Adverse Drug Reactions in Hospitalised Children

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by

Signe Thiesen

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Abstract

Adverse Drug Reactions (ADRs) are a global health problem and a leading cause of death, illness and injury in economically developed countries. Therapeutic response, as well the occurrence of undesired effects, can differ significantly between children and adults and many drugs have not been sufficiently studied in the paediatric population. Existing studies on ADRs in children differ widely in study design and outcome reporting, and many are methodically problematic.

The incidence and characteristics of ADRs in hospitalised children and factors associated with an increased risk of experiencing and ADR, were assessed in a large, prospective, observational study. 17.7% of all children experienced at least one ADR. Opiate analgesia and drugs used in general anaesthesia (GA) accounted for more than 50% of all drugs implicated in ADRs. Less than 1% of ADRs caused permanent harm or required admission to a higher level of care. Children post GA were more than six times more likely to experience an ADR than children who had not received a GA (HR 6.38; 95%CI 5.3-7.7). Other risk factors identified were increasing age (HR 1.05 for each year; 95%CI 1.04-1.07), increasing number of medicines (HR 1.25 for each additional medicine; 95%CI 1.22-1.28) and being an oncology patient (HR 1.89; 95%CI 1.36-2.63). The proportion of ADRs caused by GA agents and opiate analgesia has previously been underestimated.

The cost of excess bed days due to ADRs, has been estimated to be £2 Million per year for a 400-bed adult hospital. The cost of excess bed days in our study was only £35,000 per year for a 300-bed hospital. Other parameters and methods might need to be considered when assessing the financial impact of ADRs in children.

Cisplatin is used in cancer treatment and causes irreversible hearing loss in 42-88% of children. Cathechol-O-Methyltransferase (COMT) and Thiopurine-S-Methyltransferase (TPMT) genetic variants have been associated with hearing loss in paediatric patients, but findings from subsequent studies are contradictory. The occurrence of COMT and TPMT genetic variants in a UK population was examined in a retrospective, multicentre cohort study. Known risk factors for ototoxicity were confirmed; increasing cumulative dose of cisplatin younger age ($p< 0.01$), cranial radiotherapy ($p <0.028$) and exposure to vincristine $p< 0.091$). The association with COMT and TPMT genetic polymorphisms could not be replicated.

ADRs in children are common and improving medicines safety in children remains a vital aspect of clinical care. New assessment tools are aiming to address some of the challenges faced by clinicians and researchers. Our knowledge of pharmacokinetics and pharmacodynamics in children is still limited, but advances in the field of paediatric pharmacogenomics are beginning to translate into improved medicines safety and efficacy for children. Further work about non-Oncology ADRs would benefit from a focus on high impact events that are described in this thesis.

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The specific contribution of the author of this thesis to each study is outlined in detail below.

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Abbreviations

6-MP	6-Mercaptopurine
ABR	auditory brainstem response
ADE	adverse drug event
ADR	Adverse drug reaction
ADRIC	Adverse Drug Reactions in Children
AE	adverse event
ASHA	American Speech-Language-Hearing Association
BNFC	British National Formulary for Children
BP	Prof Barry Pizer
CI	confidence interval
CNS	central nervous system
COMT	Cathechol-O-Methyltransferase
CRF	case record form
CTCAE	Common Terminology Criteria for Adverse Events
db	decibel
db HL	decibel hearing level
EDTA	ethylenediaminetetraacetic acid
eMC	Electronic Medicines Compendium
FDA	Food and Drug administration
GA	general anaesthesia
GCT	germ cell tumour
GRiP	Global Research in Paediatrics
GST	Glutathion-S-transferase
Gy	Gray
HF	high frequency
HR	hazard ratio
HW	Hardy-Weinberg
ICD	International Classification of Disease
ICH	International Council (previously Conference) on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IQR	Interquartile range
kHz	kilo Hertz
LCAT	Liverpool Causality Assessment Tool
LREC	local research ethics committee
MAF	minor allele frequency
MAGIC	Molecular Genetics of Adverse Drug Reactions in Children

MHRA	Medicines and Healthcare Products Regulatory Agency
min	minute
NHS	National Health Service
NIHR	National Institute for Health Research
OR	odds ratio
Osteo	osteosarcoma
PD	pharmacodynamics
PICU	paediatric intensive care unit
PIP	paediatric investigation plan
PK	pharmacokinetics
PNET	primitive neuroectodermal tumour
PON	post-operative nausea
PONV	post-operative nausea and vomiting
POV	postoperative vomiting
POG	Paediatric Oncology Group
PROMs	Patient reported outcome measures
PTA	pure tone audiometry
Rhabdo	rhabdomyosarcoma
RT	room temperature
SAM	S-adenosylmethionine
SIOP	International Society of Paediatric Oncology
SmPC	summary of product characteristics
SNHL	sensorineural hearing loss
SNP	single nucleotide polymorphism
ST	Signe Thiesen
TE	Tris-EDTA
TPMT	Thiopurin-S-Methyltransferase
TPN	total parenteral nutrition
UMC	Uppsala Monitoring Centre
VRA	visual reinforcement audiometry
WHO	World Health Organisation

Publications and presentations arising from work in this thesis

The work detailed in chapter 2 was published in BMC Medicine (Thiesen et al. 2013) and in Programme Grants for Applied Research (Smyth et al. 2014). Some of the work described in chapter 2 was also presented at the European Society for Developmental Perinatal and Paediatric Pharmacology Congress (Oslo 2011) and at the annual meeting of the European Society for Paediatric Research (Porto 2013).

The work detailed in chapter 4 was presented at the Stratified medicine & prevention of adverse drug reactions joint meeting between the British Toxicological Society and the British Pharmacological Society (Edinburgh 2015) and has been accepted for publication in Pharmacogenetics and Genomics (Thiesen et al. 2017).

1 Introduction

Adverse drug reactions (ADRs) are an important cause of iatrogenic morbidity and mortality in patients of all ages.

In the in the United Kingdom (UK), ADRs account for 6.5% (95% confidence interval (CI), 6.2-6.9%) of acute hospital admissions in adults (Pirmohamed et al. 2004) and 2.9% (95% CI, 2.5-3.3%) in children (Gallagher et al. 2012). In comparison, coronary heart disease accounted for 4.9% of emergency admissions in England in 2001 (The British Thoracic Society 2006) and asthma for 3.8% of emergency admissions in children in Scotland in 2005/06 (Information Services Division (ISD) Scotland 2009).

A large, prospective, in-patient study in adults in the United Kingdom (UK) found that in 1 of 7 hospital episodes at least one ADR occurred (incidence 14.7%; 95% CI, 13.6-15.9%) (Davies et al. 2009) and that ADRs contributed to 14 of 184 deaths (0.4% of patients admitted). Based on an average cost of bed days, the financial impact of ADRs per (adult) hospital per bed per year has been estimated to be £5000 in the UK, € 5580 in France and $8000 in the US (Bates et al. 1997; Davies et al. 2009; Moore et al. 1998).

There are no comparable paediatric in-patient data. As part of the ADRIC programme, Smyth et al. conducted a systematic review of ADR studies in children (Smyth et al. 2014; Smyth et al. 2012) which is summarised below (1.5). Firstly, however, it would seem important to understand how ADRs and some aspects of drug safety are different in children compared to adults. Secondly, comparing data from different studies (in children as well as adults) can be problematic due to different study designs. Therefore, key aspects of ADR assessment and definitions of drug safety will also be discussed.

The evidence presented and discussed in this chapter will focus on the literature available at the time the author of this thesis commenced her studies. An exception are citations from webpages and results of studies that were part of the ADRIC programme as these were available to the author of this thesis prior to their publication.

1.1 ADRs in children

ADRs in children can differ from those in adults due to age dependent physiological characteristics which affect pharmacokinetics (PK) and pharmacodynamics (PD) of medication. There are numerous examples to illustrate this. Tetracyclines for example are contraindicated in young children and pregnant women as they cause staining of teeth in the newborn or growing child (Demers et al. 1968). Chloramphenicol caused 'grey baby-syndrome' (Sutherland 1959) due to impaired metabolism in neonates. Dystonia and dyskinesia due to metoclopramide is significantly more common in young adults (Bateman, Rawlins & Simpson 1985). In an analysis of 42 cases of sodium valproate induced fatal hepatitis, 69% of patients were 10 years old or less at presentation (Powell-Jackson, Tredger & Williams 1984). More recently, the death of three infants aged $\leq$ 6 months has been associated with pseudoephedrine in cough and cold medications (Srinivasan et al. 2007).

The importance of developmental pharmacology has been recognised for more than a century (Kearns et al. 2003), but only recent changes in legislation have provided and incentive to collect specific data in the paediatric population (Hawcutt & Smyth 2008). A hallmark in European legislation was the regulation on medicinal productions for paediatric use in 2007 (Union 2006). A paediatric investigation plan (PIP) is now mandatory for all medicines under development in Europe. Waivers are granted for medicines unlikely to benefit children such as medication used in the treatment of Alzheimer's disease. In addition, the new legislation also offers incentives for the development of off-patent medicines. However, such changes in legislation are likely to take some time to show an effect as the majority of medications, currently prescribed for children, predate this legislation.

1.2 Drug safety

To improve the safety of medicines in adults as well as children, post marketing evaluation is as important as well conducted clinical trials prior to marketing. To identify ADRs that occur at a rate of 1/3,000-6,000, statisticians estimate that 10,000-20,000 patients would need to be monitored (Severino & Del Zompo 2004), therefore the full ADR profile of new medicine can often only be known after many years and it is generally accepted that at least 2-3 years of post-marketing surveillance are required (Lasser et al. 2002). Many countries have therefore established spontaneous post-marketing reporting systems, such as the MHRA's yellow card scheme in the UK, but underreporting remains common (Hazell & Shakir

2006). Although international collaborations such as the World Health Organisation's (WHO) Uppsala Monitoring Centre (UMC) are addressing this issue, systematic studies into the incidence and character of ADRs occurring in the population are of particular importance, not least to highlight areas where further research is required.

The branch of pharmacological science concerned with all aspects of drug safety is Pharmacovigilance, '*the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem*' (World Health Organisation 2004).

1.3 Definitions

One of the earliest aims within the field of pharmacovigilance has been to agree on definitions of the terms 'adverse event', 'adverse drug reaction' and 'adverse drug event', to improve communication in clinical trials, research and in the clinical context. Although worldwide accepted definitions now exist, they still do not necessarily reflect their application in clinical practice (Smyth et al. 2014). It is important to take this into account when conducting clinical research (outside clinical trials) or translating research into clinical practice.

1.3.1 Adverse event (AE)

The following definition has been agreed by the International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) with input from the WHO: '*Any untoward medical occurrence in a patient […] administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.*' (ICH 1995). The relationship between medicinal product and event is therefore temporal and may or may not be causative.

1.3.2 Adverse drug reaction (ADR)

A widely accepted definition of ADR is WHO's definition from 1972: '*A response to a drug which is noxious and unintended, and which occurs at doses normally used in man for the prophylaxis, diagnosis, or therapy of disease, or for the modification of physiological function*.' (World Health Organisation 1972)

Edwards and Aaronson developed this definition further by defining the word 'noxious' more clearly and including excipients or contaminants as potential causes of reactions rather than

the drug alone by replacing the word 'drug' with the 'medicinal product'. In their definition, which is also widely used, an ADR is *'an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dose regimen, or withdrawal of the product'* (Edwards & Aronson 2000). Both definitions of ADR imply causality between medicinal product and reaction and exclude reactions due to medication error, prescribing error or overdose.

In clinical practice, the terms 'side-effect' and 'ADR' are often used interchangeably. However, 'side-effect' may refer to desirable as well as undesirable effects. It may also result in prescribers and patients considering it unavoidable, that a harmful, unintended reaction occurs in addition to the intended, therapeutic effect. Although it would perhaps be desirable to avoid the term 'side-effect' altogether, it remains the most commonly used term for ADRs in clinical practice. This is reflected in the its use in WHO fact sheets on vaccinations and medicines (World Health Organisation 2016), and in the explanation given on the MHRA website: '*Suspected side effect, also known as adverse drug reaction. These are unwanted effects that you consider are linked to taking a medicine. Side effects also include any effects from: misuse, abuse, an error in the way the medicine has been given or overdose'*(MHRA 2016).

1.3.3 Adverse drug event (ADE)

The generally accepted definition of ADE is 'an injury due to medication' or in the original wording of Bates et al. (Bates et al. 1995) '*injury resulting from medical intervention related to a drug*' . It implies a causal relationship between drug and event. Harm may occur due to medication use as well as due to dose reduction or discontinuation. Furthermore, prescribing and administration errors, as well as accidental or deliberate overdoses, are included in this definition. Therefore, all ADRs are ADEs but the reverse is not true.

1.4 Evaluation of ADRs

When investigating reactions suspected to be ADRs, there are three important concepts:

1. Establishing a causal probability (causality) between the suspected medicinal product(s) and the reaction.
2. Assessment and classification of the seriousness and severity of the ADR.
3. Assessing the preventability (or avoidability) of the ADR.

