
**DIVISION OF MEDICAL SCIENCES & GRADUATE ENTRY MEDICINE**

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# VARIATION IN CLEARANCE AND INVASIVENESS OF PHARMACOKINETIC STUDIES IN CHILDREN

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## ABSTRACT

Inter-individual variation in pharmacokinetic parameters of drugs can have profound effects on drug safety in children. Midazolam and morphine are among the most commonly used drugs in critically children. Theophylline has seen several cycles of enthusiasm and unpopularity over the years, although oral theophylline is now rarely used, IV aminophylline is still used regularly in severe asthma. These drugs are metabolised by hepatic enzymes (CYP3A4, CYP1A2 and glucuronidation) which have variable expression. Three systematic reviews were conducted in order to explore the inter-individual variation of clearance of these drugs in children.

The first systematic review evaluated the inter-individual variability of midazolam clearance in children. Midazolam is predominantly metabolised by CYP3A4. Twenty two PK studies were identified. The mean clearance of midazolam varied between 0.78 to 3.5 ml/min/kg in neonates and 1.1 to 15 ml/min/kg in children. Age was a statistically significant predictor of clearance ($p<0.05$). Critical illness was however not a statistically significant predictor of midazolam clearance after adjusting for other covariates ($p=0.279$). There was a statistically significant difference between the coefficient of variation of midazolam clearance in preterm neonates (91%) and children (40%) ($p=0.002$). However, there was no significant difference between the CV in critically ill and non-critically ill children.

A second systematic review evaluated the variability of theophylline clearance. Theophylline is metabolised by CYP1A2. Twenty nine studies were identified. Mean clearance of theophylline varied between 0.2 and 2 ml/min/kg. Age was a significant predictor of theophylline clearance ($p<0.05$). There was, however, no significant difference between the CV of theophylline in any age group. The CV of theophylline clearance was not significantly different between critically ill (35%) and non-critically ill (39%) ($p=0.403$). A sub-analysis of children also did not show any significant difference between critically ill and non-critically ill ($p=0.418$).

A third systematic review evaluated the variability of morphine clearance in children. Morphine is metabolised by UGT. Twenty studies were identified. The mean clearance of the studies identified varied between 2 and 16 ml/min/kg in neonates and 19 to 52 ml/min/kg in children. Critical illness was not a statistically significant predictor of morphine clearance. Analyses of the limited data showed no statistically significant differences in CV between any age groups. There was also no statistically significant difference between the CV in critically ill and non-critically ill children.

In all the studies, a major limitation was the limited number of PK studies in children. Invasive studies should be avoided in children therefore, a final systematic review evaluated the invasiveness of PK studies over two decades. The number of blood samples collected per

child was significantly lower in studies carried out between 2004-2014 than those between 1981-1990 (p=0.013). Furthermore, the total volume of blood collected in 24 hours for PK studies was significantly lower in new decade than old (p=0.025). However, there was no difference in the volume of blood collected per sample. There were 35 population PK studies, all of which were new studies. The median number of blood samples in population PK studies (median 6, [IQR: 4-9]) was significantly lower than non-population PK studies (median: 8, [IQR: 6-10]) (p=0.007).

In conclusion, age is a risk factor for inter-individual variation of midazolam clearance in children. It is also an important predictor of midazolam, morphine and theophylline clearance in children. Therefore, age appropriate dosing is important. More PK studies are required to determine the effect of critical illness on the variability of clearance of these drugs. The utilisation of population PK methods should be encouraged to minimise invasiveness of PK studies. New methodologies for reducing sample volumes and frequency should be considered in all studies.

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## PUBLICATIONS AND PRESENTATIONS RELATED TO THIS THESIS

### PUBLICATIONS:

- Altamimi MI, Sammons H, Choonara I. Inter-individual variation in midazolam clearance in children. A systematic review, Arch Dis Child. 2015 Jan.
- Altamimi MI, Choonara I, Sammons H. Inter-individual variation in morphine clearance in children. A systematic review, Eur J Clin Pharmacol. 2015 Apr 8.
- Altamimi MI, Choonara I, Sammons H. Invasiveness of pharmacokinetic studies in children. A systematic review, BMJ Open in press.

### ORAL PRESENTATION:

- Inter-individual variation in midazolam pharmacokinetics in children, National Child Health Workshop, 11 September 2012, Derby, UK.
- Inter-individual variation in midazolam pharmacokinetics in children: a systematic review the 14$^{th}$ Biannual European Society for Developmental Perinatal & Paediatric Pharmacology (ESDPPP) Congress, 4$^{th}$ – 7$^{th}$ June, 2013, Salzburg, Austria.
- Inter-individual variation in theophylline pharmacokinetics in children, National Child Health Workshop, 18 September 2013, Derby, UK.

- Inter-individual variation in morphine pharmacokinetics in children, National Child Health Workshop, 16 September 2014, Derby, UK.
- Invasiveness of pharmacokinetic studies in children, a systematic review the 15th Biannual European Society for Developmental Perinatal & Paediatric Pharmacology (ESDPPP) Congress, 23th – 26th June, 2015, Belgrade, Serbia.

**POSTER PRESENTATIONS:**

- Inter-individual variation in morphine pharmacokinetics in children: a systematic review, NPPG Annual Conference, 7th - 9th November, 2014, University of Nottingham, Nottingham, UK.
- Inter-individual variation in theophylline pharmacokinetics in children: a systematic review, NPPG Annual Conference, 6th - 8th November, 2015, University of Nottingham, Nottingham, UK.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| **AUC** | Area Under the Concentration Curve |
| **BPD** | Bronchopulmonary Dysplasia |
| **BSA** | Body Surface Area |
| **cAMP** | Cyclic Adenosine Monophosphate |
| **CDH** | Congenital Diaphragmatic Hernia |
| **CNS** | Central Nervous System |
| **CPB** | Cardiopulmonary Bypass |
| **CV** | Coefficient of Variation |
| **CYP** | Cytochrome P450 |
| **DBS** | Dried Blood Spots |
| **DMS** | Dried Matrix Spots |
| **DPS** | Dried Plasm Spots |
| **DUS** | Dried Urine Spots |
| **ECMO** | Extracorporeal Membrane Oxygenation |
| **EMA** | European Medicines Agency |
| **FCS** | Facial Coding System |
| **GABA** | Gamma Amino Butyric Acid |
| **GFR** | Glomerular Filtration Rate |
| **GI** | Gastrointestinal Tract |
| **ICH** | International Conference of Harmonization |
| **IL** | Interleukin |
| **IM** | Intramuscular |
| **IN** | Intranasal |
| **IPA** | International Pharmaceutical Abstracts |
| **IV** | Intravenous |
| **LPS** | Lipopolysaccharides |
| **LTA** | Lipotechoic Acid |
| **M3G** | Morphine-3-Glucuronide |
| **M6G** | Morphine-6-Glucuronide |
| **MAS** | Meconium Aspiration Syndrome |
| **MRC** | Medical Research Joint Ethics Committee |
| **NAT** | N-acetyltransferase |
| **NICU** | Neonatal Intensive Care Unit |

| | |
|---|---|
| **NONMEM** | Nonlinear Mixed-effects Model |
| **PD** | Pharmacodynamics |
| **PICU** | Paediatric Intensive Care Unit |
| **PK** | Pharmacokinetics |
| **PPHN** | Persistent Pulmonary Hypertension of the Newborn |
| **RCPCH** | Royal College of Paediatric and Child Health Ethics Advisory Committee |
| **RDS** | Respiratory Distress Syndrome |
| **SNP** | Single Nucleotide Polymorphism |
| **TBV** | Total Blood Volume |
| **TNF** | Tumour Necrosis Factor |
| **TPMT** | Thiopurine S-methyltransferase |
| **UGT** | Uridine Diphosphate Glucuronosyltransferases |
| **Vd** | Volume of Distribution |
| **VSD** | Ventricular Septal Defect |
| **WHO** | World Health Organisation |

# CHAPTER ONE
# GENERAL INTRODUCTION AND OVERVIEW

## 1.1. Introduction

Pharmacokinetics (PK) and pharmacodynamics (PD) describe how a drug interacts with the human body. PK describes the process of absorption, distribution, metabolism and elimination (ADME) of drugs (1);while PD is concerned with the relationship between drug concentration and its pharmacological response (2). The human body handles drugs in different ways; hence, the variability in PK parameters for different people. Since individuals may vary in how they absorb, metabolise and eliminate drugs, the dosing of some drugs should be individualised. This is the core principle of clinical PK, which is the application of PK principles for managing patients individually (3). These types of practices will improve a drug's efficacy and reduce its toxicity (3).

## 1.2. Pharmacokinetic Mechanisms

### 1.2.1. Absorption

Most drugs prescribed for children are administered by an oral formulation. The process of absorption is complex and several factors are responsible for the inter-individual variation. Gastric pH is a major determinant of drug absorption. Gastric pH determines the degree of ionisation and stability of drugs. It is age dependant and usually neutral at birth before decreasing to 3 at 24-48h postpartum. It

increases to become neutral at about 10 days with subsequent decline to acidity at two years (4, 5). There is a gradual decline in intestinal permeability, which is initially high at birth. The decline is seen in the first week of life possibly due to reduced surface area (6). Weakly basic drugs such as Itroconazole tend to dissolve slowly at a higher pH (7). Gastric emptying time and intestinal transit time were also noted to be the major determinants of absorption and bioavailability. For example theophylline is a poorly soluble drug therefore its absorption is lower in young children with rapid intestinal time (7). Furthermore, gastric pH in neonates is usually higher than the pH in infants and children (5).

### 1.2.2. Distribution

Drug distribution occurs after absorption when the drug enters the systematic circulation. The volume of distribution (Vd) is the apparent volume into which the drug would have to distribute to achieve the measured concentration (8).

Distribution is affected by a combination of several factors and varies considerably with age, protein binding, body fat, body water and differences in membrane permeability. Lipid and water solubility affect the volume of distribution (8). It is lower for drugs that are water soluble, for example gentamicin. In contrast water soluble drugs for example chloroquine have a high volume of distribution (8). There is a marked difference in total body water and fat content in

neonates and adults (Figure 1.1). Total body water in neonates is about 85-90% and fat content is 10-15%, while total body water reduces to about 60% in adults (9, 10). Furthermore, extracellular water in neonates is higher (45% of body weight) compared to adults (20%)(9, 10). Therefore lipid soluble drugs, such as diazepam, have a lower volume of distribution in neonates than adolescents (7).

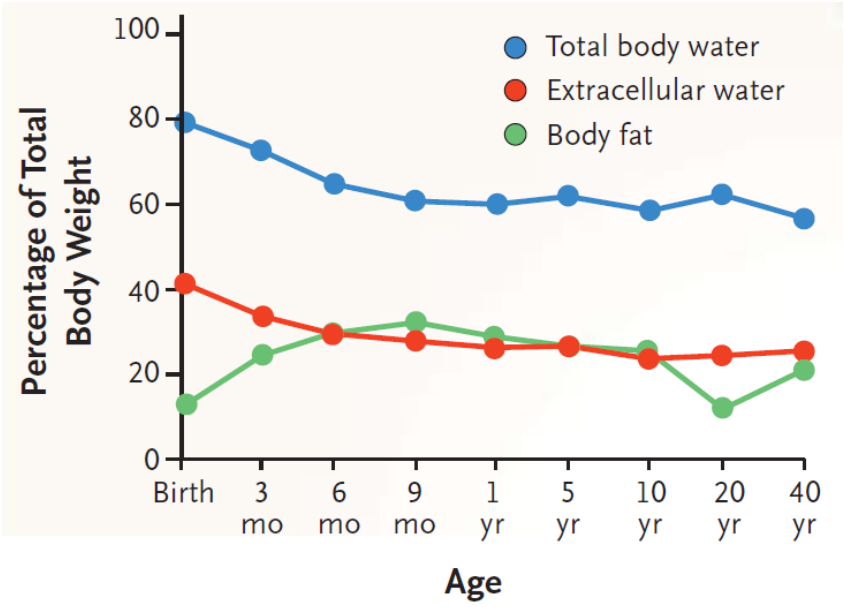


**Figure 1. 1: Developmental Changes in Distribution Sites**

*This figure adapted from Kearns et al. (2003) (10).*

### 1.2.3. Metabolism

#### 1.2.3.1. The metabolising enzymes

Several enzymes are involved in drug metabolism (Table 1.1). Although the liver is the most important organ for drug metabolism, several other organs such as the kidneys and the intestines also contribute to the process. Developmental changes in these organs

affect metabolism. While the hepatic metabolising enzymes are mainly located in the smooth endoplasmic reticulum of the hepatocytes, some are also present in the mitochondria as well as the cytosol. Oxidation by the cytochrome P450 (CYP) enzymes contribute to the majority of the metabolic process in the liver, accounting for up to 75% of drug metabolism (11).

CYPs are a large family of enzymes with different subfamilies playing overlapping and sometimes distinct roles in drug metabolism, as well as the metabolism of other endogenous compounds. Other important enzymes in the metabolic process include: the reductive enzymes (aldo-ketoreductases), hydrolytic enzymes (esterases), N-acetyl transferases, methyl transferases, glucronyltransferases, sulfotransferases and thiopurine S-methyltransferase (TPMT)(12, 13). Genetic influences can also been seen in these enzyme group; acetylation by N-acetyltransferase (NAT) depends on two genes, NAT1 and NAT2. Fast and slow acetylators can be identified by NAT2 gene analysis (14).

**Table 1. 1: Examples of substrate drugs for selected drug-metabolising enzymes**

| CYP1A2 | CYP2C9 | CYP2C19 | CYP2D6 | CYP3A4/5 | UGT2B7 | TPMT | NAT2 |
|---|---|---|---|---|---|---|---|
| Haloperidol | Diclofenac | Omeprazole | Haloperidol | Midazolam | Morphine | Azathioprine | hydralazine |
| Caffeine | Ibuprofen | Diazepam | Risperidone | Alprazolam | Chloramphenicol | 6-mercaptopurine | Isosorbide |
| Theophylline | Naproxen | Phenytoin | Amitriptyline | Cyclosporine | Ketoprofen | 6-thioguanine | Isoniazid |
| Naproxen | Celecoxib | Phenobarbitone | Clomipramine | Tacrolimus | All- trans retinoic acid | Cisplatin | - |
| Clozapine | Phenytoin | Cyclophosphamide | Desipramine | Indinavir | - | - | - |
| Duloxetine | Tolbutamide | Amitriptiylline | Imipramine | Ritonavir | - | - | - |
| Fluvoxamine | Losartan | Clomipramine | Paroxetine | Saquinavir | - | - | - |
| - | Fluvastatin | Voriconazole | Dextromethorphan | Nifedipine | - | - | - |
| - | Sulfamethoxazole | - | Flecainide | Amlodipine | - | - | - |
| - | Warfarin | - | Codeine | Verapamil | - | - | - |
| - | - | - | Tramadol | - | - | - | - |
| - | - | - | - | - | - | - | - |
| - | - | - | - | Clarithromycin | - | - | - |
| - | - | - | - | Erythromycin | - | - | - |

TPMT, thiopurine S-methyltransferase; UGT, UDP-glucuronosyltransferases; NAT, N-acetyltransferases; CYP, cytochrome P450

*This table adapted from de Wildt et al. (2014)(15).*

#### 1.2.3.2. CYP drug metabolism

CYP450 is involved in the metabolism of both endogenous and exogenous substances. Hormones, growth factors, prostaglandins and bile are all examples of the endogenous substrate of CYP. Although there are about 57 functional enzymes in the CYP superfamily, seven of the isoenzymes are involved in about 95% of CYP metabolism. These isoenzymes are: CYP1A2, CYP1A6, CYP2D6, CYP3A4, CYP2B6, CYP2C9 and CYP2C19 (16). These enzymes are mainly localised in the liver, the chief organ for drug metabolism.

There are inter-individual differences in the expression of these enzymes, with a resultant effect on drug metabolism and efficacy. Furthermore, there are developmental changes in CYP expression throughout the course of a human's lifetime.

#### 1.2.3.3. Drug interactions

CYPs are involved in several clinically important drug-drug interactions that can increase the risk of adverse reactions or reduce drug efficacy. CYP3A4 is the main enzyme involved in drug-drug interaction (Tables 1.2 and 1.3). Several drug interactions have been described in the literature. Examples of frequently prescribed enzyme inducers include phenobarbital, carbamazepine and phenytoin; while enzyme inhibitors include clarithromycin, erythromycin and omeprazole.

**Table 1. 2: Frequently prescribed CYP inducing drugs used by children**

| Inhibitor | Enzyme inhibited |
|---|---|
| Clarithromycin | CYP3A4 |
| Erythromycin | CYP3A4 |
| Itroconazole | CYP3A4 |
| Ketoconazole | CYP3A4 |
| Voriconazole | CYP3A4 |
| Esomeprazole | CYP2C19 |
| Pantoprazole | CYP2C19 |
| Omeprazole | CYP2C19 |
| Grapefruit juice | CYP3A4 |

*This table adapted from de Wildt et al. (2014) (15).*

**Table 1. 3: Frequently prescribed CYP inhibiting drugs used by children**

| Inducer | Enzyme induced |
|---|---|
| Phenobarbital | Multiple CYPs |
| Rifampin | Multiple CYPs |
| Dexamethasone | CYP2D6 |
| Carbamazepine | Multiple CYPs |
| Phenytoin | Multiple CYPs |
| Ethanol | CYP2E1 |
| St John's wort | CYP3A4s |

CYP,Cytochrom P450

*This table adapted from de Wildt et al.(2014) (15).*

CYP3A is the only subfamily of CYP3. It has four different genes, CYP3A4, CYP3A5, CYP3A7 and CYP3A43, which are located on chromosome 7 (17). They account for the metabolism of up to 50% of drugs in clinical use (18). CYP3A4 is the most expressed of the four genes; it is preferentially involved in the metabolism of large lipophilic drugs, such as midazolam and erythromycin (19). Population variability of CYP3A4 has been reported to be more than 100-fold (20). Multiple of factors such as genetic polymorphism,

environmental factors, circulating endogenoeous molecules, gender, induction and age are responsible for this variability (21). Wolbold et al. (2003) and Cotreau et al. (2005) reported differences in CYP3A4 expression in males and females (22, 23). Females usually have higher enzyme activity than males (24).

Therefore, polymorphism in CYP3A4 gene expression is a major cause of inter-individual variation. Single nucleotide polymorphism (SNP) in the $5^{I}$–flanking region of the CYP3A4 gene have been reported (25). The most common mutation is CYP3A4*1B, which is frequently reported among African-Americans (up to 50%), while it is often absent among people of Japanese and Chinese descent (16). CYP3A4 and CYP3A5 are almost identical, with more than 85% similarity in their amino acid sequences. Therefore, these enzymes also share similar substrate selectivity (19). The CYP3A5*3 allele is the most common type of CYP3A5 mutation, and similar to CYP3A4*1B, it is more prevalent among African-Americans (55%). Between 10-20% of Caucasians have been reported to have this mutation (21). CYP3A5 highly expressed in children, its level decreases to about 30% in adults (26, 27). Similarly, CYP3A7 is fully expressed in early neonatal life but becomes undetectable in adults. However, CYP3A4 is not fully expressed until after birth. It reaches 30% of adult levels one month after birth and to adult levels within three months of birth (28).

There are two subfamilies of CYP1: 1A and 1B. CYP1A has two isoforms, 1A1 and 1A2. Age, gender and enzymatic induction are common causes of variability in the expression of these enzymes. CYP1A2 metabolises several clinically important drugs, such as theophylline: acetaminophen, clozapine, propranolol and duloxetine (29-31). It is inducible by both phenobarbital and phenytoin (32) and for different substrates, including caffeine, the activity of CYP 1A2 has been reported to be higher in males than females (33). Although, polymorphism is common in the CYP1 family, its effect on drug metabolism is still largely unclear. CYP1A is extremely low in the neonates, and increases to about 25% of adult levels when the child is 1-year old; by the third year of life adult levels are attained (34). Theophylline is mainly metabolised by CYP1A2 (31). Therefore, in accordance with the level of hepatic expression, and subject to the presence of other minor metabolising enzymes, its clearance in neonates is about 50% of the adult level, increasing to 150% of adult levels by the age of 5 years before subsequently declining to adult levels in adolescence (35).

The two most common genes in the CYP2C subfamily are the CYP2C9 and CYP2C19. Both of these genes have a similar position on the chromosome. CYP2C9 and CYP2C19 are highly polymorphic. CYP2C9*2 and CYP2C9*3 are the two most common variants of the 2C9, with prevalence varying across different ethnicities. CYP2C9*2 is extremely rare among Chinese and Japanese people, but seen in 7-

16% of Caucasians (36). However, CYP2C19 polymorphism is more common in the Asian population than Caucasians (37). Despite their close genetic similarity, these enzymes have substrate specificity: CYP2C9 for weakly acidic molecules, e.g. diclofenac and phenytoin; and CYP2C19 for neutral or weakly basic molecules, e.g. proton pump inhibitors (19). Poor metabolisers have been reported to have as much as 15-fold increase in the omeprazole concentration than extensive metabolisers and consequently have a better H.Pylori eradication rate (38). Phenytoin is a substrate that is common to both enzymes, Odani et al. (1997) found that the elimination rate of phenytoin was 33% lower among individuals with CYP2C9*1/*3 alleles compared with those with homozygous CYP2C9*1. This effect was not seen with CYP2C19 polymorphism (39).

#### 1.2.3.4. UGT drug metabolism

UDP-glucuronosyltransferase (UGT) is one of the major drug metabolising enzymes. It catalyses the glucuronidation reaction, which involves the conjugation of glucuronic acid to substrate drugs (40). Glucuronic acid is derived from the cofactor UDP-glucuronic acid. UGT enzymes are a superfamily of enzymes, with distinct or overlapping substrate specificity. They are located in the smooth endoplasmic reticulum and the cell nucleus mainly in the liver, but are also expressed in the kidneys and the gastrointestinal tract (GI) (41). The majority of UGTs are expressed in the human liver, with

UGT1 and UGT2 being the most important family of enzymes (42). There is inter-individual variability in the expression of UGT, although the factors responsible for this are mostly unknown. Epigenetic regulation has been identified as a cause of variability of UGT1A10 in the liver and UGT1A1 in the kidneys (43). Age is also a major determinant of UGT expression and the time taken for the different forms of the enzymes to attain full maturity varies from 3-6 months. The maturation rates also vary between individuals (43). Early neonatal UGT deficiency is responsible for the developmental of adverse events like the grey baby syndrome, which is associated with chloramphenicol (44).

UGT substrates are as varied as the enzymes. Although the most commonly expressed isoforms are UGT2B4 (34-55%) and UGTB10 (5-19%) (45, 46), several clinically important drugs are metabolised by the less predominant forms. UGT1A3 and UGT1A4 metabolises lamotrigine (47), UGT1A6 and UGT1A9 metabolises paracetamol and propofol respectively (48, 49); morphine and naloxone are substrate drugs for UGT2B7 (50). Morphine is glucuronidated to an active metabolite (M6G) and an inactive metabolite (M3G) by UGT. Some drugs are capable of inducing the enzymes and they are involved in clinically relevant drug-drug interactions, such as the interactions between valproic acid (an inducer) and lamotrigine (a substrate)(51).

### 1.2.3.5. Ontogeny of hepatic metabolism

Hepatic metabolising enzymes undergo developmental transition. Several of these enzymes are present in the foetal liver as early as 8 weeks of gestation and their activity generally increases throughout pregnancy and beyond the postnatal period (Figure 1.2)(28). CYP3A7 is the most common enzyme expressed during the embryonic and foetal period. Its expression increases throughout gestation and peaks during the perinatal period (28).

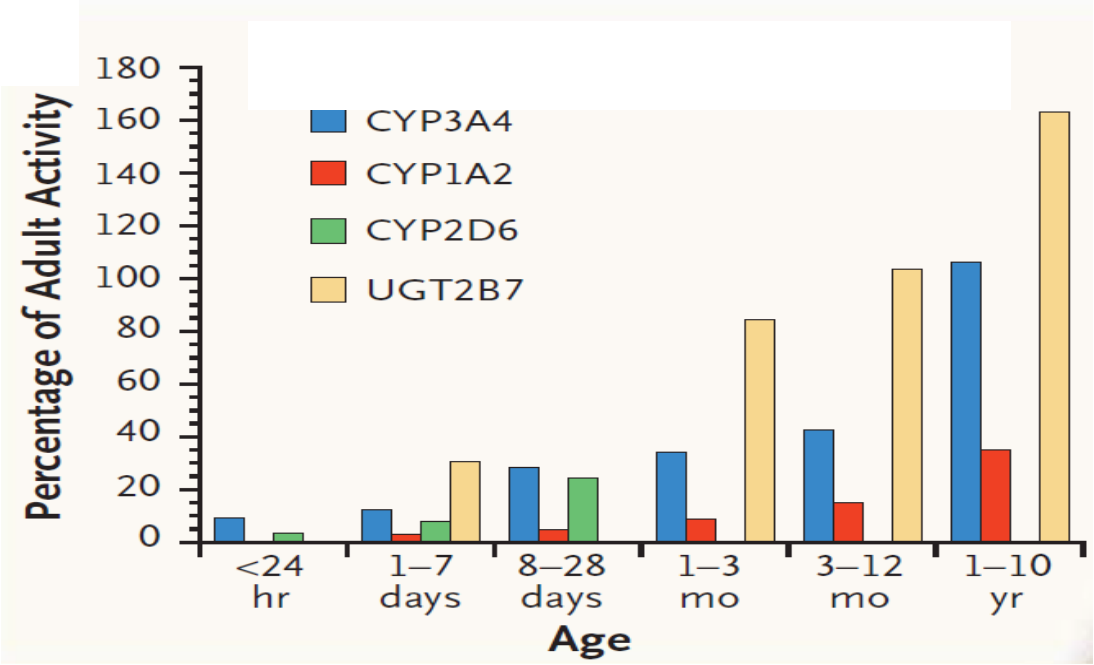


**Figure 1. 2: Metabolic capacity in children**

*This figure adapted from Kearns et al.(2003) (10).*

A shift towards greater CYP3A4 expression also occurs during the immediate postnatal period, and the enzyme ultimately becomes the most predominant hepatic enzyme expressed postnatally. Lacroix et al. (1997) reported that CYP3A4 levels in foetuses of <30 weeks was

about 10% of adult levels; while expression increased to 20% of adult levels beyond 30 weeks gestational age (28). By 6-12 months, CYP3A4 rises to about half the adult levels (28). Delayed CYP3A4 expression results in decreased clearance of CYP substrates, such as midazolam, seen in early infancy (52).

CYP1A enzymes are expressed at very low levels in the foetal liver. Bieche et al. (2007) were unable to demonstrate the expression of the enzymes in foetus aged 22-44 weeks (53). Postnatally, CYP1A2 expression has been shown to increase to 5% of adult levels in neonates and rises to 10% of adult levels in infants 1-3 months old; 25% of the adult levels is attained by 12 months (34). Several factors, including nutrition, influence the rate of enzyme maturation postnatally. Enzyme activity has been shown to be faster among formula fed infants than those receiving breast milk. The half-life of caffeine is shorter in formula fed than breast fed infants (54).

During foetal life, CYP2C19 is the dominant isoform of the CYP2C subfamily with 10-20% of adult levels expressed at birth. It increases to about 75% of adult levels by 5 months of life (55). CYP2C9 level also rises rapidly postnatally and adult levels are attained by 1-6 months of age. Adult levels are exceeded in children 3-10 years old, before returning to adult levels at puberty (56).

UGT activity is low in foetuses, but increases rapidly postnatally. In vitro studies of foetal liver (15-27 weeks) have shown UGT2B7 levels

(involved in morphine metabolism) to be up to 20% of adult levels; while adult levels are attained by 3 months of life (57). This developmental change may be partly responsible for the much lower morphine clearance in preterm neonates than children and adolescents (58). UGT1A3 levels in the foetus are 30% of adult levels; while the expression of UGT1A6 in foetal liver is less than 10%, reaching 50% at 6 months. UGT1A1, responsible for bilirubin conjugation is largely undetectable in the foetal liver (55).

CYP2D6 is a polymorphic enzyme involved in the metabolism of about 12% of clinically important drugs (55). Similar to most CYP enzymes, there is an age dependent increase in enzyme levels. It is minimally expressed in preterm neonates and in early neonatal life. About 1% of adult levels seen in foetuses rises to 20% by 28 days (59). By age 5, 70% of adult levels is attained (60) and full adult levels are seen by age 10 (56). Adult levels of CYP2E1 are also attained by the age of 10 years up from 10% seen at birth and 30% in infancy (61).

### 1.2.4. Excretion

There are three major components of drug excretion. These are the glomerular filtration rate (GFR), tubular secretion and passive reabsorption. Developmental changes in renal function occur across paediatric age groups (62) and these changes may affect plasma clearance. Generally, the kidneys are immature at birth but renal function improves over time (Figure 1-3)(63). For instance, 50% of

adult GFR is attained by 10 weeks of life and about 90% of adult level seen by the first year of life (62). Apart from age-related changes in GFR, there have also been reports of gender differences in GFR, plasma creatinine in male children higher than females (64).

Changes in tubular secretion also occur with maturity. The renal tubules attain full adult capacity by seven months of age (65, 66). The tubular secretion of certain drugs, such as imipenem, has been reported to exceed adult levels in children and adolescents (67). The effects of renal maturation on tubular reabsorption is unclear, however, it is believed that it increases with age. Critical illness can also worsen renal function in children. For example, hypoxia in children with asphyxia can result in renal dysfunction and cortical necrosis (68). These three mechanisms involved in drug excretion can have a complex relationship on renal clearance of drugs in children across the paediatric age groups.

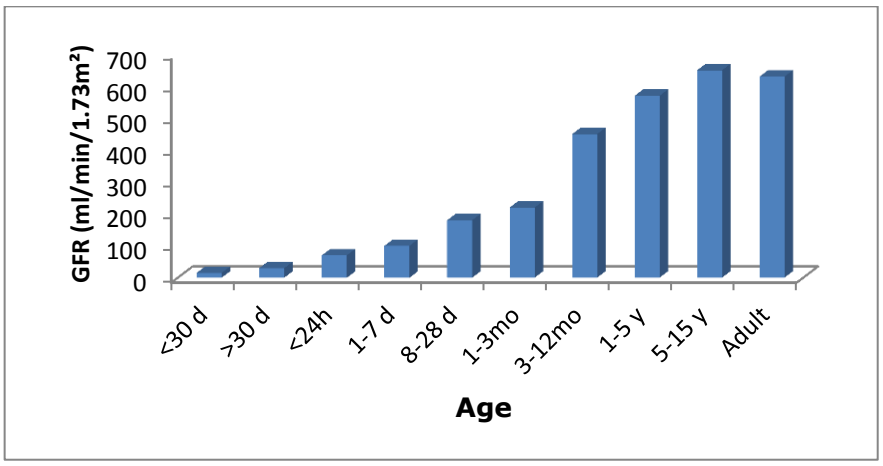


**Figure 1. 3: Developmental changes in GFR**

*This figure adapted from Hines (2008)(55).*

### 1.3. Drug clearance

Clearance refers to the removal of a drug from the body over time. It is usually calculated in relation to the volume of blood or plasma that will be totally free of the drug.

