses as follows:

- 1 mcg/kg of dexmedetomidine over 10 minutes followed by an infusion of dexmedetomidine at 1 mcg/kg/h

- Loading dose of remifentanil 1 mcg/kg over 2 minutes followed by an infusion starting at 0.1 mcg/kg/min or greater

- Sevoflurane can be used both in induction and maintenance with tidal concentrations of 0.6-0.8% or less.

The choice of the above mentioned doses was based on the following considerations:

- Drugs half-life is a confounded parameter that is dependent on clearance and volume. Both change independently during growth and cannot be used to determine infusion rates

- Most of the studies use a two-compartment model. Consequently, there is redistribution between compartments that must be taken in account. Thus, a simulation model was applied to determine loading doses and rates

- Pharmacokinetic parameters were estimated by Potts et al. (54) who describe how clearance changes with age, and which has been confirmed later in other studies

- A target concentration strategy was used to determine the dose of dexmedetomidine. The target concentrations originally chosen were increased in the pilot study because the initial analysis for target concentration was based on adult data. Later, Li et al. confirmed this higher target concentration [e.g., EC_{50} 0.903 (95% CI 0.450-2.344) mcg/L] (3).

Caudal block was often added to obtain optimal anesthesia, nevertheless in some cases it did not sufficiently inhibit the stimuli. As a result, a valid alternative was identified: low doses of sevoflurane.

Thanks to dexmedetomidine, sevoflurane can be used both in induction and maintenance with tidal concentrations of 0.6-0.8% or less. Specifically, Dexmedetomidine reduces the incidence of frequent adverse sevoflurane effects such as emergence agitation.

The knowledge base regarding this anesthesia association certainly needs to be expanded, starting from pharmacokinetic (3).

In addition to these considerations, the current dosage was developed after changes in the protocol. These were necessary because, from the beginning, it was understood that the doses initially chosen were insufficient.

Consequently dose increased from a loading dose of 0.6 mcg/kg over 10 min with maintenance 0.6 mcg/kg/h (Version 1) to 1 mcg/kg over 10 min with maintenance 1 mcg/kg/h (Version 2) to 1 mcg/kg over 10 min with maintenance 1-1.5 mcg/kg/h (Version 3) (3).

Coadministration of Dexmedetomidine with remifentanil has been shown to be 87.5% effective in the pilot study. In some cases the regimen had to be adjusted for signs of

light anaesthesia, but adjusting anaesthesia depth in response to signs of light anaesthesia is not unusual in anaesthetic practice.

The pilot study thus concluded that an anaesthetic regimen based on dexmedetomidine/remifentanil plus low doses of sevoflurane (when needed) is safe (3).

Moreover, loading and maintenance doses for dexmedetomidine were appropriate for children younger than 2 years of age (3).

The pilot study provides provisional results and application of different target concentrations for the same population of the TREX study (3).

It is worth mentioning that the above cited doses do not cause excessive side effects while avoiding light anaesthesia. This phenomenon was indeed frequently encountered in previous studies.

1.6 Pharmacokinetics and Pharmacodynamics

Pharmacokinetics and pharmacodynamics must be extensively studied in order to avoid administering doses of drugs that may have negative effects but ensure the clinical effect necessary for the patient (7).

Pharmacokinetics is a discipline that studies the absorption, distribution, metabolism and elimination of drugs. These are the processes that the drug undergoes within the body and that allow the achievement and maintenance of an adequate concentration in the various compartments.

Pharmacodynamics, which is always part of pharmacology, studies the effects of the drug on the body.

As mentioned above, pharmacokinetics has several phases:

- Absorption is the first phase in which the drug passes from the site of administration to the systemic circulation. Drugs absorption depends on several

factors such as route of administration, blood circulation at the place of administration, fat solubility of the drug that is directly proportional to the ease of passage from the biological membranes, body temperature and action of the drug itself

- Distribution is always influenced by blood flow and by the characteristics of the drug such as fat solubility, if it is very high it implies that the drug must circulate in the blood bound to protein, in particular albumin

- Biodistribution, or the metabolism of the drug, can occur in different organs but it is above all the liver to be responsible for this phase. There are two phases that characterize the metabolism of drugs:
    - Phase I reactions in which oxyreduction and hydrolysis processes take place
    - Phase II reactions in which conjugation processes take place

- Elimination is the last phase, the assigned organs to this task are:
    - Kidneys for water-soluble substances
    - Liver for the non-water-soluble and are then eliminated at the intestinal level
    - Lungs for volatile substances.

PK is determined by body composition and organ function, the first depending on changes occurring with growth. For example, in infants and small children the percentage of body water is higher than in older ages and, as a result, drugs have lower plasma levels if administered in weight-based dose (14,15,55).

Equally important is the development of organ functions; for example, kidney modifies plasma clearance and drug elimination with age. This is an essential element in choosing the dose to administer (55).

Pharmacodynamics studies the effects that the drug has on the body and this depends on the receptors with which it interacts. Different types of interactions are possible because the drug can bind reversibly or irreversibly (7).

In addition to this, the effect that the drug has on the receptor can be agonist (activating the receptor and the process that follows), or antagonist (partially or totally inhibiting the effect of the agonist).

PD is influenced by maturity of the receptors, signal transduction and responsiveness of organs like brain, heart, liver, skeletal muscle, etc., which are all still characterized by anatomical and/or functional immaturity in pediatric population.

All these important items must be considered in depth when dealing with pediatric anaesthesia.

1.6.1   PKPD modelling

To analyze pharmacokinetics and pharmacodynamics a model of analysis is needed. Population PKPD modelling is a statistical method in which mathematical equations are used to describe the typical (or population) time–concentration and time–response relationships observed after drug administration. There is a focus on quantifying and understanding variability in drug responses for individuals who represent the population in whom the drug will be used (56).

PKPD models can be directly translated into clinical anaesthesia: target-controlled infusions (TCI) are programmed with PKPD parameter sets, drug interaction models describe the effects of drugs given in combination and simulation studies are used optimize study design and drug dosing. The major differences between children and adults concern growth and development. Most enzyme systems responsible for metabolic clearance are immature at birth and mature within the first few years of life.

Clearance changes after maturation can be attributed to the nonlinear relationship between size and function and explained using allometric theory (57,58).

Pharmacodynamic endpoints in children are often difficult to define, although the electroencephalograph has proven extremely useful in anaesthesia in children out of infancy (58,59).

Drug interactions involve pharmacokinetic interactions and pharmacodynamic interactions. Their inclusion in PKPD models may increase applicability and usefulness, and can provide the opportunity to describe the time course of multiple drug effects (58,60–62).

1.6.2   PK of Dexmedetomidine

Dexmedetomidine is a lipophilic drug, it has a large distribution volume crossing the blood brain barrier and penetrating in extravascular sites. Dexmedetomidine for 94% is protein-bound drug in plasma, proteins are albumin and α1-glycoprotein (4).

Dexmedetomidine clearance is based on hepatic metabolism by hydroxylation and glucuronidation (particularly made by cytochrome P450 system), so the hepatic blood flow has an important influence. In healthy volunteers elimination half-life of 2.1–3.1 h is valued (4,54).

Important evidence is the influence that body weight has on plasma and steady-state concentration (54).