Reaching agreement on these aspects remains a challenge of pharmacoepidemiologic research. Significant disagreement can be observed whenever judgement is made without referring to agreed definitions and/or the structure of validated assessment tools or classifications (Arimone et al. 2005; Aronson & Ferner 2005; Ferner & Aronson 2010).

1.4.1 Causality of ADRs

Numerous methods to assess the causal probability between a drug and a suspected ADR have been published, none of which has been universally accepted (Agbabiaka, Savović & Ernst 2008). The approaches used can be divided into three categories: Expert judgement, algorithms and probabilistic approaches.

1.4.1.1 Expert judgement

Traditionally, most studies have used expert judgement to determine ADR causality often involving only a single assessor. This method is dependent on knowledge and experience and renders poor reproducibility and reliability (Arimone et al. 2005). Structured communication approaches, such as the Delphi technique, employing a panel of experts and using a systematic procedure order to reach consensus, are more reliable (Arimone et al. 2005) However, this is time consuming and therefore not feasible as first line assessment in larger studies or in post-marketing surveillance operations. Furthermore, a panel of experts may reach consensus but the judgement may still be wrong.

1.4.1.2 Algorithms

In a landmark address to the '*newly-founded Section of Occupational Medicine*', Sir Austin Bradford Hill discussed nine concepts of association between environmental factors and disease that should be examined when making a judgement on causation (Hill 1965). He considered them viewpoints rather than necessary conditions that could bring 'indisputable evidence for or against the cause and effect hypothesis'(Hill 1965). These viewpoints are now generally referred to as (Bradford) Hill criteria:

1. Strength of association
2. Consistency of the observed association
3. Specificity
4. Temporality
5. Biological gradient (dose-response curve)
6. Plausibility
7. Coherence (data should not conflict with the natural history of the disease),
8. Experiment (experimental evidence)
9. Analogy (ready to accept slighter but similar evidence with another disease or drug)

Algorithms, often depicted as flow-charts, are based on a set of steps that need to be followed in order to arrive at a conclusion or solution of a problem, building on several, but rarely all, of the above criteria. However, In their systematic review of methods of causality assessments, Agbabiaka et al. (Agbabiaka, Savović & Ernst 2008) examined 26 algorithms and found that the majority of authors included questions about the temporal sequence (17/26), re-challenge (17/26) and the response pattern (17/26). About 50% also included questions about drug levels (14/26) and de-challenge (12/26) and at least one third incorporated previous exposure (11/26) and confirmation by laboratory evidence (10/26) as part of their algorithms. Algorithms are reproducible, relatively easy to use and have demonstrated higher rates of inter-rater agreement (Hutchinson et al. 1983) However there is no way to include additional information and a degree of clinical judgement is usually still required for some questions e.g. when considering alternative causes. In addition, specific problems have been identified in individual algorithms, for example:

- Naranjo (Naranjo, Busto & Sellers 1981): Weighting of questions without providing a rationale and inclusion of questions that are no longer relevant (Gallagher et al. 2011)

- Karch and Lasagna (Karch & Lasagna 1977): Inability to identify new ADRs (Agbabiaka, Savović & Ernst 2008)
- Kramer (Kramer et al. 1979): Level of expertise and experience required (Agbabiaka, Savović & Ernst 2008; Leventhal et al. 1979)

In an attempt to overcome some of these issues and as part of the ADRIC programme, Gallagher et al. developed a new causality assessment tool (Liverpool CAT or LCAT), that is easy to use and has shown good inter-rater reliability (Gallagher et al. 2011).

1.4.1.3 Probabilistic approaches

Probabilistic approaches are mostly based on Bayes' theorem, which means that the probability of an ADR having occurred is calculated by firstly considering the probability of the reaction (occurring with and without exposure to the drug) and by secondly considering the available evidence given for each case. Likelihood ratios are calculated for each factor; a final estimate of the reaction having occurred as a results of the exposure to the drug is then calculated (Arimone et al. 2005). Although these approaches are reliable and highly reproducible, probabilistic methods are time consuming, complex and resource intense (Agbabiaka, Savović & Ernst 2008) which limits their use as general ADR assessment tool .

In the absence of a single, universally accepted causality assessment, combining methods is perhaps the best possible approach to yield the most reliable results. It would for example be feasible to employ a suitable algorithm in the first instance and use the Delphi technique or a probabilistic approach for cases of particular interest.

1.4.2 Seriousness and Severity of ADRs

Classifying the seriousness of an ADR is important as it is a measure of describing the harm an ADR has caused or has the potential to cause. According to ICH guidelines, a serious ADR is '*any reaction that results in death, is life-threatening (i.e. the subject was at risk of death at time of the event), requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, is a congenital anomaly or birth defect or is medically significant'* (ICH 1995). These outcome categories for seriousness are used in clinical trials (NETSCC 2016) as well as post-marketing reports of ADRs (MHRA 2016).

Severity or intensity of an ADR assesses the extent of the reaction with regards to the individual patient. Serious reactions are usually severe, but severe reactions need not be serious. The classic example used to illustrate the difference between those terms is 'headache'. A headache may be experienced as severe by the patient and therefore rate highly on a visual analogue pain scale, but is usually not serious. Severity scales using categorise such as 'trivial', 'mild', 'moderate' and 'severe' are of little value, as they are subjective and no satisfactory definitions exist (Aronson & Ferner 2005). In 1992, Hartwig et al. proposed a numbered, 7-level rating scale (Hartwig, Siegel & Schneider 1992) (Table **1.1**). In levels 1-3 the effect the ADR has on the treatment is considered (no change in treatment, change in treatment, change in treatment and antidote/additional treatment), whereas levels 4-7 incorporate categories of increasing seriousness (admission or prolonged stay,

admission to intensive care, permanent harm, death). One could therefore argue that the Hartwig scale is a combined seriousness and severity assessment scale.

More recently, Ferner and Aaronson proposed a classification that is firstly based on alteration of the dosage regimen and secondly on the response to treatment if this was required (Aronson & Ferner 2005) (Table **1.2**). In contrast to Hartwig's classification, terms referring to the seriousness of the ADR are avoided altogether. Most clinical studies investigating ADRs that have made use of severity and/or seriousness assessment tools to date are using a modified form of the Hartwig scale. This is unsurprising, given that the Hartwig scale has been established much longer. From a clinical and pharmacovigilance point of view, it is also perhaps more important to describe the seriousness of an ADR than purely assessing its severity.

Table 1.1 ADR Severity Assessment Scale (Hartwig, Siegel & Schneider 1992)

Level	Description
1	An ADR occurred but required no change in treatment with the suspected drug.
2	The ADR required that treatment with the suspected drug be held, discontinued, or otherwise changed. No antidote or other treatment required, no increase in length of stay (LOS).
3	ADR required that treatment with suspected drug be held, discontinued, or otherwise changed AND/OR an antidote or other treatment was required. No increase in LOS.
4	(A) Any level 3 ADR which increases LOS by at least 1 day. OR: (B) The ADR was the reason for admission.
5	Any level 4 ADR which requires intensive medical care.
6	The adverse reaction caused permanent harm to the patient.
7	The adverse reaction either directly or indirectly led to the death of the patient.

Table 1.2 ADR Severity (Intensity) Classification (Aronson & Ferner 2005)

Grade	Change in dosage regimen of the offending drug	Treatability of the reaction
1	No change in dosage regimen required	A. No treatment required B. Relieved or partly relieved by treatment C. Not relieved by treatment
2	Altered dosage regimen required or desirable	A. No treatment required B. Relieved or partly relieved by treatment C. Not relieved by treatment
3	Withdrawal required or desirable	A. No treatment required B. Relieved or partly relieved by treatment C. Not relieved by treatment

1.4.3 Avoidability of ADRs

Several approaches to determine the preventability of ADRs exist. The most commonly used definitions are those described by Schumock & Thornton (Schumock & Thornton 1992) and Hallas (Hallas et al. 1990) (Table 1.3 and Table 1.4). They are both based on appropriateness of prescribing and both include medication errors (questions 2-4 and 6 in Table 1.3 and definition of 'definitely avoidable' in Table 1.4). Although harm arising from, for example, prescription or administration error is preventable and hence constitutes a crucial aspect of drug safety, as per definitions discussed above (1.3) this concern ADEs but not ADRs. Assessing the appropriateness of the prescription is not necessarily straightforward, in particular in the context of paediatric prescribing. From the outset, it is not clear whether the appropriateness of prescribing should be considered prospectively or retrospectively. For example, a febrile infant, with a later confirmed viral infection, may well have been prescribed an antibiotic, on admission, to which the child developed a reaction. The reaction may therefore either be considered avoidable, as antibiotic treatment was not indicated or unavoidable, if prescribing an antibiotic was considered appropriate in view of the clinical presentation. Furthermore, if one concludes that the prescription was not indicated in the first place this could then be considered an error and hence a preventable ADE.

Schumock also includes assessment of the appropriateness of dose, route or frequency (question 2 in Table 1.3) which represents a further challenge in the paediatric context, where off-label and unlicensed prescribing is very common and hence might be impossible to answer this question. Hallas' criteria require further explanation as terms like 'reasonable means' and 'effort exceeding obligatory demands' leave considerable room for interpretation.

Table 1.3 Criteria to determine the preventability of ADRs (Schumock & Thornton 1992)

An answer of 'yes' to one or more of the following questions indicates that the ADR may indeed have been preventable.

1. Was the drug involved in the adverse drug reaction not considered appropriate for the patient's clinical condition?
2. Was the dose, route, and frequency of administration not appropriate for the patient's age, weight and disease state?
3. Was required therapeutic drug monitoring or other necessary laboratory testing not performed?
4. Was there a history of allergy or previous reactions to the drug?
5. Was a drug interaction involved in the reaction?
6. Was a toxic serum drug level documented?
7. Was poor compliance involved in the reaction?

Table 1.4 Criteria for avoidability (Hallas et al. 1990)

Definitely avoidable	The event was due to a drug treatment procedure inconsistent with present-day knowledge of good medical practice or was clearly unrealistic, taking the known circumstances into account
Possibly avoidable	The prescription was not erroneous, but the event could have been avoided by an effort exceeding the obligatory demands
Not avoidable	The event could not have been avoided by any reasonable means, or was an unpredictable event in the course of a treatment fully in accordance with good medical practice
Unevaluable	The data for rating could not be obtained or the evidence was conflicting

Ferner and Aronson recently conducted a systematic review of preventability of ADRs and concluded that *'there is no simple method'* to characterise preventability of ADRs (Aronson & Ferner 2010). In addition, reliability for any of the currently existing criteria is not ideal and none of the current criteria can be applied universally (Ferner & Aronson 2010). Consequently they proposed a new and different approach, taking into account four key aspects (Aronson & Ferner 2010):

- Preventability according to mechanism
- Preventability according to individual susceptibility
- Preventability according to time-course
- Preventability according to dose-response pattern

Their approach can be applied retrospectively, e.g. on ADR case reports, as well as prospectively. Although a new approach to define avoidability is clearly needed, Ferner and Aronson's approach is complex and requires knowledge which might not be readily available for paediatric patients, such as (age dependent) PK and PD data. In addition, the amount of in depth knowledge required overall to assess a reaction, will make it unlikely that this tool can be easily used in large scale ADR studies involving many different medications and patients. Furthermore, clinicians without a background in pharmacology or pharmacy are unlikely to be able to use this approach independently.

There remains an urgent need for a novel assessment approach for avoidability of ADRs, ideally taking into account the unique conditions of paediatric prescribing.

1.5 Studies investigating ADRs in children

Taking the above into account it is unsurprising that after analysing 101 paediatric studies investigating ADRs, Smyth et al. concluded, that although a large number of studies exists, they differ widely, a high proportion has major shortcomings in design and/or reporting and hence only limited and cautious conclusions and recommendations can be made. (Smyth et al. 2014; Smyth et al. 2012). The review confirmed, that ADRs are a significant problem in children.