As a major determinant of plasma drug concentration, clearance is one of the most important PK parameters (69). Other important factors include the rate of drug administration and the volume of distribution. In addition to being a determinant of plasma concentration, clearance also determines the half-life of drugs (69). Although drug clearance occurs mainly through the liver and kidneys, other organs such as the lungs and the GI can also contribute. The summation of clearance from all of the different organs is referred to as total body clearance (70). This is represented as:

$$CL_{tot} = CL_{renal} + CL_{hepatic} + CL_{other}$$

Others: sites include the lungs, muscle and blood.

Renal clearance is determined by three major factors: the glomerular filtration rate, tubular secretion and tubular reabsorption. Tubular reabsorption is dependent on pH and renal tubular urine flow. Hepatic clearance refers to the elimination of drugs after transformation in the liver.

## 1.4. The Effect of Critical illness on PK in Children

### 1.4.1. The critically ill child

Critically ill children are not a homogenous population. Apart from the variation in clinical presentations (due to the severity of the disease and the presence of end organ failure), there are also differences in the types of diseases. Patients loosely grouped into this category would normally be admitted to the Paediatric Intensive Care Unit (PICU). However, there is no practical definition for critical illness.

The most common types of pathology for critically ill children include sepsis and respiratory diseases (71). Inter-individual differences in pathology have profound implications on the patients' PK behaviour. In addition to intrinsic factors, iatrogenic factors also contribute to inter-individual differences in PK behaviour. These extrinsic factors include mechanical ventilation, use of vasopressor drugs, intravenous fluid administration and extracorporeal membrane oxygenation (ECMO)(72). Critical illness may cause hepatic dysfunction, renal impairment and cardiac failure, which may lead to alteration in drug clearance.

### 1.4.2. Physiological changes during critical illness

Sepsis is a common reason for PICU admission. Early sepsis is characterised by a hyperdynamic state, which may result in increases in basal metabolism, cardiac output and oxygen consumption. As a result of increased cardiac output, renal and hepatic perfusion is

increased during the early phase of sepsis (73), resulting in an increase in renal drug delivery and excretion (72). However, in the late stages of sepsis, cardiac output decreases and renal perfusion and drug clearance are diminished. Furthermore, diminished tissue perfusion can also reduce the efficacy of drugs (74).

### 1.4.3. The impact of pro-inflammatory factors on PK

Critical illnesses, such as sepsis and advanced cancer, are accompanied by inflammatory responses. Evidence from both animal and human studies have shown significant downregulation of CYP activity after inflammatory response. Some of the inflammatory mediators associated with these effects include interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 8 (IL-8), tumour necrosis factor (TNF) and interferon gamma. These mediators induce the liver's production of acute phase proteins, such as C-reactive protein and fibrinogen, while suppressing the production of the normal export proteins, including CYP (75). In a study by Morgan (1997), the expression of CYP enzymes was shown to be downregulated following the exogenous administration of pro-inflammatory cytokines and lipopolysaccharides (LPS) (76). In another study, the clearance of midazolam declined after proinflammatory LPS was administered to rats (77). Other studies have reported the effect of these mediators on specific CYPs. For example, in vitro studies on human hepatocytes have shown that IL-1, IL-6, interferon gamma and TNF specifically

down-regulates CYP3A4, while IL-6 and transforming growth factor β are specific to CYP2C9 and CYP2C19 (78).

Only a few studies have examined the effect of inflammation on PK in critically ill children. A study by Petterson et al. (2009) showed a significant association between pantoprazole clearance and systemic inflammatory response in children (79). Several studies have explored this relationship in adults. Jones et al. (2010) showed a 50% decline in midazolam metabolism in HIV positive adults in comparison to healthy adults (80). Another study also showed a decrease in midazolam metabolism following advanced cancer (81). A decline in CYP1A2 activity with a corresponding increase in IL-6 and TNF levels, was similarly observed after caffeine was administered to adults with congestive cardiac failure (82).

### 1.4.4. Critical illness and drug absorption

The extent to which orally administered drugs are absorbed depends on the condition of an individual's GI tract and the chemical properties of the drug. GI tract hypomotility is common in critically ill patients; moreover, the use of narcotics for pain relief in these patients may also impair GI motility. Hypomotility reduces the absorption of drugs; therefore, enteral administration of drugs may cause variability in drug bioavailability during the acute phase of critical illness (83). This challenge is overcome by intravenous drug administration in critically ill children, which precludes the absorption

of drugs via the intestinal membranes and also prevents first pass metabolism (84).

### 1.4.5. Critical illness and drug distribution

Drug distribution is affected by several factors such as lipid solubility, water solubility, protein binding, tissue permeability and pH. These factors interact in complex ways to alter drug distribution, which may change during critical illness. For example, respiratory failure causes pH changes and consequently changes in drug ionisation and tissue distribution (12). Endotoxins, such as LPS from gram negative bacteria and lipotechoic acid (LTA) from gram positive bacteria during sepsis, can result in vascular dilation, endothelial damage and increased capillary permeability, which causes a shift of intravascular fluids into the interstitium. A fluid shift into the interstitial space causes an increase in the volume of distribution of hydrophilic drugs, such as β-lactam antibiotics, aminoglycosides and vancomycin (85). However, in general the volume of distribution in lipophilic drugs, such as macrolide antibiotics is unchanged because they are mainly sequestrated in adipose tissue (86). Plasma proteins decrease as a result of increased vascular leakage and decreased hepatic production. Hypoalbuminemia, increases the level of free unbound drugs that normally bind to albumin. Although alteration in plasma protein binding of drugs may cause changes in individual PK parameters, it does not affect the clinical exposure of the patient.

Therefore, changes in drug dosing may not be required (87). Interventions, such as fluid replacement and ECMO can also increase the volume of distribution. ECMO is the use of pumps to facilitate blood circulation via an artificial lung in critically ill children. In two separate studies, Mulla et al. (2005 and 2003) reported increased volume of distribution and reduced clearance in neonates receiving vancomycin and theophylline while on ECMO (88, 89). These changes are likely due to increased circulatory volume as a result of ECMO.

### 1.4.6. Critical illness and drug metabolism

Critical illness affects the two main physiological processes involved in hepatic metabolism: hepatic blood flow and hepatic enzyme activity. Hepatic clearance is a function of hepatic blood flow and the extraction ratio as follows:

$Cl_H = Q \times E$

Where CL is the hepatic clearance, Q is the hepatic blood flow and E is the extraction ratio.

Drugs, such as morphine and midazolam, have high extraction ratios (>0.7) thus, their hepatic clearance depends more on hepatic blood flow than on how they are metabolised. In late sepsis, blood flow to the liver is significantly reduced leading to a reduction in hepatic drug clearance (12). However, for drugs with low extraction ratios <0.3,

hepatic clearance depends mainly on hepatic metabolism and the level of the unbound fraction of the drug, as follows:

$CL_H = f_u \times Cl_{int}$

Where $f_u$ is the unbound fraction and $Cl_{int}$ is the intrinsic clearance (hepatic metabolism)(85).

Inflammatory mediators are induced by the stress of a critical illness or are released during sepsis, and they decrease the level of the metabolising enzymes (90). In addition, hypoxia, which may occur in some critically ill children, has been shown to affect CYP, to varying degrees. While some studies have reported reduced CYP1A1, CYP1A2 and CYP3A4 expression, others have reported induced CYP2C9 expression (91).

### 1.4.7. Critical illness and drug excretion

Renal dysfunction is common in critically ill children. This can be due to acute kidney injury or a pre-existing renal compromise. Renal clearance is an important means of drug elimination. The extent to which critical illness affects kidney functions varies. Patients with renal dysfunction usually have diminished renal clearance; however critically ill patients also exhibit augmented renal clearance, which decreases the plasma concentration of drugs. For example, septic patients without kidney dysfunction initially can have increased cardiac output leading to increased renal blood flow (73). Augmented

renal clearance has also been reported in patients receiving vasoactive drugs, which are commonly used in some critically ill children (86). Burns patients have also been reported to have increased renal clearance, while those with medical and surgical conditions have more variable clearance (12).

### 1.5. Dosing in children

Dosing of drugs in children is normally based on body weight (92-94). It has usually been extrapolated from pharmacokinetic studies in adults, initially performed on healthy volunteers. Before a medicine is used in children, there has usually been clinical experience in adults, alongside scientific studies including pharmacokinetic studies in adult patients receiving the medication. These are adults with illnesses who may handle the drug differently to healthy adult volunteers. Pharmacokinetic studies in children are of benefit in ensuring that appropriate doses are given. However, doses are usually calculated from mean pharmacokinetic values. It is important to be aware that there is often significant inter-individual variation in the pharmacokinetics, and in particular the clearance of a medicine, due to factors such as age (95), weight (96), disease (97) and ethnicity/genotype (98) .

In early childhood, especially in neonates and infants, physiological growth is rapid and pharmacokinetic changes are also rapid. Hence, it is important to adjust drug dosage to reflect these changes (99).

Dosing in children is often extrapolated from adult studies and paediatric dose scaling by weight and body surface area (BSA) is common (94, 99). Adult dose scaling based on BSA predicts a higher dose for children than weight based dosing. Weight based dose estimation generally underestimates drug clearance in younger children, which may result in under dosing. Up to 10% underestimation of paediatric clearance values from adult values has been reported, hence children often require relatively higher doses per kg than adults (94, 100).

BSA is a surrogate for body size and a commonly used biometric unit for the estimation of dose. It is useful for normalising physiologic parameters such as, cardiac output, left ventricular mass and renal clearance; because they are better correlates than other indices of body size (101). While this measure can be used with reasonable accuracy for older children, it may also cause excessive dosing in neonates and infants (102).

### 1.6. Methods of estimation of PK parameters

There are two approaches to the estimation of PK parameters. Until more recently, the traditional (non-population) PK was the most common method. However, population PK is now increasingly utilised.

Non-population PK relies on the estimation of concentration-time profile from each study subject, before the mean estimate is calculated for the population. The traditional PK is poor at identifying

the effects of covariates like age and weight on inter-individual variability. Generally, large numbers of blood samples are required per person and it often involves a group of healthy volunteers or carefully selected patients. The process is highly standardised and participants tend to be homogenous (103). The overall estimates may be inaccurate if data is sparse or missing (104).

Population PK involves PK parameter estimation at the population level. This method studies the population and not the individual subject. Population PK estimates can be determined with or without PK models and the concentration-time profile for each subject can be described with a mathematical equation. Pharmacokinetic parameters are estimated for the entire population by collecting samples from subjects taking different doses over different periods of time (104). A major advantage of population PK modelling is the ability to utilise sparse samples (between 2 and 3 samples). A larger number of subjects, than non-population PK are however required. The subjects are heterogeneous and are generally more representative of the general population. Using the appropriate approach, sources of intra and inter-individual variability in drug PK as well as the covariates, causing the variability can be easily identified (105).

### 1.6.1. PK models

PK models use data generated from PK studies. They are of value in predicting the time concentration profiles of other doses of the drug (106). There are three main methods used in modelling data that has been collected from patients/volunteers.

#### 1.6.1.1. Naïve pooled data approach

This involves the pooling of time concentration data together. The mean plasma concentration of the drug at each time point is used to calculate parameter estimates (106). This method is only satisfactory if there are extensive data for each subject. It assumes there is minor inter-individual variability. It is not useful for determining inter-individual variability or the cause of such variations (106).

#### 1.6.1.2. Standard two-stage approach

Analysis of individual profiles is undertaken. Individual PK parameters are then treated as variables and combined to produce mean values. Weighting is required if there are differences in the number of measures for each individual or if there is significant inter-individual variability (107). It may be possible to identify covariates to explain some of the variability (107). This is however dependent upon having good independent data to ensure that each individual parameter is calculated accurately.

#### 1.6.1.3. Mixed effect model

These models are called "mixed effects" because they describe the data using a mixture of fixed and random effects (106). Fixed effects predict the effect of a variable such as weight. Random effects describe the remaining variability between subjects that is not predictable from the fixed effects average (106). Whereas the naive and standard two-stage approaches rely on a large amount of data from the small number of subjects, the mixed effects models can be used to analyse sparse data from a larger number of subjects (107). The most commonly used statistical program is NONMEM (the non-linear mixed effects model). Such models allow one to study a large number of covariates which may affect different PK parameters.

#### 1.6.1.4. PK compartments

The choice of appropriate compartment models that best describe the PK behaviour of drugs in the body is essential to the estimation of PK parameters. One compartment model is based on the premise that the body is a homogenous unit. This model assumes that after administration, a drug is instantly distributed inside body tissues; this assumption implies that a change in the plasma concentration of a drug is accompanied by a commensurate change in tissue concentration, and a linear relationship is shown (108). The model also assumes that elimination is first order and those pharmacokinetic parameters, such as the elimination rate constant (ke) volume of

distribution and clearance, are not affected by the drug dose. In reality, however, only very few drugs are immediately distributed inside the body (108).

The two-compartment model assumes that the body is divided into a central and a peripheral compartment. The central compartment consists of the heart, kidneys, brain, lungs and liver (109). The assumption of the two-compartment model is that following administration, a drug is distributed to both the central and peripheral compartments (109). The distribution rate constants out of the central compartment to the peripheral compartment ($k_{21}$) and into the central from the peripheral compartment ($k_{12}$) are slower than the elimination rate constant. In this model, the plasma concentration–time profile has two phases following intravenous bolus injection (109). The first is an initial phase (α- phase), which is the distribution phase, and the second is the terminal phase (β- phase), which is the post-distribution phase. The terminal phase is longer because the drug must first diffuse back from the peripheral compartment to the central compartment before it can be eliminated (109).

Pharmacokinetics data can also be summarised without fitting into any type of compartment model. An advantage of the non-compartmental model over the compartmental approaches is the requirement of fewer assumptions. With this method, the area under

the concentration curve (AUC), clearance and half-life can be calculated from drug plasma concentration data. The AUC is the area under the plasma drug concentration versus time curve calculated by means of the linear trapezoidal rule or by the log-linear trapezoidal rule (110). Drug clearance is calculated from the dose and AUC; while the maximum plasma concentration ($C_{max}$) and time to maximum plasma concentration ($t_{max}$) can also be determined from concentrations and their associated time points. Furthermore, the last two to four sampling time points can be used to determine the half-life (111).

#### 1.6.1.5. Model validation

Validation of a model is required. The model should ideally be validated on a different group of patients to those from which the model was developed. Validation may also be achieved by utilising a randomly selected fraction (25%-50%) of the test dataset, while the development of the model continues with the remaining dataset. The results observed from validation can subsequently be compared with those predicted by the model (112). Internal validation is an important component of population PK modelling (113). Zhao et al. (2013) reported that all population pharmacokinetics model used in neonatal vancomycin studies were internally validated (114). In addition to internal validation, external evaluation is equally important as it examines the modelling procedure as well as identify the effect of other study-related factors.

## 1.7 Ethical considerations for invasive PK studies in children

Like adults, children need to have research performed on the drugs they receive to provide a robust evidence base. Many medicines used in children are either off-label or unlicensed (115, 116). While it is important to perform invasive clinical research with children, this should be done in an ethical manner.

The design and methods of PK studies should be optimised to allow the smallest number of patients to be studied (117). According to the Royal College of Paediatric and Child Health (RCPCH) Ethics Advisory Committee, clinical research in children must be of a minimal or low risk. Research of greater risk may be considered if it involves diagnostic procedures or treatment which are important to the child and may help with the understanding of the child's condition. Generally the assent of a child, in addition to the consent of their parents, over 7 years old should be sought (118).

### 1.7.1. Blood sampling for PK studies

The main methods of blood sampling include: venepuncture, arterial, finger prick and heel prick. The choice of blood sampling site depends on the age, weight and the volume of blood required. Heel prick is usually done for infants less than six months old or between 3-10 kg. Finger prick is done for children over six months and more than 10kg (119). A study involving 100 neonates determined the tolerability of both methods using the Facial Coding System (FCS). This study

reported greater pain for heel prick (median FCS score of 58) than for venepuncture (FCS score 23, $p<0.001$) (120). However, heel pricks are still widely used in neonates. Several other studies have also supported that venepuncture is associated with less pain than heel pricks in neonates (120, 121). Drug measurement after venepuncture has also been shown to be less prone to errors than finger pricks, due to the squeezing associated with sampling and blood specimen contamination with extracellular fluid (122).

### 1.7.2. Blood sampling volume and frequency

Different institutions have different guidelines on the safe limit for blood sampling. The European Medicines Agency (EMA) recommends that not more than 3% of the total blood volume (TBV) of neonates should be withdrawn during a period of four weeks, and a single time blood draw should not exceed 1% of TBV (123).

The United States Department of Health and Human Services on the other hand, recommend that not more than 3.8% (3ml/kg) of the TBV should be withdrawn at one time. In addition, it was recommended that not more than 50 ml in total should be withdrawn within eight weeks (124)(Table 1.4). The Medical Research Joint Ethics Committee (MRC), in partnership with the government in Gambia, developed a more age specific guideline (125) which allows for up to 5ml of blood to be withdrawn at once in children between 0-4 years, 10ml for 5-9 years, 15ml for 10-14 years, and 30ml in

adolescents over 15 years. There are also recommendations based on baseline haemoglobin. For example, the University of California guideline recommends that 2.5% of TBV can be withdrawn in a single draw only if the child's haematocrit is at least 7g/dl or between 9-10g/dl, if cardiorespiratory compromise is present (126).

The number of samples required for PK studies vary with different research methods. There is no standard guideline on the frequency of blood sampling in children, some studies have collected as many as 20 samples (127). Raoof et al.(1995) reported that 11 blood samples may be sufficient for the estimation of PK parameters (128), however, Long et al.(1987) reported that just three samples may be sufficient for practical purposes in neonates (129).

**Table 1. 4: Guidelines on safe blood sample volume for paediatrics**

| **Institution** | **Maximum volume for a single sample % TBV** | **Maximum cumulative volume % TBV** |
|---|---|---|
| US Dept of Health and Human Services, Office for Human Research Protections | 3.8 | ≤50ml in eight weeks |
| Toronto Hospital for Sick Children Research Ethics Board | 5 | 5% in 3 months |
| Gambia Government–MRC Joint Ethics Committee | 0.3-2.4 | 0.3-2.4 in 3 months |
| University of California | 2.5 | 5% in 1 month |

*This table adapted from Stephen RC Howie,(2011)(125).*

### 1.8. Physiological, physical and psychological effect of blood sampling in children

Venepuncture is the most common invasive procedure for PK studies. This procedure has been shown to cause both psychological and physical pain in children (130). In one study, 58% of children considered venepuncture painful while about 25% had some form of psychological distress before sampling and 8% afterwards (130). Rodin et al. (1983) demonstrated an increased level of anxiety in children undergoing invasive procedures without previous psychological preparation. As well as the psychological risk, invasive procedures may also cause physical risk, such as bruising (131). Other risks associated with frequent venous blood sampling include: infection, excessive blood loss and possibly anterior intraosseous syndrome blood loss (132).

The EMA recommends that both physical and psychological pain should be reduced as much as possible when conducting clinical research in children. When pain is unavoidable, it should be effectively treated during and after the procedure (123). They also recommended that the intensity of pain and distress should be monitored and assessed using an appropriate scale (123). An example of such scale is the COMFORT scale which is used for detection of treatment related change in pain and discomfort intensity (133). Furthermore, local anaesthetic agents should be used, especially when inserting an indwelling catheter (134).

### 1.8.1. Physiological effect of blood sampling in neonates

Besides the psychological and physical effects of drug sampling in children, there are also physiological implications. Preterm infants generally have a TBV of about 90-105ml/kg and in term neonates the TBV reduces to about 80 ml/kg (135). This implies that a 3kg term neonate has a TBV of about 240ml; therefore excessive blood sampling may compromise circulation.

In a prospective study of 99 preterm infants by Madsen et al. (2000), there was a significant correlation between the volume of blood sampled and the volume of blood transfused (136). A further study of 253 preterm neonates confirmed this (137). A study of 20 preterm neonates, in which umbilical blood was sampled rapidly (20 seconds), showed a significant decrease in both oxygenated haemoglobin and total oxygenation index, with a corresponding increase in oxygenated haemoglobin in the brain (138). Similarly, another study of neonates with blood sampled from the umbilical vein showed a significant decrease in cerebral oxygenation and cerebral blood volume. Acute drop in cerebral oxygenation can lead to brain injury such as intraventricular haemorrhage and periventricular leukomalacia (139).

### 1.9. Microanalytical methods

#### 1.9.1 Dried matrix spots (DMS)

This involves the collection of biological fluid samples on blotting paper. Examples of DMS include dried blood spots (DBS), dried urine spots (DUS) and dried plasma spots (DPS). Usually, small volume of fluids ranging between 5-30µl are collected. The commonest DMS method is the DBS which is increasingly being used, in the last 10 years, in drug development process, therapeutic drug monitoring, as well as PK studies in infants (140). The degree of partitioning of drugs into red blood cells determines the differences observed between blood and plasma concentrations. In addition to minimal samples other advantages of DBS include low cost, potential for storage at room temperature and ease of transport (141, 142). A major limitation of DBS is that it provides estimates of PK data for whole blood, whereas many PK studies estimate plasma or serum concentrations. Therefore, DBS drug concentration should be corrected in order to obtain an accurate plasma level (143). DBS may not be suitable for unstable drugs, which may be degraded during the drying process (144, 145).

#### 1.9.2. Ultra-low volume assays

This method minimises the volume of blood used for PK studies. Plasma volume of <50µl can be used for drug assay compared to the

traditional 1-2ml required for PK studies. Several studies have reported the use of this method in paediatrics (146, 147).

### 1.9.3. Opportunistic PK studies

This method can be used for PK studies of drugs that are prescribed in routine care. Such samples can be taken when routine laboratory blood samples are collected. Therefore, subjects are not subjected to further blood collection. Parents are less likely to object to this type of study than the traditional PK method. A drawback of this method is that samples are sparse and untimed. In order to mitigate the effect of sparse sampling, a larger number of subjects are required (147).

### 1.9.4. Alternative biological matrices

Saliva has been used as an alternative to blood sampling for the PK analysis of some drugs. A study of PK profile of voriconazole showed a similar PK profile between saliva and plasma (148). Other alternatives such as urine or breath samples should be considered when suitable (117). A correlation between salivary and plasma concentration has been reported for theophylline, phenobarbital and phenytoin (149, 150). However, it is difficult to obtain optimally sufficient salivary samples from neonates and young children. The distribution of drug in the saliva however depends on the physiochemical properties of the drug and the characteristics of the saliva. Rapid saliva flow rate lowers the variation in the ratio of drug saliva to plasma, hence stimulated saliva is preferred over resting

saliva (151). Exhaled breath is another biological matrix that can be used for PK analysis. It is however highly dependent on the ability of the drug to diffuse across the alveoli (152).

### 1.10. Thesis aims

It is important to explore the variation in PK of medicines in children. Different enzymes are involved in the metabolism of the many different medicines used in paediatrics. These drugs can also have different PK properties in different age groups. The series of systematic reviews performed will explore several different drugs which are metabolised by different enzymes.

The medicines that will be reviewed are; midazolam, theophylline and morphine.

- Midazolam is used as a sedative in paediatrics and CYP3A4 is the main metabolic pathway.
- Theophylline is prescribed mainly as aminophylline, which is the intravenous formulation of the drug. It is used in acute asthma and is often prescribed in low income countries. It is metabolised by CYP1A2.
- Morphine is a commonly prescribed opiate that is widely used in paediatrics. It is glucuronidated to an active metabolite (M6G) and an inactive metabolite (M3G) by UGT.

The aims of the systematic reviews are to:

– Determine the extent of inter-individual variation of clearance of these drugs in children.

- Determine the factors such as age, weight or critical illness that may be responsible for inter-individual variations of clearance for these drugs.

Blood sampling is an important component of PK studies. It is however, an invasive process. A systematic review of the frequency and volume of blood sampling was conducted in order to determine whether PK studies in paediatric patients are becoming less invasive by determining the volume and number of samples taken.

# CHAPTER TWO
# METHODS FOR SYSTEMATIC REVIEWS OF INTER-INDIVIDUAL VARIABILITY OF CLEARANCE

## 2.1. Methods

Systematic reviews of the inter-individual variation in clearance of midazolam, theophylline and morphine were conducted.

## 2.2. Search strategy

An electronic search of five databases was conducted. The databases were MEDLINE, EMBASE, International Pharmaceutical Abstracts (IPA), CINAHL and the Cochrane Library. All studies that involved children aged less or equal <18 years that described the pharmacokinetics of midazolam, theophylline and morphine were included. Paediatric patients were grouped according to the guidelines of the International Conference of Harmonization (ICH)(153):

- Preterm neonates (born at less than 37 weeks of gestation)
- Term neonates ( born at 37 weeks of gestation to 27 days old)
- Infants (28 days to under 2 years old)
- Children (2 to 11 years old)
- Adolescents (12 to under 18 years old)

Paediatric keywords were selected based on the recommendations of Kastner et al. (2006)(154). The final PK keywords were selected after optimisation to generate the highest number of relevant studies. This is because PK systematic reviews in these subjects have not been performed before.

### 2.3. Database and keywords for midazolam

The following sources of information were utilised: MEDLINE (1946 to May 2012), EMBASE (1974 to May 2012), IPA (1970 to April 2012), CINAHL and Cochrane library. These databases were searched separately, the results were combined and the duplications were removed.

The search strategy included all languages and involved the keywords "midazolam" AND "preterm neonate*" OR "term neonate*" OR "neonate*" OR "new-born*" OR "child*" OR "children" OR "p*ediatric*" OR "infant*" OR "adolescent*" AND "pharmacokinetic*" OR "clearance" OR "half-life" OR "absorption" OR "distribution" OR "metabolism" OR "elimination" OR "pharmacodynamic*".

### 2.4. Database and keywords for theophylline

The following sources of information were utilised: MEDLINE (1946 to January 2013), EMBASE (1974 to January 2013), IPA (1970 to January 2013), CINAHL and the Cochrane Library. These databases were searched separately, the results were combined and the duplications were removed.

The search strategy included all languages and involved the keywords "theophylline" OR "aminophylline" AND "preterm neonate*" OR term neonat*" OR "neonate*" OR "new-born*" OR "child*" OR "children" OR "p*ediatric*" OR "infant*" OR "adolescent*" AND "pharmacokinetic*" OR "clearance" OR "half-life" OR "absorption" OR

"distribution" OR "metabolism" OR "elimination" OR "pharmacodynamic*".

### 2.5. Database and keywords for morphine

The following sources of information were utilised: MEDLINE (1946 to May 2013), EMBASE (1974 to May 2013), IPA (1974 to May 2013), CINAHL and the Cochrane Library. These databases were searched separately, the results were combined and the duplications were removed.

The search strategy included all languages and involved the keywords "morphine" AND "preterm neonate*" OR term neonat*" OR "neonate*" OR "new-born*" OR "child*" OR "children" OR "p*ediatric*" OR "infant*" OR "adolescent*" AND "pharmacokinetic*" OR "clearance" OR "half-life" OR "absorption" OR "distribution" OR "metabolism" OR "elimination" OR "pharmacodynamic*".

### 2.6. Exclusion criteria

- Review articles
- Editorials
- Conference abstracts
- Studies in adults aged over 18 years
- Studies that involved adults and paediatric patients but did not present the paediatric data separately.
- Studies in which the drug of interest (midazolam, theophylline or morphine) was not administered intravenously.

### 2.7. Inclusion criteria

- Inclusion criteria were original research studies assessing the pharmacokinetics of the drug of interest in children up to the age of 18 years.

### 2.8. Quality assessment

There is no formal quality assessment for use in PK studies. Therefore the quality of papers was assessed using the following criteria include:

- Studies with ≤ 2 patients or with number of patients not stated were excluded.
- Studies with ≤2 samples or with the number of samples not stated were excluded.

### 2.9. Data extraction

All relevant articles were read carefully and the required data were extracted onto tables. A second reviewer independently verified the eligibility of 25% of randomly selected studies. The data extracted include:

- Number of patients
- Age
- Weight
- Study year and reference
- Dose (single, multiple or continuous infusion)
- Drug-drug interaction

- Single or multi-site
- Medical condition
- Number of blood samples
- Blood, plasm or serum
- PK analysis method
- Clearance
- Values of mean/median clearance and the minimum and maximum clearance.

The variation ratio was calculated from the range of clearance maximum clearance divided by the minimum clearance. The coefficient of variation (CV) was extracted from the paper if given. If individual data were presented, then the CV was calculated using the formula CV= √ ((e SD2) -1)(155), which allows for the fact that clearance usually display a lognormal distribution in children. We contacted original authors by email if their paper did not provide individual data of clearance. If individual data were not available, then the CV was estimated by dividing the standard deviation by the mean of clearance, i.e., normal distribution was assumed. Patients were divided into two groups: (1) critically ill if they were in an intensive care unit and (2) non-critically ill, which included other groups.

## 2.10. Statistical Analysis

The coefficients of variation between studies in critically and non-critically ill patients were compared using Students t-test (or Mann-Whitney test, if not normally distributed). Differences in CV for different age groups were also determined using ANOVA. The mean drug clearance of studies in all patients was pooled, in a meta-analysis (StataSE14). For each population, the extent of association between the CL values and differences between the primary studies was assessed by conducting a heterogeneity test, using the Q statistic (156). A non-significant result ($P > 0.1$) indicated lack of significant difference between the studies (157). Additionally, I-squared statistic, which measures the percentage of the variability in effect estimates due to heterogeneity rather than sampling error was carried out. Where the I-squared was low (i.e. < 40%), the fixed-effect model was deemed suitable. The random effect model was utilised for heterogeneous data.

To identify potential influences on the CL, a meta-regression was conducted, utilising age group, weight, study type (i.e. whether it was population PK-based or not) and sample size as covariates. The random effects model was also used so as to account for possible heterogeneity.

The correlation between weight and CV was evaluated and the effect of all covariates was evaluated with multiple linear regression.