Several studies have demonstrated that to predict Dexmedetomidine PK three-compartment PK models with first order elimination is better than two-compartment model, even if most of the studies in based on the second one. Three-compartment model is based on clearance, intercompartmental clearance and volume (4).

Volumes of distribution is related to age, body weight, fat free mass, serum albumin level and maybe to the type of surgery. The elimination and distributional clearance

are influenced by height, body weight, or fat (free) mass, age, cardiac output, plasma albumin level and/or alanine aminotransferase activity (63–67)

Adults pharmacokinetic model could be enforced in children, accounting for size and age. Size and age are two essential elements to understand specially variability of the data.

1.6.3   BIS (Bispectral Index)

A model of pharmacodynamics for Dexmedetomidine has not yet been defined. One of the outcomes of TREX study, linked to pharmacokinetics, is to study the pharmacodynamics (PD) of this drug and also related to the other medications administered.

The use of the bispectral index (BIS) during the surgical procedure could provide an essential parameter for studying a model of pharmacodynamics for Dexmedetomidine. The use of BIS under anesthesia was introduced in order to monitor the depth of anesthesia. A reliable depth of anesthesia monitoring helps to achieve a adequate level of anaesthesia and potentially avoid problems with underdosing and overdose. The utility of BIS as a monitor of the level of sedation and consciousness has been already demonstrated for other medication, such as propofol, midazolam, and isoflurane (68,69).

BIS is based on the evaluation of electroencephalogram (EEG), the monitor provides a unique empirical dimensionless number, the BIS value, which varies from 0 to 100. In adults correlates well with the level of hypnosis during sedation and general anesthesia. EEG data have been entered into a multivariate statistical model to produce a number on a scale from 0 to 100, where 0 = EEG silence and 100 = EEG of a fully awake adult (70).

The ideal depth level has been identified between a value of 40 and 60, if lower it means that the anaesthesia is too deep if higher than 60 too light.

Monitoring through BIS the titration of drugs has been shown to reduce the incidence of intraoperative awareness (71).

Brain maturation and synapse formation continues after birth for up to 5 years, with most of it occurring in the earlier part of life. EEG changes with maturation have been noted from birth through puberty.

The BIS on data for adults does not express perfectly the data of the pediatric population, especially if under six months, because the analysis algorithm is standardized on the adult and therefore on a mature organism (72).

Further studies in children of various ages, comparing BIS with standardized sedation scores, may help in the development of a separate algorithm for use in pediatrics (73).

1.7   Aim of TREX study

TREX study, in addition to submit a primary outcome, has set up the objective to analyze numerous secondary outcomes.

1.7.1   Primary objective

The aim of the TREX study is to determine if low-dose sevoflurane/dexmedetomidine/remifentanil anaesthesia is associated with superior neurodevelopmental outcome compared to standard dose sevoflurane anaesthesia in children less than 2 years of age having anaesthesia expected to last 2 hours or longer. This possible superiority of the experimental arm is in terms of the global cognitive function as assessed by the full scale IQ score of the Wechsler Preschool and Primary School Intelligence Scale assessed at 3 years of age.

The first objective is developed, in addition to the full scale IQ, in several primary endpoints in terms of:

- Neurodevelopmental tests performed at 3 years of age including subscales of general cognitive functioning, language, executive function, memory, adaptive behavior, clinical behavior and social skills

- Diagnosis of any neurodevelopment disorder at 3 years of age.

1.7.2   Secondary objectives

Secondary objectives also involve determining if, in children less than 2 years of age having anaesthesia expected to last 2 hours or longer, low-dose sevoflurane/dexmedetomidine/remifentanil anaesthesia is superior to standard dose sevoflurane anaesthesia in terms of:

- Anaesthesia endpoints:

    o Incidence of intra-operative hypotension

    o Intraoperative bradycardia

    o Postoperative pain

    o Time to recovery

    o Determination of PK/PD profile only in patients undergoing treatment with dexmedetomidine. Four samples of blood will be taken for PK determination of dexmedetomidine in children recruited in the treatment group, when consent is provided.

- Safety: nature and incidence of adverse events and serious adverse events.

1.7.3   Aim of the study

While the TREX is ongoing, with active recruitment and neurological follow-up at three years of age, analysis on pharmacokinetic of dexmedetomidine have been performed ad interim. In fact, one of the secondary outcomes of the TREX study is to

investigate pharmacokinetic (PK) of dexmedetomidine and the complex pharmacodynamic (PD) interactions between dexmedetomidine, remifentanil and sevoflurane in those children. The aim of this work is to present PK data of children participating the TREX and allocated in the interventional arm of the study.

In particular, the correlation between plasma concentration and various variables (such as patient aspects and co-administered drugs) will be investigated in order to validate the anaesthesia model currently administered in the experimental arm of the TREX study.

The pharmacokinetic analyses from experimental arm of the TREX will also be compared to the analyses of the pilot study and to the estimates results derived in a larger pooled 'universal' analysis that used data from neonates to adults.

Dose of dexmedetomidine chosen is greater than in the first versions of the pilot study and in intensive care after cardiossurgery study, so another important objective will be to assess whether it is more adequate in terms of clinical effects, adverse effects and events of light anesthesia.

2 Patients and Methods

The scope of this chapter is to describe the patients population and the methods exploited to conduct the study. In more detail, this chapter comprises five sections.

The first section describes the population enrolled (how many patients and where they come from). Eligibility criteria, to be assigned to study treatment, are also explained. At last, in the first section the method by which patients are enrolled is presented.

In the second section the study design is introduced and outlined, followed by the third section concerning the protocol steps. The fourth and fifth sessions focus on the presentation of the database and its results.

2.1 Population

Pursuant to this study, population has been enrolled from different Italian Hospitals.

The population of this study is formed by patients belonging to the experimental arm of the TREX study. The TREX study is a Phase III, randomized, active controlled, parallel group, blinded evaluator, multicentre, superiority trial.

There are two arms:

- The standard dose sevoflurane arm

- The dexmedetomidine/remifentanil/low-dose sevoflurane arm (experimental arm).

Participants will be randomly assigned to the standard dose sevoflurane arm or the low-dose sevoflurane/dexmedetomidine/remifentanil arm, using a web-based database system.

2.1.1 Eligibility Criteria

Patients will be assigned to study treatment only if they meet all of the inclusion criteria and none of the exclusion criteria.

2.1.1.1 Inclusion criteria

Each patient must meet all of the following criteria to be enrolled in this study:

- Younger than 2 years old (chronological age)

- Scheduled for anaesthesia that is expected to last at least 2 hours (and/or total operating room time is scheduled to be $\geq$ 2.5 hours) and for a maximum of 6 hours

- Presence of a legally acceptable representative capable of understanding the informed consent document and providing consent on the participant's behalf

- Type of surgery that can be included, assuming patients do not meet exclusion criteria, are the following:

    o Craniofacial (i.e., isolated cleft lip, craniosynostosis, dermoid cysts of the scalp, etc.)

    o Thoracic (i.e., isolated lung malformations, diaphragmatic hernia Morgagni type, etc.)

    o Abdominal (i.e., Hirschsprung disease, Meckel resection, etc.)

    o Urologic (i.e., hypospadias, laparoscopic/robotic hydronephrosis repair, etc.)

    o Orthopaedic (i.e., congenital hip dislocation, bilateral congenital clubfoot, etc.)

    o Neurosurgical (i.e., isolated spine tethering, brachial plexus repair, etc).