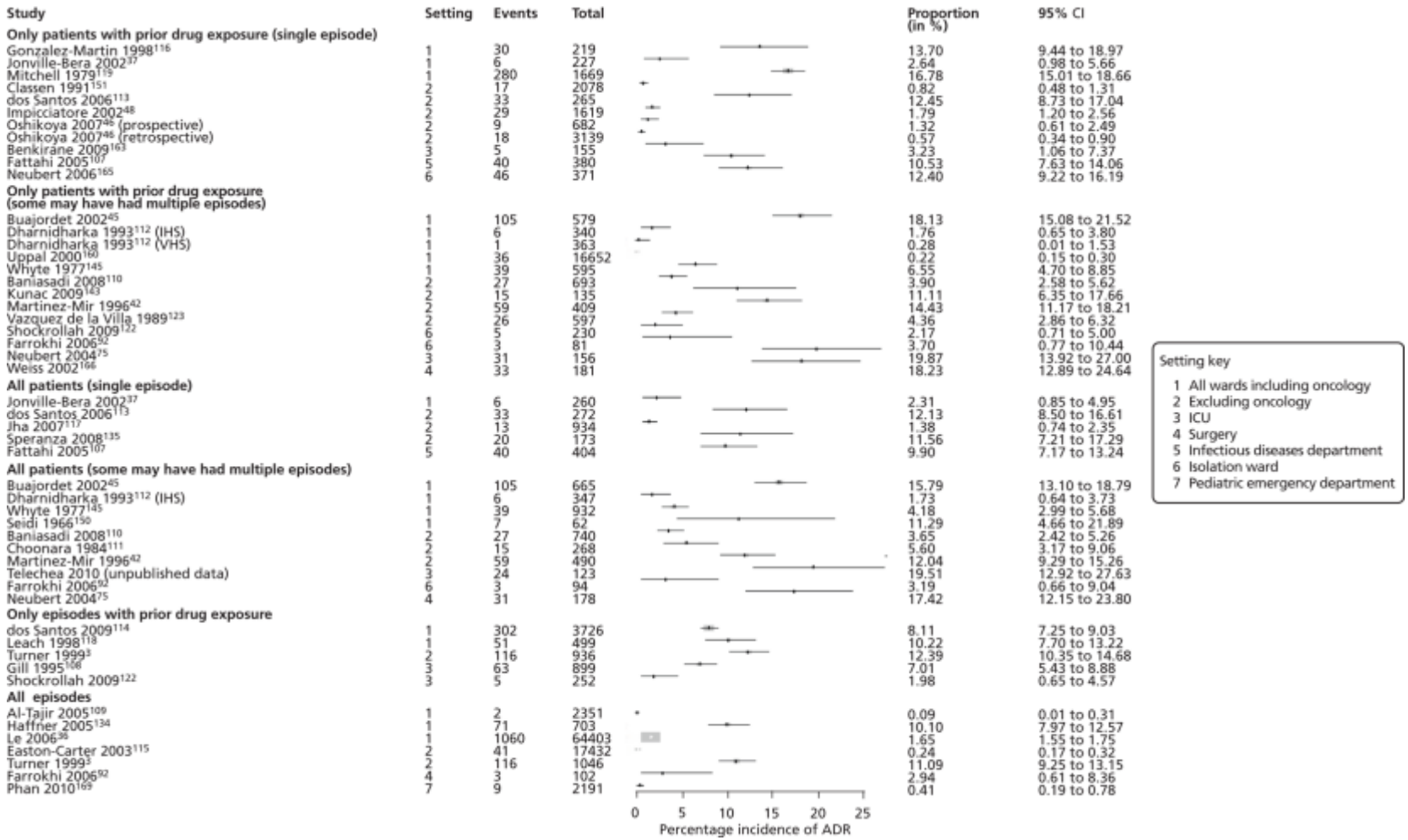
Smyth et al. analysed the studies according to 1) study design, methods and setting 2) definition, incidence and evaluation of ADRs and 3) clinical presentation, drug classifications and risk factors. The observed study periods ranged from 1 day to 11 years. 84 studies were carried out prospectively, 14 retrospectively, two used both approaches and for one study

this information was not obtainable. Studies investigated ADRs in community settings (n=35), in patients admitted to hospital (n=53) or as cause of admission to hospital (n=42), with several studies using combined settings. 62 studies used a clinician, i.e. a medical doctor, nurse or pharmacist, to identify ADRs and 30 studies included children and/or carer(s) in the identification process. Methods for detection of ADRs varied considerably. 58 studies used a combination of different sources such as parental/patient/clinician interviews, case note reviews, computerised reporting systems or attendance ward round. 41 used only a single source and for one study there was no information given.

Overall, a large proportion of the studies examined by Smyth et al. did not report data on seriousness, causality and avoidability of ADRs in a paediatric population: 71 studies commented on causality assessments but only 36 studies reported complete causality data. 34 studies reported a severity assessment, however, 14 of these did not provide details of the classification system used. Severity of ADRs was assessed in 34 studies, with 0-67% of ADRs reported as severe. Usually ADRs considered serious under ICH criteria (1.4.2) were reported as severe. Only 14 of 101 studies provided avoidability data and this might well reflect the lack of suitable preventability assessment tools. The proportion of ADRs classified as possible or definitely avoidable ranged widely from 7% to 98%.

36 of 51 studies investigating ADRs in hospitalised children provided incidence rates, ranging from 0.6% to 16.8% of patients as shown in Figure **1.1**.

Figure 1.1 ADR incidence in hospitalised paediatric patients taken from Smyth et al. 2014



1.6 Pharmacogenomics of ADRs

Regardless of definitions, study design and reporting practices, it is obvious that only a proportion of all patients receiving medication experience ADRs. By identifying the influencing factors for this person-to-person variability, it may be possible to ameliorate or even avoid ADRs all together. Contributing factors are non-genetic, such as environmental factors, age, gender, co-morbidities, drug-drug interactions and other clinical factors or genetic. The '*study of variability in drug response due to heredity*' was first called pharmacogenetics (Nebert 1999). Later, the broader term pharmacogenomics was introduced, with both terms often being used interchangeably in the literature (Pirmohamed 2001). Pharmacogenomics can be defined as '*the study of the variability of the expression of individual genes relevant to the disease susceptibility as well as drug response at cellular individual or population level'(European Medicines Agency 2002)*.

Classical examples of the clinical importance of pharmacogenomics are the discovery of pseudocholinesterase as cause of prolonged muscle relaxation (Kalow & Gunn 1959) and drug-induced haemolytic anaemia in patients with Glucose-6-phosphate-dehydrogenase (G-6-PD) deficiency (Carson et al. 1956). The identification of these variants started with the observation of a distinct ADR, a 'phenotype', occurring only in a percentage of patients receiving the drug. In the case of G6PD deficiency, it had been noted that haemolysis occurred in about 10% of individuals of African origin but only rarely in those of Caucasian origin. This lead to the hypothesis of an intrinsic, inherited cause for this ADR and subsequently to the discovery of the enzyme, G6PD.

1.6.1 Genetic variants

The most common inherited genetic variants are polymorphisms. A polymorphism, that is the occurrence of two or more alleles at one locus, originates from a mutation. In its simplest and most common form this is a single base mutation, where one nucleotide (A, G, C or T) has been exchanged by another. If such a mutation occurs in 1% or more of the population, it is then called a 'single nucleotide polymorphism' (SNP) (Schork, Fallin & Lanchbury 2000). SNPs occur approximately every 300 nucleotides. Considering that there are 3 billion nucleotides in the human genome (The International Human Genome Sequencing Consortium 2010), there are an estimated 10 million SNPs. It is estimated that less than 1% of these are found within exons and hence may or may not affect the amino-acid (AA) sequence and the resulting protein (Sachidanandam et al. 2001). However, SNPs do not have

to change the AA sequence of a protein to lead to increased susceptibility to a reaction or disease (Sparsø et al. 2009; Xiong et al. 2009).

1.6.2 Candidate gene approach and genome wide studies

Many pharmacogenomic discoveries have been made by candidate gene approaches. Typically, this approach is used for predictable (from known pharmacology) and dose-dependent ADRs. The DNA of patients who develop a certain ADR (cases) and unaffected patients (controls) is tested for genetic variants (mutations or SNPs) in genes known to be involved in the pathway of the drug (e.g. receptors, transporters, enzymes, or channel proteins). One of the limitations of a candidate gene based approach is, that ADRs which are unpredictable and do not exhibit a simple dose-response relationship, also called idiosyncratic reactions, can only be tested for genetic variants that are already known (and associated with the same type of reaction). To identify new associations, much larger, ideally genome-wide approaches need to be employed. This has been facilitated through advances in genetic sequencing techniques and the development of genetic microarrays. A well-characterised phenotype is crucial for genome wide association studies (GWAS), which allows more than one million of SNPs to be tested simultaneously for association with the observed ADR. The results are then compared to the SNP frequencies in DNA samples of unaffected individuals (controls) or against the results of publicly available databases such as the HapMap project (The International HapMap Consortium). The GWAS approach is bound to generate a multitude of false positive results, therefore associations below a certain p-value threshold are generally considered significant and associations above this p-value of are considered false positives (Becquemont 2009). Currently p-values of $\leq 5x\ 10^{-8}$ are used as significance level for replication (Hoggart et al. 2008) and corrected p-values of $\geq 1x10^{-7}$are generally not considered significant (Becquemont 2009). However, this is based on the assumption that the study is sufficiently powered to detect the association between SNP and ADR in the first place. Sample size calculations for GWASs are based on assumptions regarding the prevalence of the expressed phenotype, the minor allele frequency (MAF), the model of inheritance (dominant, recessive, additive etc.), the effect size of the actual genetic variant (expressed as relative risk or odds ratio) and error rates (Gail et al. 2008; Pfeiffer & Gail 2003; Spencer et al. 2009). Furthermore, linkage disequilibrium (LD) between SNPs has to be taken into account (Spencer et al. 2009). Any errors in the study size calculation leading to an under-powered study, may lead to true genotype-phenotype associations not being detected as they remain 'hidden' within a large group of false positive results. GWAS

approaches are therefore limited in their power to detect rare genetic variants if the effect size is small (Spencer et al. 2009).

1.6.3 Genotype-phenotype relationship

Genotype-phenotype relationships are mostly complex. Of several patients carrying the same genetic variant, some might develop the ADR and others might not. Conversely, within a group of patients without the genetic variant, some might still develop the same ADR. Even if an ADR has been shown to be associated with a particular genotype, carrying this genotype usually only increases the likelihood of developing the ADR.

Differences in drug response and/or the occurrence of an ADR, can often be related to various pharmacokinetic and/or pharmacodymanic factors and therefore several genetic as well as non-genetic factors are influential. Depending on how many other factors influence the occurrence of the ADR or drug-response and how much these contribute each, the effect size of a given genetic variant may be small or large.

The example of primaquine-induced acute haemolytic anaemia in individuals with G6PD deficiency illustrates some of the aspects that can influence a given genotype-phenotype relationship:

- One phenotype might be associated with several different genotypes. Example: G6PD deficiency leads to reduced enzymatic activity of G6PD and may be caused by one of many genetic variants (Hoffbrand & Moss 2011)
- Different genetic variants of the same gene may lead to variation in phenotype. Example: The degree of activity reduction in G6PD can vary depending on the genotype with some forms causing milder forms of haemolysis
- Ethnicity may account for or at least contribute to observed differences in drug response. Example: the prevalence of G6PD deficiency is much higher in individuals of South-East Asian, African, and Middle-Eastern origin (Hoffbrand & Moss 2011)
- The reaction (phenotype) may be dose-dependent phenotype. Example: The degree of haemolysis can vary depending on the drug dose given.
- The same drug given to the same patient might not produce the same reaction every time (variable phenotype): Example: G6PD activity decreases with time, even in 'normal' patients (red cell aging). A large proportion of young red cells in the peripheral blood e.g. once the patient is recovering from an episode of acute haemolysis, can therefore give the appearance of falsely high G6PD activity (Hoffbrand & Moss 2011). In addition,

if the patient is exposed to the drug again at this stage, haemolysis can be expected to be less severe.

- Other drugs in the same drug class as well as drugs from other drug classes may cause the same reaction - Example: chloroquine can also cause haemolyis (Youngster et al. 2010) as well as sulphonamides or rasburicase (Youngster et al. 2010).
- Factors other than medication may produce the same phenotype and could potentially lead to misclassification. Example: Acute illnesses often cause haemolysis independent of drug exposure (Youngster et al. 2010).

Given the variety of influencing factors, some of which are listed above, it is unsurprising, that is has often proven difficult to replicate findings of both, candidate gene approaches and GWAS (Siontis, Patsopoulos & Ioannidis 2010). Defining accurate phenotypes remains the most crucial prerequisite of pharmacogenomic studies (Gurwitz & Pirmohamed 2010).

1.6.4 Pharmacogenomics of ADRs in children

An added challenge in the paediatric population is 'ontogeny', the process of growth and development of an individual from embryo to mature adult. As much as children change in their appearance from infancy to adulthood, there are age related differences in body proportions and composition, functional body system and organ functions as well as individual enzymes, transporters and carriers, all of which can affect drug metabolism. Although the individual DNA sequence remains fixed, the expression of genes can vary with age. A well-known example is the cytochrome P450 (CYP) superfamily of oxidative enzymes. In addition to variable inter-individual expression of in CYP enzymes, there is significant age dependent differences in expression of some CYP genes. CYP enzymes are predominantly found in the liver and total the amount of cytochrome P450 in the fetal liver is about 30-60% of that observed in adults (Hines & McCarver 2002). Although fetal liver contains fewer CYP forms, some CYP genes are only found in the fetal liver (Hakkola et al. 1994). Some CYP genes are expressed at low levels in the fetal liver and expression increases shortly after birth, the expression of other CYP genes increase gradually over time and reaches adult levels around puberty (Hines & McCarver 2002). CYP1A2 activity , for example, is nearly undetectable in fetal liver, increases after the neonatal period and reaches about 50% of adult activity after the first year of live (Dorne, Walton & Renwick 2001). Caffeine and Theophylline metabolism for example is predominantly driven by hepatic CYP1A2. Therefore, neonates, are at much higher risk of caffeine toxicity (Dorne, Walton & Renwick 2001).

There numerous additional examples to illustrated that PK data for children cannot simply be extrapolated from adult data and that the lack of available data is likely to put children at higher risk of toxicity as well as treatment failure. In addition, some diseases e.g. Kawasaki's disease, and some forms of cancer e.g. neuroblastoma, occur predominantly in children whereas other disease are exclusively found in adults such as Alzheimer's disease or Parkinson's disease.