# CHAPTER THREE
# A SYSTEMATIC LITERATURE REVIEW OF INTER-INDIVIDUAL VARIATION IN MIDAZOLAM CLEARANCE IN CHILDREN

## 3.1. Introduction

Midazolam is a 1, 4-benzodiazepine derivative that was first synthesized in 1975 by Walser and Fryer (158). It is a short acting benzodiazepine that is used as a central nervous system depressant (CNS)(158). It reversibly potentiates the effects of gamma-amino butyric acid (GABA) receptors in the central nervous system (159). As a result of this characteristic, it exhibits sedative, anxiolytic, anticonvulsive, muscle relaxant and amnesic effects, which can be seen in both adults and children (158). Midazolam can be administered via different routes: IV, intramuscular (IM), buccally, intranasally (IN), and orally (160).

Following IM injection, more than 90% of midazolam is absorbed. However, oral bioavailability is only about 27% in children (161). The lower oral bioavailability is due to first pass metabolism in the liver and small intestine. Its Vd varies between 0.95 and 6.6L/kg, and is generally higher in females than males (161). Obese children also have a higher Vd. A significant decrease in central and peripheral Vd has been reported after weight loss in morbidly obese adults (162).
Midazolam is metabolised by the cytochrome P450 isoforms such as CYP3A4, CYP3A5, and CYP3A7 to a major hydroxylated metabolite (1-OH-midazolam) and several minor metabolites (163). 1-OH-

midazolam is equipotent to midazolam and is metabolised to 1-OH-midazolam-glucuronide by UGTs. 1-OH-midazolam-glucuronide is subsequently excreted via the kidneys (163).

Therefore developmental differences in CYP3A activity may alter the pharmacokinetics of midazolam in paediatric patients of different ages (28). Clearance is reduced in neonates and infants under 6 months of age. Thereafter, it is similar to that in adults. In addition, the pharmacokinetics of midazolam can be altered by dysfunction of the kidneys or the liver (164, 165).

The dose of midazolam used varies with the indication, route of administration and age. For sedation in stable patients, IV midazolam is given at an initial dose 25-50 μg/kg in those aged 12-18 years (166). This may be increased gradually if necessary. However, higher doses are needed for sedation in intensive care unit and this varies according to the age of the patients. Preterm neonates less than 32 weeks require about 30 μg/kg, IV for sedation in intensive care unit, while a much higher IV dose of 60 μg/kg is recommended for patients > 32 weeks to 6 months (166). Children 6 months-12 years require up to 200 μg/kg while the dose in adolescents can be as higher as 300 μg/kg given by slow IV injection (166). Dose also varies according to route of administration with a lower dose required for the IV route whereas oral, rectal and buccal require higher doses (166).

Prolonged administration of midazolam has been shown to result in tolerance and the development of withdrawal symptoms such as hypertension, tachycardia and seizures (167).

Some studies have suggested that factors such as age (95), route of administration (168), gender and weight (169), and critical illness (170) may affect the pharmacokinetics of midazolam in children.

## 3.2. Aims

The purpose of this systematic review is to determine the variability of midazolam clearance in children and establish the factors which are responsible for this variability.

## 3.3. Objectives

- Determine the extent of inter-individual variation in midazolam clearance by estimating the CV.
- Evaluate the effect of factors such as age, birth weight and critical illness that may be responsible for the inter-individual variation in midazolam.

## 3.4. Methods

The methods for this chapter are described in chapter two.

## 3.5. Results

A total of 1,654 articles were short listed by five search engines (Figure 3.1). Only 26 articles (including two identified by manual search) met the inclusion criteria. Table 3.1 shows the details of papers excluded from the systematic review.

**Table 3. 1: Details of excluded articles from systemic review**

| Exclusion Reason | |
|---|---|
| Review studies | 245 |
| Editorials | 8 |
| Conference abstracts | 16 |
| Studies in which midazolam was not administered intravenously | 3 |
| Studies in adults aged over 18 years | 123 |
| Combined adult and paediatric studies where paediatric data was not presented separately | 1 |
| Studies where data for midazolam was not presented | 614 |
| Studies that did not evaluate the pharmacokinetics of midazolam | 358 |
| Total | 1,368 |

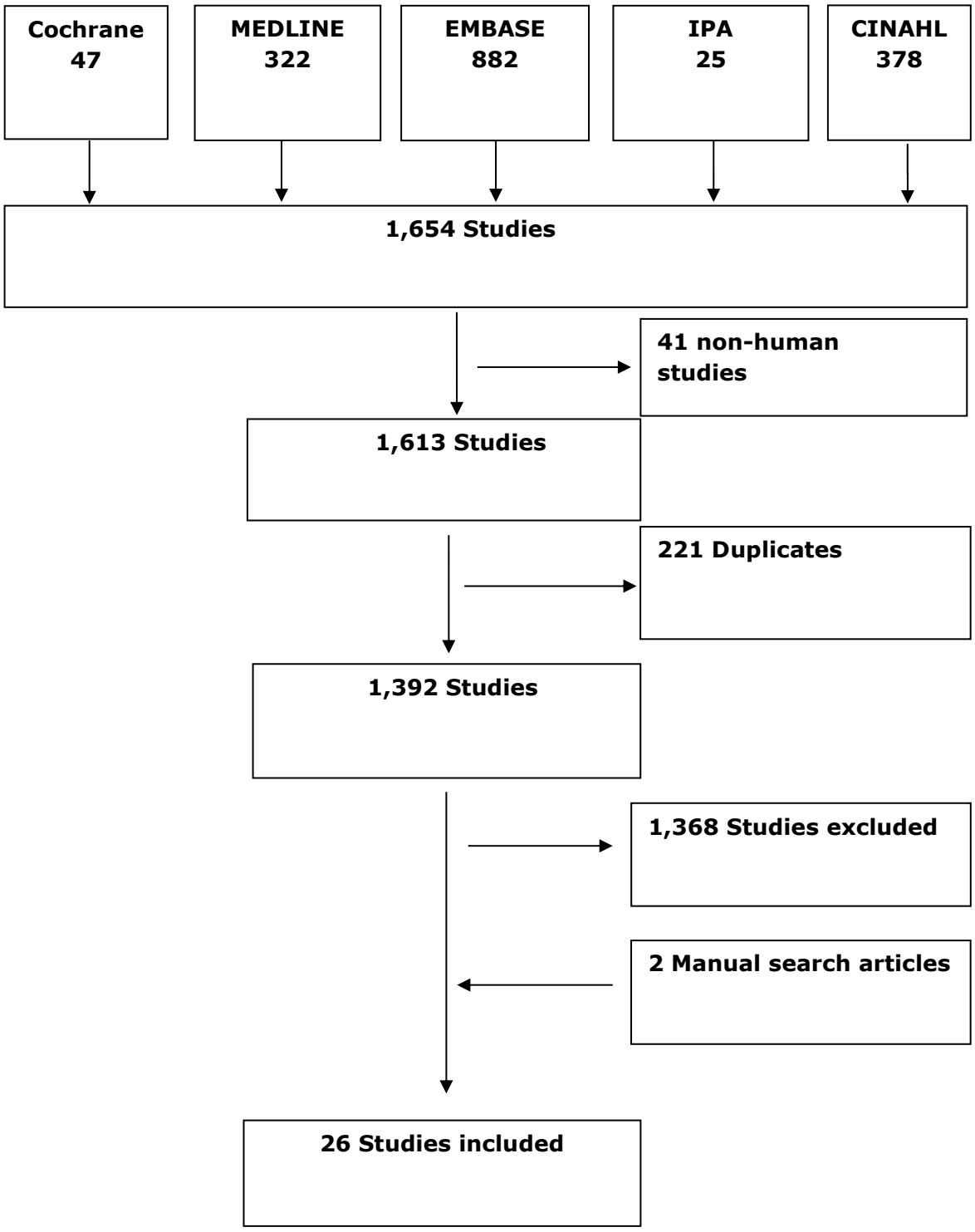


**Figure 3. 1: Search strategy of midazolam**

### 3.5.1. Quality assessments

Four studies were excluded after quality assessment, described in section (2.8) of chapter 2. Two studies were excluded because of the small number of blood samples (171, 172). One study was excluded because of lack of information on the number of blood samples (173) and one study was excluded because of the small number of patients (174) (Table 3.2).

**Table 3. 2: Quality assessment of midazolam PK studies in paediatric**

| Study | Age group | †Number of patients | +Number of blood samples collected | Verdict |
|---|---|---|---|---|
| Jacqz-Aigrain et al.(1992)(175) | Preterm neonates | 15 | 7* | Included |
| Jacqz-Aigrain et al.(1990) (176) | | 5 | 6* | Included |
| de Wildt et al.( 2001) (177) | | 24 | 8* | Included |
| Harte et al.(1997) (178) | | 9 | 4* | Included |
| Lee et al. (1999)(179) | | 60 | 4‡ | Included |
| Jacqz-Aigrain et al. (1990) (176) | Term neonates | 5 | 6* | Included |
| Mulla et al. (2003)(180) | | 11 | 7* | Included |
| de Wildt et al. (2003)(164) | | 5 | 3* | Included |
| Burtin et al. (1994)(181) | | 187 | 3-6* | Included |
| Ahsman et al.(2010)(182) | | 20 | 5-9* | Included |
| Mulla et al.(2003)(183) | | 19 | 7 | Included |
| de Wildt et al.(2003)(164) | Infants | 5 | 3* | Included |
| Hughes et al.(1996)(171) | | 25 | 1 | Excluded |
| Peeters et al.(2006)(184) | | 24 | 7-10* | Included |
| Minagawa (2006)(174) | | 2 | 3 | Excluded |
| Mathews et al.(1988)(185) | | 5 | 12-14* | Included |
| Reed et al.(2001)(186) | | 5 | 12* | Included |
| de Wildt et al. (2003)(164) | 2-11 years | 6 | 3* | Included |
| Minagawa (2006)(174) | | 2 | 3 | Excluded |
| Roberts et al.(2009)(187) | | 5 | 5* | Included |
| Muchohi et al.(2008)(188) | | 12 | 13* | Included |
| Hughes et al. (1996)(171) | | 12 | 1 | Excluded |
| Mathews et al. (1988)(185) | | 12 | 12-14* | Included |
| Reed et al. (2001)(186) | | 14 | 12* | Included |
| Rey et al.(1991)(189) | | 6 | 10* | Included |
| Jones et al. (1993)(190) | | 12 | 9* | Included |
| Kraus et al.(1989)(191) | | 6 | 13* | Included |
| Payne et al.(1989)(192) | | 8 | 9* | Included |
| Salonen et al.(1987)(193) | | 21 | 12* | Included |
| Tolia et al.(1991)(194) | 12-18 years | 20 | 7* | Included |
| Vet et al.(2012)(173) | 2 dy to 17 yrs | 21 | NA | Excluded |
| Nahara et al.(2000)(97) | 2dy-16yrs | 22 | 5* | Included |
| Hartwig et al.(1991)(172) | 26 dy to 5 yrs | 24 | 1‡ | Excluded |

†Studies with ≤ 2 patients or with number of patients not stated are excluded; **+**Studies with ≤2 samples or with the number of samples not stated are excluded, *plasma, ‡serum

### 3.5.2. Clearance of midazolam in neonates (0 - 27 days)

Ten studies reported midazolam clearance in 360 critically ill neonates undergoing sedation for mechanical ventilation (175-179, 181-183, 195). All were single centre studies. The number of participants varied between 5 and 181 (Tables 3.3 and 3.4). The number of collected blood samples was between 3 and 8.

Six of the studies were 2 compartment studies; there were 2 one compartment studies and 1 non-compartment study. In the one other study, one, two and three compartment models were used. Midazolam loading dose varied between 0.03 and 0.2 mg/kg and maintenance dose between 0.01 and 0.6 mg/kg/h (Table 3.5). Age and weight were covariates in half of these studies. Other important covariates were route of administration and co-medication. None of the studies reported the effect of drug-drug interaction.

The CV in the four studies of preterm neonates ranged from 78% to 109% (Table 3-6). The CV in the four studies of term neonates ranged from 18% to 77%. Five studies gave data regarding the range of clearance values for individual neonates (175-177, 183, 195). The three studies in preterm neonates suggested a 4.5 fold, 5-fold and 10-fold variation in clearance in 15 and 5 and 24 neonates respectively. One study involving five term neonates reported a 2-fold variation in clearance. The mean clearance in preterm neonates

ranged from 0.78 to 1.7 ml/min/kg and in term neonates ranged from 1.17 to 3.5 ml/min/kg.

**Table 3. 3: Characteristics of studies in preterm neonates**

| Study | Number of patients | Gestational age (weeks) | Postnatal age (days) | Range of weight (kg) | Type of patients | Covariates | Comments |
|---|---|---|---|---|---|---|---|
| Jacqz-Aigrain et al. (1992)(175) | 15 | 29-41 (32)* | NA | 1-3 (1.9)* | Mechanical ventilation | NA | Liver and renal function normal. Period of illness NA. |
| Jacqz-Aigrain et al. (1990)(176) | 5 | 34-37 (35)‡ | NA | 2-3.6 (2.8)* | Respiratory distress syndrome or infection | Gestational age and weight | Liver and renal function normal. Period of illness NA. |
| de Wildt et al. (2001)(177) | 24 | 26-34 (29)* | 3-11 (5.5)* | 0.8-1.6 (1)* | ICU | Age, co-medication (indomethacin and caffeine) and mechanically ventilated | Excluded patients receiving morphine, dobutamine, dopamine or those with primary renal, hepatic, hemodynamic, or neurologic dysfunction. Period of illness NA. |
| Harte et al. (1997)(178) | 9 | 25-30 (28)* | NA | 0.7-1.4 (1)* | Mechanical ventilation | NA | Excluded patients with cardiovascular instability, neurologic, hepatic or renal dysfunction. Period of illness was over two months. |
| Lee et al. (1999)(179) | 33<br>27 | 24-31 (27)* | 2-15 | <1kg<br>>1kg | Mechanical ventilation | Single/multiple birth pregnancy and birth weight | Excluded patients with cardiovascular instability, neurologic, hepatic or renal dysfunction. Period of illness NA. |

*Mean, ICU, intensive care unit, NA, not available

**Table 3. 4: Characteristics of studies in term neonates**

| Study | Number of patients | Gestational age (week) | Postnatal age (days) | Range of weight (kg) | Type of patients | Covariates | Comments |
|---|---|---|---|---|---|---|---|
| Jacqz-Aigrain et al. (1990)(176) | 5 | 38-41 (39)* | NA | 3-3.9 (3.5)* | Neonatal infection or respiratory distress syndrome | Gestational age and weight | Liver and renal function was normal. Period of illness NA. |
| Mulla et al. (2003)(180) | 11 | 38–41.6 (40)* | 0.5-16 (3.6)* | (3.6)* | ECMO | Route of administration (IV vs. extracorporeal) | Patients were diagnosed, MAS, PPHN, CDH, Sepsis, post cardiac surgery and metabolic. Period of illness NA. |
| de Wildt et al. (2003) (164) | 5 | NA | 1-16 (9)* | 2.8-3.8 | Cardiac | Age and disease | Duration of illness ranged from 3.8 hours to 25days. |
| Burtin et al. (1994)(181) | 181<br>7 | < 39/40<br>>39/40 | NA | 0.7-5.2 | ICU | Gestational age, birth weight and co-medication | 138 patients received aminoglycosides, 135 aminopenicillins,91 third generationcephalosporins,34 sympathomimetic amines,31 opioids,20 fentanyl,6 received nalbuphine,5 phenoperidine,12 colfibrate,10 neuromuscular blocking agents and 6 other benzodiazepines . Period of illness NA. |
| Ahsman et al.(2010) (182) | 20 | 37-42 (40)* | 0.17-5.6 | 2.7-3.9 (3)* | ECMO | Weight, PNA, time after cannulation, and concomitant inotrope use | 10 patients with MAS,7-CDH,2 respiratory insufficiency,1-sepsis Duration of ECMO ranged from 70-275h. |
| Mulla et al. (2003)(183) | 19 | 39.5* | 0.5-18 (3.8)* | 3.4* | ECMO | Route of administration (IV vs. extracorporeal) and time dependency | 12 patients with MAS, 1 sepsis, 4 CDH, 2 post cardiac surgery and 1 metabolic. Length of ICU stay 6 ±8.6 days |

*Mean, ECMO, extracorporeal membrane oxygenation, MAS ,Meconium aspiration syndrome, PPHN, Persistent pulmonary hypertension of the newborn, CDH, congenital diaphragmatic hernia , PNA, postnatal age, NA, not available,

**Table 3. 5: Midazolam doses in neonates**

| Age group | Loading dose or Single dose (SD) Mg/kg | Midazolam infusion Mg/kg/h | Number of blood samples collected | Method | Study |
|---|---|---|---|---|---|
| Preterm neonates | 0.2 | 0.06 | 7 | Two compartment | Jacqz-Aigrain et al.(1992)(175) |
| | 0.2 | NA | 6 | Two compartment | Jacqz-Aigrain et al.(1990)(176) |
| | NA | 0.1 | 8 | Non-compartmental | de Wildt et al. (2001)(177) |
| | 0.1 | NA | 4 | Two compartment | Harte et al. (1997)(178) |
| | 0.1* | NA | 4 | Two compartment | Lee et al.(1999) (179) |
| Term neonates | 0.2 | NA | 6 | Two compartment | Jacqz-Aigrain et al.(1990)(176) |
| | 0.05-0.1 | 0.05-0.25 | 7 | One compartment | Mulla et al. (2003)(180) |
| | NA | 0.05-0.4 | 3 | Two compartment | de Wildt et al. (2003)(164) |
| | 0.03-1.6 | 0.01-0.6 | 3-6 | One and two compartment | Burtin et al. (1994)(181) |
| | NA | 0.1† (0.10-0.30) | 5 | One compartment | Ahsman et al.(2010)(182) |
| | 0.05-0.1 | 0.05-0.25 | 7 | One, two and three compartment | Mulla et al. (2003)(183) |

* Single dose or multiple doses when necessary, †Median (range), NA, not given

**Table 3. 6: Midazolam clearance in critically ill neonates**

| Age group | Number of patients | Mean Clearance (ml/kg/min) | SD | Coefficient of variation (CV %) | Range of clearance (ml/kg/min) | Variation ratio in clearance | Comments | Study |
|---|---|---|---|---|---|---|---|---|
| Preterm neonates | 15 | 1.7 | 1.8 | 106 | 0.6-2.7 | 4.5 | Non-pop-Pk | Jacqz-Aigrain et al.(175) |
| | 5 | 1.6 | 0.9 | 109 | 0.74-3.5 | 5 | Individual data available ,Non-pop-Pk | Jacqz-Aigrain et al.(176) |
| | 24 | 1.8* | NA | NA | 0.7 - 6.7 | 10 | Non-pop-Pk | de Wildt et al. (177) |
| | 9 | 1.7 | 1.4 | 81*f* | NA | NA | Pop-PK | Harte et al. (178) |
| | 33 | 0.78 | 0.6 | 83*f* | NA | NA | The only population PK study with omega$^2$ /eta values, Pop-PK | Lee et al. (179) |
| | 27 | 1.24 | 0.9 | 78*f* | NA | NA | | |
| Term neonates | 5 | 2.5 | 0.75 | 30 | 1.8-3.7 | 2 | Two stage PK study Individual data available, Pop-PK | de Wildt et al. (164) |
| | 5 | 2.5 | 1.2 | 77 | 0.86-3.7 | 4 | Individual data available Non-pop-Pk, | Jacqz-Aigrain et al. (176) |
| | 20 | 2.6 | NA | 18*f* | NA | NA | Pre-ECMO , Pop-PK | Ahsman et al. (182) |
| | 19 | 1.4 | 1 | 73*f* | NA | NA | Post –ECMO, Pop-PK | Mulla et al. (183) |
| | 11 | 3.5 | 4.9 | 71*f* | 0.4–17.5 | 44 | Post –ECMO, Pop-PK | Mulla et al. (180) |
| | 181<br>6 | 1.17<br>1.84 | 0.7<br>1.2 | 65*f* | NA | NA | Pop-PK | Burtin et al.(181) |

*Median, SD, standard deviation, CV, Coefficient of variation, POP-PK, population pharmacokinetics study, non-pop-Pk, non-population pharmacokinetics study,*f*, CV provided by author, NA, not available

### 3.5.3. Clearance of midazolam in infants (>28 days -23 months)

Four studies reported midazolam clearance in 40 infants (177, 184-186). Three of these studies were in critically ill infants. There were 3 single and 1 multicenter study. The number of participants varied between 5 and 24 (Table 3.7). The number of collected blood samples was between 3 and 14.

Two of the studies were 2 compartment studies and there was 1 non-compartment studies. In 1 other study, one and two compartment models were used. Midazolam loading dose varied between 0.05 and 1 mg/kg and maintenance dose between 0.05 and 0.4 mg/kg/h. Age was a covariate in 3 of these studies. Other covariates were weight and disease condition. None of the studies reported the effect of drug-drug interaction.

The CV ranged from 31% to 89%. The CV was lowest in the study where the infants were not ventilated (184). Two studies gave the full range of clearance values for individual infants. There was a 6 to 9 fold variation in clearance values.

**Table 3. 7: Characteristics of studies in infants**

| Study | Number of patients | Loading dose or Single dose (SD) Mg/kg | Midazolam infusion Mg/kg/h | Number of blood samples collected | Method | Type of patients | Covariates | Comments |
|---|---|---|---|---|---|---|---|---|
| de Wildt et al. (2003)(164) | 6 | NA | 0.05-0.4 | 3 | Two compartment | ICU | Age and disease | 1 Patient With respiratory insufficiency, 3 upper airway infection,1 meningitis and 1 pneumonia. Duration of illness (3.8 hours to 25days). |
| Peeters et al. (2006)(184) | 24 | 0.1 | 0.05 | 7-10 | Two compartment | Surgical | Age | Major craniofacial surgery. Period of illness NA. |
| Mathews et al.(1988)(185) | 5 | 0.05 | 0.05 | 12-14 | Non-compartment | Cardiac | Intra and post-operative treatment | No patients prescribed benzodiazepines. No liver dysfunction. 3 patients with transposition of great vessels, 1 AV canal defect, 1 mitral stenosis. Period of illness NA. |
| Reed et al. (2001) (186) | 5 | 0.25-1 | NA | 12 | One and two compartment | Minor surgery | Age and weight | Exclusion of any patients with upper airway disease, CNS dysfunction, gastroesophageal dysmotility, or taking hepatic inducer, inhibitors or substrate. |

ICU, intensive care unit, AV, atrioventricular, CNS, central nervous system, NA, not available

### 3.5.4. Clearance of midazolam in children (2-11 years)

Ten studies reported midazolam clearance in 102 children. Four studies reported clearance values in critically ill children (164, 185, 187, 188). All but one of the studies were single center studies. The number of children participating in each study varied between 5 and 21 (Table 3.8). The number of blood samples taken between 3 and 14.

The majority of the studies (6 studies) utilised non-compartment models. Two studies were two compartment and 1 three compartment study. In 1 other study, one and two compartment models were used. Midazolam loading dose varied between 0.05 and 1 mg/kg and maintenance dose between 0.01 and 0.4 mg/kg/h (Table 3.10). Age was a covariate in half of the studies. In 3 studies, weight and route of administration were covariates. Drug-drug interaction was not reported in any of the studies.

The CV ranged from 21% to 72% (Table 3.11). Three studies gave full range of values for individual children. There was a 2 to 5 fold variation in clearance (164, 187, 188). Six studies were in non-critically ill children (186, 189-193). Their CV ranged from 13% to 54%. Four of these studies provided data regarding the full range of clearance values and the degree of inter-individual variation was between 2 to 10.5 fold (189-191, 193).

### 3.5.5. Clearance of midazolam in adolescents (12-18 years)

Only one study reported midazolam clearance in 20 non-critically ill adolescents (Table 3.12). This was a non-compartmental study involving 20 patients receiving midazolam maintenance dose of 0.1 mg/kg/h. No loading dose or covariates were reported in this study. The CV for this study was 50%.

### 3.5.6. Clearance of midazolam in paediatric patients of mixed ages

Only one study reported midazolam clearance in 22 critically ill paediatric patients across all age groups (196)(Table 3.11). This was a non-compartmental study. This study did not report neither mean or the CV of clearance.

**Table 3. 8: Characteristics of studies in children**

| Study | Number of patients | Type of patients | Covariates | Comments |
|---|---|---|---|---|
| de Wildt et al. (2003)(164) | 6 | ICU | Age and disease | 1-Empyema ,1-Acute bronchitis ,2-Pulmonary hypertension<br>1-Staphylococcal and1-Measles pneumonia.<br>Duration of illness ranged from 3.8 hours to 25days. |
| Roberts et al. (2009)(187) | 5 | ICU | Age | 1-severe head injury, 1-sepsis, 1-meninigitis, 1-alveolar haemorrhage, 1-addisonian crisis.<br>Exclusion of patients with hepatic impairment, renal impairment or co-medication with CYP3A4 or CYP3A5 inducer or inhibitor.<br>Period of illness NA. |
| Muchohi et al. (2008)(188) | 12 | High dependency unit | Route of administration (IV,IM and buccal) | Severe malaria and convulsion.<br>Patients who received diazepam were excluded.<br>Period of illness NA. |
| Mathews et al. (1988)(185) | 12 | Cardiac | Intra and post-operative treatment | None prescribed benzodiazepines and no liver dysfunction.<br>5 coarctation of aorta,1 tricuspid valve shunt, 3 Fallot's tetralogy,1 atrial septum defect, 1 mitral valve stenosis, 1 Pulmonary valve infundibular stenosis. Period of illness NA. |
| Reed et al. (2001)(186) | 14 | Minor in –hospital or day procedures | Age and weight | Exclusion of upper airway disease, CNS dysfunction, gastroesophageal dysmotility, or taking hepatic inducer, inhibitors or substrate. |
| Rey et al. (1991)(189) | 6 | Healthy children | Route of administration (IV,IN) | Mino genito- urinary |
| Jones et al. (1993)(190) | 12 | Healthy children | NA | Children undergoing circumcision. Exclusion for hepatic, renal, respiratory, cardiac or haematological disease.<br>Period of illness NA. |
| Kraus et al. (1989)(191) | 6 | Minor elective surgery | Age and weight | - |
| Payne et al. (1989)(192) | 8 | Minor surgery | Route of administration (IV,IM,rectal and oral) | Minor inguinal area surgery. |
| Salonen et al. (1987)(193) | 21 | Minor surgery | Age and weight | - |

ICU, intensive care unit, IN, intranasal, CNS, central nervous system, NA, not available,

**Table 3. 9: Characteristics of studies in adolescents and mixed age groups**

| Study | Number of patients | Type of patients | Covariates | Comments |
|---|---|---|---|---|
| Tolia et al. (1991) (194) | 20* | Endoscopy | NA | Esophagogasterodudenal endoscopy.<br>Children with cardiac, renal, liver or neurologic disease were excluded. |
| Nahara et al. (2000) (97) | 22 | ICU | NA | Period of illness NA. |

*Adolescents, ICU, intensive care unit, NA, not available

**Table 3. 10: Midazolam doses in children**

| Age group | Loading dose or Single dose (SD) Mg/kg | Midazolam infusion Mg/kg/h | Number of blood samples collected | Method | Study |
|---|---|---|---|---|---|
| 2-11 yrs | NA | 0.05-0.4 | 3 | Two compartment | de Wildt et al.(2003)(164) |
| | 0.1-0.5 | 0.01-0.06 | 5 | Two compartment | Roberts et al. (2009)(187) |
| | 0.3 | NA | 13 | Non-compartment | Muchohi et al.(2008)(188) |
| | 0.05 | 0.05 | 12-14 | Non-compartment | Mathews et al.(1988)(185) |
| | 0.25-1 | NA | 12 | One and two compartment | Reed et al.(2001)(186) |
| | 0.2 | NA | 10 | Non-compartment | Rey et al.(1991)(189) |
| | NA | 0.5 | 9 | Three-compartment | Jones et al.(1993)(190) |
| | NA | 0.1 | 13 | Two compartment | Kraus et al.(1989)(191) |
| | NA | 0.15 | 9 | Non-compartment | Payne et al.(1989)(192) |
| | NA | 0.15-0.45 | 12 | Non-compartment | Salonen et al.(1987)(193) |
| 12-18yrs | NA | 0.1 | 7 | Non-compartment | Tolia et al.(1991)(194) |
| 6dys-16yrs | NA | NA | 5 | Non-compartment | Nahara et al.(2000)(97) |

NA, not given

**Table 3. 11: Midazolam clearance in critically ill children**

| Age group | Number of patients | Range of age | Range of weight (kg) | Mean Clearance (ml/kg/min) | SD | Coefficient of variation (CV %) | Range of clearance (ml/kg/min) | Variation ratio in clearance | Comments | Study |
|---|---|---|---|---|---|---|---|---|---|---|
| >28 dy-23 Mos | 6 | 1-8mos (3)* | 4-20 (8) * | 4.8 | 5 | 89 | 1.8 - 16 | 9 | Two stage PK study, Individual data available, Pop-PK | de Wildt et al. (164) |
| | 5 | 1.3* yrs | 8.8* | 9 | 3.4 | 37 | NA | NA | Non-pop-Pk | Mathews et al. (185) |
| | 24 | 3-24.7mos (11) * | 5-12 | 16.7 | 5.2 | 31ƒ | 0.1-0.6 | 6 | Not ventilated, ml/min (not $kg^{-1}$),CV Pop-PK | Peeters et al. (184) |
| 2-11 yrs | 5† | 7-14 (8) * | 13-88 (38)* | 1.1 | NA | NA | 0.9-3.8 | 4 | Individual data available, Non-pop-Pk | Roberts et al. (187) |
| | 6 | 2.8-9 (7)* | 13-25 (20)* | 6 | 3.8 | 72 | 2.3-11 | 5 | Two stage PK study, Individual data available, Pop-PK | de Wildt et al.(164) |
| | 12 | 7-39mos (27)* | 8.7-12 (10)* | 14 | NA | NA | 9.2-19.7 | 2 | Non-pop-Pk | Muchohi et al. (188) |
| | 6 | 5* | 18* | 12 | 6.6 | 55 | NA | NA | Non-pop-Pk | Mathews et al. (185) |
| | 6 | 4.7* | 16* | 8.5 | 1.8 | 21 | NA | NA | - | |
| 8 dy to 16 yrs | 22 | 8dys-16yrs | NA | NA | NA | NA | 1.6-51.6 | 32 | Non-pop-Pk | Nahara et al. (97) |

*Mean,†only one patient with 14 years old, SD, standard deviation, CV, Coefficient of variation, POP-PK, population pharmacokinetics study, non-pop-Pk, non-population pharmacokinetic study, ƒ,CV provided by authors, NA, not available.