2.1.1.2 Exclusion criteria

- Known neurologic, chromosomal or congenital anomaly which is likely to be associated with poor neurobehavioral outcome

- Existing diagnosis of behavioral or neurodevelopmental disability

- Prematurity (defined as < 36 weeks gestational age at birth)

- Birth weight less than 2 kg

- Congenital cardiac disease requiring surgery

- Intracranial neurosurgery and intracranial craniofacial surgery (isolated cleft lip is not an exclusion)

- Previous cumulative exposure to general anaesthesia exceeding 2 hours

- Planned future cumulative exposure to anaesthesia exceeding 2 hours before the age of 3 years

- Any specific contraindication to any aspect of the protocol

- Previous adverse reaction to any anaesthetic

- Circumstances likely to make long-term follow-up impossible

- Living in a household where the primary language spoken at home is not a language in which we can administer the Wechsler Preschool and Primary School Intelligence Scale

- Planned postoperative sedation with any agent except opioids (e.g., benzodiazepines, dexmedetomidine, ketamine, barbiturates, propofol, clonidine, chloral hydrate, and other non-opioid sedatives). For example, if such sedation is planned for postoperative ventilation

- Known hypersensitivity to the study investigational medicinal products (IMPs) and their excipients

- Renal impairment, defined as GFR < 60 mL/min/1.73 m^2, as calculated with Schwartz formula (eGFR $= 0.5$ height (cm)/plasma creatinine)

- Hepatic impairment, defined as levels of AST or ALT three times above the upper limit of the range of normality, or levels of total bilirubin two times above the upper limit of the range of normality, as reported by the local lab

- Advanced heart block (grade 2 or 3), unless paced

- Uncontrolled hypotension

- Acute cerebrovascular conditions.

2.1.2 Assignment of participant identification code

Participants will be assigned a participant identification code (Participant ID) as they consent to take part in the study. The Participant ID will be created by the dedicated secure web-based database system that will be used to collect study data. The Participant ID will consist of two sections of four digits each: NNNN-NNNN. The first section will represent the site at which the participant was created (site ID). The second section will represent a sequential number for participants at that site.

2.2 Study design

In the structure of the study was defined:

- Anaesthesia regime to which the children have been subjected, and in this case the experimental arm is analyzed

- Indications for the collection and analysis of blood in order to analyze the dexmedetomidine concentration

- How to deal with the most predictable adverse events.

2.2.1 Experimental arm

Induction may be with inhaled sevoflurane/and or propofol at the discretion of the anaesthetist. After induction the child will have a loading dose of 1mcg/kg of dexmedetomidine over 10 minutes followed by an infusion of dexmedetomidine at 1 mcg/kg/h. The loading dose should start immediately after induction (or as soon after induction as practicable).

Dexmedetomidine will be discontinued 10 minutes before the end of surgery. All children should have a loading dose of remifentanil 1 mcg/kg over 2 minutes followed by an infusion starting at 0.1 mcg/kg/min or greater. The loading dose should be

completed within 10 minutes of induction. The infusion should cease after last stitch/dressing is applied.

After induction and after dexmedetomidine loading is completed the child receives sevoflurane aiming for an end tidal concentration of 0.6-0.8% or less. Sevoflurane will be discontinued after last stitch and/or dressing applied. Morphine or other long-lasting opioids may be given 15 minutes prior to end of the case as decided by anaesthetist.

2.2.2 Sample blood

For patients, belonging to the experimental arm, was asked parental permission to take blood samples to evaluate the concentration of dexmedetomidine. Consequently infants allocated to the dexmedetomidine group will contribute with a maximum of six blood samples. Samples are taken throughout the duration of the intervention at six different intervals:

- T0: baseline

- T1: 10-15 minutes after loading dose

- T2: 30 minutes after loading dose

- T3: mid surgery

- T4: end of surgery

- T5: 20 minutes after infusion end of dexmedetomidine infusion.

The amount of blood collected is approximately 3 mL in total, samples are taken from an indwelling cannula following dexmedetomidine administration and collected into a lithium heparin tube.

Internal standard (tolazoline) will be added to plasma aliquots, followed by liquid/liquid extraction of buffered plasma with ethyl acetate. Plasma is separated by centrifugation (at 2500 g for 10 minutes at 4 ℃) and stored at -80 ° C at the site, until

assay. Following centrifugation, the organic extracts are dried and reconstituted with aqueous/organic solvent, vortexed, sonicated and injected onto the column.

Liquid chromatography and mass spectrometry analysis was performed by a UHPLC Agilent 1290 Infinity II 6470 (Agilent Technologies) equipped with an ESI-JET-STREAM source operating in the positive ion (ESI+) mode for dexmedetomidine.

The software used for controlling this equipment and analyzing data was MassHunter Workstation (Agilent Technologies).

The mobile phase consisted of 10 mM ammonium formate plus 3 mL formic acid buffer (A) and acetonitrile (B), run as a gradient at 200 μL/min. The precursor/product ion transition for dexmedetomidine is m/z 201 to m/z 95 (protonated molecular ion of dexmedetomidine). The precursor/product ion transition for tolazoline (internal standard) was m/z 161 to m/z 91. The lower limit of quantification for this method is 0.015 mcg/L.

The intra-batch mean value for dexmedetomidine at each concentration level usually varies from the theoretical values by less than 15% (accuracy). The intra-batch precision at each dexmedetomidine concentration level is usually less than 15%, as measured by the coefficients of variation (CV %).

The assay analysis was performed by an accredited central laboratory, located at IRCCS Ospedale Pediatrico Bambino Gesù in Rome (Italy), thanks to the contribution of the doctor Goffredo.

2.2.3 Rescue treatments

Any abnormal reaction or event is to be reported. In particular, based on previous studies on these drugs, there are some more likely events. For which indications have been developed indications on how to intervene.

Most predictable adverse events are:

- Hypotension

- Light anaesthesia

- Bradycardia.

2.2.3.1 *Hypotension*

Hypotension and hypertension are defined according to weight band (MAP = mean arterial pressure).

< 5 kg	MAP < 35 mmHg MAP > 70 mmHg
> 5 kg	MAP < 40 mmHg MAP > 75 mmHg

Hypotension must be defined with two different registers.

If hypotension is likely solely due to, or partly due to, excessive anaesthesia depth:

- If in low dose sevoflurane/dexmedetomidine/remifentanil arm, decrease remifentanil infusion rate by 0.05 mcg/kg/min and if hypotension persists decrease the dexmedetomidine infusion rate to 0.5 mcg/kg/h

- If in the standard dose sevoflurane arm, decrease the sevoflurane to aim for an end tidal concentration of 1.6-2.0%. Decrease further if hypotension persists

- If hypotension persists after the above then give fluid bolus and/or phenylephrine 1-5 mcg/kg or epinephrine 1-5 mcg/kg or ephedrine 0.25-0.5 mg/kg or metaraminol 5-10 mcg/kg

- Note that at any stage, the anaesthetist can at their discretion give phenylephrine 1-5 mcg/kg or epinephrine 1-5 mcg/kg or ephedrine 0.25-0.5 mg/kg or metaraminol 5-10 mcg/kg and/or further fluid to manage hypotension, particularly if the hypotension is due to factors other than excessive anaesthesia depth

- If at any stage the anaesthetist is concerned that the child has significant hypotension due to any reason and the hypotension could put the child at risk then they may decrease the sevoflurane, remifentanil and/or dexmedetomidine at their discretion.