Paediatric specific pharmacogenomic studies are therefore required to improve overall drug safety and efficacy in children.

1.6.5 Pharmacogenomics and paediatric oncology

One area of paediatric medicine with particularly high rates of serious ADRs is paediatric oncology medicine. Cytotoxic drugs account for over 40% of ADRs causing hospital admission (Gallagher et al. 2012) and 40% of cancer survivors have experienced a life-threating ADR or sustained permanent harm from an ADR (Geenen et al. 2007). Numbers are on the rise as survival rates of childhood cancer have improved significantly over the last few decades. The overall 5 year survival rates for children between 1 and 14 years increased from around 60 % in 1975 - 1978 to over 80% in 1999-2002 (Smith et al. 2010) . In 2005 24% of all survivors of childhood cancer had survived more than 30 years (Mariotto et al. 2009) and ADRs are contributing significantly to long term morbidity and reduced life expectancy (Geenen et al. 2007) in this patients. Drug specific examples for serious cytotoxic ADRs in paediatric cancer patients are anthracycline induced cardiotoxicity (Kremer et al. 2002) which may require heart-transplantation; cisplatin induced irreversible ototoxicity (Skinner et al. 1990), which may lead to significant lifelong disability (Berg, Spitzer & Garvin Jr 1999); and vincristine induced peripheral neuropathy , which may even be fatal (Tarlaci 2008).

On the other hand, paediatric oncology is an area of paediatric medicine in which pharmacogenomic research has successfully been translated into clinical practice: 6-Mercaptopurine (6-MP), used in the treatment of all children with acute lymphoblastic leukaemia (ALL), is metabolised by TPMT. Approximately 0.3% of the population is TPMT deficient (Weinshilboum & Sladek 1980), which leads to severe reduction in TPMT enzyme activity ('slow metabolisers'). The resulting accumulation of 6-MP can cause severe myelosuppression (Lennard et al. 1993; McLeod et al. 2000) and maybe prevented by reducing the dose to 10% of the standard 6-MP dose. Genetic testing to assess the TPMT

activity status for each patients was introduced in the UKALL 2003 protocol (Medical Research Council 2009). Noticeably, there is a 'translational time lag' of over 20 years between discovery of inherited TPMT deficiency and introduction of routine genetic testing prior to treatment with 6-MP or other thiopurines.

1.7 Aims and objectives of the thesis

Having highlighted the lack of reliable data with regards to ADRs in hospitalised children and having described the emerging role of pharmacogenomics in reducing the occurrence of serious ADRs specific to the paediatric population, the aim of my thesis was to contribute to improved drug safety in paediatric patients by

- Determining the incidence of ADRs in hospitalised children and identifying risk factors for the occurrence of ADRs in this population. Characterising those ADRs identified in terms of causality, reaction types and medication implicated.
- Assessing the contribution of ADRs in hospitalised children to duration of hospital stay in order to estimate the economic burden of ADRs in children.
- Examining the association between described genetic variants and cisplatin induced hearing loss in a UK paediatric cohort, an important example of a high impact ADRs.

2 Incidence, characteristics and risk factors of Adverse Drug Reactions in hospitalised children – a prospective observational cohort study of 6601 admissions

2.1 Introduction

Reducing the impact of paediatric ADRs needs precise estimates of the incidence and nature of ADRs. A recent systematic review of 102 studies of ADRs in children by Smyth et al. (Smyth et al. 2012) showed that previous studies have differed widely in their definition of ADRs, clinical settings, and age range of children studied and a high proportion had major shortcomings in design and/or reporting. A large proportion did not report data on incidence, severity and causality of ADRs or drugs and reaction types implicated. Study sizes for the 21 prospective paediatric inpatient studies ranged from 81 to 3726 patients – 3/21 of these studies were large (n>1000). Reported incidence rates for hospitalised children experiencing an ADR ranged from 0.6% to 16.8%. A recent prospective analysis of 3695 patient-episodes in adults (Davies et al. 2009) reported an ADR incidence rate of 14.7% with estimated rates in earlier studies ranging from 0.86% (Simmons, Georgeson & Hill 1998) to 37% (Van Kraaij et al. 1994) depending on study population, design and setting.

Data on the drugs associated with ADRs were only available in 52 of 102 studies investigated in the systematic review by Smyth et al. and many did not report the associated clinical presentations. Although 70% of studies analysed in this systematic review referred to a causality assessment, less than one third reported this in detail. Of 34 studies which assessed the severity of ADRs, only 20 provided a reference for the assessment tool used, with proportions of severe reactions reported from 0 to 66.7%. Only 14 studies provided data on avoidability of ADRs and outcomes differed widely, with 7-98% of ADRs deemed definitely/possibly avoidable. Furthermore, few studies to date have investigated risk factors for ADRs in children. In the systematic review, female gender (10/19 studies), increasing number of drugs (16/17 studies), off-label use (3/3 studies), and oncological treatment (2 studies) were identifiable risk factors (Bellis et al. 2013; Smyth et al. 2012). The methodology of this study aimed to avoid these shortcomings and provide robust findings.

2.2 Aim

The aim of this study was to determine the incidence of ADRs in paediatric medical and surgical inpatients, to characterise those ADRs identified in terms of type, medication implicated, causality, and severity, and to identify factors which increase the risk of ADRs.

2.3 Methods

2.3.1 Development of methods and author's contribution

This study was part of the wider ADRIC programme, planned by Prof Sir Munir Pirmohamed, Prof Anthony J Nunn, Prof Rosalind L Smyth, Prof Paula R Williamson, Prof Matthew Peak and Dr Mark A Turner who were also the senior investigators of this study.

The contribution of the author of this thesis (ST) to this study, was to develop a workable methodology for identification, assessment and evaluation of ADRs. Methods based on those used by Gallagher et al. (Gallagher et al. 2012) and Davies et al. (Davies et al. 2009) were piloted. The most significant finding was time constraint. With three investigators, the same number as used by Gallagher et al. for their study, it was not possible to collect the target data identified by the senior investigators, and hence first attempts at conducting a pilot study had to be abandoned early, after incomplete data collection. This was somewhat surprising but could be partly explained by the following significant differences between this and the two studies cited above. In this study, ADRs were identified throughout the stay, in Gallagher et al.'s study only at one single time point, the time of admission. In contrast to Davies et al., patients in this study, who were admitted over the weekend were included and medication data were collected for all patients thought the stay rather than only for patients with ADRs with a random control sample of 1:10.

To understand the impact and duration of the different steps involved, such as collection of patient data, collection of medication data, ADR identification, ADR evaluation, the author of this thesis conducted a step-by-step analysis with special consideration of the time involved. This analysis identified that the evaluation of suspected ADRs was one of the most time-consuming steps and could often only be completed after discharge. It was therefore decided to separate this step from the daily collection of data and prospective identification of ADRs by allocating study team members (research pharmacist, research nurse or the author of this thesis in regular rotation) to either the task of compiling ADR case reports or

collecting patient data and identifying potential ADRs as outlined below. Another outcome of the step-by-step analysis was that the study team felt, that there was a lot of redundancy of clinical information. When the author of this thesis analysed this aspect further in a sample of 24 suspected ADRs, it became apparent, that only a 4 of 26 suspected ADRs were identified from scrutinising the medical notes but these reactions were also identified from prescription chart review and/or review of the nursing notes. The majority of suspected ADRs, that is 21 of 26, were identified from the nursing notes, more than half, 14 of 26, from the prescription charts and 12 of 26 from review of observation charts. It was therefore decided to limit the time-consuming step of reviewing of medical notes as outlined below.

Furthermore, the author of this thesis identified the exclusion of PICU as a necessary adaption for the final methodology. The implications of these steps are discussed under limitations. Primarily, the assessment of ADRs occurring in a PICU setting is far more complex and therefore requires different methodologies for detection compared to the rest of the study. Ideally, paediatric intensive care specialists – clinicians, pharmacists and nurses – should be part of the research team identifying and assessing ADRs in this environment. Secondarily the assessment of ADRs in patients admitted to PICU is more time consuming, owing to the inevitable complexity of the clinical background and management.

The author of this thesis then also established that neither the Hallas' avoidability tool as used by Gallagher et al., nor any other available avoidability tool was suitable for this study and avoidability could therefore not be assessed. The implications of this step are discussed under limitations.

In addition to the above, the author of this thesis was part of the study team that collected patient data, medication data and ADR details prospectively over one year as outlined below. From a practical point of view this meant daily visits to the hospital wards, in order to review and enter data prospectively into the study database using study laptops on. The author also compiled ADR case reports, assessed the causality of every ADR case (process outline below), oversaw the MeDRA coding and assessed all ADRs that had occurred prior to a patient being transferred to PICU or HDU, to evaluate their contribution to the patient being transferred to a higher level of care. Furthermore, the author of this thesis worked with the team of statisticians to provide the clinical context for the analysis as outlined below.

2.3.1 Study design and setting

This was a prospective observational cohort study conducted over one year in a single secondary and tertiary paediatric referral centre, Alder Hey Children's NHS Foundation Trust, which treats 200,000 children a year from the North West of England, North Wales, Shropshire and the Isle of Man. The Accident and Emergency department treats over 60,000 children every year. There are 274 inpatient beds, including PICU. Although neonates were included in the study, the hospital does not have a designated neonatal intensive care unit (NICU), since a NICU exists in a nearby tertiary maternity centre. Any neonates requiring surgical management (including cardiac surgery) are transferred to Alder Hey, and cared for on PICU (if ventilated), the cardiac ward, or on the neonatal ward (surgical patients not requiring ventilation).

2.3.2 Participants

The study population comprised children aged between 0 and 16 years 11 months on admission, who were inpatients between 1st October 2009 and 30th September 2010. Extensive pilot work before the study established that the study team did not have the resources to carry out a detailed review of every inpatient every day. In 2008, a total of 39,747 inpatient admissions were recorded (emergency admissions, elective admissions and day-case attendances); of those 10,943 stayed longer than 24 hrs and 5357 stayed longer than 48 hours. A pragmatic decision was thus made to include only those children who had been inpatients for >48 hours. Admissions included in this study were elective and emergency admissions of all paediatric medical and paediatric surgical specialities. Observations were carried out on 17 wards, including oncology wards and the high dependency unit (HDU). Patients were not observed whilst admitted to the paediatric intensive care unit (PICU), theatre, recovery or the department of radiology.

2.3.3 Participant selection and data collection

The established hospital database Meditech (MEDITECH 3.0 Health Care Information Systems (HCIS), Westwood, USA) was used for the recruitment of patients to the study. Electronic files containing a list of all children in the study population who met the inclusion criteria were automatically generated every 12 hours.

2.3.5 Data collection and handling

Patients were identified by name, hospital number and admission number. Data were entered directly into the study database created for this study. The study database was stored securely on the Trust server. For the purpose for statistical analysis an anonymised copy of the database was created after completion of data entry. At study entry, the data set outlined in Table 2.1 was recorded.

Table 2.1 Dataset recorded for each patient at study entry with format and source of data

Data	Source	Format of entry into study data base
Age*	Meditech	Automatic download**
Gender	Meditech	Automatic download**
Weight (kg)	Prescription chart, Observation chart, A&E documentation	Manual entry
Surface area (SA) m^2	Prescription chart, medical notes	Manual entry
Drug allergies	Prescription chart(s), Medical notes, nursing notes	Not known (tick box) or free text
Date of Admission	Meditech	Automatic download*
Reason for admission	Medical notes, nursing notes	Free text entry
Past medical History	Medical notes, nursing notes	Free text entry
Medication History	Medical notes, Prescription chart(s)	Drug name, route, dose, frequency (drop down menu)
Current Medication	Prescription chart(s), Emergency department document	Drug name, route, dose, frequency (drop down menu)

*Data files containing a list of all children in the study population who met the inclusion criteria were automatically generated every 12 hours and imported into the study database. The files contained patient name, hospital number, admission number, DoB, age and date of admission.
** Age was given as mm/dd up to one year, as yy/mm up to 6 years and as years from 6 years.

Children who did not receive any medication during their admission were highlighted in the database (tick box "no medication") to avoid misclassification. Any days of admission to PICU or HDU were also recorded in the study database. After discharge, patients with an oncology or haemato-oncology diagnosis and on-going medical treatment were highlighted through an additional entry in the database. Patients who had been admitted to theatre and had undergone general anaesthesia (GA) were identified through the hospital database Meditech and the date of admission to theatre was transferred into the study database through file import. For the duration of the hospital stay and within the study period, each child was followed up every 48 or 72 hours on weekdays and weekends respectively by one member of a multidisciplinary team of researchers comprising two research pharmacists, one research nurse, and a paediatrician (ST).

2.3.5.1 Inclusion and exclusion of medicines

Medicines were included in this study if they were prescribed and administration was recorded on the medical prescription sheet, the anaesthetic charts, the emergency department document, the prescription sheet for post-operative pain or the prescription sheet for opioids on the oncology ward. Suspected reactions to certain blood products, total parental nutrition and intravenous hydration fluids were excluded from this study (Table 2.3).