**Table 3. 12: Midazolam clearance in non-critically ill children**

| Age group | Number of patients | Range of age | Range of weight (kg) | Mean Clearance (ml/kg/min) | SD | Coefficient of variation (CV %) | Range of clearance (ml/kg/min) | Variation ratio in clearance | Comments | Study |
|---|---|---|---|---|---|---|---|---|---|---|
| >28 dy-23Mos | 5 | 6mos-<2yrs | 3-13 | 11 | 6 | 53 | NA | NA | Two stage PK study, Pop-PK | Reed et al. (186) |
| 2-11 yrs | 6 | 1.8-4 (2.5) | 11-17 (15)‡ | 13 | 4 | 41 | 9.5-17.8 | 2 | Individual data available | Rey et al. (189) |
| | 12 | 5-9 (6.5)‡ | 14-39 (22)‡ | 15 | 3 | 20 | 11-23 | 2 | Non-pop-Pk | Jones et al. (190) |
| | 6 | 3-7 | 15-30 | 3.2 | 1.1 | 54 | 1.1-6.5 | 6 | Individual data available, Non-pop-Pk | Kraus et al. (191) |
| | 21 | 6‡ | 23‡ | 7 | 3 | 42 | 1.3-13.7 | 10.5 | Non-pop-Pk | Salonen et al. (193) |
| | 14 | 2-<12 | 19.5‡ | 10 | 3.8 | 38 | NA | NA | Two stage PK study, Pop-PK | Reed et al. (186) |
| | 8 | 5.5‡ | 17‡ | 9 | 1.2 | 13 | NA | NA | Non-pop-Pk | Payne et al. (192) |
| 12-18 yrs | 20 | 8-17 (13.5)* | NA | 10 | 5 | 50 | NA | NA | Non-pop-Pk | Tolia et al. (194) |

‡Mean;*Median, SD, standard deviation, CV, Coefficient of variation, POP-PK, population pharmacokinetics study, non-pop-Pk, non-population pharmacokinetic study, NA not available,

**Table 3. 13: Coefficient of variation for midazolam clearance in paediatrics**

| Age | Critically ill % (n) | Non-critically ill % (n) |
|---|---|---|
| Preterm neonates | 78-109 (113) | - |
| Term neonates | 18-77 (247) | - |
| Infants | 31-89 (35) | 53 (5) |
| Children | 21-72 (35) | 13-54 (67) |
| Adolescents | - | 50 (20) |

(n), number of patient

### 3.5.7. Effect of covariates on midazolam clearance and variability

All studies in neonates were performed in those that were critically ill. It was therefore not possible to compare the effect of critical illness. Also, there was only one study performed in 20 adolescents who were not critically ill (194), so it was not possible to compare the effect of critical illness in this age group either.

Three studies were performed in 34 critically ill infants (164, 184, 185), but only one study in those not critically ill. (186). This study only included five infants and so it was therefore felt inappropriate to try and perform statistical comparison.

Statistical comparison was therefore only performed between children aged 2-11 years, who were critically ill and those who were non-critically ill. The coefficient of variation between these studies was compared using Students t-test. Mean CV for critically ill children was 49% while the mean CV from non-critically ill children was 35%. However, this difference was not statistically significant p=0.261. Meta-analysis is inappropriate to compare coefficient of variations.

Mean CV of midazolam clearance in preterm neonates 91% was significantly higher than children 40% (p=0.002). There were no significant differences between other age groups.

A total of 17 PK studies across all age groups were identified. The mean clearance for these studies was 5.89 ml/min/kg (95% CI 4.69-6.67). The effect of covariates, such as age, critical illness, weight and sample size on midazolam CL was evaluated in a meta-regression. Weight, age and critical illness were statistically significant co-variates (p=0.007, 0.001 and 0.016 respectively). Sample size and study type were not significant. Critical illness was however not a statistically significant predictor of midazolam clearance after adjusting for age and weight (p=0.279). After adjusting for critical illness and weight, age remained a statistically significant predictor of midazolam clearance (Table 3.15).

**Table 3. 14: Unadjusted meta-regression for covariates**

| Covariates | P value |
|---|---|
| **Age** | |
| Term neonates | 0.737 |
| Infants | < 0.001 |
| Children | < 0.001 |
| Adolescents | 0.016 |
| **Critical illness** | 0.007 |
| **Weight** | <0.001 |
| **Study type** | 0.156 |
| **Sample size** | 0.295 |

As a result of the heterogeneity of midazolam clearance for preterm and term neonatal studies, meta-analysis using the random effect model was performed. Pooled mean clearance were 1.25 (95% CI 0.83-1.67) and 1.75 (1.2 -2.29) respectively for critically ill preterm and term neonates (Figures 3.2 and 3.3).

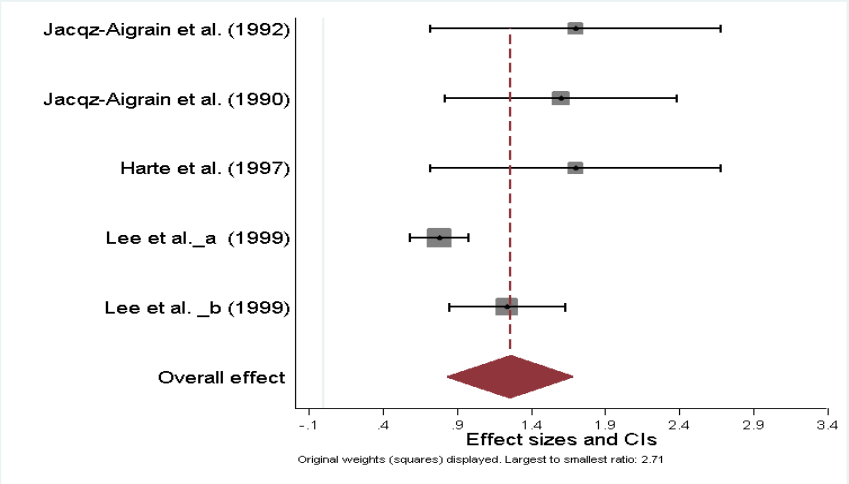


**Figure 3. 2: Mean CL and CIs for preterm neonates**

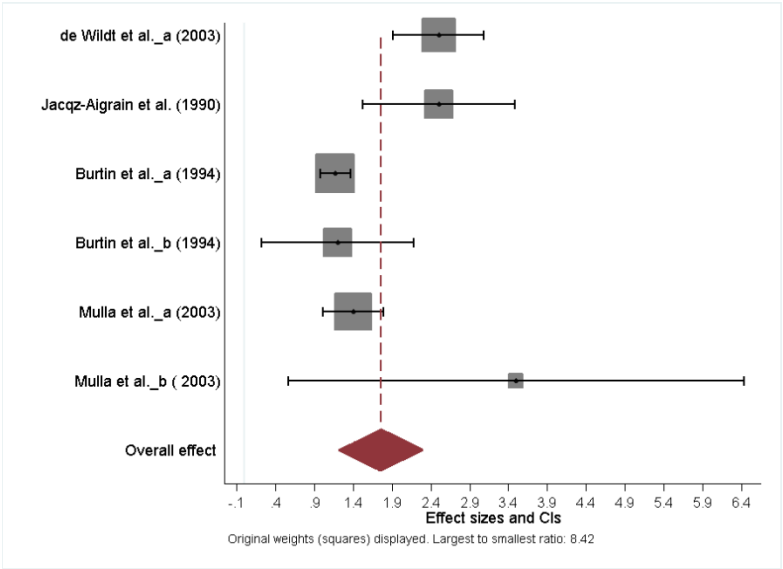


**Figure 3. 3: Mean CL and CIs for term neonates**

The mean clearance from studies involving infants and children was highly heterogeneous (I square>80%); therefore the mean clearance was not pooled.

Multiple factors such as age (p=0.003), weight (p=0.002) and critical illness (p=0.031) are significantly associated with CV. A multiple linear regression analysis of the effects of these covariates however shows that age group alone is independently associated with the CV (p<0.05).

### 3.6. Discussion

This systematic review shows that the CV of midazolam clearance varied between 13% and 109% across all age groups. In neonates, it ranged between 78% and 109% and in children CV was between 21% and 72%. Critical illness significantly increased the variability of midazolam in all pediatric studies. However, after adjusting for weight and age, this was not significant. There was no significant difference in variability between critically ill and non-critically ill children (2-11years old) and the relationship between critical illness and variability in other age groups (neonates, infants and adolescents) could not be determined due to the non-availability of data for non-critically ill patients.

A study by Ince et al. (2012) had previously demonstrated the effect of critical illness on the clearance of midazolam. They reported 93% and 86% reduction in CYP3A4/5 mediated midazolam clearance among

ICU patients when compared with non-critically ill patient groups (197). Critical illness is however a broad categorisation including, diverse disease states such as sepsis, renal failure, respiratory failure. It was impossible to determine the effect of specific disease conditions on midazolam clearance due to the limited number of studies and the clinical details provided about the patients they included. Van den Anker (2003) have however previously shown that covariates such as renal failure, hepatic failure, and concomitant administration of CYP3A inhibitors affect midazolam PK among critically ill children (198). However, many of the studies in this review excluded patients on co-medication, as well as hepatic and renal failure.

Age was a significant predictor of CV and clearance. Clearance is generally lower in neonates than older children and adolescents (199) due to the immaturity of drug metabolism process in neonates (176, 181). The CV was greater in preterm neonates (78%-109%) than term neonates (18%-77%), probably due to the developmental changes in metabolising enzymes, which is more prominent during this stage of life. The major pathway of midazolam metabolism is oxidation which involves the cytochrome P450 dependent (CYP) enzymes (200) and the enzymes involved are CYP3A4, CYP3A5 and CYP3A7 (175, 200). CYP34A activity is significantly reduced in early neonatal life, but gradually increases as the liver matures (Figure 1.2) (26-28). CYP3A7 has greatest activity in the neonatal period (28). This variability in maturation will account for some of the variability in PK.

A limitation of this review is the lack of sufficient studies involving non-critically patients. Thus, it was impossible to establish the relationship between critical illness and CV or clearance, for the different age categories. Furthermore, some studies did not report the CV and some others did not provide the required details, such as the standard deviation and individual clearance values, for the calculation of CV.

Midazolam is administered by a continuous intravenous infusion in critically ill children and the dose is titrated in relation to the response. With about 44-fold variability in clearance in children, the development of dosing strategies based on midazolam plasma concentrations in critically ill patients may be difficult (201). Therefore, midazolam dosing should be individualised. This is particularly important in neonates with high inter-individual variation during the first few days of life. Therefore, after the initial dosing of midazolam, maintenance dosing should be based on the individuals response (5). An approach to achieving this is by therapeutic drug monitoring (5, 201). This involves the measurement of drug concentration at designated intervals to maintain a constant concentration in a patient's bloodstream, in order to optimise individual dosage regimens and efficacy (202).

Furthermore, optimisation of dosing can also be achieved by PK/PD modelling, which links dose-concentration relationship (PK) with concentration-effect relationship. The effect of inter-individual variability and other covariates can be established by utilising the

population PK approach (203, 204). Such studies have been used to predict the disposition of midazolam and theophylline in paediatrics (205).

Overall, it is important clinicians are aware of this variation and should titrate dose based on clinical response and adverse effects.

# CHAPTER FOUR
# A SYSTEMATIC LITERATURE REVIEW OF INTER-INDIVIDUAL VARIATION IN THEOPHYLLINE CLEARANCE IN CHILDREN

## 4.1. Introduction

Theophylline is 1, 3-dimethylxanthine; it was first used clinically in the early 20th century (206). Although its mechanism of action is not fully known, it has been hypothesised that its bronchodilator effect is due to phosphodiesterase inhibition, which causes an increase in cyclic adenosine monophosphate (cAMP) (207). Other possible mechanisms include inhibition of adenosine receptors (208), interleukin-10 release (209) and prevented transport of proinflammatory transcription factor (KB) into the nucleus (210) .

Theophylline can be administered IV or orally. For the treatment of acute asthma in children, theophylline is administered as IV aminophylline which is a combination of theophylline and ethylendiamine (211). For chronic asthma in children, oral theophylline may be used.

After IV administration, theophylline is rapidly distributed throughout the extracellular fluid. Its volume of distribution is between 0.4 to 0.7 L/Kg (212). Theophylline reversibly binds to plasma proteins, with about 40% bound (212). It is metabolised in the liver by CYP1A2, CYP2E1 and CYP3A3 to an active metabolite (3-methylxantheine) (212). The half-life of theophylline varies with age. In preterm

neonates it ranges between 14 to 58 hours. In children (1-4 years old) half-life varies between 2 to 5 hours while in adults it is between 3 to 9 hours (213). About 10% is excreted unchanged in the urine (214).

Theophylline has a narrow therapeutic range. As result, patients on the drug may require monitoring of theophylline plasma concentration (213). Generally, a plasma concentration of 10-20 mg/L is required to produce a therapeutic effect (213).

Theophylline is currently not licensed for neonatal use. It can, however, be given to neonates for apnoea as an aminophylline intravenous injection. The dose varies according to age. In chronic asthma, infants between 6 months and two years require 12mg twice daily orally, children between 2 and 6 years can receive up to 120mg twice daily and children of 6-12 years can be given up to 250mg twice daily. A dose of up to 500mg twice daily is recommended for adolescents (215).

Common side effects in children include tremor, insomnia, nausea vomiting, abdominal distension, dyspepsia, palpitations (216, 217).

Factors such as age , diet , disease , obesity and other drugs can influence the clearance of the drug and contribute to the extensive inter-individual variability of theophylline clearance (211, 218).

## 4.2. Aims

The purpose of this systematic review is to determine the variability of theophylline clearance in children and establish the factors which are responsible for this variability.

## 4.3. Objectives

- Determine the extent of inter-individual variation in theophylline clearance by estimating the CV.
- Evaluate the effect of factors such as age, birth weight and critical illness that may be responsible for the inter-individual variation in theophylline.

## 4.4. Methods

The methods for this chapter are described in chapter two.

## 4.5. Results

A total of 2,663 articles were short listed by five search engines (Figure 4.1). Only 48 articles (including 9 identified by manual search) met the inclusion criteria. Table 4.1 shows the details of papers excluded from the systematic review.

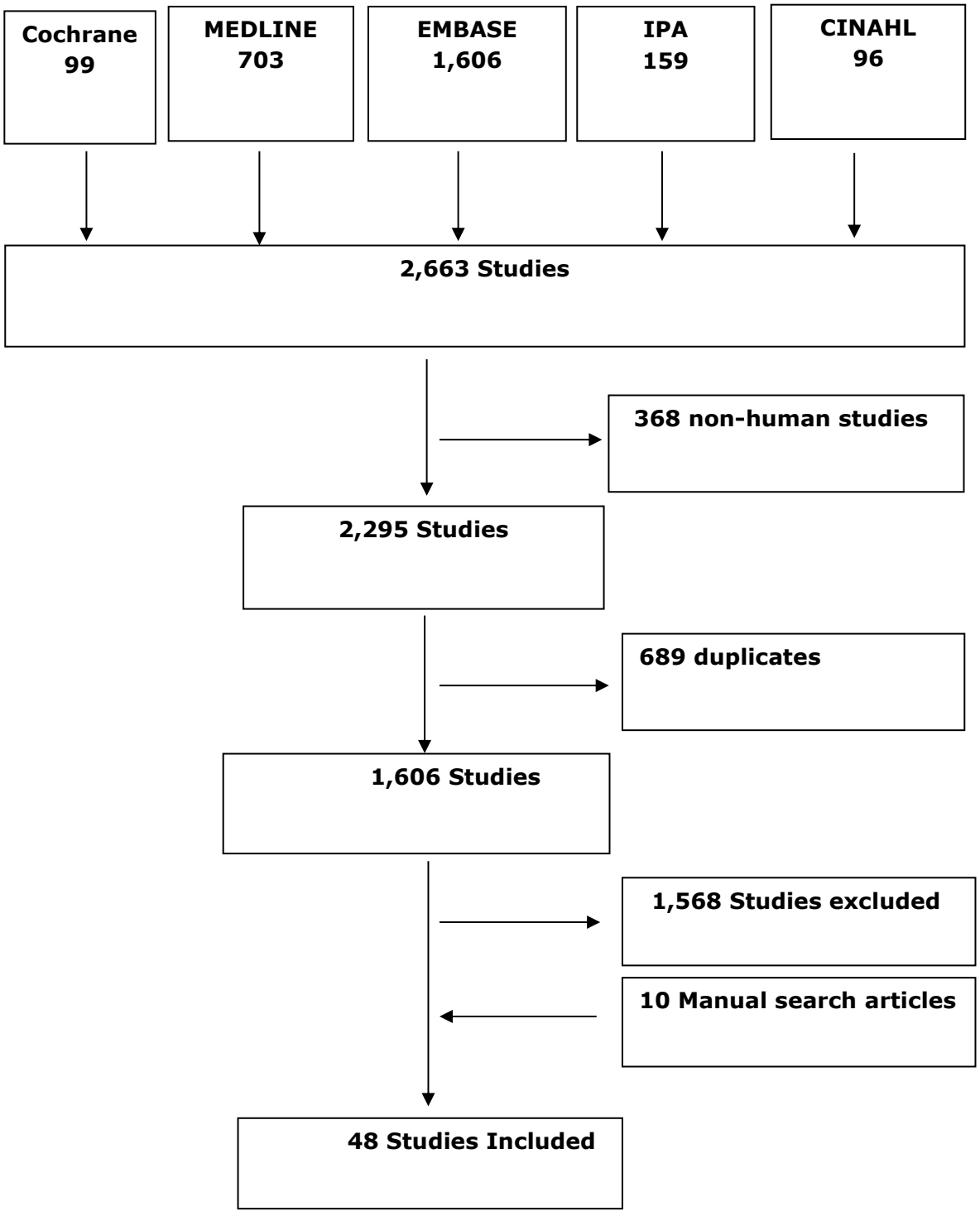


**Figure 4. 1: Search strategy of theophylline**

**Table 4. 1: Details of excluded articles from systemic review**

| Exclusion Reason | |
|---|---|
| Review studies | 383 |
| Editorials | 5 |
| Case reports | 12 |
| Conference abstracts | 4 |
| Studies in adults aged over 18 years | 180 |
| Combined adult and paediatric studies where paediatric data was not presented separately | 5 |
| Studies where data for theophylline was not presented | 442 |
| Studies in which theophylline not administered intravenously | 51 |
| Studies that did not evaluate the pharmacokinetics of theophylline | 486 |
| Total | 1,568 |

### 4.5.1 Quality assessments

Twenty nine studies were included after excluding 19 studies. Eleven of these did not report the number of blood samples collected (219-229) and 8 studies collected less than three samples (230-237)(Table 4.2).

**Table 4. 2: Studies excluded after Quality assessment**

| Study | Age group | †Number of patients | +Number of blood samples collected | Method |
|---|---|---|---|---|
| Robert et al. (1990)(219) | Preterm neonates | 12 | NA | Non-compartmental |
| Nahata et al. (1989)(230) | | 9 | 1 | Non-compartmental |
| Narui et al. (2003) (220) | | 24 | NA | Non-compartmental |
| Lowry et al. (2001) (221) | | 3 | NA | One compartment |
| Bonati et al. (1981) (222) | | 9 | NA | Non-compartmental |
| Samira et al. (2004)(231) | | 50 | 2 | Non-compartmental |
| Gilman et al. (1986)(223) | | 179 | NA | Non-compartmental |
| Gal et al. (1982)(232) | Term neonates | 30 | 1 | Non-compartmental |
| Imai et al. (2012) (233) | Infants | 30 | 1 | Non-compartmental |
| Igarashi et al. (2009)(224) | | 69 | NA | Non-compartmental |
| Mayo (2001) (234) | Children | 31 | 2‡ | Non-compartmental |
| Odajima (225) | | 33 | NA | Non-compartmental |
| Yano et al. (1993) (226) | | 66 | NA | Non-compartmental |
| Slotfeldt et al.(1979) (235) | | 3 | 1 ‡ | Non-compartmental |
| Ginchansky et al.(1977) (227) | | 23 | NA | Non-compartmental |
| Weinberger et al. (1976)(236) | Adolescents | 6 | 1‡ | Non-compartmental |
| Reiter et al. (1992)(228) | 3mos-14yrs | 61 | NA | Non-compartmental |
| Muslow et al. (1992) (229) | 6mos-4yrs | 58 | NA | One compartment |
| Itazawa et al. (2006) (237) | 0-6yrs | 21 | 2 | Non-compartmental |

†Studies with **≤** 2 patients or with number of patients not stated are excluded; **+**Studies with **≤**2 samples or with the number of samples not stated are excluded, ‡serum

**Table 4. 3: Studies included after Quality assessment**

| Study | Age group | Number of patients | Number of blood samples collected | Method |
|---|---|---|---|---|
| Latini et al. (1978)(238) | Preterm neonates | 7 | 8-10 | One compartment |
| Aranda et al. (1976)(239) | | 6 | 10 | Two compartment |
| Stile et al. (1986)(240) | | 9 | 8 | One/Two compartment |
| Jones and Baillie. (1979) (241) | | 12 | >2 | One compartment |
| Dothey et al. (1989) (242) | | 9 | 6 | Non-compartmental |
| Moustogiannis et al. (1997) (243) | | 15 | 6 | Non-compartmental |
| Kraus et al. (1993) (244) | | 52 | 7‡ | Non-compartmental |
| Ahn et al. (1999) (245) | | 7 | 5‡ | Non-compartmental |
| Ni Y et al. (2009) (246) | | 11 | 5‡ | Non-compartmental |
| Bolme et al. (1979) (247) | Infants | 5 | 12‡ | Two compartment |
| Simons et al. (1978) (248) | | 13 | 6‡ | One compartment |
| Franko et al. (1982) (249) | | 12 | 6‡ | One compartment |
| Eriksson et al.(1983)(250) | | 6 | 6* | Non-compartmental |
| Desoky et al. (1997)(251) | Children | 15 | 3* | One compartment |
| Hill et al. (1988) (252) | | 7 | 11 | Two compartment |
| Scott et al. (1989) (253) | | 7 | 8‡x | Non-compartmental |
| Loughnan et al.(1976) (254) | | 10 | 7* | Two compartment |
| Uematsu (1993) (255) | | 16 | 6‡ | One compartment |
| Arnold et al. (1981) (256) | | 8 | 9* | Non-compartmental |
| Vichyanond et al. (257)(2011) | | 13 | 11‡ | One compartment |
| Leung et al. (1977) (258) | | 8 | 3*x | Non-compartmental |
| Prats (1991) (259) | | 16 | 3 | Non-compartmental |
| Principi et al. (1987) (260) | | 5 | 8‡ | One/Two compartment |
| Kubo et al. (1986)(261) | | 13 | 3*x | Non-compartmental |
| Eriksson et al.(1983)(250) | | 25 | 6* | Non-compartmental |
| Larsen et al. (1980)(262) | Adolescents | 18 | 14‡ | Two compartment |
| Leung et al. (1977) (258) | | 22 | 3*x | Non-compartmental |
| LaForce et al. (1981)(263) | 9-19 Yrs | 14 | 3‡x | Non-compartmental |
| Walson et al. (1977) (264) | 7-16 Yrs | 7 | 5‡x | Non-compartmental |
| Ellis et al. (1976)(265) | 6-18 Yrs | 30 | 9 * | Two compartment |
| Wells et al. (1984)(266) | 1-14yrs | 29 | 3‡x | One compartment |

*plasma, ‡serum, x, at steady state

### 4.5.2. Clearance of theophylline in neonates (0 - 27 days)

Ten studies reported theophylline clearance in 131 neonates. All were single centre studies and involved critically ill neonates (238-246, 259). The majority treated apnoeic neonates. The number of participants varied between 3 and 20. Age and weight were the most common covariates (Table 4.4). The number of collected blood samples was between 3 and 10. Theophylline loading dose varied between 5 and 7 mg/kg and maintenance dose between 1 and 6 mg/kg/h (Table 4.5).

Seven of the studies were non-compartment studies; there were 2 one compartment studies and 1 two compartment studies. None of the studies reported the effect of drug-drug interaction.

The mean clearance from all preterm neonatal studies ranged between 0.2 and 0.9 ml/min/kg.

The CV ranged between 9% and 58% in studies involving preterm neonates. The lowest of these values was reported in a study involving 6 preterm neonates receiving aminophylline for apnoea (239). The highest was recorded for a study involving 5 preterm neonates treated for apnoea.

Eight studies provided data regarding the range of clearance values for individual neonates. The variation ratio of clearance in preterm neonatal studies ranged between 1.5-7 fold.

**Table 4. 4: Characteristics of studies in preterm**

| Study | Number of patients | Gestational age (weeks) | Postnatal age (days) | Range of weight (kg) | Type of patients | Covariates | Comments |
|---|---|---|---|---|---|---|---|
| Latini et al. (1978)(238) | 7 | 26-33 (31)* | 4-8 (7)* | 0.87-2.7 (1.66)* | Severe apnea | Age | Patients with hypoglycemia, CNS infection or sepsis were excluded. Duration of illness was 13 days. |
| Aranda et al. (1976)(239) | 6 | 25-32 (27)* | NA | 0.6-1.2 (0.9)* | Apnea | Age | Duration of illness between 3-15 days. |
| Stile et al. (1986)(240) | 9 | 25-30 (28)* | NA | 0.8-1.5 (1)* | Apnea | Age and weight | - |
| Jones and Baillie (1979)(241) | 12 | 25-30 (28)* | 1-3 | 0.7-1.4 (1.1)* | Apnea | NA | Any patient with infection was excluded. Duration of illness NA. |
| Dothey et al. (1989)(242) | 9 | 25-30 (28)* | 12-191 (57)* | 0.9-2.4 (1.3)* | Apnea | Age and weight | Patient who received drugs such as phenobarbital or cimeditine as well as patients with cholestatic hepatitis were excluded. Duration of the illness NA. |
| Ahn et al. (1999)(245) | 7 | 28.5-34 (32)* | 2-26 (8)* | 0.6-1.5 (1.2)* | NICU | NA | Patients were apnea and/or bradycardia. The neonates with hypoxemia, patent ductus arteriosus, anemia, infection, metabolic abnormalities, or abnormal liver and kidney functions were excluded. Duration of illness NA. |
| Moustogiannis et al. (1997)(243) | 5<br>5<br>5 | 33*<br>33*<br>34* | 10*<br>9*<br>9* | 1.55*<br>1.6*<br>1.7* | Apnea | Fluid and calorie intakes | Neonates with perinatal asphyxia, respiratory distress syndrome, hemodynamic instability or hepatic or renal failure were excluded. Nine patients were given ampicillin and gentamicin while sepsis and /or necrotizing enterocolitis were evaluated as secondary causes of apnea no other medications were given. Duration of illness NA. |
| Kraus et al. (1993)(244) | 20<br>15<br>17 | 29*<br>30*<br>31* | 2-9 | NA<br>NA<br>NA | Apnea | Age, weight, co-medication and race | Patients with known bleeding abnormalities, renal and hepatic impairment were excluded. Duration of illness NA. |
| Ying-hua et al. (2009)(246) | 11 | 30-33 (31)* | NA | 1.1-2 (1.5)* | Apnea | Age | Duration of illness NA. |
| Prats (1991) (259) | 3 | NA | 6-20 (12)* | 1-3 (1.7)* | NICU | Age | Duration of illness NA. |

*Mean ,NICU, neonatal intensive care unit, NA, not available

**Table 4. 5: Theophylline doses in neonates**

| Loading dose or Single dose (SD) Mg/kg | Theophylline infusion Mg/kg/h | Number of blood samples collected | Study |
|---|---|---|---|
| NA | 3.3-6 | 4-8 | Latini et al. (1978) (238) |
| 5.5 | 2.5-5 | 10 | Aranda et al. (1976) (239) |
| 5.5 | 2.5 | 8 | Stile et al. (1986) (240) |
| 5-7 | 4.4 | >2 | Jones and Baillie (1979)(241) |
| NA | NA | 6 | Dothey et al. (1989) (242) |
| 5 | NA | 6 | Moustogiannis et al. (1997)(243) |
| NA | 2.1-26.4* | 7 | Kraus et al. (1993) (244) |
| 5 | 1 | 5 | Ahn et al. (1999 (245) |
| NA | 5 | 5 | Ying-hua et al. (2009)(246) |
| 5* | 1.4* | 3 | Prats (1991 (259) |

*Mean, NA, not given

**Table 4. 6: Theophylline clearance in critically ill preterm neonates**

| Number of patients | Mean Clearance (ml/min/kg) | SD | Coefficient of variation (CV %) | Range (ml/min/kg) | Variation Ratio | Comments | Study |
|---|---|---|---|---|---|---|---|
| 7 | 0.2 | NA | NA | 0.11-0.49 | 1.5 | Individual data available | Latini et al. (238) |
| 6 | 1.1 | 0.1 | 9 | 0.7-1.5 | 2 | - | Aranda et al. (239) |
| 9 | 0.7 | 0.23 | 45 | 0.5-1 | 2 | Individual data available | Stile et al. (240) |
| 12 | 0.3 | 0.08 | 25 | 0.2-0.5 | 2.5 | Individual data available | Jones and Baillie (241) |
| 9 | 0.4 | 0.23 | 50 | 0.15-0.98 | 6 | Individual data available | Dothey et al. (242) |
| 7 | 0.4 | 0.1 | 25 | NA | NA | - | Ahn et al. (245) |
| 11 | 0.23 | 0.1 | 10 | 0.1-0.4 | 4 | Individual data available | Ying-hua et al. (246) |
| 20 | 0.3 | 0.1 | 33 | NA | NA | - | Kraus et al. (244) |
| 15 | 0.5 | 0.2 | 40 | NA | NA | | |
| 17 | 1.1 | 0.2 | 18 | NA | NA | | |
| [a]5 | 0.3 | 0.07 | 23 | 0.23-1.6 | 7 | - | Moustogiannis et al. (243) |
| [b]5 | 0.5 | 0.25 | 50 | - | - | | |
| [c]5 | 0.9 | 0.53 | 58 | - | - | | |
| 3 | 0.5 | 0.1 | 11 | 0.4-0.7 | 2 | Individual data available | Prats (1991) (259) |

SD, standard deviation, CV, Coefficient of variation, all studies were non-population pharmacokinetics ,Categorized according to caloric intakes, a) 150 Kcal, b) 98 kcal, c) 38 Kcal, NA, not available

### 4.5.3. Clearance of theophylline in infants (>28 days -23 months)

Four studies reported theophylline clearance in 37 infants (247-250). All were single centre studies involving between 6 to 13 participants. The most common covariate was age. The number of blood samples varied between 6 to 12 (Table 4.7). There were two 1 compartment and 1 two compartment studies.