2.2.3.2   Light anaesthesia

As light anesthesia we mean an inadequate anesthesia that results in events requiring drug doses to be increased or additional drugs administred. Light anaesthesia events result mainly in hypertension or movements. Hypertension without movement need a confirm trough repetition of BP measurement.

Indications in case of light anesthesia on how to manage:

- If light anaesthesia is suspected due to movement and/or confirmed hypertension (repeat BP measurement) give a remifentanil bolus of 0.25 mcg/kg and/or increase the remifentanil infusion by 0.1-0.2 mcg/kg/min

- Repeat bolus if hypertension persists after 5 minutes. If hypertension or movement persists after remifentanil bolus then the inspired sevoflurane may be increased to aim for an end tidal concentration that is increased by 0.3%. Repeat increase if light anaesthesia persists

- If movement and/or confirmed hypertension give an opioid at discretion of anaesthetist (except remifentanil) and/or increase the concentration of sevoflurane by 0.3%, repeat if necessary

- NDNMB Non depolarizing neuro muscular blocker alone may be given if movement occurs without hypertension and the anaesthetist judges that anaesthesia depth is not inadequate

- If movement interferes with surgery or risks compromising adequate ventilation/oxygenation in a way that puts the child at significant risk of an adverse

event then a repeat dose of NDNMB should be given immediately. If NDNMB cannot be given due to nearing completion of surgery then a bolus of propofol should be given.

2.2.3.3 Bradycardia

For HR less than 90 BPM for over one minute administer atropine 10-20 mcg/kg or glycopyrrolate 5 mcg/kg. If bradycardia persists after the above and is less than 70 bpm or is associated with hypotension then decrease the dexmedetomidine to 0.5 mcg/kg/h if in the low dose sevoflurane/dexmedetomidine/remifentanil arm.

2.3 Protocol steps

The protocol of the TREX study provides several steps that correspond to six visits:

- Visit 1: informed consent and screening, up to 24 hours prior to surgical procedure. Explain to the parent(s)/legal guardian(s) the objectives of the study. Informed consent must be signed by the parent(s)/legal guardian(s). Benefits and risks for the participant and will answer all questions regarding the study

- Visit 2: pre-randomization, up to 24 hours prior to surgical procedure. Baseline demographic data will be collected prior to anaesthesia, including: age, birth weight, multiple pregnancy, weight at time of surgery, gender, gestational age at birth, any prior medical history and indication for surgery. Primary language at home, maternal education, maternal age, family structure, rurality, ethnicity, birth order and number of siblings will also be collected

- Visit 3: surgical procedure. During the procedure, MAP, HR, oxygen saturation (SpO2), end tidal CO2 (ETCO2), temperature, end tidal sevoflurane concentration (ET sevo) (where applicable) will be collected from electronic anaesthesia records at 3/5-minute intervals intraoperatively. HR, SpO2, MAP will be recorded in

PACU every 5 minutes (from electronic record if possible). Other data, such as change in drug concentration or in drug administration and events of light anaesthesia, will be collected by research assistants on a paper case report form (CRF) and then entered into the web-based database system. Any hypertensive or bradycardia event requiring treatment, or any episode of light anaesthesia requiring intervention will be noted along with the intervention/treatment used

- Visit 4: short term follow up 1, 24 hours after surgery to determine if any major events happen

- Visit 5: short term follow up 2, 5 days after surgery to determine if any major events happen

- Visit 6: long term follow up, 3 years of age plus 6 months to evaluate neurodevelopment assessment.

2.4   Database structure

By analyzing pharmacokinetic studies of dexmedetomidine, a database was constructed with data deemed important to obtain a correct analysis of the drug of patients belonging to the experimental arm. Most of the data needed for the database was collected in visit 2 and 3 provided by the protocol. The dexmedetomidine concentrations in the patients' blood were entered into a database.

Elements have been included in this database:

1. ID patient: sequential number to identify the patient

2. ID centre: sequential number to identify the centre.

   The number code is:

   a. 021 Giannina Gaslini Institute, Genoa

   b. 026/126 Mangiagalli Center, Milan

   c. 027 Bambino Gesù Children's Hospital, Rome

    d.   028 Children's Hospital 'Cesare Arrigo', Alessandria

    e.   029 Universital Hospital, Pisa

    f.   031 Meyer Children's Hospital, Florence

    g.   125 Children's Hospital 'Vittore Buzzi', Milan

3. Time: is calculated from the start of drug administration. Each time a change occurs the corresponding minute is marked

4. Rate Dexmedetomidine: represents the concentration at which the drug is infused. Except the first value which is calculated from bolus data, through this formula

$$[\text{AMT Dex }'[\text{mcg/kg}']\ ]/[\text{Duration Dex }'[\text{min}']]$$

5. Rate Dexmedetomidine multiplied by the weight

$$[\text{RATE Dex }'[\text{mcg/min/kg}']] * [\text{WT }'[\text{kg}']]$$

6. Amt Dexmedetomidine: for each patient first data corresponds to bolus that is administered at the beginning of anesthesia. Subsequent data, inserted in this column, are obtained through a formula from the concentration with which the drug is infused

$$[\text{RATE Dex }'[\text{mcg/min/kg}']] * [\text{Duration Dex }'[\text{min}']]$$

7. Amt Dexmedetomidine multiplied by the weight

$$[\text{AMT Dex }'[\text{mcg/kg}']\ ] * [\text{WT }'[\text{kg}']]$$

8. Duration of Dexmedetomidine: duration for each concentration that was administered

9. DV: plasma concentration that was measured in the samples in the different ranges, in first column it is expressed in mcg/ml, in second one in mcg/L

10. Duration Remifentanil: duration for each concentration that was administered

11. Amt Remifentanil: for each patient first data corresponds to bolus that is administered at the beginning of anesthesia. Subsequent data, inserted in this

column, are obtained through a formula from the concentration with which the drug is infused

$$[\text{Rate Remi } '[\text{mcg/min/kg}']] * [\text{Duration Remi } '[\text{min}']]$$

12. Amt Remifentanil multiplied by the weight

$$[\text{AMT Rem } '[\text{mcg/kg}']] * [\text{WT } '[\text{kg}']]$$

13. Rate Remifentanil: represents the concentration at which the drug is infused. Except the first value which is calculated from bolus data

$$[\text{AMT Rem } '[\text{mcg/kg}']]/[\text{Duration Remi } '[\text{min}']]$$

14. Rate Remifentanil multiplied by the weight

$$[\text{Rate Remi } '[\text{mcg/min/kg}']] * [\text{WT } '[\text{kg}']]$$

15. Sevoflurane: percentage to which it was infused

16. Duration of Sevoflurane: duration of every percentage

17. Light anaesthesia: event requiring drug doses to be increased or additional drugs administered, such as rocuronium or propofol. Causes have been movement, hypertension or laryngospasm