2.3.5.2 Data of medicinal products and suspected ADRs

At each visit the following details were recorded for all medication administered on the ward: Drug name, route of administration, dose and daily frequency. In addition, children were reviewed for occurrence of new symptoms or those that had worsened and for abnormal results, that may have indicated the occurrence of an ADR, taking into account the case history, the ADR profiles of medication and the temporal relationship between drug exposure and reaction. Data reviewed to detect new or worsening symptoms or abnormal results are summarised below.

Table 2.2 Data used to identify reactions suspected to be an ADR and their sources

Type of data	Source
Medical notes*	Handwritten paper records
Emergency department document**	Handwritten paper records
Nursing notes	Electronic (Meditech)
Prescription charts including specialist prescription charts such as pain charts and chemotherapy prescription charts	Handwritten paper records
Clinical charts e.g. observation chart, fluid balance chart	Handwritten paper records
Care pathways e.g. for children admitted with diabetic ketoacidosis or asthma; post-operative pathways	Handwritten paper records
Imaging results	Electronic report (Meditech)
Laboratory results	Electronic reports (Meditech)

*Medical notes were only reviewed at study entry and to assess suspected ADRs.

** If the patient had been admitted via the accident and emergency department.

Key reference used, were the Summary of Product Characteristics (SmPC) in the electronic Medicines Compendium (eMC)(DataPharm Communications Ltd. 2010) or the British National Formulary for Children (BNFC) (Paediatric Formulary Committee 2008/2009). Reactions suspected to be an ADR were highlighted and then followed up separately (2.3.7). This two-step approach was taken because detailed ADR assessments are more time consuming and relevant information (e.g. results of investigations, response to withdrawal

or reduction of medicine) was often only available at time of or after discharge. Some highlighted reactions could not be assessed because of missing or conflicting information, for example if the nursing notes stated bowels opened but the clinical chart for the same day stated bowels not opened.

Members of the study team, consisting of research nurses (KB and HM), research pharmacists (JB, LB and JC) and a paediatrician (ST) took it in turns to collect data and follow up ADRs. However, KB left the study team and was replaced by HM. JB and ST were part of the team throughout and LB and JC joined the study team once data collection had commenced.

2.3.6 ADR definition

In this study the following definition of Edwards and Aronson (Diez 1998; Edwards & Aronson 2000) was used: An ADR is "an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dose regimen, or withdrawal of the product." Prescribing or administration errors as well as accidental or deliberate overdoses were thus not considered ADRs in this study.

ADR cases in this study were defined as suspected reactions to any systemic or topical medicinal product administered in hospital and presenting after admission to the ward or in the accident and emergency department prior to admission to the ward. This included reactions to medicinal products administered in PICU, theatre, recovery, the department of radiology, provided the reaction became apparent after transfer to a ward. Reactions to a medicinal product had been started prior to admission were included if (1) it was continued in hospital and (2) the reaction was not apparent on admission

.

Table 2.3 List of drug groups or non-medicinal products which were not considered in this study (excluded) and exceptions (included) with rationale.

	Excluded	Included	Rationale
Topical anaesthetics	Lidocaine 2·5%, prilocaine 2·5% cream (EMLA®) or tetracaine 4% gel (Ametop®)	LAT gel (lidocaine 4% & adrenaline 0·1% & tetracaine 0·5% gel)	Topical anaesthetics were not always prescribed on the medication prescription charts.
Ranitidine	Ranitidine added to TPN.	Ranitidine administered otherwise	Ranitidine added to TPN is not prescribed on the regular medication prescription chart.
Heparin	Heparin administered as intermittent intravenous heparin flush.	Intermittent intravenous injection other than heparin flush, heparin administered as continuous intravenous infusion or as subcutaneous injection.	Administration of Heparin administered as intermittent iv heparin flush was not reliably recorded.
Total parenteral nutrition (TPN)	Total parenteral nutrition (TPN)	N/A	The individualised nature of TPN and iv fluid prescribing means that a large amount of additional data would need be collected for every patient.
Intravenous hydration fluids	Intravenous hydration fluids.	Any drugs added to intravenous fluids	
Rectal washouts	Rectal washouts with Sodium Chloride 0·9%.	N/A	Administration was not reliably recorded.
Blood products	Red cells Platelets Cryoprecipitate Albumin solutions Fresh Frozen Plasma	Antithrombin III Concentrate; Dried Prothrombin Complex; Drotrecogin Alfa (activated); Factor VIIa (recombinant) Factor VIII Fraction, dried; Factor VIII Inhibitor ; By-passing Fraction; Factor IX Fraction, Dried; Factor XIII Fraction, Dried; Protein C Concentrate	The excluded products are not medicines. They are obtained from the transfusion service. The included products are listed in the BNF and some were under intensive surveillance by the MHRA
Oxygen therapy	Oxygen therapy	N/A	Oxygen is not prescribed. It would have been difficult to obtain and record data of the amount of oxygen administered

2.3.7 Assessment of ADRs including causality

Each reaction suspected to be an ADR was followed up with an assessment by one research team member who compiled an ADR case report. This report included a detailed description of the suspected reaction and its time frame, results of any relevant investigations, details of the clinical management and the outcome for the patient.

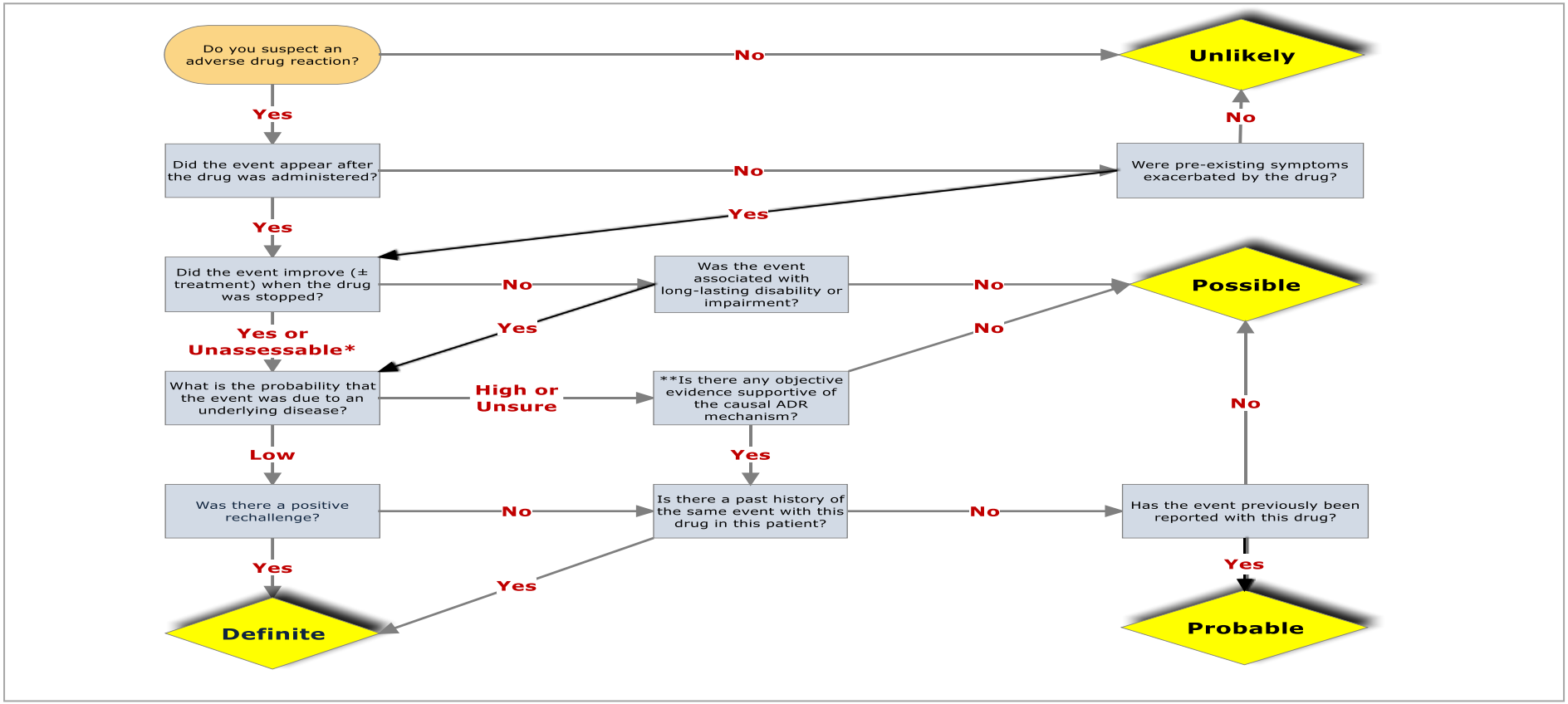
2.3.7.1 Causality assessment

Each ADR case report was then assessed independently by (1) a research nurse, (2) a research pharmacist and (3) a paediatrician (ST) using the Liverpool adverse drug reaction causality assessment tool (Gallagher et al. 2011) as unlikely, possible, probable or definite . Outcome reporting was based on consensus agreement between the three assessors, if agreement could not be achieved, the case was referred to a panel of two senior investigators who reached a joint consensus about the causality outcome. For ADRs with a high or uncertain probability that the reaction is due to an underlying disease, the causality outcome is “possible” unless objective evidence of the causal ADR mechanism is available. For the analysis of results and incidence calculation we considered probable and definite ADRs as they were deemed to have a low probability of the underlying disease causing the reaction.

2.3.7.2 Coding of reaction type

To standardise reaction types and facilitate analysis of results, all possible, probable and definite ADRs were coded using the Medical Dictionary for Regulatory Activities terminology (MedDRA). MedDRA is the international medical terminology developed under the auspices of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (International Federation of Pharmaceutical Manufacturers and Associations (IFPMA), Geneva, Switzerland).

Figure 2.1 Liverpool ADR causality tool taken from Gallagher et al. 2011



*Unassessable refers to situations where the medicine is administered on one occasion (e.g. Vaccine), the patient receives intermittent therapy (e.g. Chemotherapy), or is on medication which cannot be stopped (e.g. Immunosuppressants). **Examples of objective evidence: positive laboratory investigations, supra-therapeutic drug levels, good evidence of dose-dependent relationship with toxicity in the patient

2.3.7.3 Assessment of severity

Severity of ADRs was assessed by the researcher compiling the case report using a modified form of the Hartwig scale (Hartwig, Siegel & Schneider 1992). In addition, all ADRs that occurred prior to a patient's admission to PICU or HDU were also assessed by a paediatrician (ST) and, if required, reviewed by a panel of two senior investigators in order to evaluate their contribution to the patient being transferred to a higher level of care (Hartwig level 4). Reactions classified as Level 4 and above were considered severe.

Table 2.4 Modified Hartwig ADR Severity Assessment Scale (Hartwig, Siegel & Schneider 1992)

Severity level	Level definition
1	Required no change in treatment
2	Drug dosing or frequency changed
3	Required treatment, or drug administration discontinued
4	Result in patient transfer to higher level of care
5	Caused permanent harm to patient or significant haemodynamic instability
6	Directly or indirectly resulted in patient death

2.3.7.4 Contribution to duration of stay

ADRs were assessed for their potential contribution to the duration of stay. This is presented and discussed in chapter 3.

2.3.8 Incidence

The incidence of ADRs was calculated to describe (1) the burden of inpatient ADRs to the hospital (incidence) and (2) the burden of paediatric inpatient ADRs to the patient (incidence per child). It was calculated by dividing the number of admissions with at least one ADR by the total number of admissions regardless of drug exposure and the number of children with at least one ADR by the total number of children respectively.

2.3.9 Outcomes

The observed outcome was the occurrence of an ADR. For the risk factor analysis time from admission to first ADR was calculated in days. For patients admitted to PICU this was time to first ADR prior to PICU admission.

ADRs occurring after discharge from PICU were included in the overall count of events, but time from admission to PICU was censored for the risk factor analysis.

2.3.10 Risk factors

Age, gender, number of medicines, admission to theatre and oncology patient status were assessed as risk factors. Age on admission (in years) was treated as a continuous variable and gender as a categorical variable. Both were treated as time-invariant risk factors in the multivariate model. The number of medicines count refers to the daily number of medicines administered to the patient on the ward. As this risk factor was counted daily throughout the admission period, it was treated as a continuous, time-varying covariate in the multivariate model. The factor "admission to theatre" was considered to be present from day of (first) undergoing a general anaesthetic (GA) until discharge from hospital. This risk factor was treated as a binary, time-varying variable in the multivariate model that takes the value zero on days up to an admission to theatre and unity thereafter for the remaining days of a patient's admission. Oncology patient status, had emerged as a significant risk factor for ADRs leading to hospital admission and was therefore considered as risk factor in this study. It was considered a binary, time invariant factor to distinguish oncology and non-oncology patients.

Off-label and unlicensed medicine use in this study population was investigated and analysed separately (Bellis et al. 2013)

2.3.11 Statistical methods

The analysis plan for for this study was designed by JJK, EC, LC and PW. Statistical analysis was carried out by EC, who used the statistical software package R (version 2.13.2). The role of the author of this thesis was to provide the clinical context for example by explaining the data collection process and limitations, to ensure data quality and finally to provide clinical interpretation of the data.