All studies were in non-critically ill infants. The mean clearance in these infants varied between 0.6 and 2 ml/min/kg. All studies gave data regarding the range of clearance values for individual infants. The variation ratio ranged between 3-10 fold. The CV ranged from 22% to 52% (Table 4.8).

**Table 4. 7: Characteristics of studies in infants**

| Study | Number of patients | Loading dose or Single dose (SD) Mg/kg | Theophylline infusion Mg/kg/h | Number of blood samples collected | Type of patients | Covariates | Comments |
|---|---|---|---|---|---|---|---|
| Simons et al. (1978)(248) | 13 | NA | 0.65 | 6 | Asthma | Age | Duration of illness was between 24-192h. |
| Franko et al. (1982)(249) | 12 | 5-8 | 2.5-5 | 6 | Bronchiolitis | Age | Duration of illness NA. |
| Bolme et al. (1979)(247) | 6 | 5-6 | NA | 12 | Asthma | Route of administration | Duration of illness NA. |
| Eriksson et al.(1983)(250) | 6 | NA | 5 | 6 | Asthma | Nutritional status | 6 underweight patients. |

NA, not given

**Table 4. 8: Theophylline clearance for infants (>28 days-23 months)**

| Number of patients | Range of age | Range of weight (kg) | Mean Clearance (ml/min/kg) | SD | Coefficient of variation (CV %) | Range | Variation Ratio | Comments | Study |
|---|---|---|---|---|---|---|---|---|---|
| 12 | 3wks-6.5mos (3.6mos)* | 2.6-10 (5)* | 0.6 | 0.23 | 22 | 0.3-1 | 3 | Individual data available | Franko et al. (249) |
| 6 | 2-14mos (8)* | NA | 2 | 0.9 | 45 | 0.3-3 | 10 | Individual data available | Bolme et al. (247) |
| 13 | 3-20mos (10)* | NA | 1.07 | 0.55 | 52 | 0.47-2.4 | 5 | Individual data available | Simons et al. (248) |
| 6 | 1.9 yrs | 7-11 (8)* | 1 | 0.15† | NA | NA | NA | - | Eriksson et al.(1983)(250) |

*Mean, †SE, slandered error, SD, standard deviation, CV, Coefficient of variation, all studies were non-population pharmacokinetics study, NA, not available

### 4.5.4. Clearance of theophylline in children (2-11 years)

Twelve studies reported theophylline clearance in 143 children (250-253, 255-261, 267). All studies were single centre studies. The number of participants in each study varied between 5 to 25. The most common covariate was age (Table 4.9). Maintenance dose varied between 1-13.6 mg/kg/h (Table 4.10).

The number of blood samples collected was between 3 to 11. The majority (5 studies) were non compartment. There were 3 and 2 one and two compartment studies respectively. Drug-drug interaction was not reported in any of the studies (Table 4.3).

The mean clearance from all these studies ranged between 1 and 2 ml/min/kg. The CV ranged between 20% and 50% in studies involving non-critically ill children (Table 4.11). The lowest CV was reported in a study involving 7 patients who received treatment for moderate to severe asthma. The highest value was documented in a study involving 7 patients who received theophylline for chronic asthma (253). Four studies involved critically ill children with acute asthma, and the CV in those studies ranged between 29% and 72%. Nine studies gave data regarding the range of clearance (Table 4.12). The variation ratio of theophylline clearance in all children involved in these studies ranged between 1.3 and 5 fold.

**Table 4. 9: Characteristics of studies in children**

| Study | Number of patients | Type of patients | Covariates | Comments |
|---|---|---|---|---|
| Scott et al. (1989) (253) | 7 | Asthma | NA | Duration of treatment NA. |
| Loughnan et al. (1976) (254) | 10 | Chronic asthma | Age | Other drugs prednisone, orciprenaline, phenobarbital, ephedrine, penicillin and erythromycin. |
| Desoky et al. (1997)(251) | 15 | Acute asthma with dyspnoea and cough | NA | Normal liver and kidney function.<br>Duration of treatment NA. |
| Hill et al. (1988) (252) | 7 | Moderate to severe asthma | NA | All patients were free of cardiac, renal and hepatic disease.<br>Duration of treatment NA. |
| Uematsu (1993) (255) | 16 | Asthma | Age | No patents showed hepatic or renal dysfunction also No patents's body temperature exceeded 37.5 °C. |
| Arnold et al. (1981)(256) | 8 | Acute asthma | NA | All patients were with normal hepatic and renal function |
| Vichyanond et al. (257) (2011) | 13 | Asthma | NA | Patients with upper respiratory tract infection and acute asthma were excluded.<br>Duration of illness NA. |
| Leung et al. (1977)(258) | 8 | Severe asthma | Age | - |
| Prats (1991) (259) | 16 | ICU | Age | Duration of illness NA. |
| Principi et al. (1987)(260) | 5 | Chronic asthma | NA | The clearance was not altered by miocamycine administration. |
| Kubo et al. (1986) (261) | 13 | Severe asthma | NA | Exclusion patients with viral infection, hepatic or renal disease also patients who received $\beta_{2-}$ adrenergic agonists or hydrocortisone were excluded.<br>Duration of illness NA. |
| Eriksson et al.(1983)(250) | 25 | Asthma | Nutritional status | 12 patients were normal, 8 Marasmus and 5 Kwashiorkor. |

ICU, intensive care unit, NA, not available

**Table 4. 10: Theophylline doses in children**

| Loading dose or Single dose (SD) Mg/kg | Theophylline infusion Mg/kg/h | Number of blood samples collected | Study |
|---|---|---|---|
| 6 | 1 | 3 | Desoky et al. (1997) (251) |
| NA | 4-6 | 11 | Hill et al. (1988) (252) |
| NA | 13.6 | 8 | Scott et al. (1989) (253) |
| 4* | 0.9* | 3 | Prats (1991) (259) |
| NA | 3 | 7 | Loughnan et al. (1976) (254) |
| NA | 3-4 | 6 | Uematsu (1993) (255) |
| NA | 2-4 | 9 | Arnold et al. (1981) (256) |
| NA | 5 | 11 | Vichyanond et al. (2011) (257) |
| NA | 1 | 3 | Leung et al. (1977) (258) |
| NA | 4.5 | 8 | Principi et al. (1987) (260) |
| NA | 5-7 | 3 | Kubo et al. (1986)(261) |
| NA | 5 | 6 | Eriksson et al.(1983)(250) |

*Mean, NA, not given

**Table 4. 11: Theophylline clearance for non-critically ill children (age 2-11 years)**

| Number of patients | Range of age (year) | Range of weight (kg) | Mean Clearance (ml/min/kg) | SD | Coefficient of variation (CV %) | Range (ml/min/kg) | Variation Ratio | Comments | Study |
|---|---|---|---|---|---|---|---|---|---|
| 15 | 2-12 (6.4)* | 12-30 (20)* | 1 | 0.20 | 46 | 0.7-1.5 | 2 | Individual data available Pop-PK | Desoky et al. (251) |
| 7 | 2-7 | NA | 2 | 0.44 | 20 | 1.5-3 | 2 | Individual data available, Non-PK | Hill et al. (252) |
| 7 | 7-11 | NA | 1.5 | 0.43 | 50 | 0.9-2 | 2 | Individual data available, Non-PK | Scott et al. (253) |
| 10 | 1.3-4 (2.5)* | 9-16 (13)* | 1.6 | 0.6 | 38 | 0.95-3 | 3 | Non-Pop PK | Loughnan et al. (254) |
| 16 | 6-11 (8.7)* | 15-45 (28)* | 1 | 0.4 | 38 | 0.5-1.8 | 3.5 | Individual data available Non-pop-PK | Uematsu (255) |
| 8 | 7-12 (10)* | NA | 1 | 0.55 | 45 | 0.6-2.3 | 4 | Individual data available Non-pop-PK | Arnold et al. (256) |
| 13 | 7-12 (9)* | 29* | 1.5 | NA | NA | 0.6-3 | 5 | Non-pop-PK | Vichyanond et al. (257) |
| 12‡ | 2.7* | 13* | 1.2 | 0.2† | NA | NA | NA | - | Eriksson et al. (1983)(250) |
| 8‡ | 4.4* | 7* | 1.45 | 0.17† | NA | NA | NA | - | |
| 5‡ | 2.4* | 9.5* | 1.49 | 0.22† | NA | NA | NA | - | |

*Mean, ‡ three different groups, normal, Marasmus and Kwashiorkor, †SE, slandered error, SD, standard deviation, CV, Coefficient of variation, POP-PK, population pharmacokinetics study, non-pop-Pk, non-population pharmacokinetics study, NA, not available

**Table 4. 12: Theophylline clearance for critically ill children (age 2-11 years)**

| Number of patients | Range of age (year) | Range of weight (kg) | Mean Clearance (ml/min/kg) | SD | Coefficient of variation (CV %) | Range (ml/min/kg) | Variation Ratio | Comments | Study |
|---|---|---|---|---|---|---|---|---|---|
| 8 | 6-12 (8)* | 19-48 (34)* | 1.3 | 0.4 | 29 | 1.18-1.5 | 1.3 | Individual data available, Non-Pop PK | Leung et al.(258) |
| 16 | 3-11 (6)* | 14-49 (30)* | 1 | 0.4 | 44 | 0.68-1.6 | 2.5 | Individual data available Non-Pop PK, | Prats (259) |
| 5 | 7-10 | NA | 1.7 | 0.3 | 72 | 0.36-2 | 2 | Individual data available, Non-Pop PK | Principi et al.(260) |
| 13† | 4-12.8 (7.5)* | 15-40 (24)* | 1 | 0.6 | 60 | 0.79-3 | 4 | Individual data available, Non-Pop PK | Kubo et al. (261) |

*Mean, †,Only one patient with 12.8 years old, SD, standard deviation ,CV, Coefficient of variation, non-pop-Pk, non-population pharmacokinetics study, NA, not available

### 4.5.5. Clearance of theophylline in adolescents (12-18 years)

Two studies (1 critically ill and 1 non-critically ill) reported theophylline clearance in 30 adolescents. Age was a covariate in one study while the other did not report any covariate. Theophylline infusion dose was 1 and 4 mg/kg/h for critically and non-critically patients respectively (Table 4.13). None of the studies reported the effect of drug-drug interaction.

The CV of these patients was 29% and 43% (Table 4.14). Both studies gave the range for theophylline clearance. The variation ratio in critically ill adolescents was 1.4 and 5-fold.

**Table 4. 13: Characteristics of studies in adolescents**

| Study | Number of patients | Loading dose or Single dose(SD) Mg/kg | Theophylline infusion Mg/kg/h | Number of blood samples collected | Type of patients | Covariates |
|---|---|---|---|---|---|---|
| Larsen et al. (1980)(262) | 8 | NA | 4 | 14 | Cystic fibrosis | NA |
| Leung et al. (1977)(258) | 22 | NA | 1 | 3 | Severe asthma | Age |

NA, not given

**Table 4. 14: Theophylline clearance for adolescents (age 12-18 years)**

| Number of patients | Range of age (Year) | Range of weight | Mean Clearance (ml/min/kg) | SD | Coefficient of variation (CV %) | Range (ml/min/kg) | Variation ratio | Comments | Study |
|---|---|---|---|---|---|---|---|---|---|
| 8‡ | 12-18<br>(14.5)* | 26-53<br>(45)* | 1.5 | 0.5 | 29 | 0.9-2.5 | 3 | Individual data available | Larsen et al. (262) |
| 22† | 12-17 | 31-61 | 1.3 | 0.6 | 43 | 0.60-2.93 | 5 | Individual data available | Leung et al. (258) |

‡Non-critical, †Critical ill, *mean, SD, standard deviation, CV, Coefficient of variation, all studies were non-population pharmacokinetics

### 4.5.6. Clearance of theophylline in paediatric patients of mixed ages

Four studies reported theophylline clearance in 80 children across all age categories. These involved a combination of different age groups. All were single centre studies and the number of participants varied between 7 to 30. Covariates in these studies included age, sex and weight. Maintenance dose varied between 4 to 30 mg/kg/h and the number of blood samples collected was between 3 to 9 (Table 4.15). None of the studies reported the effect of drug-drug interaction.

All studies except one were conducted with non-critically ill patients. The mean clearance for these studies ranged between 0.8 and 1.5 ml/min/kg (Table 4.16).

The CV ranged between 5% and 45% in all studies. The lowest CV of 5% was reported in a study involving 29 patients (aged 1 - 14 years), while the highest CV of 45% was documented in a study involving 7 patients (aged 7-16years)(264, 266).

All studies documented the range of clearance values. The variation ratio of theophylline clearance ranged between 2 to 8 fold.

**Table 4. 15: Characteristics of studies in mixed age groups**

| Study | Number of patients | Loading dose or Single dose (SD) Mg/kg | Theophylline infusion Mg/kg/h | Number of blood samples collected | Type of patients | Covariates | Comments |
|---|---|---|---|---|---|---|---|
| LaForce et al. (1981) (263) | 14 | NA | 16-30 | 3 | Asthma | Age, sex and weight | Duration of illness NA. |
| Walson et al. (1977) (264) | 7 | NA | 4-6 | 5 | Moderate to severe asthma | NA | Duration of illness NA. |
| Ellis et al. (1976) (265) | 30 | NA | 4 | 9 | Asthma | Age | Duration of illness NA. |
| Wells et al. (1984) (266) | 29 | 4.56* | 0.86* | 3 | Acute asthma | NA | Patients with hepatic, renal or cardiovascular diseases were excluded. Duration of treatment NA. |

*Mean, NA, not given

**Table 4. 16: Theophylline clearance for paediatric patients with unspecified age groups**

| Number of patients | Age Range (Year) | Range of weight (kg) | Mean Clearance (ml/min/kg) | SD | Coefficient of variation (CV %) | Range (ml/min/kg) | Variation Ratio | Comments | Study |
|---|---|---|---|---|---|---|---|---|---|
| 14 | 9-19 (14.5)* | 30-73 | 0.8 | 0.18 | 43 | 0.48-1.1 | 2 | Individual data available Non-PK | LaForce et al. (1981)(263) |
| 7 | 7-16 | NA | 1.8 | 0.8 | 45 | 0.95-3 | 3 | Individual data available | Walson et al. (1977) (264) |
| 30 | 6-16 (11)* | NA | 1.5 | 0.58 | 39 | 0.5-4 | 8 | - | Ellis et al. (1976) (265) |
| 29‡ | 1-14 | NA | 1.5 | 0.08 | 5 | 0.8-2.4 | 3 | - | Wells et al. (1984) (266) |

*Mean,‡ critical ill, SD, standard deviation ,CV, Coefficient of variation ,all studies were non-population pharmacokinetic, NA, not available

**Table 4. 17: Coefficient of variation for theophylline clearance in paediatrics**

| Age | Critically ill % (n) | Non-critically ill % (n) |
|---|---|---|
| Preterm neonates | 9-58 (131) | - |
| Infants | - | 22-52 (37) |
| Children | 29-72 (42) | 20-50 (101) |
| Adolescents | 43 (22) | 29 (8) |

(n) Number of patients

### 4.5.7. Effect of covariates on theophylline clearance and variability

The mean clearance of theophylline in all patients was 1 ml/min/kg. A meta regression analysis was performed to determine the effect of covariates on theophylline clearance. Covariates including weight ($p<0.001$), age (infants, children and adolescents ($p<0.05$)) and critical illness ($p<0.001$) were independent predictors of theophylline clearance. However, sample size did not significantly affect clearance (Table 4.18). After adjusting for confounding covariates, critical illness did not significantly affect clearance ($p=0.768$). The mean clearance from studies involving neonates, infants and children was highly heterogeneous (I square>80%); therefore the mean clearance was not pooled.

The CV of theophylline clearance was not significantly different between the critically ill with a mean CV of 35% (95% CI 26-43) and the non-critically ill patients with a mean CV of 39% (95% CI 33-45)($p=0.403$). A sub-analysis of children also did not show any statistically significant difference between critically ill and non-

critically ill (p=0.418). Subgroup analysis in neonates was not carried out because all neonatal studies were in the critically ill. There was insufficient data on critically ill infants and adolescents, hence comparison of CV within this age group was also not performed.

**Table 4. 18: Unadjusted meta-regression for covariates**

| Covariates | P value |
|---|---|
| **Age** | |
| Infants | 0.021 |
| Children | < 0.001 |
| Adolescents | 0.002 |
| **Critical illness** | <0.001 |
| **Weight** | <0.001 |
| **Sample size** | 0.803 |

## 4.6. Discussion

Theophylline is used mainly for the treatment of apnoea in neonates and asthma in children and adolescents. This systematic review suggests that age, weight and critical illness are independent predictors of theophylline clearance. However, critical illness did not significantly affect clearance after adjusting for confounding factors.

The CV in preterm neonates was higher than in children and adolescents. Theophylline clearance is significantly reduced in neonates, but increases rapidly in the first year of life and reaches its peak value by age 1-2 years, when clearance is twice that of the

adult level (34). The major factor responsible for age-related differences in the pharmacokinetics of theophylline is the altered metabolism of the drug across paediatric age groups. Studies have shown differences in the oxidising capacity of CYP in neonates, infants, and children. CYP1A2 synthesis is very negligible in utero and very low in neonates before rising to adult level by 4 months postpartum (56). Furthermore, CYP1A2 activity is very highly variable between individuals because of difference in genetic constitution, with up to 15 alleles identified (16).

The effect of critical illness in all the age categories could not be determined. All studied neonates were critically ill, all studies in infants were in the non-critically ill and there were too few adolescent studies for comparison. Although CV varied more among critically ill children (29%-72%) than non-critically ill (20-50%), there was no significant difference in the CV of theophylline between the two patient groups. The major indication for theophylline is airway obstruction, which may be accompanied by hypoxia and/or hypercapnia, both of which have been shown to reduce the clearance of theophylline in vivo (268). Hypoxia alters expression of CYP1A1 and CYP1A2, thereby affecting theophylline clearance (269). Similar to CYP3A4, CYP1A2 can also be down-regulated by inflammatory mediators such as interleukin and interferon gamma, which are produced in inflammatory airway disease (270-272).

Theophylline has a narrow therapeutic index; hence, an increase in plasma concentration beyond therapeutic range can result in toxicity. Extensive variation in metabolism among patients also increases the risk of fluctuation in plasma concentration. To avoid toxicity, therapeutic monitoring of theophylline concentration in plasma is therefore necessary (273).

This study is limited by insufficient studies in non-critically ill subjects. Therefore, the relationship between critical illness and CV in the different age categories could not be determined. Studies involving patients with renal and hepatic failure were not identified and it was impossible to establish how these conditions influenced theophylline clearance. Similarly the effect of drug interaction on theophylline CV could not be determined. Similar to midazolam studies, CV or individual clearance values were not reported in some studies.

In conclusion, age and weight are the most important covariates of theophylline PK and these should be considered when treating children.

# CHAPTER FIVE
# A SYSTEMATIC LITERATURE REVIEW OF INTER-INDIVIDUAL VARIATION IN MORPHINE CLEARANCE IN CHILDREN

## 5.1. Introduction

Morphine is a naturally occurring opioid alkaloid. It was first isolated in 1805 and synthesized in 1952 (274, 275). The World Health Organization (WHO) recommends morphine as the first choice analgesic for cancer pain (276). It is also one of the most commonly used analgesics in neonates (277). Apart from its analgesic effect, morphine can also be used for preoperative sedation. Studies have suggested that morphine binds to specific μ opioid receptors in the brain to exert its analgesic effect. $\mu_1$ receptors mediate analgesic effect while $\mu_2$ is involved in gastrointestinal and respiratory adverse effects (278).

Morphine can be administered via different routes IV, IM, SC, orally and rectally. Dosing varies according to age and route of administration. Intravenous morphine is administered to neonates at an initial dose of 100μg/kg six hourly, initially by slow bolus over five minutes then by continuous intravenous infusion of 10-20μg/kg. The subcutaneous dose of morphine is 100 μg/kg. Oral, rectal and IM morphine are not recommended for neonates. Dosing for children and adolescents can be up to 200 μg/kg (maximum 10mg ) four hourly (279). Since patients' responses to pain vary, the morphine dose is usually titrated according to clinical response.

Oral morphine undergoes first pass metabolism and extensive inter-individual variation in metabolism. Its bioavailability is approximately 20-30% (278). The volume of distribution is 2.8 ±2.6 L/kg and protein binding about 15-35% (278, 280).

It is metabolised primarily in the liver by UGT to M3G and MG6. M3G is an inactive metabolite while M6G is very active and more potent than morphine itself. MG6 is responsible for the opioid effects observed during treatment. M3G however, does not have any effect on opioid receptors (281). Hence, it does not have analgesic properties. Some studies have reported its anti-analgesic and respiratory depressant effect (282). Plasma concentrations of both metabolites are usually higher than morphine itself and are mainly excreted via the kidneys (58, 280). About 10% is excreted unchanged and only a small amount is excreted as glucuronide in bile (283).

The elimination half-life in preterm neonates and term neonates is 9 ±3.4 and 6.5 ±2.8 hours respectively. The half-life in infants and children is 2 ±1.8 hours (280).

Some studies have suggested that age contributes to the variability of morphine pharmacokinetics in children. Clearance of the drug in neonates increases with age and reaches adult levels at three month (57). The rate of glucuronidation is also affected by age (284). Critically ill neonates have also been shown to have inter-individual

variation of morphine clearance (285). There is also evidence to suggest that there may be a racial difference in morphine clearance (286, 287).

Common side effects associated with morphine include, nausea, vomiting, constipation, sedation and respiratory depression. Less common side effects include  hyperalgesia delayed gastric emptying and muscle rigidity (288).

### 5.2. Aims

The purpose of this systematic review is to determine the variability of morphine clearance in children and establish the factors which are responsible for this variability.

### 5.3. Objectives

The purpose of this systematic review is to:

- Determine the extent of inter-individual variation in morphine clearance.
- Evaluate the effect of factors such as age, birth weight and critical illness that may be responsible for the inter-individual variation in morphine PK.

## 5.4. Methods

The methods for this chapter are described in chapter two.

## 5.5. Results

A total of 2,040 articles were short-listed from five search engines (Figure 5.1). Only 28 articles met the inclusion criteria. Table 5.1 shows the details of papers excluded from the systematic review.

**Table 5. 1: Details of excluded articles from systematic review**

| Exclusion Reason | |
|---|---|
| Review articles | 590 |
| Editorials | 33 |
| Case report | 32 |
| Conference abstracts | 23 |
| Studies in adults aged over 18 years | 284 |
| Combined adult and paediatric studies where paediatric data was not presented separately | 26 |
| Studies where data for morphine was not presented | 312 |
| Studies that did not evaluate the pharmacokinetics of morphine | 424 |
| Studies in which morphine not administered intravenously | 6 |
| Total | 1,730 |

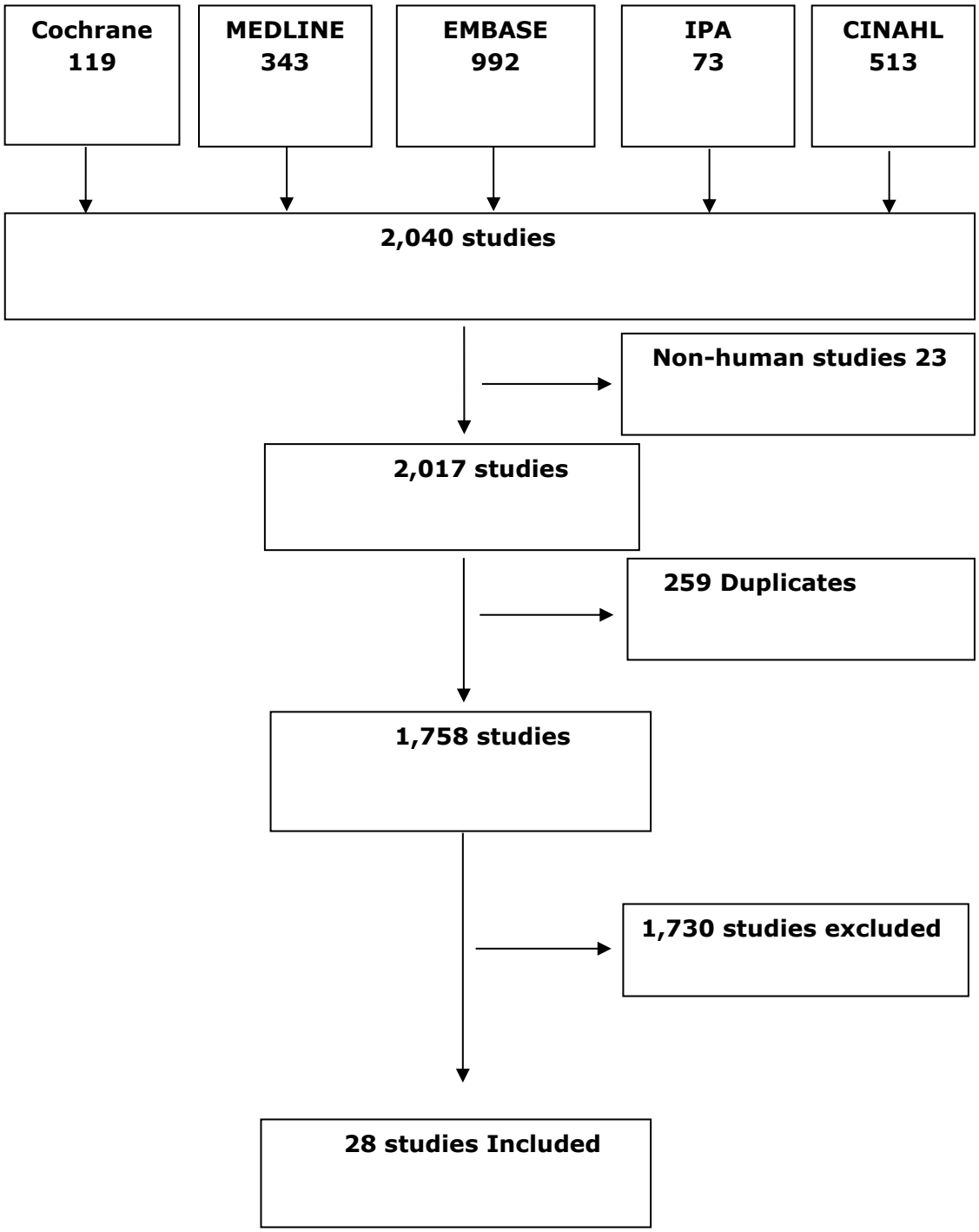


**Figure 5. 1: Search strategy of morphine**

### 5.5.1. Quality assessments

Twenty eight studies were identified, however only twenty studies fulfilled the inclusion criteria. Three studies were excluded for collecting less than 3 blood samples (289-291) and another three for not reporting number of blood samples (58, 292, 293). One study involving less 3 participants was therefore excluded (294) (Table 5.2).

**Table 5. 2: Studies excluded after quality assessment**

| Study | Age group | †Number of patients | +Number of blood samples collected | Method |
|---|---|---|---|---|
| Hartley et al. (1993)(292) | Preterm neonates | 10 | NA | Non compartment |
| Lynn et al. (1998)(289) | Term neonates and Infants | 26 | 2 | Non compartment |
| Choonara et al. (1992) (290) | | 8 | 1 | Non compartment |
| Lynn et al.(2003)(295) | | 10<br>10 | 2 | Non compartment |
| Shelly et al.(1986)(294) | Children | 2 | 9 | Non compartment |
| Robie et al. (1992)(293) | 3-18yrs | 24 | NA | Non compartment |
| Choonara et al. (1989) (58) | Preterm neonates and children | 17 | NA | Non compartment |
| Mashayekhi et al. (2009)(291) | 2.5-16 yrs | 7 | 2 | One compartment |

†Studies with ≤ 2 patients or with number of patients not stated are excluded
+Studies with ≤2 samples or with the number of samples not stated are excluded

**Table 5. 3: Studies included after quality assessment**

| Study | Age group | Number of patients | Number of blood samples collected | Method |
|---|---|---|---|---|
| Hartley et al. (1993) (296) | Preterm neonates | 9 | 6*$^{x}$ | Non compartment |
| Mikkelsen et al. (1994) (297) | | 8 | 5-7 | Non compartment |
| Barrett et al. (1991) (298) | | 24 | 12-15*$^{x}$ | Non compartment |
| Chay et al. (1992) (299) | | 10 | 4-19* | Non compartment |
| Saarenmaa et al. (2000)(300) | | 31 | 5‡$^{x}$ | Non compartment |
| Barrett et al. (1996) (301) | | 18 | 4-9*$^{x}$ | Non compartment |
| Pokela et al. (1993) (302) | | 4 | 14 $^{x}$ | Non compartment |
| Bhat et al. (1990)(303) | | 10<br>7 | 8 $^{x}$ | Non compartment |
| Scott et al. (1999) (304) | | 9<br>13<br>13<br>3 | 6‡$^{x}$ | Non compartment |
| Geiduschek et al. (1997) (305) | Term neonates | 11 | 4‡$^{x}$ | Non compartment |
| Bouwmeester et al. (2003) (306) | | 52 | 4 $^{x}$ | Non compartment |
| Lynn et al. (1987) (307) | | 6 | 11*$^{x}$ | Non compartment |
| McRorie et al. (1992) (308) | | 14 | 10*$^{x}$ | Non compartment |
| Mikkelsen et al. (1994) (297) | | 5 | 5-7*$^{x}$ | Non compartment |
| Chay et al. (1992) (299) | | 5 | 4-19* | Non compartment |
| Koren et al. (1985) (309) | | 12 | 3-5* | Non compartment |
| Bhat et al. (1990)(303) | | 3 | 8 | Non compartment |
| Pokela et al. (1993) (302) | | 16 | 14 $^{x}$ | Non compartment |
| Roka et al. (2008) (310) | | 16 | 5‡$^{x}$ | Non compartment |
| Olkkola et al. (1988)(311) | Infants | 5 | 11* | Non compartment |
| Lynn et al. (1987) (307) | | 3 | 11 | Non compartment |
| Bouwmeester et al.(2003)(306) | | 97 | 4 $^{x}$ | Non compartment |
| Pokela et al. (1993) (302) | | 5 | 14 $^{x}$ | Non compartment |
| Olkkola et al. (1988) (311) | 2-11 yrs | 9 | 11* | Non compartment |
| Nahata et al. (1985) (312) | | 3 | 3 | Non compartment |
| Nahata et al. (1985) (312) | 12-18 yrs | 3 | 3 | Non compartment |
| McRorie et al. (1992) (308) | 31dys-2.5yrs | 35 | 10 | Non compartment |
| Collins et al. (1996)(313) | 1-15yrs | 10 | 3 | Non compartment |
| Dagan et al. (1993)(314) | 7mos-7yrs | 8<br>8 | 5 | Non compartment |
| Dampier et al. (1995)(315) | 6-19 yrs | 18 | 13* | Two compartment |

*plasma, ‡serum, x, at steady state

### 5.5.2. Clearance of morphine in neonates (0 - 27 days)

Fourteen studies reported morphine clearance in 247 neonates (Table 5.2) (292, 296-305, 307-309). Only one study, involving 52 neonates, reported M6G/M ratio but did not report clearance (306). All were single centre studies. The number of participants varied between 3 and 52. Age and weight were the most common covariates (Table 5.4 and 5.5). The number of collected blood samples was between 3 and 19. Morphine loading dose varied between 50 and 200 μg/kg and maintenance dose between 5 and 100 μg/kg/h (Table 5.6). All studies were non-compartment studies. None reported the effect of drug-drug interaction.