18. Gestation age: week of gestation in which infant was born

19. PNA: post natal age, most useful element if we talk about premature babies born, which in this case were excluded from the protocol

$$[\text{Age } '[\text{months}']] * (52/12)$$

20. PMA: post menstrual age, most useful element if we talk about premature babies born, which in this case were excluded from the protocol

$$[\text{GA gestational age } '[\text{week}']] + [\text{PNA weeks}].$$

21. Age in months

22. Weight in kg

23. Sex: 0 = female, 1 = male

24. BIS: depth of sedation, in some patients, was measured through the bispectral index (BIS). The BIS, to monitor the appropriateness of general anesthesia, is measured through four electrodes are applied at the child's forehead, the first at half and the last at eye level. This allows to record the electroencephalogram that is translated into a number

25. Pathology: this kind of sedation has been applied in different surgery, such as craniofacial, thoracic, abdominal, urologic, orthopedic, neurosurgical. Consequently there are different pathology for example:

    a. Tethered cord Syndrome

    b. Supernumerary finger

    c. Cleft palate

    d. Megaureter

    e. Scaphocephaly

    f. Ureteral reflux

    g. Hirschsprung disease

    h. Anorectal malformation

    i. Megacolon

    j. Obstetric paralysis

    k. Renal mass

    l. Duplex collecting system with megaureter

    m. Monolateral testicular retention

    n. Right hand central longitudinal defect associated with simple IV/V fingers syndactyly

    o. Hypospadias

    p. Cryptorchidism

    q. Hydronephrosis

r. Kidney cancer

s. Congenital pulmonary airway malformation.

2.5   Results of the database

In this section the first quantitative results from the database are presented.

Data were gathered when a blood sample was collected and/or an element of anaesthesia was modified.

From the analysis of the database we have obtained the results that we will illustrate:

- There were 138 blood samples from 32 patients (22 males and 10 females), that have been analysed. Four to five samples were collected for each child

Patients distribution by gender
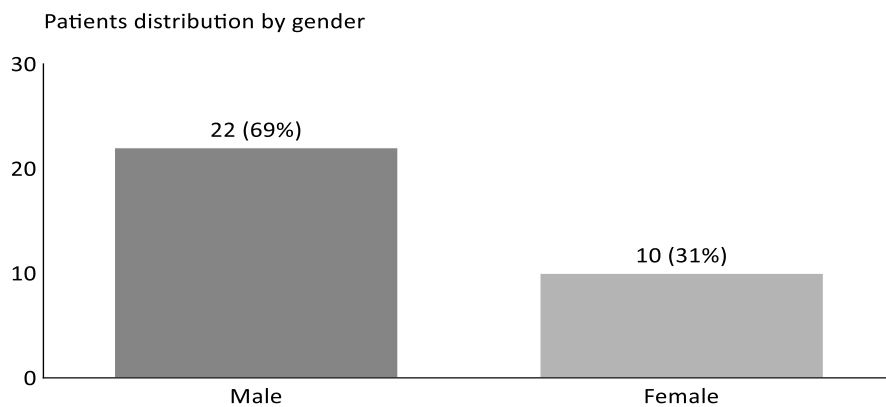
- Patients came from eight centers in Italy

Patients and samples by center
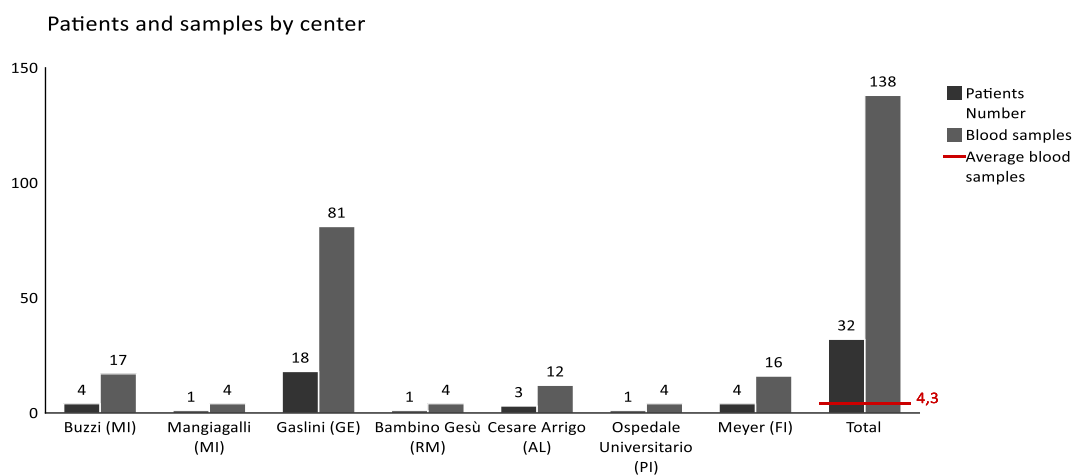
- Patients, in this study, underwent anaesthesia for at least 2 hours, with the longest lasting 6 hours

- The infusion rates of dexmedetomidine ranged from 0.5 to 1.0 mcg/kg/h, while remifentanil dose ranged from 0.1 to 0.8 mcg/kg/min

- Dexmedetomidine measurements in blood samples range from undetectable levels (baseline) up to 0.01073 mcg/mL; of note the dataset comprises an outlier measurement (0.1211 mcg/mL), probably caused by sampling from the same infusion arm

Dexmedetomidine measurements by age (mcg/mL)
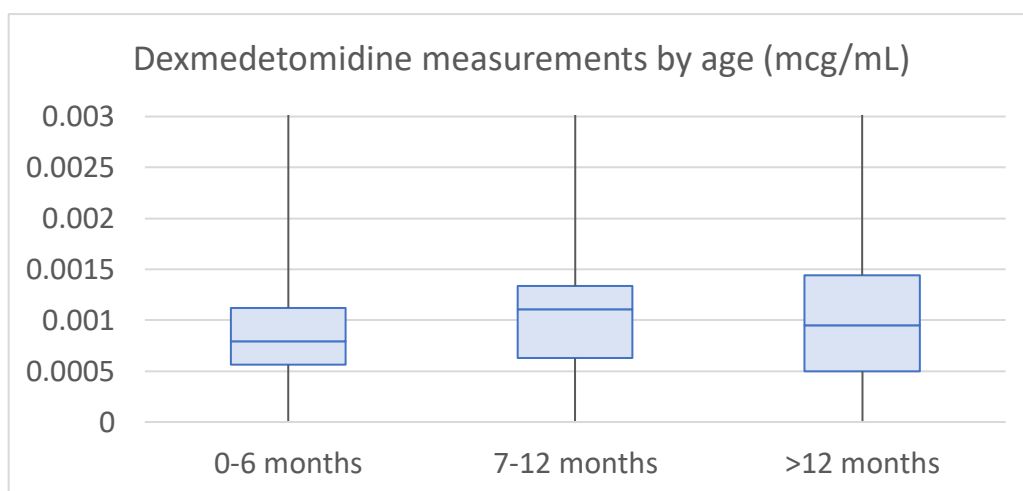
- Measurements accuracy was reported as Bias % meanwhile precision was defined as % Coefficient of variation (CV) for High, Medium and Low QC. In particular, Bias % was -1.02, 4.14 and 6.08 for High, Medium and Low QCs, respectively. % CV was 2.05 for Low, 1.93 for Medium and 4.19 for High QC. Limit of quantification was 0.15 ng/mL