A time-to-event model of analysis model was chosen to allow inclusion of patients into the analysis, who were admitted to PICU during their hospital stay, where ADRs were not observed. Time-to-event analysis models allow this through inclusion of censored data.

A two-sided significance level of 0.05 (5%) was used throughout the analysis.

A univariate analysis on data collected for each patient during their first admission was used to assess the affected patient population. A univariate analysis of the risk factors was performed to explore the effect that patient characteristics has on the likelihood of an ADR. Kaplan Meier (KM) curves were produced for each level of a categorical prognostic factor and ADR-free survival data compared between groups using a log-rank test (extending to a log rank test for trend when appropriate). However, comparison of KM curves can only conclude that that a difference between survival curves exists. In contrast, a regression model of analysis allows to measure the risk arising from individual clinical factors. The most commonly used multivariate approach for this is the Cox regression model, which relies on the assumption of proportional hazards between survival curves. Both a single event and a multi event Cox proportional hazards regression model was fitted to the data. Results for both models are given in terms of the hazard ratio (HR) together with accompanying 95% confidence intervals (95% CI). Due to their clinical importance, all the risk factor variables are included in both models.

The proportional hazards assumption for each covariate was investigated using log cumulative hazard plots and Schoenfeld residual plots. The assumption was also tested for significant violations through incorporation of a time-dependent covariate.

Deviance residuals were plotted against the linear predictor to look for mis-modelling of the data and empirical validation of the model was done using a data splitting technique to assess model accuracy.

2.3.12 Missing data

Patients with missing prescription details for the entire duration of the admission could not be assessed for ADRs and were therefore withdrawn from the study. Patients with partially missing prescription details (e.g. prescription details for day of discharge) or missing clinical observations were assessed on a case-by-case basis by the research team and a rationale for inclusion or withdrawal was recorded for each case. Patients were only included if it was considered unlikely that the missing data would have led to missing an ADR. Furthermore, any potentially missed ADRs towards the end of the stay are unlikely to have had an impact on the risk factor analysis as time to first ADR was the observed outcome.

2.3.14 Potential sources of bias

Investigations were based on clinical indication and observations dependent on documentation by the clinical team. Hypertension could e.g. only be observed if a child's blood pressure was monitored for clinical reasons.

Only ADRs observed between 1st October 2009 and 30th September 2010 were recorded. Patients admitted between 28th and 30th September 2009 or discharged after 30th September 2009 who experienced an ADR before 1st October 2009 or after 30th September 2010 were counted as admissions without ADR in this study. Consequently, there are 180 admissions that lie outside the observation period where an ADR may have occurred that has not been recorded.

2.3.15 Ethical considerations

This study used routinely collected clinical data in an anonymised format. The Chair of Liverpool Paediatric Local Research Ethics Committee (LREC) therefore declared that this study did not require individual patient consent or review by an Ethics Committee.

2.3.16 Funding

This study was funded by the National Institute of Health Research (NIHR) as part of a research programme grant awarded to the University of Liverpool and Alder Hey Children's Hospital to investigate Adverse Drug Reactions in Children (ADRIC).

2.4 Results

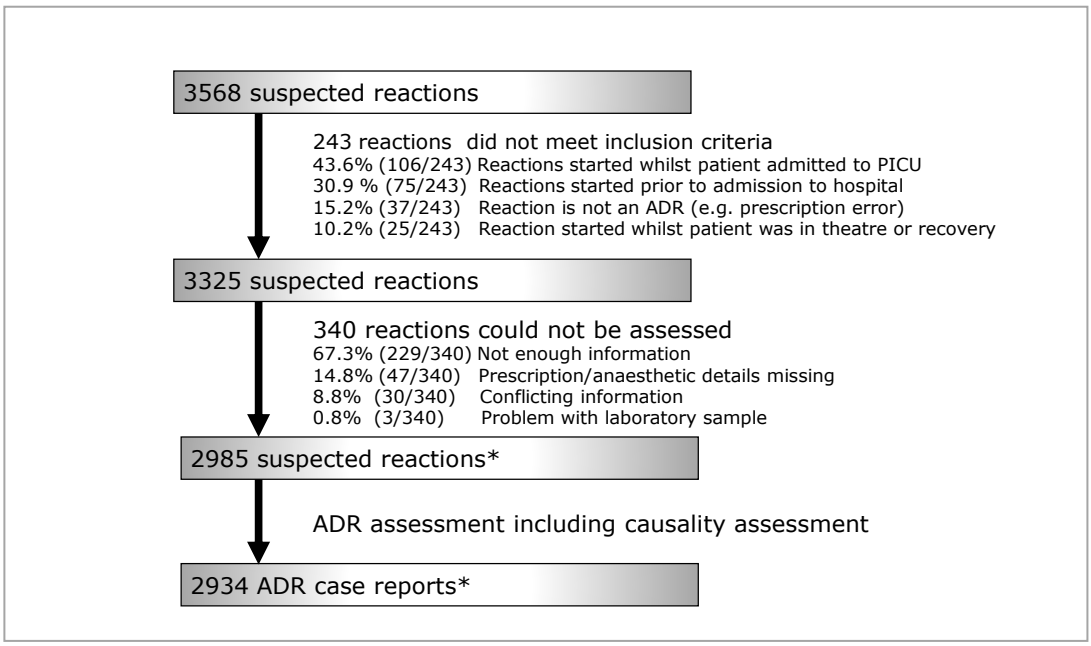
2.4.1 Participants and descriptive data

6825 eligible admissions were identified. 181 (2.7%) admissions could not be included due to missing data. Forty-three patients spent their entire admission on PICU and were thus excluded. Consequently 6601 admissions of 5118 children were included in the study; of these, 827 were also admitted to PICU with 45.2% being cardiology or cardiothoracic patients. The median duration of follow up time across admissions was 5 days (IQR 3.8 days, range 2 - 280 days). The median age on admission was 3.4 years (IQR 0.6-10.7); 2297 (44.8%) were female. 4284 (83.7%) of children had one admission and 834 children had more than one admission. 2856 children (55.8%) underwent at least one GA during 3265 admissions (49.4%); 114 children (2.2%) were oncology patients. 126 children (2.4%) did not receive any medicines during 150 admissions (2.3%). In 98.7% of admissions with medicine, more than one medicine was administered and 98.5% of children who received medicines during their admission(s), received more than one medicine in at least one admission (4919/4992). The median daily number of medicines administered was 3 (IQR 1-5).

2.4.2 Causality and severity of ADRs

3568 suspected reactions were assessed and a total of 2934 ADR case reports completed. The review process is outlined in Figure 2.2. After causality assessment, 213 (7.3%) of the suspected ADRs were deemed definite, 1233 (42.0%) probable, 896 (30.5%) possible, and 592 (20.2%) unlikely. Consensus was reached independently in 1805 cases (61.5%) and by panel decision in 1128 cases (38.5%). All definite and probable ADRs were included in the further analysis (total number 1446). 0.8% of ADRs were severe and required patient transfer to a higher level of care. One patient sustained permanent harm (peripheral neuropathy due to vincristine). No ADR resulted in death. Details of all severe reactions by reaction type and associated drugs are listed in Table 2.6.

Figure 2.2 Summary of review process of reactions highlighted during patient visits



*Different suspected reaction types highlighted in the same patient due to the same medication(s) would have been reported as one ADR case e.g. A patient with respiratory depression and bradycardia = 2 suspected reactions highlighted and assessed in one ADR case report

Table 2.5 Assessment of Severity using a modified Hartwig scale (2.3.7.3)

Severity level	Description	Number of ADRs at each severity level*	
		n	%
1	Required no change in treatment	322	22.3%
2	Drug dosing or frequency changed	66	4.6%
3	Required treatment, or drug administration discontinued	1046	72.3%
4	Resulted in patient transfer to higher level of care	12	0.8%
5	Caused permanent harm to patient or significant haemodynamic instability	1	0.1%
6	Directly or indirectly resulted in patient death	0	0%

*Denominator was the total number of probable or definite ADRs

Table 2.6 Severe reactions (Hartwig scale ≥ 4) by reaction type and medication implicated

Severity level	ADR Type (count)	Medication implicated (count)	Admission to PICU or HDU (count)
4	Cardiac failure (1)	Bisoprolol (1), Carvedilol (1)	HDU (1)
	Sedation withdrawal (1)	Fentanyl (1), Midazolam (1), Promethazine (1) , Chloral hydrate (1)	PICU (1)
	Raised INR and haemorrhage (1)	Warfarin (1)	HDU (1)
	Pulmonary oedema (1)	Diazoxide (1)	HDU (1)
	Respiratory depression (5)	Fentanyl (4), Ketamine (2), Midazolam (1),	PICU (3)*, HDU (2)
	Respiratory arrest (2)	Fentanyl (2), Sevoflurane (1), Isoflurane (1), Ketamine (1)	PICU (1), HDU (1)
5	Peripheral neuropathy (1)	Vincristine (1)	N/A

* ADR was not the only factor leading to PICU admission; other, clinical factors may also have contributed

2.4.3 Incidence and Risk factor analysis

The overall incidence of definite and probable ADRs based on admissions was 15.9% (95% CI 15.0-16.8), and 17.7% when based on numbers of patients (95% CI 16.7-18.8). The ADR incidence for patients with only one admission was 14.8% (95% CI 13.7-15.9). For patients with more than one admission, the incidence per admission was 18.0% (95% CI 13.7-15.9) but 32.7% per patient (95% CI 29.6-35.9).

Only first admissions were included in the univariate and multivariate analysis (Figure 2.3). Results of the univariate analysis are shown in

Table 2.7. Multivariate risk factor analysis of first admissions is shown in Table 2.8 and indicated that the risk of an ADR was associated with a GA, more than one medicine, being an oncology patient and age.

Figure 2.3 Flowchart outlining the number of admissions included in the univariate and multivariate risk factor analysis

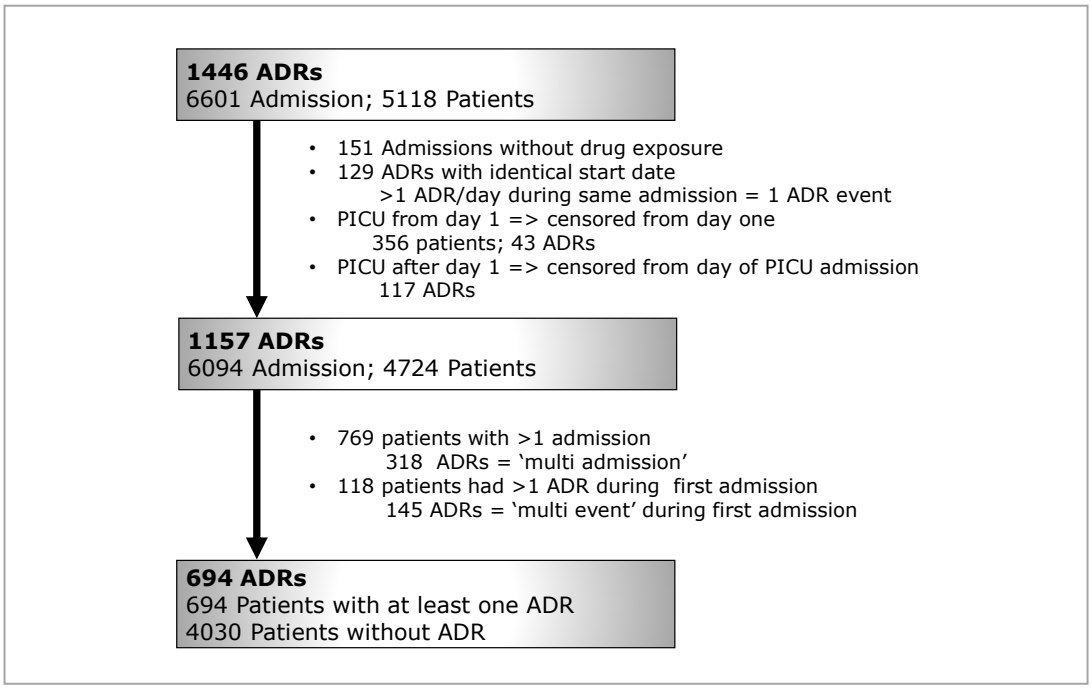


Table 2.7 Univariate analysis by categorical time invariant risk factor

Covariate		N of Patients (%) (total N = 4724)	N of patients with ADR* (%)	p-value
Gender	Male	2602 (55.1%)	382 (14.7%)	0.900
	Female	2122 (44.9%)	312 (14.7%)	
Age on admission	<1 year	1369 (29.0%)	78 (5.7%)	<0.001
	1-5 years	1259 (26.6%)	155 (12.3%)	
	5-11 years	1105 (23.4%)	231 (20.9%)	
	>11 years	991 (21.0%)	230 (23.2%)	
Oncology	Yes	103 (2.2%)	45 (43.7%)	<0.001
	No	4621 (97.8%)	649 (14.4%)	

* Only the first ADR was included in this analysis

Table 2.8 Risk factors for ADRs assessed by multivariate analysis

Covariate		HR	95% CI	p-value
Gender	Female	1		0.301
	Male	0.93	(0.8-1.08)	
Age on admission *		1.06	(1.04-1.07	<0.001
Number of drugs		1.25	(1.22-1.28)	<0.001
Received a GA	No	1		<0.001
	Yes	6.38	(5.30-7.68)	
Oncology	No	1		<0.001
	Yes	1.89	(1.36-2.63	

HR = hazard ratio; CI = confidence interval ; * in years

2.4.4 Reaction types, drug classes implicated in ADRs

Reaction types are listed in Table **2.9**. The three most common reaction types together (nausea and/or vomiting, pruritus and constipation) accounted for 54.8% of all ADRs. Drug classes implicated in ADRs are listed in Table 2.10. The three most common drug classes were opioid analgesics, drugs used during GA and cytotoxic drugs and together accounted for 67.1% of all drugs implicated in ADRs.