All studies, except two, involved critically ill neonates who were receiving treatment in a NICU. The mean clearance for all preterm neonatal studies ranged between 2 and 10 ml/min/kg and the mean clearance for term neonates varied between 2 and 12 ml/min/kg.

The CV ranged between 16% and 97% in studies involving preterm neonates. The lowest CV of 16% was recorded in a small study involving 9 preterm neonates receiving morphine to encourage synchronisation of their respiratory effort with the ventilator, as well as for analgesia (296). The highest CV of 97% was documented in a study involving 10 postoperative preterm neonates during the first week of life (303).

The range of CV was similar in term neonates, (24%-75%) to preterm neonates (16%-97%). The variation ratio of clearance was also similar in term neonatal studies (2-20 fold) and preterm neonatal studies (2-14 fold).

**Table 5. 4: Characteristics of studies in preterm neonates**

| Study | Number of patients | Gestational age (weeks) | Postnatal age (days) | Range of weight (kg) | Type of patients | Covariates | Comments |
|---|---|---|---|---|---|---|---|
| Hartley et al. (1993) (296) | 9 | 26-34 (29.6)* | <1 | 0.9-2 (1)* | RDS | Age | Duration of illness NA. |
| Mikkelsen et al. (1994) (297) | 8 | 25-32 (25)* | NA | 2-4 (1)* | RDS | Age | Duration of illness NA. |
| Barrett et al. (1991) (298) | 24 | 26-36 (30)* | 1-37 | 0.7-4 (1.4)* | The majority of patients with RDS | Age | 20 Patients with RDS, 2 pneumothorax, 1 apnea, 1 perforated caecum. Duration of illness NA. |
| Chay et al.(1992) (299) | 10 | 28-36 (33)* | >2 | 1-3.5 (2)* | RDS | Age and weight | Duration of illness NA. |
| Saarenmaa et al. (2000) (300) | 31 | 30† | NA | 1† | Ventilator-treated | Age and weight | 27 Patients with RDS and other patients with infection, hypertension and intraventricular hemorrhage. Duration of illness NA. |
| Barrett et al. (1996) (301) | 18 | 24-35 (29)* | <1 | 0.8-3 (1.5)* | Ventilated | Age and dose | Duration of illness NA. |
| Pokela et al. (1993) (302) | 4 | 28-36 (33)* | 1-3 | 2-3 (2)* | Surgery | Age | Duration of illness NA. |
| Bhat et al. (1990) (303) | 10<br>7 | 28*<br>33* | NA | 1†<br>2† | Ventilated | Age | Duration of illness NA. |
| Scott et al. (1999) (304) | 9<br>13<br>13<br>3 | 24-27(27)*<br>28-31(30)*<br>32-35 (32.5)*<br>36-39 (35)* | 1.1*<br>1.3*<br>6.1*<br>16* | 1*<br>1*<br>2*<br>3* | Mechanical ventilation | Age | Duration of illness NA. |

*Mean, †Median, RDS, Respiratory distress syndrome, NA ,not available

**Table 5. 5: Characteristics of studies in term neonates**

| Study | Number of patients | Gestational age (weeks) | Postnatal age (days) | Range of weight (kg) | Type of patients | Covariates | Comments |
|---|---|---|---|---|---|---|---|
| Geiduschek et al. (1997) (305) | 11 | 36-41 (40)* | NA | 2-4 (3)* | ECMO | ECMO | 5 patients with MAS, 2sepsis, 3 CDH and 1 PPHN. Duration of ECMO from 4-19 days. |
| Bouwmeester et al. (2003) (306) | 52 | 38* | 0-28 | 2.9 | Mechanical ventilation | Age, sex and weight | Patients with hepatic or renal dysfunction were excluded. Duration of illness NA. |
| Lynn et al. (1987) (307) | 6 | NA | 2-4 (2)† | 3-4 (3)* | Ventilated | Age | 3 patients with RDS, 2 MAS and 1 resection lung malformation. All patients were normal cardiovascular and renal function. Duration of illness NA. |
| McRorie et al. (1992) (308) | 14 | >36 | 1-19 | 2-5 | Cardiac surgery | Age | All patients undergo cardiac surgery and all were with normal hepatic and renal function. Duration of illness NA. |
| Mikkelsen et al. (1994) (297) | 5 | 37-40 (39)* | NA | 2-4 (3)* | Mechanical ventilation | Age | 2 patients with congenital heart disease,1 neonate of diabetic mother,1 persistent foetal circulation and 1 asphyxia |
| Chay et al. (1992) (299) | 5 | 37-41 (39)* | <4 | 3-4 (4)* | Mechanical ventilation | Weight | 3 patients with RDS, 2paients MAS Duration of illness NA. |
| Koren et al.(1985) (309) | 12 | 35-41 (39)* | 1-49 | 2-4 (3)* | Postoperative | NA | 3 patients with total anomalous pulmonary drainage,3 duodenal atresia and down syndrome, 2 complex cyanotic heart disease 1 transposition of great arteries,1 pulmonary arteries, 1 tracheoesophageal fistula and 1 omphalocoele. Duration of illness NA. |
| Bhat et al.(1990)(303) | 3 | 40* | NA | 3* | Ventilated | Age | Duration of illness NA. |
| Pokela et al.(1993) (302) | 6 | 38-42 (40)* | 3-35 | 3-5 (4)* | Surgery | Age | Duration of illness NA. |
| Roka et al. (2008)(310) | 16 | 36-40 (38)† | ≤3 | 2-4 (3)† | Asphyxia and hypothermia | Disease | Duration of illness NA. |

*Mean, †median, ECMO, extracorporeal membrane oxygenation, MAS, meconium aspiration syndrome, CDH, congenital diaphragmatic hernia, PPHN, persistent pulmonary hypertension, ICU, intensive care unit, NA, not available

**Table 5. 6: Morphine doses in neonates**

| Loading dose or Single dose (SD) µg/kg | Morphine infusion µg/kg/h | Number of blood samples collected | Study |
|---|---|---|---|
| 100 | 12.5 | 6 | Hartley et al. (1993) (296) |
| 200 | 50 | 6 | |
| 150 | NA | 5-7 | Mikkelsen et al. (1994) (297) |
| 50 | 15 | 12-15 | Barrett et al .(1991) (298) |
| 10-100 | 7.5-30 | 4-19 | Chay et al. (1992 (299) |
| 140 | 20 | 5 | Saarenmaa et al. (2000)(300) |
| 50 or 200 | 15 | 4-9 | Barrett et al. (1996) (301) |
| 100 | 10-20 | 4 | Geiduschek et al. (1997)(305) |
| 100 | NA | 8 | Bhat et al. (1990 (303) |
| 50 | 20-30 | 6 | Scott et al. (1999) (304) |
| 100 | 10-30 | 4 | Bouwmeester et al. (2003) (306) |
| NA | 20-100 | 11 | Lynn et al. (1987) (307) |
| NA | 20 | 10 | McRorie et al. (1992) (308) |
| 150 | NA | 5-7 | Mikkelsen et al. (1994) (297) |
| 10-100 | 7.5-30 | 4-19 | Chay et al. (1992) (299) |
| 100 | NA | 14 | Pokela et al. (1993 )(302) |
| 50-100 | 6-40 | 3-5 | Koren et al. (1985)(309) |
| 100 | NA | 8 | Bhat et al. (1990) (303) |
| 50 | 20-30 | 6 | Scott et al. (1999) (304) |
| 50-150 | 5-30 | 5 | Roka et al. (2008) (310) |

NA, not given

**Table 5. 7: Morphine clearance in critically ill neonates**

| Age group | Number of patients | Mean Clearance (ml/min/kg) | SD | Coefficient of variation (CV %) | Range of clearance (ml/min/kg) | Variation ratio in clearance | Comments | Study |
|---|---|---|---|---|---|---|---|---|
| Preterm neonates | 9 | 2 | 0.4 | 16 | 2-3 | 2 | Individual data available | Hartley et al. (296) |
| | 8 | 5 | 1.8 | 47 | 2-6 | 3 | Individual data available | Mikkelsen et al. (297) |
| | 24 | 3 | 0.8 | 26 | 2-6 | 3 | Individual data available | Barrett et al. (298) |
| | 10 | 3 | 1.8 | 96 | 0.5-7 | 14 | Individual data available | Chay et al. (299) |
| | 31 | 2 | 1 | 50 | 0.8-6 | 8 | - | Saarenmaa et al. (300) |
| | 18 | 5 | 3 | 71 | 1-14 | 14 | Individual data available | Barrett et al. (301) |
| | 4 | 2 | 0.7 | 35 | 1-2 | 2 | Individual data available | Pokela et al. (302) |
| | 10‡<br>7‡ | 3<br>10 | 3<br>4 | 97<br>42 | NA | NA | - | Bhat et al. (303) |
| | 9‡<br>13‡<br>13‡<br>3‡ | 2<br>3<br>5<br>8 | 1<br>2<br>2<br>3 | 47<br>66<br>40<br>37 | NA<br>NA<br>NA<br>NA | NA<br>NA<br>NA<br>NA | - | Scott et al. (304) |
| Term neonates | 11 | 12 | 9 | 75 | 3-35 | 12 | - | Geiduschek et al. (305) |
| | 6 | 6 | 2 | 35 | 4-10 | 3 | Individual data available | Lynn et al. (307) |
| | 14 | 7 | NA | NA | 3-14 | 5 | - | McRorie et al. (308) |
| | 5 | 5 | 1.8 | 55 | 2-7 | 4 | Individual data available | Mikkelsen et al. (297) |
| | 5 | 2 | 1.4 | 74 | 0.8-4 | 5 | Individual data available | Chay et al. (299) |
| | 6 | 8 | 2.5 | 30 | 4-13 | 3 | Individual data available | Pokela et al. (302) |
| | 12 | 8 | 2 | 24 | 2-39 | 20 | - | Koren et al. (309) |
| | 3 | 16 | 10 | 65 | NA | NA | - | Bhat et al. (303) |
| | 10 | 0.7* | NA | NA | 0.6-1 | 2 | - | Roka et al. (310) |

‡, Two or four groups of preterm neonates, *median, SD, standard deviation, CV, Coefficient of variation, all studies were non-population pharmacokinetics, NA, not available

### 5.5.3. Clearance of morphine in infants (>28 days -23 months)

Three studies reported morphine clearance in 13 infants (302, 307, 311). One study, involving 110 infants, reported M6G/M ratio but did not report clearance (306). All were single centre studies. The number of participants varied between 3 and 97 (306). Age and weight were the most common covariates (Table 5.8). The number of collected blood samples was between 4 and 14. Morphine loading dose varied between 50 and 150μg/kg and maintenance dose between 5 and 100 μg/kg/h. All studies were non-compartment studies. None of the studies reported the effect of drug-drug interaction.

Two studies involved critically ill infants (307, 311) and one non-critically ill (302). The mean morphine clearance for these infants varied between 21 and 24 ml/min/kg (Tables 5.10 and 5.11).

The CV varied between 35% to 134%. The highest CV of 134% was documented for a study involving 5 patients who received morphine after thoracic and orthopedic surgery (311). Only one study in non-critically infants reported the CV (44%) (302). Variability of morphine clearance in critically ill infants was 3 to 11 fold (Table 5.10).

**Table 5. 8: Characteristics of studies in infants**

| Study | Number of patients | Loading dose or Single dose (SD) µg/kg | Morphine infusion µg/kg/h | Number of blood samples collected | Type of patients | Covariates | Comments |
|---|---|---|---|---|---|---|---|
| Olkkola et al. (1988)(311) | 5 | NA | 50 | 11 | Thoracic or orthopedic surgery | NA | Duration of illness NA. |
| Lynn et al. (1987) (307) | 3 | NA | 20-100 | 11 | Ventilated | Age | 2 patients with viral pneumonia and 1 VSD. Duration of illness NA. |
| Bouwmeester et al. (2003)(306) | 97 | 100 | 10-30 | 4 | Mechanical ventilation | Age, sex and weight | Patients with hepatic or renal dysfunction were excluded. |
| Pokela et al. (1993) (302) | 5 | 100 | NA | 14 | Surgery | Age | Duration of illness NA. |

VSD, ventricular septal defect, NA, not given

### 5.5.4. Clearance of morphine in children and adolescents

Two studies reported morphine clearance in critically ill children aged 2-12 (311, 312). These studies involved a total of 12 children. Both were non-compartmental studies (Table 5.9). The mean clearance was 32 and 52 ml/min/kg. The CV of clearance was 39% and 55% (Table 5.10).

Only one small study involving 3 adolescents with malignancy aged 12-18 was identified (312). The mean clearance was 19 ml/min/kg. In this study the CV was 74%.

### 5.5.5. Clearance of morphine in paediatric patients of mixed ages

Four studies reported morphine clearance in 79 critically ill paediatric patients across all age groups (291, 293, 313-315). All were single centre studies. The number of participants in each study varied between 10 to 35. The number of blood samples collected was between 3 to 13. All but one study were non-compartmental studies.

Two of these studies were in patients with cardiac surgery, one study was in patients with sickle cell disease and the last was in patients with malignancy. Mean clearance varied between 14 and 36 ml/min/kg. The highest CV of 39% was reported in a study of 18 patients between 6 and 19 years old (315). The lowest CV of 25% was reported in 8 patients who received morphine after cardiac surgery (314). The CV was not documented in two studies. Four

studies reported the range of morphine clearance. The variation ratio was between 2-10 fold.

**Table 5. 9: Characteristics of studies in paediatric groups**

| Study | Number of patients | Loading dose or Single dose (SD) µg/kg | Morphine infusion µg/kg/h | Number of blood samples collected | Type of patients | Covariates | Comments |
|---|---|---|---|---|---|---|---|
| Nahata et al. (1985) (312) | 3* | 90-150 | NA | 3 | Hematologic-oncologic disorder | Age and disease | Liver and renal function was normal. |
| Olkkola et al. (1988) (311) | 9* | NA | 50 | 11 | Thoracic or orthopedic surgery | NA | Duration of illness NA. |
| Nahata et al. (1985) (312) | 3† | 90-150 | NA | 3 | Hematologic-oncologic disorder | Age and disease | Liver and renal function was normal. |
| McRorie et al. (1992) (308) | 35‡ | NA | 30 | 10 | Cardiac surgery | Age | All patients undergo cardiac surgery and all were with normal hepatic and renal function. Duration of illness NA. |
| Collins et al. (1996) (313) | 10‡ | 28-100 | 10 | 3 | Hematologic malignancy | NA | Duration of illness NA. |
| Dampier et al. (1995) (315) | 18‡ | NA | 35-195 | 13 | Sickle cell disease | NA | Duration of illness NA. |
| Dagan et al. (1993) (314) | 16‡ | NA | 20-40 | 5 | Cardiac surgery | NA | 8 patients with Fontan repair and 8 tetralogy of Fallot. Duration of illness NA. |

*Children, †adolescents, ‡mixed age, NA, not given

**Table 5. 10: Morphine clearance in critically ill paediatric patients**

| Age group | Number of patients | Range of age | Range of weight (kg) | Mean Clearance (ml/min/kg) | SD | Coefficient of variation (CV %) | Range of clearance (ml/min/kg) | Variation ratio in clearance | Comments | Study |
|---|---|---|---|---|---|---|---|---|---|---|
| Infants | 5 | 1-6mos (3)* | 4-8 (5)* | 22 | 17 | 134 | 4-45 | 11 | Individual data available | Olkkola et al. (311) |
| | 3 | 17-65dys (42)* | 3-6 (4)* | 24 | 14 | 35 | 13-39 | 3 | Individual data available | Lynn et al. (307) |
| 2-11yrs | 9 | 2-7 (5)* | 13-26 (19)* | 52 | 18 | 39 | 26-76 | 3 | Individual data available | Olkkola et al. (311) |
| | 3 | 11-12 (12)* | 21-40 (31)* | 32 | 18 | 55 | 21-53 | 3 | Individual data available | Nahata et al. (312) |
| 12-18yrs | 3 | 13-16 (15)* | 50-60 (56)* | 19 | 8 | 74 | 9-24 | 3 | Individual data available | Nahata et al. (312) |
| 31dys-2.5yrs | 35 | 31dys-2.5yrs | 3-14 | NA | NA | NA | 6-29 | 5 | - | McRorie et al.(308) |
| 1-15yrs | 10 | 14* | NA | 34 | NA | NA | 19-58 | 3 | - | Collins et al. (313) |
| 6-19yrs | 18 | 15* | NA | 36 | 14 | 39 | 6-59 | 10 | - | Dampier et al. (315) |
| 8mos-7yrs | 8†<br>8‡ | 8mos-7yrs | NA | 14<br>23 | 5<br>6 | 37<br>25 | 8-22<br>18-33 | 3<br>2 | Individual data available | Dagan et al. (314) |

*Mean, †Fontan repair;‡ Tetralogy of Fallot (TOF), SD, standard deviation, CV, Coefficient of variation, all studies were non-population pharmacokinetics ,NA; not available

**Table 5. 11: Morphine clearance in non-critically ill paediatric patients**

| Age group | Number of patients | Range of age | Range of weight (kg) | Mean Clearance (ml/min/kg) | SD | Coefficient of variation (CV %) | Range of clearance (ml/min/kg) | Variation ratio in clearance | Comments | Study |
|---|---|---|---|---|---|---|---|---|---|---|
| Term neonates | 10 | 38-42wks (36)* | 3-5 | 10 | 3.5 | 37 | 6-16 | 3 | Individual data available | Pokela et al. (302) |
| | 6 | 36-40wks (39)† | 2-4 | 1† | NA | NA | 0.6-1 | 2 | - | Roka et al. (310) |
| Infants | 5 | 2-6mos (1.5)* | 4-8 | 21 | 8 | 44 | 10-31 | 3 | Individual data available | Pokela et al. (302) |

*Mean, †median, SD, standard deviation, CV, Coefficient of variation, all studies were non-population pharmacokinetics, NA, not available

### 5.5.6. M6G/M ratio in paediatric patients

Three studies reported the ratio of M6G to morphine in 188 patients. (Table 5.12) (301, 306, 313) . One of these studies involved preterm neonates, with mean plasma M6G/M of 0.8 and the variation ratio was 29 fold. The variation ratio of M6G/M was between 2 and 29 fold (306).

There was only one infant study. This involved 110 infants and provided the plasma M6G/M range (0.9-2.3) from which a variation ratio of 3 fold was estimated. A further study involved 10 mixed aged children from 1 to 15 years and the variation ratio was 2 fold. The difference in the variation of M6G/M for the different age groups could not be established due to insufficient number of studies in each age categories.

**Table 5. 12: Range and variation ratio of critically ill patients in relation to M6G/M**

| Age group | Number of patients | Mean plasma concentration (ng ml$^{-1}$) | | Mean Plasma M6G/M | Range (M6G/M) | Variation Ratio (M6G/M) | Study |
|---|---|---|---|---|---|---|---|
| | | Morphine | M6G | | | | |
| Preterm neonates | 19 | 86 | 48 | 0.8 | 0.1-2.9 | 29 | Barrett et al.(301) |
| Term neonates | 52 | NA | NA | NA | 0.5-1.7 | 3 | Bouwmeester et al. (306) |
| Infants | 110 | NA | NA | NA | 0.9-2.3 | 3 | Bouwmeester et al. (306) |
| 1-15 yrs | 10 | NA | NA | 2.5 | 1.44-3.3 | 2 | Collins et al. (313) |

NA, not available

**Table 5. 13: Coefficient of variation for morphine clearance in paediatrics**

| Age | Critically ill % (n) | Non-critically ill % (n) |
|---|---|---|
| Preterm neonates | 16–97 (159) | - |
| Term neonates | 24–75 (72) | 37 (16) |
| Infants | 35–134 (8) | 44 (5) |
| Children | 39–55 (12) | - |
| Adolescents | 74 (3) | - |

(n) Number of patients

### 5.5.7. Effect of covariates on morphine clearance and variability

The mean clearance of morphine is 7ml/min/kg (95% CI: 5.97-7.92). Age is an independent predictor of morphine clearance. Neonates (p= 0.027), infants (p=<0.001), children (p=<0.001) and adolescents (p=0.017) have significantly higher clearance values than preterm neonates (Table 5.14). Similarly, weight is also a significant predictor of clearance (p=0.009). However, critical illness did not significantly affect the morphine clearance. There was no significant difference between the mean CV of morphine clearance in critically ill (52.4%) and non-critically ill patients (40.5%) (p=0.538). Subgroup analysis of the CV for the different age groups was not performed because of insufficient number of studies.

**Table 5. 14: Unadjusted meta-regression for covariates**

| Covariates | P value |
|---|---|
| **Age** | |
| Term neonates | 0.027 |
| Infants | <0.001 |
| Children | < 0.001 |
| Adolescent | 0.017 |
| **Critical illness** | 0.566 |
| **Weight** | 0.009 |
| **Sample size** | 0.338 |

## 5.6. Discussion

Morphine is one of the most commonly prescribed drugs for pain management in children. This systematic review has identified age and weight as predictors of morphine clearance in paediatric patients. A large inter-individual variation in the ratio of M6G to morphine in neonates and infants was also observed.

At birth, the renal clearance of drugs is generally underdeveloped and inefficient. Generally, this maturation (glomerular, filtration and renal tubular function) occur at different rates. However, by the first year of life, renal function matures to adult levels (316). Term neonates can have about three times preterm neonatal clearance rate (304).

Morphine is extensively metabolised by UGT2B7, to M3G and M6G, which is developed early in neonatal life. This isoform of UGT develops rapidly and reaches adult level by three months (57).

Generally, neonates and infants have relatively immature and inefficient glucuronidation compared with older children, adolescents and adults (57, 58). In addition, UGT expression is highly variable among paediatric patients (317) with consequent effect on morphine clearance (318). African American children have been reported to have higher morphine clearance than Caucasian children and this has been attributed to variability in UGT expression and increased M3G formation (287).

In this study, neonates and infants had a very high variation ratio of M6G/M clearance. In four neonatal studies, which reported M6G/M, variation ranged between 2-29 fold. M6G is an active metabolite of morphine. Studies have shown that factors such as age (304, 308) and disease (312), may affect the variability in the pharmacokinetics of morphine and its metabolites. A systematic review by Faura et al. (1998) showed that morphine glucuronide formation was much lower in neonates than children and adults. They also reported higher metabolite ratios in patients with renal impairment (319). The determination of relationship between M6G and morphine and the degree of variability in the plasma concentration is important in order to avoid morphine toxicity.

The CV of morphine clearance was not significantly different between critically ill and non-critically ill patients. All studies involving children and adolescents were in critically ill patients and only one study

involved neonates and infants, hence it was impossible to compare inter-individual variability between critically ill and non-critically ill patients in these age groups. Due to this limitation, the effect of critical illness on variability of morphine clearance could not be adequately explored. Furthermore, the effect of hepatic and renal impairment could not be determined, because most of the studies excluded patients with hepatic and renal impairment.

In conclusion, age is an important determinant of morphine clearance. It also determines the rate of metabolite formation which may influence morphine toxicity and tolerance.

# CHAPTER SIX
# A SYSTEMATIC LITERATURE REVIEW OF INVASIVENESS OF PHARMACOKINETIC STUDIES IN CHILDREN

## 6.1. Introduction

Pharmacokinetic studies require the collection of multiple blood samples, most commonly venous blood. Venous blood sampling is however invasive. In the previous chapters it was observed that the frequency of blood sampling varied considerably in paediatric PK studies. There should be a limit to the frequency and volume of blood sampling in children. Although, several institutions have proposed guidelines for the maximum acceptable volume of blood for PK studies (125), these guidelines are highly varied. Furthermore, there are no guidelines for the frequency of blood sampling in children. Invasive PK methods can cause pain in children and reduce their level of cooperation in clinical research (130). Pain should be avoided if possible and intensity and level of distress should be monitored.

Over the years, less invasive techniques have been developed for PK studies, such as the collection of micro samples (141, 320). Other methods, such as the use of scavenged clinical samples have also been encouraged. When possible, alternative non-invasive methods like the use of saliva, urine and breath samples have been utilised.

## 6.2. Aims

- To determine whether PK studies in paediatric patients are becoming less invasive

- To determine the number of samples and volume of blood collected in two decades of practice (1981-1990 and 2005-2014).

### 6.3. Objectives

- To determine the number of blood samples collected in PK studies over two decades and make statistical comparison.
- To determine and statistically compare the volume of blood samples per child and total volume of blood over two decades (1981-1990 and 2005-2014).

### 6.4. Methods

A systematic literature review was conducted electronically to identify papers describing the invasiveness of pharmacokinetic studies in children (defined as aged less than 18 years or paediatric age as defined by the authors). Paediatric patients were grouped according to the guidelines of the ICH (153) as follows:

- Preterm neonates (born at less than 37 weeks of gestation)
- Term neonates ( born at 37 weeks of gestation to 27 days old)
- Infants (28 days to under 2 years old)
- Children (2 to 11 years old)
- Adolescents (12 to under 18 years old)

Paediatric keywords were selected based on the recommendations of Kastner et al. (2006)(154). The final blood samples/PK study keywords were selected after optimisation to generate the highest

number of relevant studies, as a systematic review on invasiveness of PK has never been conducted before.

The following sources of information were utilised: MEDLINE (1946 to May 2014), EMBASE (1974 to May 2014), IPA (1970 to May 2014), CINAHL (1937 to May 2014) and the Cochrane Library. These databases were searched separately and combined together to remove duplications. The search strategy included all languages. The keywords "preterm neonate*" OR term neonate*" OR "neonate*" OR "new-born*" OR "child*" OR children OR "p*ediatric* OR "infant*" OR "adolescent*" (154) AND "pharmacokinetic*" OR blood OR plasma OR specimen OR serum OR blood sampling OR blood sample*" were used.

### 6.4.1. Inclusion Criteria

Inclusion criteria were original PK studies with documented number of blood samples in children up to the age of 18 years.

### 6.4.2. Exclusion Criteria

- Review articles
- Editorials
- Conference abstracts
- Studies in adults aged over 18 years
- Studies where data for blood sampling was not presented
- Observational studies
- Therapeutic drug monitoring studies

### 6.4.3. Data extraction

All relevant articles were read carefully and the required data were extracted onto tables. The data extracted include:

- Number of patients in PK studies
- Age group
- Mean weight
- Total number of blood samples taken
- Volume of blood per sample over 24 hours
- Total volume of blood for whole study
- Name of drug
- Year of study
- Country
- Sampling methods

### 6.4.4. Statistical analysis

The samples and volume for the two decades under consideration were not normally distributed for some age groups. Therefore, the number of samples and the volume for two decades were compared with the use of Mann–Whitney U test. A significance level of $P< 0.05$ was considered significant for all tests. Missing data were accounted for with complete case analysis. Data were summarised with the use of median and interquartile range.

## 6.5. Results

A total of 3,994 articles were identified from five search engines (Figure 6.1). Only 473 articles met the inclusion criteria 82 studies were between 1981-1990 and 152 studies between 2005-2014. Table 6.1 shows the details of papers excluded from the systematic review.

**Table 6. 1: Details of excluded articles from systematic review**

| Exclusion Reason | |
|---|---|
| Reviewed articles | 1,429 |
| Editorials | 170 |
| Case report | 147 |
| Conference abstracts | 225 |
| Studies in adults aged over 18 years | 1,352 |
| Studies where data for blood sample was not presented | 170 |
| Observational studies | 15 |
| Therapeutic drug monitoring studies | 13 |
| Total | 3,521 |

The most common drugs studied were antibiotics and analgesics. There were more studies involving children (114, 49%) than any other age group. Seventy two studies (31%) involved paediatric patients across the whole age spectrum. The number of studies in neonates and infants were lower in the later decade however, the number of patients were higher. In contrast, the number of studies in children and mixed ages were considerably higher in the later decade (Table 6.2).