- There are 9 samples in six patients where the dexmedetomidine concentration was undetectable, plus baseline samples

- From the data we have collected age and weight are certainly two of the elements that most influence the concentration of dexmedetomidine in the blood. Of these two data were calculated the medians; the median (IQR) age is 12 (5.2-15.5)

months, weight 9.9 (7.3-10.8) kg. None of children were born premature, as for inclusion criteria, the median of gestational age is 39 weeks (IQR 38-40)

- There were 11 events of light anaesthesia that occurred in 8 patients, with movements or hypertension observed during the surgical procedure. These events have been resolved by increasing two times dexmedetomidine, seven times remifentanil and three times sevoflurane, in three occasions it was decided to increase two drugs simultaneously. Propofol or Rocuronium were administered to manage light anaesthesia events only once each

- BIS data are very heterogeneous. The data collected in the experimental arm patients range from 21 to 76. The problem is that numerous studies have shown that in children the measurement is not so reliable. Our goal is to understand if the collected numbers of BIS are indicative of anything and if they allow to have a pharmacodynamic data, an essential aspect that is still under scrutiny defined with regard to dexmedetomidine.

3 Pharmacokinetic analyses

In this chapter the analysis of parmacokinetics are presented.

The first section illustrates the considerations and informations, based on validated model studies, necessary to create an efficient pharmacokinetic model.

The second section explains the formulas necessary to obtain the pharmacokinetic data. Section three describes the model selected for the analysis of this study. Finally, results are summarized and discussed in section four.

3.1 Consideration about pharmacokinetic

Pharmacokinetic analyses of dexmedetomidine, as mentioned above, have already been carried out by working groups.

In this work the collected data and in particular the weight and age of the children who have an impact on the concentration of dexmedetomidine in the blood were analysed. Age is important because it correlates to the level of maturation of the organism essential for processes such as drug clearance.

To carry out the analysis, after collecting the data of our population, Dr Anderson and PhD James Morse have been asked to cooperate. They come from department Anaesthesiology, University of Auckland, they are two of the most experienced people in the analysis of pharmacokinetics of this drug in children.

Analysis is designed to determine if observed concentrations match those predicted by the PK model used to estimate concentration in the TREX study:

- The PK model comprises a three-compartment (central and peripheral) linear model

- Population plasma predictions will be simulated in infants from the trial using their demographic data (age, weight, dose) with population modelling (NONMEM)

- The performance of the infusion regimen simulated data will be compared with observed data by calculating the median performance error, median absolute performance error

- The ability of a known PK model to predict new data from a further study can also be assessed using a visual predictive check

- Concentration prediction intervals from an earlier study are graphically superimposed on those intervals determined from observed concentrations in the new study

- Simulation is performed with parameter estimates from the earlier study using 1000 subjects with characteristics taken from new patients

- For data such as these where covariates such as dose, weight, and height are different for each patient, a prediction-corrected visual predictive check is used; observations and simulations are multiplied by the population baseline value divided by the individual-estimated baseline.

3.2 Pharmacokinetic analyzing

Data obtained in the TREX study were pooled with published dexmedetomidine time-concentration observations: Potts (54,74), Cortinez (75), Rolle (76), Talke(77). Two and three-compartment PK models with first order elimination were used to describe dexmedetomidine PK. These models were parameterised in terms of clearance (CL), volume of distribution (V) and intercompartmental clearance (Q). Allometric theory described the relationship between size, structure and function and was used to quantify size-related changes in PK parameters (6). PK parameters (e.g., CL,Q, V) were standardized to an adult measure of body size (size) with a standard weight of 70 kg using allometric scaling (Equation 1) (7-9):

$$Fsize = \left(\frac{size}{70}\right)^{EXP} \qquad\qquad \text{Equation 1}$$

Where Fsize is a variable describing the fractional difference from a standard adult value and EXP is the allometric exponent; ¾ for functional processes such as clearance and 1 for volumes.

The maturation of dexmedetomidine clearance was assessed using a maturation function (Equation 2):

$$MF = \frac{PMA^{Hill}}{TM_{50}^{Hill} + PMA^{Hill}} \qquad\qquad \text{Equation 2}$$

Where TM_{50} is the maturation halftime and Hill is exponent describing the steepness of the maturation profile.

Equation 3 shows how Fsize can be used to scale a standard value of CL (CL_{STD}) and account for maturation with age to predict the value in a given individual:

$$CL = CL_{STD} \times Fsize \times MF \qquad\qquad \text{Equation 3}$$

Population parameter estimates were obtained using nonlinear mixed effects models (NONMEM 7.5 ICON Development Solutions, USA) with first-order conditional estimation and a convergence criterion set to 3 significant digits.

Population parameter variability (PPV) was accounted for using an exponential model for the random effect variables (η). This assumes a log-normal distribution and avoids parameter estimates falling below biologically plausible values. Variables were assumed to have a mean of zero and variance denoted by ω^2 (Equation 4):

$$P_i = P_{TV}\, e^{\eta_i} \qquad\qquad \text{Equation 4}$$

Where P is the parameter (e.g. CL) for the ith individual, P_{TV} is the typical value for that parameter and η is the random effects variable.

Residual unidentified variability (RUV) was modelled using both proportional and additive residual errors (Equation 5). The between subject variability ($\eta_{RUV,i}$) of the RUV was also estimated for data. The population mean parameters, between subject variance and residual variance were estimated using the first order conditional interaction estimate method using ADVAN13 TOL=9 of NONMEM. Convergence criterion was 3 significant digits.

$$\text{SD}ij = \sqrt{\left(\left(Obs_{ij}\cdot\theta_{RUV\_CV}\right)^2 + \left(\theta_{RUV\_SD}\right)^2\right)}\cdot e^{\eta PPV_{RUV\,i}} \qquad \text{Equation 5}$$

Where Obs_{ij} is the dexmedetomidine plasma concentration in the ith individual at the jth time. Individual predictions of dexmedetomidine concentration were calculated using Equation 6 with the random effects (ε) fixed to 1:

$$Y = Obs_{ij} + \text{SD}ij\,.\,\varepsilon \qquad\qquad \text{Equation 6}$$

3.3 Model selection

The minimum value of the objective function (OBJ) [-2log-likelihood (-2LL)] provided by NONMEM served as a guide during model building. Model selection was also based on parameter plausibility and prediction-corrected visual predictive checks (PC-VPC) plots (10). For two nested models a decrease in the minimum value of the objective function (ΔOBJ) of 3.84 points for an added parameter was considered significant at the 0.05 level. Shrinkage considers the quality of the observed data. The

term η-shrinkage refers to the between-subject variability. When the observed data are informative η-shrinkage approaches zero and when the data are less informative it approaches 1. Bootstrap methods provided a means to evaluate parameter uncertainty(78). A total of 1000 bootstrap replications were used to estimate parameter means and confidence intervals. Results from the population models are presented as parameter estimates, together with their 95% CI. Between subject parameter variability is expressed as an apparent coefficient of variation obtained from the square root of the variance estimate [CV (%)].