Table 2.9 Common ADR types observed

Reaction type	All reactions (n=1457)		Reaction following GA** (n=845)	
	N	%	N	%
Nausea and/or vomiting	400	27.5%	295	73.8%
Pruritus	243	16.7%	232	95.5%
Constipation	155	10.6%	107	69.0%
Diarrhoea (9/88 with vomiting)	88	6.0%	0	0.0%
Somnolence (without cardio-respiratory symptoms)	50	3.4%	34	68.0%
Respiratory depression (41)/ arrest (3)	44	3.0%	43	97.7%
Candidiasis	41	2.8%	0	0.0%
Urinary retention	40	2.7%	37	92.5%
Rash	31	2.1%	3	9.7%
Hypokalaemia	25	1.7%	0	0.0%
Hypotension	22	1.5%	9	40.9%
Hepatotoxicity (in 12/18 transaminases increased only)	18	1.2%	1	5.6%
Stomatitis	16	1.1%	0	0.0%
Myoclonus	15	1.0%	14	93.3%
Pancytopenia	13	<1%	0	0.0%
Hyperglycaemia	12	<1%	0	0.0%
Hypertension	11	<1%	2	18.2%
Allergic reactions	10	<1%	3	30.0%
Pain (4/10 pain in jaw, 2/10 back pain)	10	<1%	0	0.0%
Other reactions (occurred < 10 times)	213	14.6%	65	30.5%

* If the same patient experienced 2 types of reactions to the same medication(s) at the same time this would have been reported as one ADR case but will be listed here as 2 reaction types e.g. A patient with respiratory depression and bradycardia = one ADR case, but listed as two reactions. **Reaction occurred post theatre AND drugs given in theatre and/or used in post-operative pain management were implicated.

Table 2.10 Drug groups implicated in ADRs by frequency with associated reaction types

Drug Group (N of ADR cases)	Total N of drugs (% of total)	Drugs (N)	ADR type* (N)
Opioid analgesics (688)	844 (27·9%)	Morphine (426), Fentanyl (267), Codeine (144), Dihydrocodeine (4), Diamorphine (2), Tramadol (1)	Pruritus (198), Nausea or Vomiting (186), Constipation (143) Respiratory arrest/ depression (3/37), Somnolence without cardio-respiratory symptoms (37), Urinary retention (28), Myoclonus (13), Hallucination (8), Rash (4), Bradycardia (3), Dizziness (3), Drug withdrawal syndrome (3), Ileus (3), Agitation (2), Delayed recovery from anaesthesia (2), Flushing (2), Visual disturbance (2), other ** (11)
Drugs used in GA (322) [excluding opiate analgesics other than remifentanyl]	779 (25·8%)	Sevoflurane (253), Propofol (200), Nitrous oxide (131), Remifentanil (83), Desflurane (54), Isoflurane (38), Ketamine (6), Atracurium (4), Rocuronium (4), Thiopental (4), Atropine (1), Vecuronium (1)	Nausea or vomiting (266), Urinary retention (21), Respiratory arrest or depression (2/6), Delayed recovery from anaesthesia (5), Flushing (4), Bradycardia (3), Allergic reaction (3), Hypotension(3), Pruritus (2), other ** (7)
Cytotoxic drugs and drugs used for cytotoxic induced side effects (179)	405 (13·4%)	Vincristine (70), Etoposide (56), Cyclophosphamide (46), Cytarabine (41), Methotrexate (31), Doxorubicin (22), Ifosfamide (21), Mesna (15), Daunorubicin (13), Carboplatin (12), Cisplatin (12), Melphalan (11), Busulfan (7), Asparaginase (6), Fludarabine (6), Clofarabine (5), Actinomycin D(5), Allopurinol (4), Mitoxantrone (4), Rasburicase (4), Idarubicin (3), Thiotepa (3), Amsacrine (2), Temozolomide (2), Cladribine (1), Gemcitabine (1), Irinotecan (1), Tretinoin (1)	Nausea or vomiting (81), Stomatitis (16), Pancytopenia (13), Diarrhoea and vomiting (9), Diarrhoea without vomiting (9), Hepatotoxicity (11; 8 increased transaminases only), Febrile neutropenia (6), Rash (6), Pain in jaw (3), Constipation (3), Pain other than jaw (2), Headache (2), Hyperglycaemia (3), Oral candidiasis (3), other **(14)
Antibiotics (162)	319 (10·6%)	Cefotaxime (56), Metronidazole (29), Gentamicin (29), Piperacillin and Tazobactam (28), Cefuroxime (18), Teicoplanin (19), Cefalexin (17), Ciprofloxacin (16), Flucloxacillin (15), Co-amoxiclav (16), Ceftazidime (13), Rifampicin (10), Amoxicillin (8), Clarithromycin (7), Vancomycin (7), Penicillin V (5), Benzylpenicillin (4), Meropenem (4), Amikacin (3), Co-trimoxazole (3), Tobramycin (3), Trimethoprim (3), Clindamycin (2), Cefradine (1) , Ceftriaxone (1)	Diarrhoea (66), Candidiasis (38) Rash (16), Nausea or vomiting (8), Clostridium difficile colitis (7), Colonisation with Candida (4), Transaminases increased (4), Anaphylactic reaction (2), Angioedema (2), Flushing (2), Hepatotoxicity (3), Pruritus (2), other** (8)

Drug Group (N of ADR cases)	Total N of drugs (% of total)	Drugs (N)	ADR type* (N)
Drugs used in epidurals, regional anaesthetics and IV drugs used in post- operative pain management other than opioids (188)	195 (6·4%)	Fentanyl & Levobupivicaine (116), Ketamine (36), Clonidine & Levobupivicaine (25), Levobupivacaine (11), Clonidine (7)	Pruritus (52), Nausea and/or Vomiting (35), Constipation (24), Urinary retention (15), Somnolence without cardio-respiratory symptoms (11), Respiratory depression/arrest (8/1), Hypotension (7), Paraesthesia (6), Bradycardia (4), Myoclonus (3), Hypoaesthesia (2), Visual disturbance (2), Hallucination (2), Hypertension (2), Urinary incontinence (2), other **(12)
Corticosteroids (51)	62 (2·05%)	Dexamethasone (24), Methylprednisolone (14), Prednisolone (14), Hydrocortisone (8), Beclomethasone (1), Fludrocortisone (1)	Hyperglycaemia (13), Hypertension (8), Candidiasis (9), Fluid retention (2), Gastritis (2), other** (17)
Bronchodilators (31)	58 (1·92%)	Salbutamol (35), Aminophylline (21), Ipratropium (2)	Hypokalaemia (15), Nausea and/or vomiting (7), Tremor (4), Tachycardia (2), other ** (3)
Antiemetics (50)	55 (1·82%)	Ondansetron (51), Levomepromazine (3), Cyclizine (1)	Constipation (45), Disorientation (2), other** (4)
Antiepileptic drugs (45)	49 (1·62%)	Midazolam (35), Pregabalin (4), Carbamazepine (3), Diazepam (3) Gabapentin (2), Lorazepam (1) Valproate (1)	Nausea and/or vomiting (24), Somnolence without cardio-respiratory symptoms (6), Abnormal behaviour (2), Constipation (2), Delayed recovery from anaesthesia (2), Respiratory depression (2), other** (7)
Diuretics (28)	41 (1·36%)	Furosemide (30), Spironolactone (8), Metolazone (2), Chlorothiazide (1)	Hyponatraemia (9), Hypokalaemia (8), Hypotension (3), Hypomagnesaemia (4), other** (5)
Drugs affecting the immune respones (supression and modulation) + cytokine modulators (31)	34 (1·12%)	Alemtuzumab (11), Ciclosporin (7) Adesleukin (5), Rabbit anti-human thymocyte immunoglobulin (3), Tacrolimus (3), Rituximab (2), Azathioprine (1), Mycophenolate (1) Tocilizumab (1)	Pyrexia (4), Candidiasis (4), Infusion associated reaction (3), Stomatitis (3), Oedema (2), Pruritus (2), Vomiting (2), other** (11)
Drugs affecting the cardiovascular system (23)	27 (0·89%)	Captopril (10), Lisinopril (4), Amlodipine (4), Milrinone (3), Bisoprolol (1), Dinoprostone (1), Enalapril (1), Hydralazine (1), Isoprenaline (1), Carvedilol (1)	Hypotension (11), Hyperglycaemia and Glycosuria (3), other**(9)

Drug Group (N of ADR cases)	Total N of drugs (% of total)	Drugs (N)	ADR type* (N)
NSAIDS (+ aspirin) (24)	24 (0·79%)	Diclofenac (15), Ibuprofen (5), Naproxen (2), Aspirin (2)	Nausea and/or vomiting (11), Haematemesis (3), Other gastrointestinal bleed (2), Constipation (2), other** (5)
Laxatives (20)	22 (0·73%)	Lactulose (12), Macrogol (6), Docusate (3), Sennoside (1)	Diarrhoea (17), Abdominal pain (2) Vomiting (1)
Antifungals and Antivirals (20)	21 (0·69%)	Amphotericin (7), Aciclovir (5), Fluconazole (4), Voriconazole(2), Itraconazole (1), Miconazole (1), Ribivarin (1)	Diarrhoea (8), Hepatotoxicity (3), Hypokalaemia (3), other** (5)
Drugs used in diabetes and hypoglycaemia (13)	16 (0·53%)	Insulin (4), Insulin aspart (4), Insulin detemir (4), Diazoxide (3), Glucagon (1)	Hypoglycaemia (7), Fluid overload (2), Hypokalaemia (2), other **
Other (69)	73 (2·41%)	-	-

* If the same patient experienced 2 types of reaction to the same medication at the same time this would have been reported as one ADR case but will be listed here as 2 ADR types e.g. patient with respiratory depression and bradycardia = one ADR case but listed as two types. ** Reactions that occurred once are listed as other

2.5 Discussion

To our knowledge, this is the largest, prospective paediatric in-hospital study investigating ADRs. The study population represents a wide range of paediatric medical and surgical specialities, given the nature of the hospital as a regional centre. The methodology included causality and severity assessments using validated tools. An avoidability assessment was not undertaken because of the lack of appropriate tools and imperfect definitions of preventability as highlighted recently by Ferner and Aronson (Ferner & Aronson 2010). The most frequently used assessment tools were Schumock and Thornton (Schumock & Thornton 1992) and Hallas (Hallas et al. 1990) which are based on appropriateness of prescribing or treatment choice. These tools might be used successfully to improve prescribing practice in specific clinical circumstances. However, they become problematic when treatment is guided by multiple sources of tertiary paediatric specialist advice such as in this study.

2.5.1 Incidence

In a prospective, observational, paediatric, multicentre cohort study of 1278 patients (1340 admissions), Rashed at al. reported an overall ADR incidence of 16.5% (95% CI, 14.5-18.7%) per patient (Rashed et al. 2012). Davies et al. conducted a comparable study in adults and observed an incidence of 14.7% per episode (admission) and 15.8% per patient (Davies et al. 2009). However, both Rashed and Davies used the Naranjo algorithm (Naranjo, Busto & Sellers 1981) for causality assessment and included possible, probable and define ADRs in their calculations. In this analysis, only probable and definite ADRs were included, as these have a low probability of the underlying disease, or other co-morbidities, causing the reaction. Had possible ADRs been included, the overall ADR incidence rate would have been more than 25% per child (data not shown) which is much higher than that reported in adults. One possible explanation is, that many common medicines have not been tested properly or at all in infants and children.

2.5.2 Severity

Although less than 1% of reactions in this study were classified as severe, this does not take into account what impact an ADR might have on the child and/or carer. For instance, a teenage patient is likely to feel very distressed about having to be catheterised because of urinary retention or receive an enema to treat constipation. The most common reaction in

our study was vomiting, mainly observed in post-operative patients. Vomiting is a common and non-specific symptom in children and thus unlikely to be regarded as being particularly significant by clinicians. However, Diez reported that parents placed a very high value on the distress caused by post-operative vomiting (Diez 1998) In addition, parents of children included in this study reported that suspected ADRs cause them concern, irrespective of the "medical" severity of the suspected reaction. On the other hand, parents of children treated on the oncology unit valued the proactive explanations of ADRs given by clinicians, suggesting that this aspect should form part of the preoperative discussion (Arnott et al. 2012).