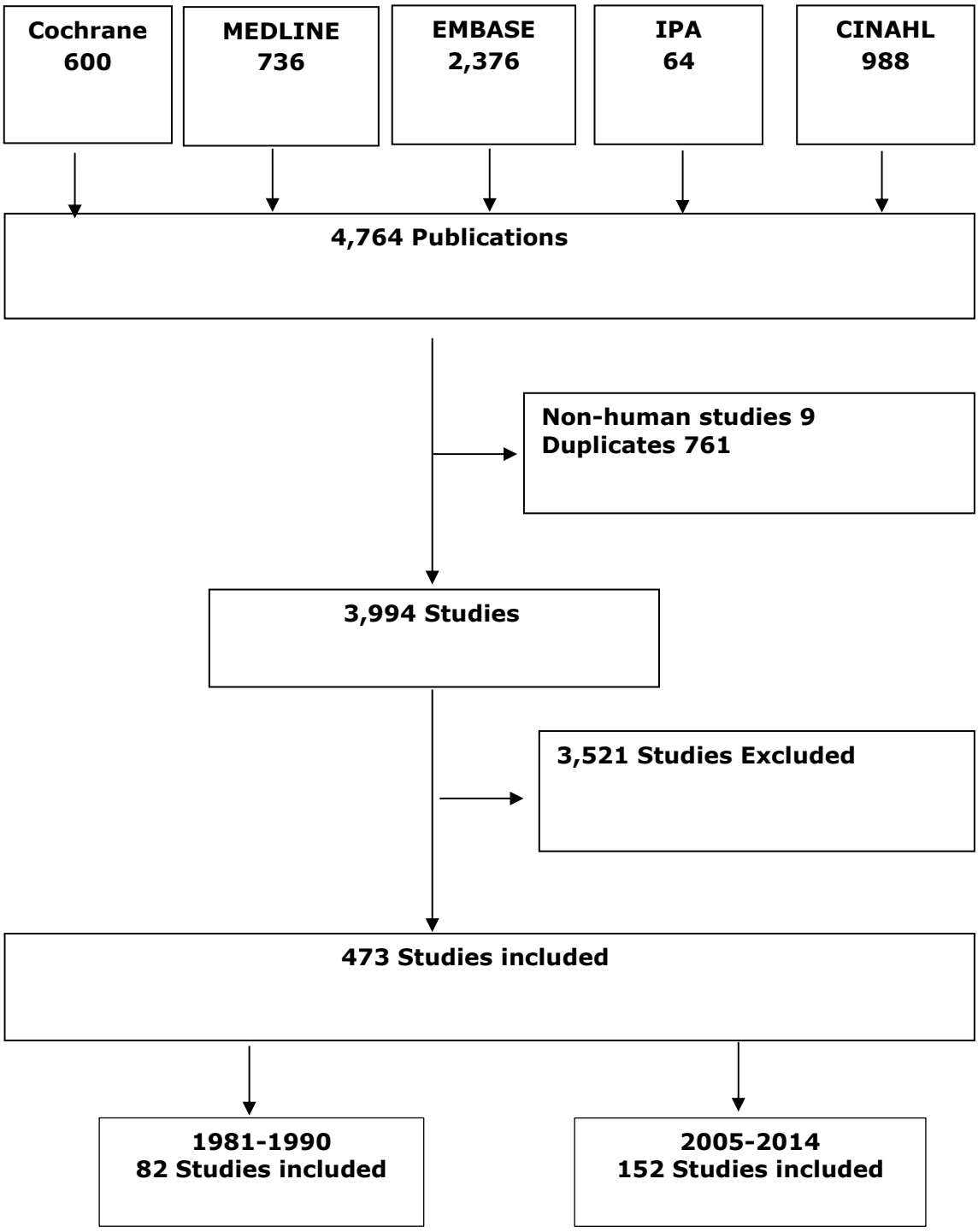


**Figure 6. 1: Search strategy of blood sampling**

**Table 6. 2: Pharmacokinetic studies in the two decades**

| Age group | Number of studies (number of cannula studies) | | Number of patients | |
|---|---|---|---|---|
| | 1981-1990 | 2005-2014 | 1981-1990 | 2005-2014 |
| Neonates | 13 (3) | 10 (1) | 193 | 300 |
| Infants | 9 (3) | 8 (1) | 184 | 365 |
| Children | 45 (18) | 69 (25) | 668 | 4,243 |
| Adolescents | 4 (2) | 4 (1) | 25 | 45 |
| Mixed age | 11 (5) | 61 (12) | 280 | 3,503 |
| Total | 82 (31) | 152 (40) | 1,350 | 8,456 |

In total 9,806 patients were enrolled in the PK studies. Eighty six percent of participants were in new decade. The lowest number of participants in a single study (1987) was 3 patients, in a study of hydrocortisone (321); while the largest study (2009) involved 690 children in a PK study of amodiaquine (322).

The route of blood collection was documented in 23% of the studies in the old decade and 35% of the new decade. For both periods, intravenous blood collection was the preferred method by investigators. Sixteen percent of studies in new decade and 24% in old decade used an indwelling catheter, while 22% in new decade and 17% in old decade used venous blood samples. Other sampling methods in both decades included arterial sampling (1%), heel prick

(1%) and finger prick (2%). In 42% of studies the method was not stated.

The majority of the studies (40%) were conducted in the United States of America (US). Other studies originated from France (8%), United Kingdom (UK) (7%), Japan (4%) and Canada (4%). Some studies were multinational and this accounted for (13%) of all studies.

Thirty five of the studies were population PK studies (Table 6.3). The number of blood samples varied between 1 and 15 samples; while the number of samples in non-population PK studies ranged between 2 to 20. The median number of samples collected in all population PK studies (median: 6, IQR [4-9]) was significantly lower than non-population PK studies (median: 8, IQR [6-10]) ($p=0.007$) (Figure 6.2).

**Table 6. 3: Blood samples in population pharmacokinetic studies**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study |
|---|---|---|---|---|---|---|
| 28 | 1* | 3 | 0.2 | 0.6 | Amphotericin B | Wurthwein et al. (2005) (323) |
| 24 | NA | 5 | 0.2 | 1 | Ganciclovir | Acosta et al. (2007) (324) |
| 20 | 3 | 5-9 | 0.5 | 7 | Midazolam | Ahsman et al. (2010) (182) |
| 58 | 10 | 5 | 2 | 10 | Desloratadine | Gupta et al. (2007)(325) |
| 104 | 20 | 3 | 2 | 6 | Tramadol | Garrido (2006)(326) |
| 79 | 25 | 1 | NA | NA | Methotrexate | Piard et al.(2007)(327) |
| 53 | 46 | 9 | NA | NA | Clofarabine | Bonate et al.(2011)(328) |
| 34 | 18 | 5 | 3 | 15 | Artemisinin | Salman et al.(2012)(329) |
| 53 | 22* | 2-7 | 3 | 6 - 21 | Naproxen | Valitalo et al.(2012)(330) |
| 70 | 11 | 4 | 1.5 | 6 | Artesunate | Hendriksen et al.(2013)(331) |
| 23 | 11 | 6 | 1.8 | 11 | Tranexamic acid | Goobie et al.(2013)(332) |
| 28 | 16 | 8 | 2.5 | 20 | Primaquine | Moore et al.(2014)(333) |
| 123 | NA | 11 | NA | NA | Azithromycin | Zhao et al.(2014)(334) |
| 105 | 28 | 4 | 0.1 | 0.4 | Abacavir | Jullien et al.(2005)(335) |
| 76 | NA | 8 | 0.3 - 0.4 | 2.4 – 3.2 | Sotalol | Laer et al.(2005)(336) |
| 24 | 16 | 10 | 0.5 | 5 | Midazolam | Peeters et al. (2006)(184) |
| 99 | 17 | 2 | NA | NA | Meropenem | Du et al.(2006)(337) |
| 73 | 52 | 4 | 0.5 | 2 | Amlodipine | Flynn et al.(2006(338) |
| 59 | 19 | 15 | 5 | 75 | Melphalan | Nath et al.(2007)(339) |
| 34 | 8 | 4 | 0.5 | 2 | Gentamicin | Seaton et al.(2007)(340) |
| 72 | 36 | 7 | NA | NA | Micafungin | Hope et al.(2007)(341) |
| 162 | 22 | 10 | 1 | 10 | Ciclosporin | Fanta et al.(2007) (342) |
| 128 | 37 | 3 | 2 | 6 | Abulterol | Maier et al.(2007)(343) |
| 24 | 23 | 2-8 | NA | NA | Busulfan | Booth et al.(2007)(344) |
| 33 | 30 | 8 | NA | NA | Actinomycin-D | Mondick et al.(2008) (345) |
| 50 | 30 | 7 | NA | NA | Tacrolimus | Zhao et al.(2009)(346) |
| 50 | NA | 7 | 2 | 14 | Lopinavir | Rakhmanina et al. (2009) (347) |
| 22 | 34 | 6 | 0.5 | 3 | Valganciclovir | zhao et al.(2009)(348) |
| 217 | 18 | 1 | NA | NA | Tebipenem | Sato et al.(2008) (349) |
| 20 | NA | 10 | 0.5 | 4.5 | Pantoprazole | Pettersen et al.(2009) (79) |

***Median, NA, not available

**Table 6.3 continued: Blood samples in population pharmacokinetic studies**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study |
|---|---|---|---|---|---|---|
| 189 | NA | 10 | 1 | 10 | Tamsulosin hydrochloride | Tsuda et al.(2010) (350) |
| 50 | 15 | 2 - 4 | NA | NA | Meropenem | Ohata et al.(2011) (351) |
| 94 | NA | 5 | NA | NA | Busulfan | Trame et al.(2011)(352) |
| 67 | 15* | 2-6 | NA | NA | Busulfan | Michel et al. (2012)(353) |
| 236 | 18 | 10 | NA | NA | Piperaquine | Tarning et al.(2012)(354) |

*Median, NA, not available

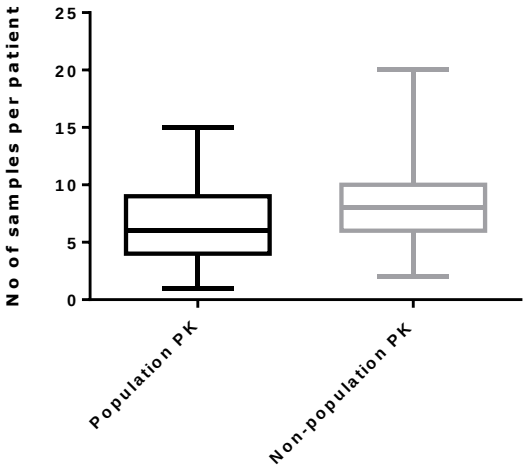


**Figure 6. 2: Number of blood samples in population PK and non-population PK studies (p=0.007)**

### 6.5.1. Blood samples in neonates

In the two time periods, 23 neonatal studies were identified. There were thirteen studies in old decade (1981-1990) involving 193 neonates; while 10 studies in the new decade (2005-2014) involving 300 neonates were identified (Tables 6.4 and 6.5). Seventy eight percent (18 studies) provided information on the volume of blood

collected from each subject. Similar volumes per sample were collected from studies in the old decade (median: 0.7ml, IQR: [0.2-1]) than the new (median: 0.3ml, IQR: [0.2-0.8ml])(p=0.283). The maximum volume of blood sampled at a single time in old studies was 2ml (355); while the minimum was 0.2ml . The range in the studies in the new decade was between 0.15ml-2ml.

Between 3-10 blood samples were collected in the old decade; while 2-9 samples were collected in the new (Figure 6.3). The median frequency of blood sampling in the new studies was slightly lower (median=4.5, IQR [3-7.2]) than the old studies (median=7, IQR [5-8]), but there was no statistically significant difference (P=0.054). During old studies, the highest volume of blood obtained from neonates over a 24 hour period was 16ml and this was a study conducted in the United States (US) in 1989 (355). In the new decade the highest volume collected within 24 hours was 7ml (182). The total volume of blood ranged from 1.4-16ml (old) and 0.6-7ml new.

**Table 6. 4: Blood samples in preterm neonates**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (1981-1990) |
|---|---|---|---|---|---|---|
| 28 | NA | 8 | 0.5 | 4 | Cefoperazone | Rosenfeld et al. (1983) (356) |
| 9 | NA | 8 | NA | NA | Theophylline | Stile et al.(1986) (240) |
| 9 | 1 | 6 | NA | NA | Theophylline | Dothey et al. (1989) (242) |
| **Number of patients** | **Mean weight (kg)** | **No. of sample** | **Vol of blood (ml)** | **Total vol of blood (ml)** | **Drug** | **Study (2005-2014)** |
| 28 | 1* | 3 | 0.2 | 0.6 | Amphotericin B | Wurthwein et al. (2005) (323) |
| 55 | 2 | 3 | NA | NA | Cefepime | Capparelliet al. (2005) (357) |
| 24 | NA | 5 | 0.2 | 1 | Ganciclovir | Acosta et al. (2007) (324) |
| 35 | NA | 7 | 0.3 | 2 | PK study | Heidmets et al. (2011) (358) |
| 42 | 3 | 8 | 0.15 | 1 | Dexmedetomdine | Chrysostomou et al. (2014) (359) |

***Median, NA, not available

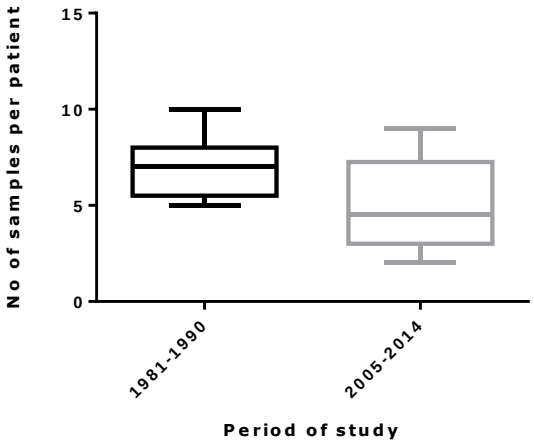


**Figure 6. 3 : Number of samples collected from each neonate during old and new periods (P=0.054)**

**Table 6. 5: Blood samples in term neonates**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (1981-1990) |
|---|---|---|---|---|---|---|
| 16 | 3 | 5 | 0.3 | 1.5 | phenytoin | Bourgeois et al. (1983) (360) |
| 12 | 2 | 8 | 0.2 | 1.6 | Cefotaxime | Lambert et al. (1984) (361) |
| 12 | 3 | 3-5 | 1 | 3-5 | Morphine | Koren et al. (1985)(309) |
| 10 | 2 | 7 | 0.8 | 5.6 | Cilastatin | Gruber et al. (1985) (362) |
| 41 | 2 | 7 | 0.2 | 1.4 | Ceftazidime | Mulhall &Louvois (1985) (363) |
| 4 | 3 | 5 | NA | NA | Teicoplanin | Tarral et al. (1988) (364) |
| 21 | NA | 10 | NA | NA | Immunoglobuline | Noya et al. (1989) (365) |
| 10 | 4 | 8 | 2 | 16 | Gentamicin | Southgate et al. (1989) (355) |
| 11 | 3 | 9 | 0.5-1 | 4.5-9 | Bupivacaine | Bricker et al. (1989)(366) |
| 10 | 3 | 6 | 0.7 | 4.2 | Midazolam | Jacqz-Aigrain et al.(1990) (176) |
| **Number of patients** | **Mean weight (kg)** | **No. of samples** | **Vol of blood (ml)** | **Total vol of blood (ml)** | **Drug** | **Study (2005-2014)** |
| 14 | 3.2 | 4 | 1.4 | 5.6 | Morphine | Peters et al. (2005) (367) |
| 20 | 3 | 5-9 | 0.5 | 7 | Midazolam | Ahsman et al. (2010) (182) |
| 15 | 1.8 | 7 | 0.2 | 0.6 | Anidulafungin | Wolkowiez et al. (2011) (368) |
| 36 | 3* | 4 | 0.6 | 2.4 | Cefazolin | Cock et al. (2014) (369) |
| 31 | 3 | 2 | 0.5- 1 | 1-2 | Amikacin | Vucicevic et al. (2014) (370) |

*Median (range), NA, not available

### 6.5.2. Blood samples in infants

There were 8 studies in the new decade and 9 in the old involving 365 and 184 infants respectively (Table 6.6). Only one old study provided information on the volume of blood, 0.5ml of blood was obtained six times in each of 12 infants (249). The volume of blood collected at a single time in the new decade varied from 0.5ml-2.5ml; with the frequency of blood sampling 4-10 times. The median frequency in both decades was similar for the new (median=7, IQR [4-8]) and old (median=7, IQR [5.5-7.5]) (p=0.999) (Figure 6.4).

**Table 6. 6: Blood samples in infants**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (1981-1990) |
|---|---|---|---|---|---|---|
| 27 | 11 | 7 | NA | NA | Amoxicillin | Ginsburg et al. (1981)(371) |
| 10 | 12 | 8 | NA | NA | Ceftriaxone | Schaad et al. (1982)(372) |
| 34 | NA | 7 | NA | NA | Potassium clavulanate | Nelson et al. (1982) (373) |
| 19 | 10 | 7 | NA | NA | Ceftriaxone | Rio et al. (1982) (374) |
| 12 | 6 | 6 | 0.5 | 3 | Theophylline | Franko et al. (1982) (249) |
| 29 | 12 | 5 | NA | NA | Aztreonam | Stutman et al. (1984) (375) |
| 20 | 15 | 7 | NA | NA | Sultamicllin | Ginsburg et al. (1985)(376) |
| 13 | 24 | 3-4 | NA | NA | Theophylline | Kubo et al. (1986)(261) |
| 20 | NA | 10 | NA | NA | Sufentanil | Davis et al. (1987)(377) |
| **Number of patients** | **Mean weight (kg)** | **No. of samples** | **Vol of blood (ml)** | **Total vol of blood (ml)** | **Drug** | **Study (2005-2014)** |
| 58 | 10 | 5 | 2 | 10 | Desloratadine | Gupta et al. (2007)(325) |
| 13 | 10 | 8 | NA | NA | Levetiracetam | Glauser et al. (2007)(378) |
| 24 | 16 | 10 | 0.5 | 5 | Midazolam | Peeters et al. (2006)(184) |
| 107 | 9 | 4 | 1.5 | 6 | Esmolol | Tabbutt et al. (2008)(379) |
| 13 | 12 | 8 | 2 | 16 | Clonidine | Almenrader et al.(2009)(380) |
| 5 | 11 | 8 | 2 | 16 | Carvedilol | Leucuta et al. (2010)(381) |
| 114 | 14 | 6 | 1.5 | 9 | Amodiaquine | Tekete et al. (2011)(382) |
| 31 | NA | 4 | 2.5 | 10 | Topotecan | Park et al. (2011)(383) |

NA, not available

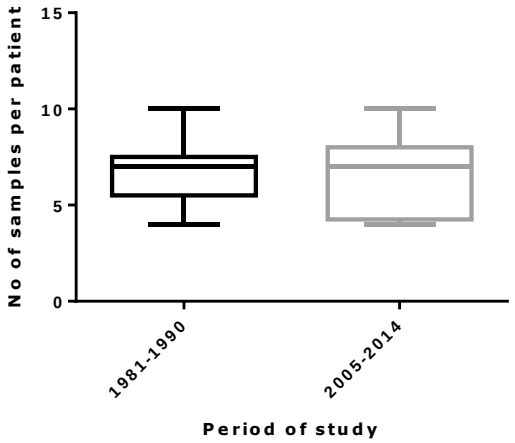


**Figure 6. 4: Number of samples collected from each infants during old and new periods (p=0.999)**

### 6.5.3. Blood samples in children

There were 45 studies in the old decade involving 668 children (Table 6-7). The total volume of blood collected during the course of a study ranged between 2.8-55ml and the number of samples between 4-20 times. The lowest frequency of blood collection, reported in several studies, was 4 times from each patient. However, the highest number of blood samples of 20, was collected in a 1985 French study of bupivacaine (384).

There were 69 studies in the new decade involving 4,243 children (Tables 6.7 and 6.8). The number of blood samples collected ranged between 1-13 times. The lowest volume of blood collected at a single time was 0.2ml, in a 2007 multicentre US study of zolpidem pharmacokinetics involving 67 children (385). The highest volume of blood was 7ml, in a PK study involving 18 children receiving

methylphenidate (386). The total volume across all study periods varied between 2-77ml.

Significantly more samples were collected during the old (median=9, IQR [7-12]) than new decade (median=8, IQR [5-9.5]) (P=0.006) (Figure 6-5). Similarly, there was significant (P=0.014) difference between the total volume of blood (median= 24ml, IQR [12-33ml]) vs. (median=15ml, IQR [8-18ml]). However, there was no significant difference between the volume of blood collected for a single sample (P=0.942), (median=2ml, IQR [1-3ml]) vs. (median=2ml, IQR [1.5-3ml]).

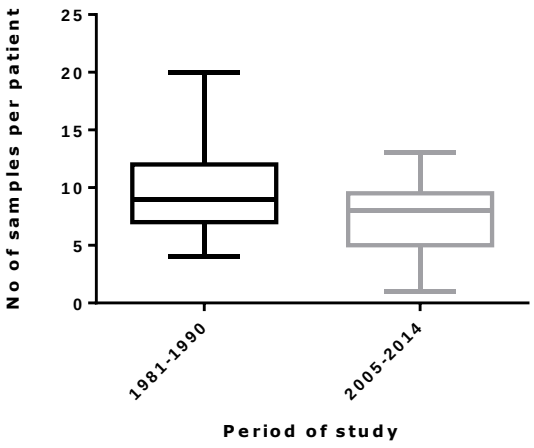


**Figure 6. 5: Number of samples collected during two periods in children (p=0.006)**

**Table 6. 7: Blood samples in children (1981-1990)**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (1981-1990) |
|---|---|---|---|---|---|---|
| 18 | 20 | 7 | NA | NA | Amikacin | Lanao et al. (1981)(387) |
| 7 | NA | 6 | NA | NA | Co-trimoxazole | Beachet al. (1981)(388) |
| 8 | 19 | 7 | NA | NA | Netilmicin | Michalsen & bergan (1981) (389) |
| 28 | NA | 7 | NA | NA | Cefadroxil | Windorfer et al.(1982)(390) |
| 13 | NA | 5 | NA | NA | Moxalactam | Romagnoli et al. (1982) (391) |
| 8 | NA | 6 | NA | NA | Theophylline | Eriksson et al. (1983)(250) |
| 25 | NA | 13 | NA | NA | Valproic acid | Otten et al. (1983)(392) |
| 15 | NA | 9 | NA | NA | Methotrexate | Balis et al. (1983) (393) |
| 12 | NA | 8 | NA | NA | Amoxicillin | Schaad et al. (1983)(394) |
| 31 | 29 | 6 | NA | NA | Mezlocillin | Kramer et al. (1984)(395) |
| 11 | 20 | 18 | 0.5 | 9 | Lignocaine | Ecoffey et al. (1984)(396) |
| 10 | 21 | 8 | NA | NA | Flunitrazepam | Iisalo et al. (1984)(397) |
| 9 | 32 | 6 | 5 | 30 | Prednisone | Gatti et al. (1984)(398) |
| 8 | NA | 8 | NA | NA | Etoposide | Sinkule et al. (1984)(399) |
| 6 | 23 | 20 | 1 | 20 | Bupivacaine | Ecoffey et al. (1985)(384) |
| 17 | NA | 13 | NA | NA | Tiazofurin | Bails et al. (1985)(400) |
| 16 | 19 | 12 | 2 | 24 | Midazolam | Maurice et al. (1986)(401) |
| 29 | NA | 11 | 5 | 55 | 6-mecaptopurine | Lennard et al. (1986)(402) |
| 11 | NA | 8 | NA | NA | Amoxcycillin | Schaad et al. (1986)(403) |
| 20 | 22 | 12 | 3 | 36 | Morphine | Attia et al. (1986)(404) |

NA, not available

**Table 6.7 continued: Blood samples in children (1981-1990)**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (1981-1990) |
|---|---|---|---|---|---|---|
| 9 | 19 | 12 | 2 | 24 | Midazolam | Saint et al.(1986)(405) |
| 20 | NA | 12 | 2 | 24 | Alfentanil | Roure et al.(1987)(406) |
| 10 | NA | 8 | NA | NA | Rimantadine | Anderson et al.(1987)(407) |
| 5 | 32 | 17 | 2 | 34 | Bupivacaine | Murat et al.(1987)(408) |
| 8 | 21 | 16 | 2 | 32 | Alfentanil | Meistelman et al.(1987)(409) |
| 5 | NA | 8 | 2 | 16 | Theophylline | Principi et al.(1987)(260) |
| 21 | 22 | 12 | NA | NA | Midazolam | Salonene et al.(1987)(193) |
| 7 | NA | 11 | NA | NA | Theophylline | Hill et al.(1988)(252) |
| 17 | 16 | 12 | 1 | 12 | Midazolam | Mathews et al.(1988)(185) |
| 9 | 38 | 10 | NA | NA | Lithium | Vitiello et al.(1988)(410) |
| 13 | 21 | 6 | 3 | 18 | Teicoplanin | Terragna et al.(1988)(411) |
| 60 | NA | 4 | 1 - 2 | 4 - 8 | Methohexitone | Forbes et al.(1989)(412) |
| 7 | NA | 8 | NA | NA | Theophylline | Scott et al.(1989)(253) |
| 6 | NA | 11 | NA | NA | Prednisolone | Choonara et al.(1989)(413) |
| 10 | 17 | 11 | 3 | 33 | Dantrolene | Lerman et al.(1989)(414) |
| 32 | 17 | 9 | 3 | 27 | Midazolam | Payne et al.(1989)(192) |
| 6 | 15 | 7 | 0.4 | 2.8 | Midazolam | Kraus et al.(1989)(191) |
| 16 | NA | 9 | 3 | 24 | Fazarabine | Heideman et al.(1989)(415) |
| 12 | 22 | 8 | 0.5 | 4 | Amphotericine B | Benson et al.(1989)(416) |
| 15 | NA | 12 | NA | NA | Amoxicillin | Jones et al.(1990)(417) |
| 20 | 34 | 4 | 1 | 4 | Esmolol | Wiest et al.(1991)(418) |
| 12 | 26 | 18 | NA | NA | Propofol | Jones et al.(1990)(419) |
| 19 | 23 | 4 | 3 | 12 | Deferoxamine | Bentur et al.(1990)(420) |
| 6 | 15 | 9 | 0.5 | 4.5 | Vigabatrin | Rey et al.(1990)(421) |
| 21 | NA | 9 | 4 | 36 | Idarubicin | Reid et al.(1990)(422) |

NA, not available

**Table 6. 8: Blood samples in children (2005-2014)**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (2005-2014) |
|---|---|---|---|---|---|---|
| 62 | 57 | 8 | 1.5 | 12 | Paroxetine | Findling et al.(2005)(423) |
| 20 | 24 | 9 | 5 | 45 | Levobupivacain | Kokko et al.(2005)(424) |
| 15 | NA | 6 | NA | NA | Levocetirizine | Cranswick et al 2005.(425) |
| 25 | NA | 9 | 3 | 27 | Montelukast | Ramakrishan et al. (2005)(426) |
| 14 | 30 | 12 | 2.5 | 30 | Levocetirizine | Simons et al.(2005)(427) |
| 20 | 27 | 12 | NA | NA | Amprenavir | Yogev et al.(2005)(428) |
| 41 | 34 | 3 | 2 | 6 | Levofloxacin | Chien et al.(2005)(429) |
| 35 | NA | 5 | NA | NA | Tinzaparin | kuhle et al.(2005)(430) |
| 31 | NA | 8 | 2 | 16 | Dactinomycin | Veal et al.(2005)(431) |
| 19 | NA | 10 | NA | NA | Ifosfamide | Willits et al.(2005)(432) |
| 6 | NA | 6 | 3 | 18 | Topotecan | Freeman et al.(2006)(433) |
| 11 | 36 | 8 | NA | NA | Hydroxurea | Montalembert et al.(2006) (434) |
| 24 | 20 | 7 | 1 | 7 | Rocuronium | Gebicka et al.(2006)(435) |
| 104 | 20 | 3 | 2 | 6 | Tramadol | Garrido (2006)(326) |
| 17 | NA | 12 | NA | NA | Quetiapine | Findling et al.(2006)(436) |
| 24 | 43 | 9 | 2 | 18 | Ziprasidone | Sallee et al.(2006)(437) |
| 48 | 38 | 2 - 3 | 3-5 | 6-15 | Valproate | Herranz et al.(2006) (438) |
| 19 | NA | 13 | 5 | 65 | Temozolomide | Baruchel et al.(2006)(439) |
| 128 | 37 | 3 | 2 | 6 | Abulterol | Maier et al.(2007)(343) |
| 15 | 16 | 10 | NA | NA | Meglumine | Cruz et al.(2007)(440) |
| 5 | NA | 12 | 1 | 12 | Treosulfan | Glowka et al.(2007)(441) |
| 79 | 25 | 1 | NA | NA | Methotrexate | Piard et al.(2007)(327) |

NA, not available

**Table 6. 8 Continued: Blood samples in children (2005-2014)**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (2005-2014) |
|---|---|---|---|---|---|---|
| 43 | 25 | 9 | 2 | 18 | Paracetamol | kulkarni et al.(2007)(442) |
| 9 | 43 | 5 | NA | NA | Risperidone | Aman et al.(2007)(443) |
| 18 | 28 | 11 | 7 | 77 | Methylphenidate | Quinn et al.(2007)(386) |
| 71 | 21 | 7 | 4 | 28 | Mefloquine | Ramharter et al.(2007)(444) |
| 49 | 33 | 9 | 2 | 18 | Sodium ferric gluconate | Warady et al.(2007)(445) |
| 21 | NA | 7 | NA | NA | Levtiracetam | Fountain et al.(2007)(446) |
| 67 | 35 | 10 | 0.2 | 2 | Zolpidem | Blumer et al.(2007)(385) |
| 26 | 11 | 12 | 0.75 | 9 | Lorazepam | Muchohi et al.(2008)(447) |
| 77 | 17 | 2 | NA | NA | Fenoldopam mesylate | Hammer et al.(2008)(448) |
| 30 | 32 | 7 | 2.5 | 17.5 | Eslicarbazepine Acetate | Almeida et al.(2008)(449) |
| 30 | 41 | 10 | 1.5 | 15 | Gadoversetamide | Wible et al.(2009)(450) |
| 22 | 46 | 11 | 0.5 | 5.5 | Doxorubicin | Thompson et al.(2009)(451) |
| 44 | 12 | 5 | 1 | 5 | Rifampin | Schaaf et al.(2009)(452) |
| 37 | NA | 13 | NA | NA | Growth hormone | Peter et al.(2009)(453) |
| 5 | 13 | 5 | 3 | 15 | Midazolam | Roberts et al.(2009)(187) |
| 690 | NA | 5 | 3 | 15 | Amodiaquine | Mwesigwa et al.(2010)(322) |
| 52 | 16 | 8 | NA | NA | Recombinant factor VIII | Bjorkman et al.(2010)(454) |
| 16 | NA | 11 | 3 | 33 | Cediranib | Fox et al.(2010)(455) |
| 39 | 43 | 9 | NA | NA | Lithium | Findling et al.(2010)(456) |
| 15 | 15 | 9 | 2 | 18 | Micafungin | Metha et al.(2010)(457) |
| 41 | 20 | 7 | NA | NA | Efavirenz | Fillekes et al.(2011)(458) |
| 899 | 14 | 5 | 1.5 | 7.5 | Lumefantrine | Djimde et al.(2011)(459) |

NA, not available

**Table 6. 8 Continued: Blood samples in children (2005-2014)**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (2005-2014) |
|---|---|---|---|---|---|---|
| 40 | NA | 7 | 1 – 2 | 7 - 14 | Voriconazole | Driscollet al.(2011)(460) |
| 20 | NA | 5 | NA | NA | Isoniazide | Thee et al.(2011)(461) |
| 47 | 48 | 8 | NA | NA | Tenofovir | king et al.(2011)(462) |
| 87 | NA | 7 | 2 - 3 | 14 - 21 | Hydroxyurea | Ware et al.(2011)(463) |
| 19 | 13 | 7 | 1.5 | 10.5 | Lopinavir | Kasirye et al.( 2011)(464) |
| 53 | 46 | 9 | NA | NA | Clofarabine | Bonate et al.(2011)(328) |
| 53 | 22* | 2-7 | 3 | 6 - 21 | Naproxen | Valitalo et al.(2012)(330) |
| 31 | 49 | 12 | 1 | 12 | Rizatriptan | Fraser et al.(2012)(465) |
| 34 | 18 | 5 | 3 | 15 | Artemisinin | Salman et al.(2012)(329) |
| 46 | 18 | 7 | 2.5 | 17.5 | Napthoquine | Batty et al.(2012)(466) |
| 25 | 34 | 8 | 1 | 8 | Roxatidine | Nakamura et al.(2012)(467) |
| 21 | 54 | 4 | 2 | 8 | Fesoterodine | Malhotra et al.(2012)(468) |
| 58 | 36 | 5 | 3 | 15 | Tigecycline | Purdy et al.(2012)(469) |
| 236 | 18 | 10 | NA | NA | Piperaquine | Tarning et al.(2012)(354) |
| 15 | NA | 9 | 1 | 9 | Sulphadoxine | Ademisoye et al.(2012)(470) |
| 27 | NA | 7 | 1.5 | 10.5 | Beclomethasine | Chawes et al.(2013)(471) |
| 40 | 35 | 8 | 0.5 - 1 | 4 - 8 | Sirolimus | Goyal et al.(2013)(472) |
| 70 | 11 | 4 | 1.5 | 6 | Artesunate | Hendriksen et al.(2013)(331) |
| 23 | 11 | 6 | 1.8 | 11 | Tranexamic acid | Goobie et al.(2013)(332) |
| 23 | 38 | 9 | 5 | 45 | Aerosol particle | Bonnelykke et al.(2013) (473) |
| 23 | 50 | 3 | NA | NA | Lyophilisate | Bruyne et al.(2014)(474) |
| 28 | 16 | 8 | 2.5 | 20 | Primaquine | Moore et al.(2014)(333) |
| 22 | NA | 6 | NA | NA | Ofloxacin | Thee et al.(2014)(475) |
| 123 | NA | 11 | NA | NA | Azithromycin | Zhao et al.(2014)(334) |
| 16 | 36 | 4 | 1.2 | 4.8 | Liposomal cytarabine | Peyrl et al.(2014)(476) |

*Median, NA, not available

### 6.5.4. Blood samples in adolescents

There were 8 studies involving 70 adolescents (Table 6.9). Four of these studies were from the new and 4 from old decade. There was no difference in the median number of blood samples collected (median: 9, IQR [5-11] vs. old (median: 7.5, IQR [5-8.7] (p=0.571)(Figure 6.6). The lowest frequency of blood sampling in the new decade was four, reported in a study conducted in Greece (477), and the largest was 11 times (478). Most studies did not provide details of volume of blood collected.