3.4 Results

Parameter estimates for the pooled data determining the dexmedetomidine model are shown in Table 1. There were 2267 dexmedetomidine concentrations that were amenable for modelling in the pooled dexmedetomidine PK analysis. A three-compartment PK model proved superior to the two-compartment model for the pooled dexmedetomidine analysis (ΔOBJ 285.2).

Table 1 - *Dexmedetomidine population pharmacokinetic parameter estimates. Bootstrap median and 95% confidence interval (CI) determined from 100 bootstrap replications.*
Volume of distribution: V; clearance: CL; intercompartmental clearance: Q; TM_{50}: maturation halftime; Hill: exponent describing the steepness of the maturation profile. Residual unidentified variability: RUV; population parameter variability: PPV%. Sh%=shrinkage. Size is accounted for using theory-based allometric scaling to a 70 kg individual with the allometric exponents of ¾ for CL and 1 for V. PPV%=√variance

	Estimate	Bootstrap median	PPV (%)	95%CI	Sh%
V1 (L/70 kg)	19	18.7	105	15.2, 24.1	25.6
V2 (L/70kg)	24.1	25.2	35.2	19.9, 31.3	27.4
V3 (L/70 kg)	50	50.6	63.4	42.8, 66.8	2.0
CL (L/min/70 kg)	0.67	0.67	37.7	0.61, 0.75	1.0
Q2 (L/min/70kg)	1.37	1.31	54.3	0.91, 1.70	14.3
Q3 (L/min/70 kg)	0.518	0.491	94.4	0.33, 0.71	3.3
TM_{50}	48.9	48.6	-	40.3, 66.8	-
Hill	1.29	1.29	-	0.76, 2.2	-
Additive residual Error (µg/mL)	0.006	0.006	η_{RUV} 0.53	-	-
Proportional Residual Error (%)	20.7	20.4	-	18.2, 22.6	-

The final model including allometric scaling pharmacokinetic parameters using total body weight. The increase in CL due to age accounted for with a maturation function. A PC-VPC for the final model using pooled data is shown in Figure 1. Figure 2 demonstrates the performance of the model with the data observed in the TREX study. This figure shows that observations from the TREX study were consistent with those predicted by the "universal" model using pooled data. Maturation of dexmedetomidine clearance scaled using total body weight is shown in Figure 3.
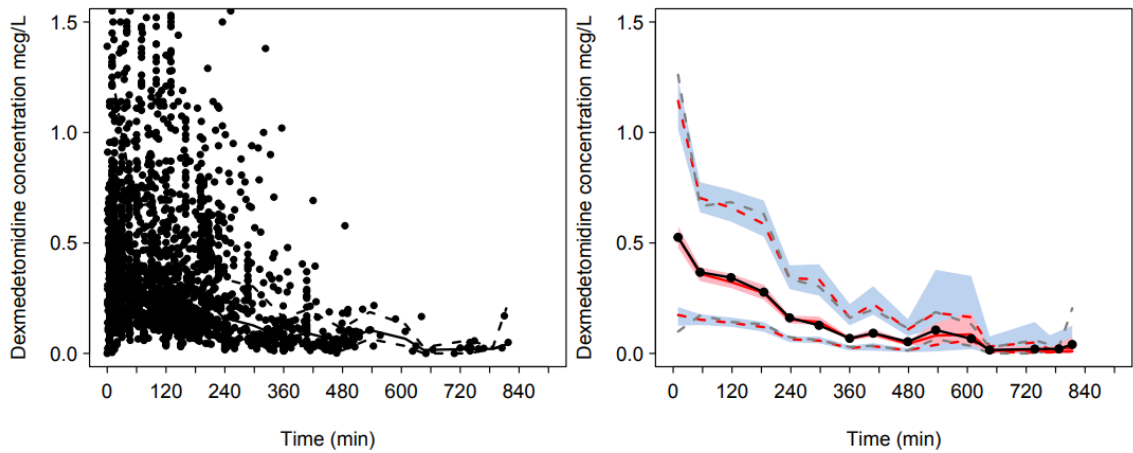
Figure 1 *– In this figure the model with the pooled data is shown. Prediction-corrected visual predictive check (PC-VPC) for the pooled dexmedetomidine pharmacokinetic model. Model developed using pooled pediatric (54) and adult (77,79) dexmedetomidine plasma concentrations. Plots show median (solid) and 90% intervals (dashed lines). The left hand plot shows all prediction corrected observed dexmedetomidine concentrations. Right hand plot shows prediction corrected percentiles (10%, 50%, and 90%) for observations (grey dashed lines) and predictions (red dashed lines) with 95% confidence intervals for prediction percentiles (median, pink shading; 5th and 95th blue shading).*
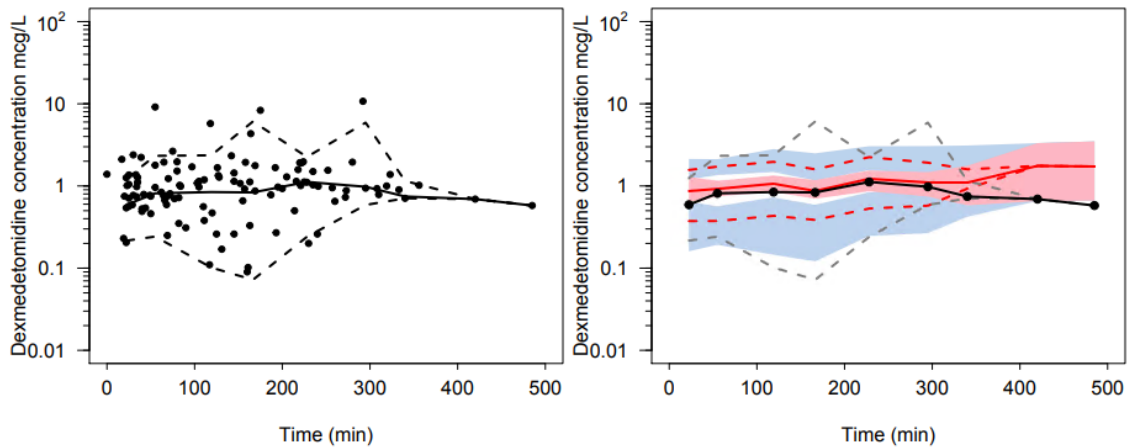
Figure 2 *– In the figure 2 the model whit the TREX data is shown. Prediction-corrected visual predictive check (PC-VPC) for the dexmedetomidine data obtained only in the TREX study using the pooled pharmacokinetic model. Plots show median (solid) and 90% intervals (dashed lines). The left hand plot shows all prediction corrected observed dexmedetomidine concentrations. Right hand plot shows prediction corrected percentiles (10%, 50%, and 90%) for observations (grey dashed lines) and predictions (red dashed lines) with 95% confidence intervals for prediction percentiles (median, pink shading; 5th and 95th blue shading).*
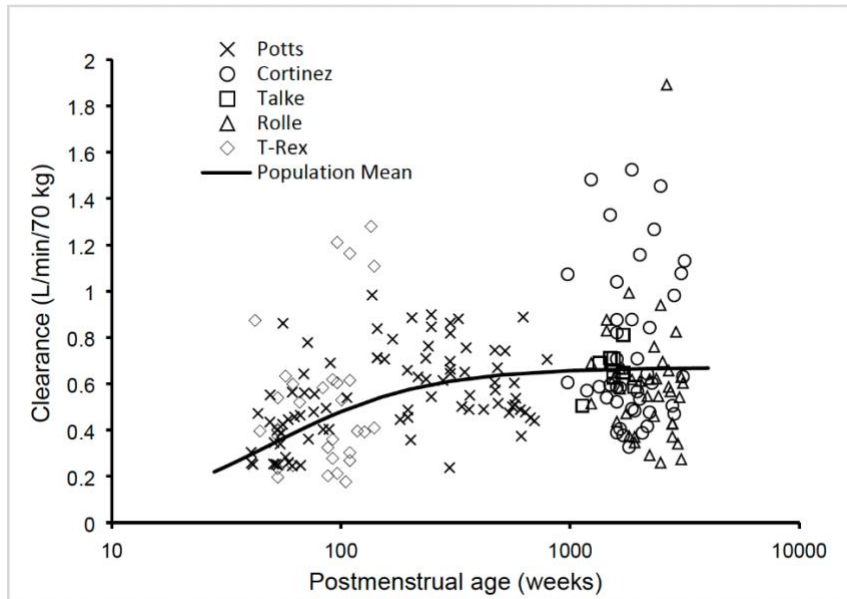
51