**Table 6. 9: Blood samples in adolescents**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (1981-1990) |
|---|---|---|---|---|---|---|
| 9 | NA | 5 | 5 - 7 | 25 - 35 | Metoclopramide | Bateman et al.(1983) (479) |
| 3 | Na | 8 | Na | Na | Hydrocortisone | Iwasski (1987)(321) |
| 5 | 48 | 7 | NA | NA | Aminosalicylic acid | Tolia et al.(1989)(480) |
| 8 | 34 | 9 | NA | NA | Theophylline | Agbaba et al.(1990)(481) |
| **Number of patients** | **Mean weight (kg)** | **No. of samples** | **Vol of blood (ml)** | **Total vol of blood (ml)** | **Drug** | **Study (2005-2014)** |
| 24 | 42 | 10 | NA | NA | Agalsidasealfa | Ries et al. (2007)(482) |
| 10 | 41 | 4 | NA | NA | Voriconazole | Markantonis et al. (2011) (477) |
| 5 | 53 | 8 | NA | NA | Ketorolac | Mohammed et al.(2013) (483) |
| 6 | NA | 11 | 1 | 11 | Cisatracurium | Gao et al.(2014)(478) |

NA, not available

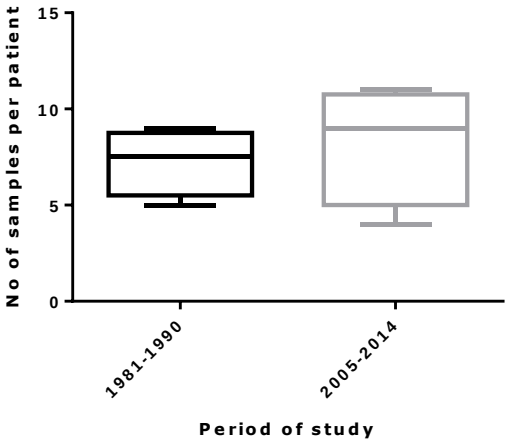


**Figure 6. 6: Number of samples collected during two periods in adolescents (P=0.571)**

### 6.5.5 Blood samples in mixed age groups

Some studies involved patients across the whole paediatric age spectrum. There were a total of 72 studies, 61 of these were from new and 11 from old decade, involving 3,503 and 280 patients respectively (Tables 6.10 and 6.11). The median number of blood samples was similar, 7 in the new decade, IQR [6-9], compared to 8, IQR [6-11] (p=0.384) (Figure 6.7). The range of samples collected in the new decade was 1-15 samples compared to 2 -18 samples.

At each time point a median volume of 1ml, IQR [0.5-2ml] compared to 2ml in the older decade, IQR [1-3ml], was collected, there was no significant difference (p=0.234). During the course of study, there was a trend towards collection of a larger volume of blood in the old decade (median=16.5ml IQR [11-25ml]) than the new (median=9.5ml, IQR [3-15ml]) (p=0.062).

**Table 6. 10: Blood samples in mixed age groups (1981-1990)**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (1981-1990) |
|---|---|---|---|---|---|---|
| 46 | NA | 9 | 2 | 18 | Ceforanide | Dajani et al.(1982) (484) |
| 15 | 25 | 6 | NA | NA | Piperacillin | Wilson et al.(1982) (485) |
| 30 | NA | 8 | NA | NA | Ceftriaxone | Steele et al.(1983) (486) |
| 16 | 23 | 18 | 0.5- 1.5 | 9 - 27 | Thiopental | Sorbo et al.(1984) (487) |
| 13 | 12 | 11 | 0.7 | 8 | Cefotaxime | Trang et al.(1985)(488) |
| 50 | 20 | 5 | 3 | 15 | Methohexitone | Quaynor et al. (1985) (489) |
| 27 | 19 | 8 | 1.5 | 12 | Bupivacaine | Rothstein et al.(1986) (490) |
| 10 | 14 | 2 | NA | NA | Amphotericin B | Starke et al.(1987) (491) |
| 40 | NA | 13 | NA | NA | Trimetrexate | Bails et al.(1987)(492) |
| 18 | 12.3 | 7 | NA | NA | Acyclovir | Sullender et al.(1987) (493) |
| 15 | NA | 8 | 2-3 | 16 - 24 | Idarubicin | Pui et al.(1988)(494) |

NA, not available

**Table 6. 11: Blood samples in mixed age groups (2005-2014)**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (2005-2014) |
|---|---|---|---|---|---|---|
| 105 | 28 | 4 | 0.1 | 0.4 | Abacavir | Jullien et al.(2005)(335) |
| 76 | NA | 8 | 0.3 - 0.4 | 2.4 – 3.2 | Sotalol | Laer et al.(2005)(336) |
| 25 | NA | 5 | 3 | 15 | Anidulafungin | Benjamin et al.(2006)(495) |
| 99 | 17 | 2 | NA | NA | Meropenem | Du et al.(2006)(337) |
| 73 | 52 | 4 | 0.5 | 2 | Amlodipine | Flynn et al.(2006(338) |
| 19 | 20* | 8 | NA | NA | Lopinavir | Lee et al.(2006)(496) |
| 35 | 19 | 12 | 0.5 | 6 | Propofol | ShanGuanet al.(2006)(497) |
| 59 | 19 | 15 | 5 | 75 | Melphalan | Nath et al.(2007)(339) |
| 34 | 8 | 4 | 0.5 | 2 | Gentamicin | Seaton et al.(2007)(340) |
| 46 | NA | 9 | 0.7 - 1.5 | 6.3 - 13.5 | Lisinpril | Hogg et al.(2007)(498) |
| 72 | 36 | 9 | NA | NA | Micafungin | Hope et al.(2007)(341) |
| 15 | NA | 9 | 2 | 18 | Tanespimycin | Bagatell et al.(2007)(499) |
| 16 | NA | 8 | 2 | 16 | Temozolomide | Horton et al.(2007)(500) |
| 33 | 28 | 7 | 2 | 14 | Itraconazole | Abdel-Rahman et al.(2007) (501) |
| 24 | 23 | 2-8 | NA | NA | Busulfan | Booth et al.(2007)(344) |
| 31 | NA | 10 | 0.1 | 1 | Pemetrexed | Malempati et al.(2007)(502) |
| 18 | 17* | 7 | NA | NA | Busulfan | Hempel et al.(2007)(503) |
| 53 | NA | 6 | NA | NA | Lonafarnib | kieran et al.(2007)(504) |
| 162 | 22 | 10 | 1 | 10 | Ciclosporin | Fanta et al.(2007)(342) |
| 50 | 28 | 7 | NA | NA | Efavirenz | Fletcher et al.( 2007)(505) |
| 64 | 30 | 7 | 2 | 14 | Nevirapine | Kabamba et al.( 2008)(506) |
| 21 | NA | 4 | 3 | 12 | Bevacizumab | Bender et al. (2008) (507) |

*Median, NA, not available

**Table 6-11 Continued: Blood samples in mixed age groups (2005-2014)**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (2005-2014) |
|---|---|---|---|---|---|---|
| 33 | 30 | 8 | NA | NA | Actinomycin-D | Mondick et al.(2008) (345) |
| 217 | 18 | 1 | NA | NA | Tebipenem | Sato et al.(2008) (349) |
| 15 | NA | 6 | NA | NA | Flomoxef | Masuda et al.(2008) (508) |
| 33 | 11 | 13 | 0.5 | 6.5 | Midazolam | Muchohi et al.(2008) (188) |
| 25 | 46 | 10 | 2 | 20 | Roflumilast | Neville et al.(2008) (509) |
| 30 | 9 | 8 | 0.1 | 0.8 | Rifampicin | Ren et al.(2008)(510) |
| 97 | NA | 10 | 5 | 50 | Temozolomide | Meany et al.(2009) (511) |
| 50 | 30 | 7 | NA | NA | Tacrolimus | Zhao et al.(2009) (346) |
| 50 | NA | 7 | 2 | 14 | Lopinavir | Rakhmanina et al. (2009) (347) |
| 22 | 34 | 6 | 0.5 | 3 | Valganciclovir | zhao et al.(2009)(348) |
| 24 | 56 | 3 | NA | NA | Montelukast | Friesen et al.(2009) (512) |
| 22 | 8 | 4 | 1.2 | 4.8 | Nevirapine | Oudijk et al.(2009)(513) |
| 20 | NA | 10 | 0.5 | 4.5 | Pantoprazole | Pettersen et al.(2009) (79) |
| 189 | NA | 10 | 1 | 10 | Tamsulosin hydrochloride | Tsuda et al.(2010) (350) |
| 71 | 26 | 4 | 5 | 20 | Busulfan | Gaziev et al.(2010) (514) |
| 11 | NA | 7 | NA | NA | Valproate | Visudtibhan et al.(2011) (515) |
| 15 | 49 | 7 | 3 | 21 | Cyclophosphamide | Chinnaswamy et al. (2011) (516) |
| 165 | 39 | 7 | NA | NA | Lopinavir | Rakhmanina et al.(2011) (517) |
| 31 | NA | 6 | 0.1 | 0.6 | Ethionamide | Thee et al.(2011) (518) |
| 50 | 15 | 2 - 4 | NA | NA | Meropenem | Ohata et al.(2011)(351) |
| 38 | 14 | 6 | 0.75 | 4.5 | Azithromycim | Liu et al.(2011)(519) |
| 35 | 7 | 9 | 1 | 9 | Pentobarbital | Zuppa et al.(2011)(520) |

NA, not available

**Table 6. 11 Continued: Blood samples in mixed age groups (2005-2014)**

| Number of patients | Mean weight (kg) | No. of samples | Vol of blood (ml) | Total vol of blood (ml) | Drug | Study (2005-2014) |
|---|---|---|---|---|---|---|
| 195 | NA | 7 | NA | NA | Atazanavir | Kiser et al.(2011) (521) |
| 94 | NA | 5 | NA | NA | Busulfan | Trame et al.(2011) (352) |
| 23 | NA | 8 | NA | NA | Sunitinib | DuBois et al.(2012) (522) |
| 38 | NA | 7 | NA | NA | Plitidepsin | Geoerger et al.(2012) (523) |
| 50 | 37 | 14 | 0.6 | 8.4 | Esomeprazole | Sandstorm et al. (2012)(524) |
| 24 | 71 | 7 | 1 | 7 | Olmesartan | Wells et al.(2012) (525) |
| 48 | NA | 5 | 2 | 10 | Lorazepam | Chamberlain et al. (2012) (526) |
| 67 | 15* | 2-6 | NA | NA | Busulfan | Michel et al. (2012) (353) |
| 47 | NA | 6 | NA | NA | Cixutumumab | Malempati et al. (2012) (527) |
| 237 | 11 | 3 | 1 | 3 | Glycopeptide | Ito et al.(2013)(528) |
| 41 | 36 | 9 | 2 | 18 | Doxylamine | Balan et al.(2013)(529) |
| 126 | 31 | 10 | 0.2 | 2 | Raltegravir | Nachman et al.(2013)(530) |
| 28 | 36 | 8 | 1 | 8 | Telbivudine | Stein et al.(2013)(531) |
| 38 | 12 | 15 | NA | NA | Tacrolimus | Min et al.(2013)(532) |
| 36 | 63 | 10 | 1 | 10 | Aliskiren | Sullivan et al. (2013)(533) |
| 6 | 24 | 7 | NA | NA | Busulfan | Okamoto et al. (2014)(534) |
| 32 | 20 | 11 | 2.5 | 27.5 | Famotidine | Madani et al. (2014)(535) |

*Median, NA, not available

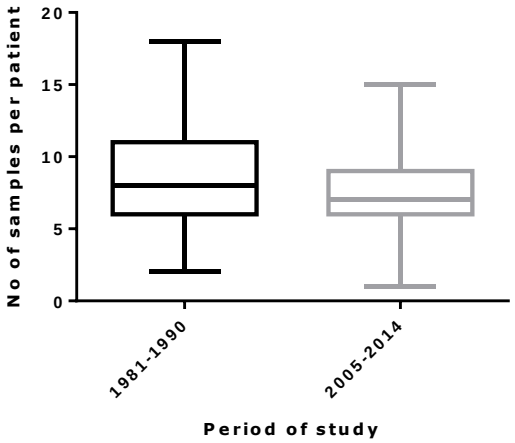


**Figure 6. 7: Number of samples collected during two periods in mixed age groups (p=0.384)**

### 6.5.6. Summary of all paediatric studies

There were 234 studies involving 9,806 patients, with more studies involving children than any other age groups. The median number of blood samples collected over all age groups was significantly higher in old decade (median: 8 [IQR: 6-11] compared to 7 [IQR: 5-9](p=0.013) (Table 6.12 and Figure 6.7). There were no significant differences in blood volume collected at a single time point or overall between the periods. The total volume of blood samples was significantly higher in old decade (median: 16 [IQR: 6-27]) than new (median:10 [IQR: 5.6-17.5]).

Thirty five of the new studies used population PK method. There was significant difference in the frequency of blood of sampling between population PK studies (median 6, IQR [4-9]) and non-population PK studies (median 8, IQR [6-10] (p=0.007) (Figure 6.2).

**Table 6. 12: Summary of all paediatric studies**

| **Number of blood samples** | | | | | |
|---|---|---|---|---|---|
| **Age group (No of studies)** | **Old studies Median [IQR]** | **Range** | **New studies Median [IQR]** | **Range** | **P value** |
| **Neonates (23)** | 7 [5-8] | 3-10 | 4.5 [3-7] | 2-9 | 0.054 |
| **Infants (17)** | 7 [5.5-7.5] | 3-10 | 7 [4-8] | 4-10 | 0.999 |
| **Children (114)** | 9 [7-12] | 4-20 | 8 [5-9.5] | 1-13 | **0.006** |
| **Adolescents (8)** | 7.5 [5-9] | 5-9 | 9 [5-11] | 4-11 | 0.571 |
| **Mixed age (72)** | 8 [6-11] | 2-18 | 7 [6-9] | 1-15 | 0.384 |
| **Total** | 8 [6-11] | 2-20 | 7 [5-9] | 1-15 | **0.013** |
| **Volume of blood samples** | | | | | |
| **Neonates (23)** | 0.7 [0.2-1] | 0.2-2 | 0.3 [0.2-0.8] | 0.15-1.4 | 0.283 |
| **Infants (17)** | NA | NA | 2 [1.5-2] | 0.5-2.5 | NA |
| **Children (114)** | 2 [1-3] | 0.4-5 | 2 [1.5-3] | 0.2-7 | 0.942 |
| **Adolescents (8)** | NA | NA | NA | NA | NA |
| **Mixed age (72)** | 1.7 [1-3] | 0.7-3 | 1 [0.5-2] | 0.1-5 | 0.234 |
| **Total** | 2 [0.7-3] | 0.2-5 | 1.5 [1-2.5] | 0.1-7 | 0.889 |
| **Total volume of blood samples** | | | | | |
| **Neonates (23)** | 4 [1.5-7] | 1.4-16 | 2 [0.8-4] | 0.6-7 | 0.117 |
| **Infants (17)** | NA | NA | 10 [6-16] | 5-16 | NA |
| **Children (114)** | 24 [12-33] | 2.8-55 | 15 [8-18] | 2-77 | **0.014** |
| **Adolescents (8)** | NA | NA | NA | NA | NA |
| **Mixed age (72)** | 16.5 [11-25] | 8-27 | 9.5 [3-15] | 0.4-75 | 0.062 |
| **Total** | 16 [6-27] | 1.4-55 | 10 [5.6-17.5] | 0.4-77 | **0.025** |

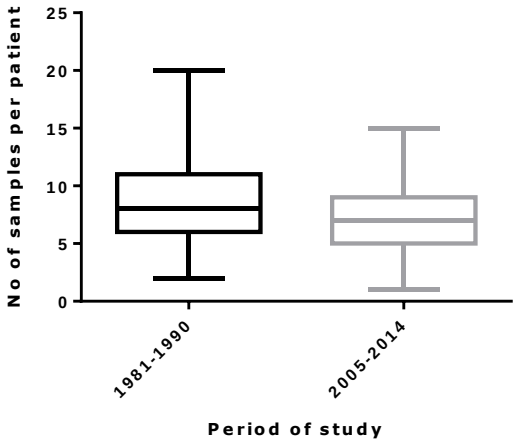


**Figure 6. 8: Number of blood samples collected in all paediatric age groups during two periods (p=0.013)**

## 6.6. Discussion

We have performed a systematic review to determine the invasiveness of pharmacokinetic studies in children over two separate decades.

Both the number of blood samples and their total volume were significantly greater in the old decade than new. The dangers of excessive blood sampling include the increased risk of infection, bruising, pain and higher rates of blood transfusion (122). A correlation between the blood volume sampled and transfusion rate has already been shown in neonates (536).

Lower sampling frequency and volume in recent studies may be due to the development of new techniques which minimise the requirement of blood for PK studies. This study showed that fewer blood samples are utilised in population PK studies than non-

population. Population PK studies have been discussed in chapter one. They involve PK parameter estimation by collecting samples from a number of subjects taking different doses over different periods of time (104). There are currently no guidelines on the frequency of blood sampling in children. For population PK studies, it has been suggested that no more than 6 samples need to be collected (537) and for neonates, 3 samples are thought to be optimal for the estimation of PK parameters (538). In this review the frequency of blood sampling in neonates was between 2-10. Despite the recommendations for less frequent blood sampling, Reed showed in his study that the greater the number of blood samples taken, the better the estimation of PK parameters (127). This conclusion was reached after comparing PK parameters obtained from 5 and 12 sampling time points (127). Therefore, this accuracy may need to be balanced against the need to limit the number of samples depending on the patient's age and disease.

The available guidelines on the volume of blood samples for PK studies in children are highly varied with most recommendations suggesting a range between 1 and 5% of TBV within 24 hours and not more than 10% of TBV within 8 weeks (125)(Table 1.4). Children's TBV can be estimated from their weight (70 ml/kg), preterm neonates 90 ml/kg and term neonates 80 ml/kg. This suggests that a three kilogram neonate will have a TBV of about 240 ml. We found the largest total blood volume drawn from preterm

neonates to be 4ml, in a PK study of cefoperazone (356). The weights of neonates in this study were not reported; hence it could not be exactly determined whether the maximum allowable total blood volume draw based on EMA recommendations for total volume of blood was exceeded. It would have represented approximately 1.5% of the TBV of a 3kg term baby, which is within the recommended limits (123). In another study of midazolam in term neonates, a sample volume of 0.5 ml was taken on nine occasions, total volume 4.5ml in 24 hours (182). Therefore each individual sample and total volume did not exceed 1% (2.4ml single draw) and 3% (7.2ml total) per EMA guideline for a 3kg baby.

The maximum recommended allowable blood draw is a percentage of blood volume per body weight. It is often difficult to ascertain whether the studies involving children and adolescents exceeded these recommendations, as they may have wide age ranges and only quote a mean or median weight. Two Canadian studies involved the collection of 77 and 65 ml respectively over a 24 hour period in children (386, 439). The maximum recommended volumes would have been exceeded in the lightest children recruited in one of these studies (386). The second study of temozolomide, however did not specify the patient's weight. It is therefore very important to look at the whole age and weight ranges of children taking part in PK studies, when deciding the blood volumes to be collected. Protocols may need

to have different regimens for different ages/weights if the population studied is wide.

Blood samples were collected mainly from a catheter or by venous sampling. Catheter sampling is the most convenient method when repeated blood collection is required and minimises the distress of repeated blood sampling. However, a catheter requires nursing care and monitoring, also it may not always continue to sample for the whole duration of the study (539, 540). The other sampling method identified was arterial sampling. Unlike venous sampling, it requires greater expertise and may result in bleeding. Venous sampling is also generally preferred to heel pricks in neonates, because it is less painful and more likely to provide the required volume (120).

In conclusion, definitive evidence guidelines should be developed regarding the maximum volume and number of samples that should be collected in children of various ages. There is presently no guideline on frequency of blood sampling and the guidelines on volume are variable. More attention needs to be given to reducing both the number of blood samples and the total volume of blood collected in an individual patient, by using micro analytical techniques and population PK methods. Consideration should be given by researchers to all ages/weights of potential children within the study, as to whether the single volume or total volume of blood exceeds current guidance.

# CHAPTER SEVEN
# GENERAL DISCUSSION AND CONCLUSION

## 7.1. General discussion

From the preceding chapters it is clear that age is a major determinant of midazolam, theophylline and morphine clearance in paediatric patients. Critical illness was a significant predictor of midazolam and theophylline clearance, but not morphine. This effect, however, was not observed after adjusting for age and weight. There were, however, very few midazolam, morphine and theophylline PK studies conducted in non-critically ill subjects.

It was also shown that significantly fewer blood samples were being utilised in population PK studies than non-population PK studies in children. Population PK methods are increasingly being utilised in children in the last 10 years and new studies are less invasive in paediatrics.

### 7.1.1. Age and variability of drug clearance

The metabolism and consequently clearance of midazolam, theophylline and morphine is low at birth before progressively rising to adult levels during infancy (28, 34, 57). The ontogeny of the different metabolizing enzymes have been described in the introductory chapter and further discussed in the individual chapters. Drug clearance is generally more variable during neonatal life than in late childhood. Hattis et al. (2003) evaluated the PK characteristics of

45 different drugs from published literature and compared PK parameters between different age groups. They reported a higher inter-individual variability in drug half-life and clearance in the first week of life than late neonatal period (2-8 weeks). They also reported lower variability in children and adolescents (541). Their findings reflect the impact of the rapid changes to drug elimination systems that occur during the neonatal period. Differences in drug pharmacokinetics between children and adults are mainly due to physiological differences and also as a result of differences in the maturity of the enzyme systems and clearance mechanisms. Variability in PK characteristics makes it necessary to titrate the drug dose according to individual requirements and also where applicable monitor therapeutic drug concentration.

In this thesis, midazolam clearance in neonates was found to be much lower in neonates than children and adolescents. Clearance ranged between 0.78 and 1.7 ml/min/kg in preterm neonates and between 1.17 and 3.5 ml/min/kg in term neonates. In children however, midazolam clearance was higher, ranging between 1.1and 15 ml/min/kg. This trend has also been previously reported in a previous study (198). However, a study by de Wildt et al. (2001) reported no significant relationship between age (gestational, postnatal, or postconceptional) and midazolam clearance (542). Higher clearance levels in children than neonates are reflective of the developmental changes in CYP3A4 activity, which has been shown to

increase with postnatal age (28). During the immediate postnatal period, there is a shift from CYP3A7 predominance to a progressive rise in CYP3A4 activity. Thus the contribution of CYP3A7 to midazolam metabolism becomes marginal (28, 542). These highly dynamic changes in CYP activity in early neonatal life may therefore contribute to the higher CV observed in preterm neonates than children.

A morphine clearance ranging from up to 10ml/kg/min in preterm neonates to 52 ml/kg/min in children was found. However, evidence from limited data showed no significant difference in CV of morphine clearance between neonates and infants. There was insufficient data for comparison of CV between children and adolescents. Morphine is metabolised by glucuronidation and complete UGT expression occurs over the first six months of life (43). During this period, especially the neonatal period, rapid maturation may cause variable expression of UGT (5). Morphine is also metabolised by sulphation which is decreased after neonatal period (543).

In neonates, theophylline clearance varied between 0.2 and 0.9 ml/min/kg; while clearance ranging between 1 and 2 ml/min/kg was reported in children. This age related increase in theophylline clearance has been similarly described by Tanaka (1998). In our findings, the CV of theophylline was not significantly different between neonates and children. However, Milsap et al.(1994)

reported a significant inter-individual variability of theophylline clearance in a review of several clinical studies involving premature neonates, full-term neonates, and infants (544). Theophylline is metabolised in the liver by CYP1A and CYP1A2. CYP1A activity level increases during the first three months, which may result in variability of clearance (249).

### 7.1.2. Critical illness and variability of drug clearance

Although no significant difference in CV of clearance was observed for midazolam, theophylline or morphine, critically ill subjects showed comparatively lower clearance than non-critically ill. We were unable to perform a subgroup analysis for the different age groups due to insufficient number of studies. For all of the drugs, very few studies were conducted in non-critically ill subjects. Critical illness alters PK in different ways. Some of this has been discussed in the different chapters for the respective drugs. The capacity of the liver to metabolise drugs and renal function are often compromised during critical illness.

Stress and infection, which are commonly associated with critical illness cause a spike in inflammatory mediators, such as IL-6 and nitric oxide (NO), causing changes in drug clearance (545). The expression of these mediators has been shown to downregulate the expression of cytochrome P450 enzymes in the liver (546) following a decline in mRNA transcription and protein translation (547). Children

with severe sepsis are reported to have as much as 2 fold reduction in drug clearance and up to four fold decline when they have multiple organ failure (548). A study by Haas et al. (2003) showed a statistically significant decline in the expression of CYP3A4 in immediate post-operative patients compared with baseline levels. The CYP3A4 level in their study was negatively correlated with IL-6 concentration (545). An inverse relationship between clearance and IL-6 and NO has also been reported in children (548).

Reduced organ perfusion also occurs in severely ill patients. Reduced liver perfusion and subsequent oxygen deprivation can result in reduction in enzyme activity in the liver cells (549). Renal hypo perfusion can cause renal failure leading to poor drug clearance (12). Mechanical ventilation is an important part of the management of severely ill children. This can reduce cardiac output with eventual liver and kidney damage (550).

**7.2. Implication of this research**

Age has been shown to be the most important determinant of PK studies in children. Age appropriate dosing is an important aspect of treatment in paediatrics. For several drugs, including those evaluated in this thesis, there are dosing guidelines specifically for neonates, children and adolescents. It is however unclear how rapidly age related changes in PK occur during childhood. Therefore, more studies evaluating the effect of age on the variability of PK

parameters are required. Furthermore, there were very few studies comparing of the effect of critical illness on clearance. Critical illness is a very heterogeneous classification and it is important that future research should focus on how specific illnesses and their affects influence drug clearance. Understanding the relationship between critical illness and drug pharmacokinetics can help clinicians to optimise treatment in these children.

One of the reasons sighted for the very few PK studies in children is the ethics of conducting such studies in this age group. It is therefore important to adopt less invasive PK techniques for future research in children. Raising awareness on the use of available minimally invasive techniques is important. Evidence based guidance is needed for the determination of the ideal frequency of blood samples for PK studies in different age groups. Finally, a standardisation of the maximum volume of blood samples collected in children of various ages is required.

### 7.3. Limitations

These systematic reviews are limited by the inconsistent reporting styles of the authors. While some provided the range and the SD which are measures of variability, some provided neither. Therefore, valuable data was lost because such results could not be included for interpretation. Additionally, some studies did not report the results for different paediatric age groups. There were very few PK studies

conducted in non-critically ill subjects. Therefore, comparison between critically ill and non-critically ill patients was impossible. A further limitation is the inability to determine the CV of the drug clearance in some studies because the individual patient data were not provided. Hence, comparison of the CV between different age groups was inadequate, because of small datasets. Furthermore, there were extensive variation in the number of participants in each PK studies and some of the studies were old, especially theophylline PK studies. Predominantly old studies made it impossible to determine the effect of population PK methods on clearance determination.

When examining invasiveness, this was limited by the lack of data on weight of patients. Therefore compliance of studies with current guidelines could not be determined. Furthermore, an accurate comparison within the paediatric age group is difficult because of the wide age range in the ICH age paediatric catagory. Finally some studies did not provide information on volume of blood sampled and methods used.

### 7.4. Final conclusion

Age is a major risk factor for inter-individual variation of PK parameters of midazolam, theophylline and morphine. It is also an important predictor of drug clearance in children. Therefore, age appropriate dosing of these drugs is important. Furthermore,

significantly less numbers and volume of blood samples are utilised in new PK studies than old studies. This trend is particularly influenced by the increasing utilisation recently of population PK studies. Population PK method should be encouraged to minimise invasiveness of PK studies. New methodologies for reducing sample volumes and frequency should be considered for all studies.

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