Figure 3 *– In this figure is represented the maturation of dexmedetomidine clearance using pooled data.*

4 Conclusions

The goal of treatment is the target effect. A pharmacodynamic (PD) model is used to predict the target concentration known to be associated with a target effect. Pharmacokinetic information is used to predict a dose that achieves the target effect. That pharmacokinetic information should include covariates such body size and age (describing clearance maturation) as these are important to predict concentration in an individual child (80).

The dosing schedule for the TREX study (3) was based on known pharmacokinetics in children with mean age of 3.8 (median 3 years, range 1 week-14 years) and weight of 16.0 kg (median 13.3 kg, range 3.1-58.9 kg) (54).

The analyzed population comprises is of 32 children, of which 10 were females and 22 males, with an age ranging from two weeks to 23 months, the median (IQR) age is 12 (5.2-15.5) months. The range of weight goes from 3.75 kg to 15 kg, whose median is 9.9 (7.3-10.8) kg. None of children were born premature, as for inclusion criteria, the median of gestational age is 39 weeks (IQR 38-40). These elements, as seen in previous studies, were the most influential on the analysis of the PK.

For children nursed in an intensive care ward after cardiac surgery and while a target concentration of 0.6 mcg.L^{-1} was identified, it remained uncertain if that target might be applicable to those sedated for invasive procedures out of intensive care (54). Dexmedetomidine dose infusion for the TREX study was initially set to establish a steady-state concentration of 0.6 mcg.L^{-1}. This target concentration has subsequently proven useful for sedation during radiological procedures (81). Higher infusion rates were entertained during study design but concern was expressed that cardiovascular adverse effects could cause compromise in children (82–84). There were few adverse

effect concentration-response relationships in children published at the time of trial initiation (85), although subsequent data has improved understanding of these adverse effects (86).

It is necessary to obtain higher target concentrations to reach the target effect.

The pilot study already confirmed the need to use higher doses, as well as ensuring the safety of these doses and effects were investigated in the TREX study.

A change in trial protocol to increase dexmedetomidine infusion rates was necessary during the pilot study because sedation was inadequate. Dose increased from a loading dose of 0.6 mcg/kg over 10 min with maintenance 0.6 mcg/kg/h (Version 1) to 1 mcg/kg over 10 min with maintenance 1 mcg/kg/h (Version 2) to 1 mcg/kg over 10 min with maintenance 1-1.5 mcg/kg/h (Version 3) (3). Hypotension increased with dose, but was generally mild and no child required vasoconstrictor rescue during the pilot study. Only one child suffered bradycardia and that episode was considered unrelated to dexmedetomidine (3). Cardiovascular changes observed in children using the experimental anaesthesia regimen (dexmedetomidine: 1 mcg/kg over 10 min with maintenance 1-1.5 mcg/kg/h) had been predicted, but in most cases did not compromise the outcome of the procedure and were managed with the increase in drugs administered.

The incidence of episodes of light anaesthesia requiring a rescue dose of anaesthetics is lower in our series of patients (11 episodes in 32 infants) than that of the pilot study (42 episodes in 60 patients). Dexmedetomidine plasma concentration when events of light anaesthesia occurred was lower (0.79 mcg.L^{-1}) than the target concentration (1 g.L^{-1}), which could contribute to the occurrence of these events. The current protocol also includes the use of low dose of sevoflurane, that was not used in the pilot study. It will be important to have a better understanding of the complex interaction between

dexmedetomidine, remifentanil and sevoflurane, with the subsequent pharmacodynamic. This is one of the follow-up steps of this current trial.

It remained uncertain if the increased dose required for sedation was due to pharmacokinetic differences in the cohort used for dose prediction or if a higher target concentration was necessary for adequate sedation. This current study confirms that pharmacokinetics in Italian children involved in the TREX study are similar to those of others (87), including those children recovering after cardiac surgery. Although only few pharmacodynamic studies in children have been performed, those in adults confirm a target concentration for sedation of 1 mcg.L^{-1} (88,89), consistent with the higher doses required in the later protocols of the pilot study (3). The cardiovascular compromise associated with rapid infusion have been demonstrated using simulation (90) and cardiovascular changes observed in those children using protocol versions 2 and 3 could also have been predicted had concentration-adverse effects been described before trial initiation.

This current PK analysis from the experimental arm of the TREX study confirms that plasma concentration of dexmedetomidine predictable using known covariates such as age and size. Fat mass can also be used as a descriptor for volume of distribution (87). Drug interactions also are an important covariant for describing pharmacodynamic variability (91,92). The complex interactions between dexmedetomidine, remifentanil and sevoflurane, drugs used in this TREX study, requires further investigation.

4.1 Contribution

In the current state of the art the two main contributions that this study gives are:

- Pharmacokinetic analyses from the experimental arm of the TREX study allows to validate the study considering that estimates are similar to initial estimates used in

the pilot study and to those estimates derived in a larger pooled 'universal' analysis that used data from neonates to adults

- The initial target concentration (0.6 mcg.L^{-1}) used to sedate children cared for in the intensive care after cardiac surgery was inappropriate for infants in the TREX study. A target concentration 1 mcg.L^{-1} proved satisfactory.

4.2 Weaknesses

Weaknesses are mainly:

- Events of light anaesthesia that still occur and the difficulty to obtain optimal sedation in some cases. This adds on to the difficulty of evaluating the depth of sedation. The evaluation of sedation can be based only on external elements such as parameters, on what we observe but it is really complex

- Difficulty in fully understanding the interactions that occur between the various drugs, this aspect needs further investigation because it is crucial to understand the pharmacodynamic.

4.3 Next steps

TREX study is still in progress, the next steps include the development of:

- Main outcome: superior or equal neurodevelopmental outcome in low-dose sevoflurane/dexmedetomidine/remifentanil anaesthesia compared to standard dose sevoflurane anaesthesia

- Secondary outcomes: anaesthesia endpoints and safety

- In particularly pharmacodynamic: BIS data would be use as a parameter to find a valid pharmacodynamic model of dexmedetomidine and also a model of the interaction between the three drugs administered to understand better the clinical effects.

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