2.5.3 Risk factors for ADRs

This study confirmed similar risk factors for ADRs to those reported previously, including increasing age, oncology treatment, and number of drugs (Gallagher et al. 2012; Rashed et al. 2011) . It is not entirely clear why ADR risk increased with age, but is likely to be due to many factors including lack of detection and the inability of a younger child to effectively communicate their symptoms (e.g. nausea, pain, hallucinations); acceptance of some common clinical manifestations such as vomiting and loose stools as being "normal" in younger children; and reaction types such as pruritus being mistaken for "unsettledness" in an infant.

Most previous paediatric inpatient studies were carried out in general paediatric settings (Smyth et al. 2012) in which only a small number of patients, if any, will have undergone GAs thus underrepresenting drugs used in paediatric peri- and post-operative management. Rashed et al. who conducted their multicentre study on general medical wards, reported that anaesthetics, which accounted for only 1% of all prescriptions, were amongst the drugs most commonly implicated in ADRs (Rashed et al. 2011). In the two previous inpatient studies investigating paediatric surgical patients and providing medication details, opiate analgesics were amongst the two most commonly implicated drugs. However, data on drugs used in GAs were not reported, perhaps because they were not specifically investigated (Farrokhi et al. 2009; Turner et al. 1999). The differences in this study population, which included a large number of surgical patients (but not those admitted to PICU immediately post-operatively), are also reflected in the spectrum and severity of common reaction types observed. Some reaction types such as urinary retention and respiratory depression/arrest occurred almost exclusively following GA. Eight of the 12 reactions classified as severe in this

study occurred in post-operative patients and led to transfer to HDU or PICU (Table 2.6). Notably, the risk of experiencing an ADR in patients undergoing a procedure under GA has not been assessed previously.

2.5.4 Type of reactions and drug groups implicated

The most commonly implicated drug groups were opioid analgesics and drugs used in GA, which together comprised 53.7% of all drugs implicated in ADRs in this study. Pruritus, respiratory depression, and urinary retention occurred almost exclusively in the post-anaesthetic setting. In over two-thirds of patients with nausea/vomiting, constipation, or somnolence, which together were contributing 41.5% of all reactions, drugs given during the anaesthetic and/or used in post-operative pain management were implicated. Morphine is one the most commonly used opioids in perioperative pain management in children, but there is significant inter-individual variability in analgesic response. This is partly explained by clinical factors such as age, gender, type and duration of surgery. However, there are also known ethic differences such higher frequency of opioid related ADRs in children of Caucasian origin (Sadhasivam et al. 2012) and there is an emerging body of evidence that genetic variability may explain differences in pharmacokinetics of morphine and other opioids in children (Fukuda et al. 2013; Venkatasubramanian et al. 2014) as well as the risk of morphine induced ADRs (Chidambaran et al. 2015; Chidambaran et al. 2016). Although these are very interesting findings, they are still a long way from becoming clinically relevant in the management of ADRs. The question at hand is, whether and how the post-operatively occurring reactions, could have been prevented. A focussed assessment of the preventability/avoidability of post-operative reactions by reaction type would provide much needed information in this area. Post-operative nausea and vomiting (PONV) alone comprised 20% of all ADRs investigated in the analysis. At the time this study was conducted, national guidelines for the prevention of post-operative vomiting (POV) in children had been published (Carr et al. 2009). The recommendation was to target children at high risk of POV. Prophylactic treatment for all children was considered “probably unnecessary, as it is financially costly and may results in excessive ADRs”(Carr et al. 2009). It would be interesting to assess, whether these guidelines were already followed in the post-operative management in this study. If not, a follow up study could ascertain whether an implementation of these guidelines has been more effective in preventing PONV. Carr et al. also highlight the lack of research focussing “on the children’s perspective of POV, and whether they perceive this symptom with the same distress and loathing as adults” (Carr et

al. 2009) This is true not only for POV but also for other ADRs. Some of the most common reactions, constipation, pruritus and urinary retention are likely to be experienced as distressing by the patient and further investigation in this area is warranted.

2.5.5 Limitations

The observational approach depends on documentation by the clinical team regarding signs and symptoms and is thus a limitation of this study. A further limitation was the exclusion of the PICU setting, which is likely to have led to a lower overall ADR incidence as the incidence of ADRs on PICU is likely to be higher than in a ward setting. Silva et al. reported 110 proven, probable or possible adverse events, using the Naranjo algorithm, in 84 of 239 PICU patients (35.1%) (Silva et al. 2013). Their case definition did however not exclude drug or administration errors. The decision to exclude PICU from this study was deliberate as the assessment of ADRs occurring in a PICU setting is more difficult and requires different methodologies for detection. Ideally, paediatric intensive care specialists – clinicians, pharmacists and nurses – should be part of the research team identifying and assessing ADRs in this environment.

The methodology was geared towards detecting known, previously described ADRs. It is therefore possible that novel ADRs were missed in this study. It is also possible that novel ADRs were detected but categorised as unlikely or possible. Furthermore ADRs with delayed onset, occurring after discharge from hospital or only developing slowly, for example after recurrent exposure, would not have been regularly identified in this study. Examples for these types of reactions are peripheral neuropathy induced by vincristine and ototoxicity induced by cisplatin. In addition, despite intense surveillance, it is possible that some detectable ADRs occurring during the inpatient stay, were missed. Any ADRs occurring in patients admitted for less than 48hrs would not have been included in this study, although these were probably the less serious ADRs. Similarly ADRs requiring readmission to hospital would not have been captured, however, we have studied the latter category in a separate study of ADRs which required admission to hospital (Gallagher et al. 2012).

It is unsurprising, that oncology treatment was confirmed as a significant risk factor for ADRs in this study, considering the significant toxicity many chemotherapeutic agents produce. However, immunosuppressant drugs like methotrexate or cyclophosphamide, which were commonly implicated in ADRs caused by cytotoxic drugs (Table 2.10) are also frequently

used in rheumatology treatment. This and other “speciality treatment” was not investigated as separate risk factor and is a limitation to the risk factor analysis.

Finally, differentiating symptoms due to an underlying condition from those caused by drugs (e.g. tachycardia in patients being treated for acute asthma) remains a challenge.

2.6 Conclusions

The data of this study show that 17.7% of all children who spent more than 48 hours as an in-patient experienced at least one ADR. This is a higher incidence than in adults, but it is likely that the true incidence was underestimated as “possible” and “unlikely” ADRs were excluded. 58% of ADRs observed in our study occurred in patients undergoing a procedure under GA, which increased the risk of developing an ADR by more than six times. Drugs used in perioperative management appear to be a major risk factor for experiencing an ADR, thus systematic monitoring of common and severe adverse effects of these drug groups would be an important step towards improving their safety. Advances in pharmacogenomics could improve drug safety of perioperative pain management further. ADRs may also be an important problem in children who are discharged home shortly after surgery. Given the ongoing strategies to increase the proportion of children having day case surgery (Koenig & Gu 2013; NHS Modernisation Agency 2004), this warrants further investigation.

2.7 Acknowledgements

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3 Estimating the financial burden of ADRs - assessment of the contribution of ADRs to the duration of hospital stay in children

3.1 Background

The economic burden of ADRs to society is significant. The annual cost of ADR related admissions for the NHS has been estimated to be £466 Million (Pirmohamed et al. 2004). For inpatients, considering excess bed days due to ADRs alone, the financial impact for adult patients in the UK has been estimated to be £5000 per bed per year, which would amount to at least £2 Million per year for a 400-bed hospital (Davies et al. 2009). Similar estimates have been given in studies in France and the US (Bates et al. 1997; Moore et al. 1998)

Economic impact may consider direct and indirect costs. Direct costs arise for example from hospital admissions due to ADRs, extension of hospital stay, additional investigations, additional treatment (including invasive and non-invasive procedures), involvement of additional clinical expertise or transfer to higher level of care and indirect costs from disability, loss of earnings or absence from studies, litigation costs and repeated hospital visits. Many of these costs are difficult to quantify, particularly in large, population based studies. Estimates of the financial impact of ADRs have therefore mainly been based on pragmatic approaches and data that are easily available (Gautier et al. 2003). Cost calculations for ADRs causing hospital admissions are relatively straight forward, as the entire duration of the hospital stay can be used for cost calculations. On the other hand, for ADRs occurring after admission to hospital, only costs that are directly attributable to the ADR can be included in any estimates. The most informative but also most resource intensive approach of making these estimates is a detailed case review with patient specific data collection ('microcosting') (Jackson 2000). A more readily available proxy measure is calculating the excess bed days due to ADRs, often using a matched case-control study design. However, without adjustment for confounding variables such as comorbidities, number of drugs etc., this approach is likely to become inaccurate (Rodríguez-Monguió, Otero & Rovira 2003). Alternatively, excess bed days can be assessed on a case-by-case basis, relying on professional judgement. Excess bed days are then translated into cost by using average bed-day cost estimates ('per diem' cost). Diagnosis related approaches have also been used, for example using international classification of diseases (ICD) or diagnosis-related group (DGR) coding, however without consideration of contributing clinical factors

this approach can also become problematic as the overall cost difference for the same diagnosis may be significant. For example, a patient who develops drug-induced acute renal failure may recover quickly and fully within a few days, whereas a different patient developing renal failure after being exposed to the same drugs may require weeks to recover fully or may even develop chronic renal failure.

3.1.1 Overview of literature investigating the financial impact of ADRs in hospitalised patients

Rodriguez-Monguio et al. reviewed studies investigating the financial impact of ADEs and found that most studies have been carried out in acute care settings in the US and are mainly considering direct costs for the hospital (Rodríguez-Monguió, Otero & Rovira 2003). They also highlighted that due to methodological differences studies may not allow comparison. Concerning the financial impact of ADEs in hospitalised patients, they identified 7 studies, all of which were based in US hospitals. Although some of these studies included paediatric patients, paediatric specific data on cost implications were not available from the original publications. In addition, data were given for ADEs and not ADRs (1.3.2 and 1.3.3). More recently Davies et al. have provided an estimate of attributable duration of stay from ADRs in adult inpatients (above) (Davies et al. 2009). In contrast to most of the US based studies employing a matched case control study design, they assessed ADRs on a case-by-case basis, taking into account clinical and social information such as underlying disease, additional medication, awaiting placement in nursing home etc. Clinical judgment was used to make the final decision.

There is a lack of paediatric studies assessing the financial impact of ADRs. Although several paediatric inpatient ADR studies (dos Santos & Coelho 2006; González-Martin, Caroca & Paris 1998; Martínez-Mir et al. 1999; Weiss et al. 2002) and one including both adult and paediatric patients (Gholami & Shalviri 1999) have identified a longer duration of stay as a risk factor for ADRs, few have attempted to describe whether the ADRs detected are actually contributing to the increased duration of stay. Weiss et al. reported that 5 of the 68 ADRs (7.8%) they identified in 214 inpatients were responsible for the prolongation of the hospital stay (Weiss et al. 2002). No details were provided on how they assessed the cases and reached this conclusion. In a prospective study of 1339 paediatric admissions, Haffner et al. found that children with ADR stayed longer in hospital (median stay 6 days vs. 4 days without ADR). However, they were unable to adjust for other clinical factors and excess bed days

attributable to ADRs were therefore uncertain (Haffner et al. 2005). Martinez-Mir et al. found that children with ADR stayed longer in hospital than those without (average 4.66 days longer; total number of admission n= 40) (Martínez-Mir et al. 1999). However, children with ADR in this study had also been exposed to a larger number of medications, which is a known risk factor for ADRs 2.5.3 and may also be an indicator for severity and/or complexity of the underlying medical condition(s).

3.1.2 Assessment of financial impact of ADRs in this study

Given the considerable financial burden of inpatient ADRs in adults and the paucity of equivalent data in the paediatric population, a cost estimation to assess the economic burden of ADRs in hospitalised children was included in this study. Employing a similar approach as Davies et al., a duration of stay assessment was included in each ADR assessment as part of the study methodology. The researcher completing the detailed ADR case report in the study database (2.3.7) answered the following questions: 'Did the ADR prolong the hospital stay (yes/no/possible); if yes, for how long (in days)?' Preliminary analysis of the results indicated that only 1.24% (18/1446) of ADR cases led to a prolonged hospital stay. Given the significant difference to Weiss et al. (3.1.1) and to adult data, a study using a similar study design and assessment methods, it was possible that this had been an underestimate and that the methodology used had not captured all ADR cases that prolonged the hospital stay.

3.1.3 Objective and Aims

The aim of this additional study was therefore re-assessing excess bed days attributable to ADRs in a paediatric inpatient population by

- developing an alternative methodology to assess the contribution of ADRs towards prolonged hospital stay.
- assessing the feasibility of this methodology in a pilot study and identifying any modifications needed.
- re-assessing all ADR cases with the alternative methodology.

3.2 Methodology

This study was planned and conducted by ST, the author of this thesis. ST also developed the methodology, contributed to data collection during all stages (see also 3.2.4) and analysed the results.

3.2.1 Stage 1a – Evaluating methods of assessment of duration of stay

3.2.1.1 Matched case control study

One advantage of using a matched case control study design is, that it is not dependant on the knowledge and experience of the assessor thus avoiding potential bias introduced by using clinical judgement. However, when comparing duration of stay in patients with and without ADR, the result is likely going to overestimate the contribution of ADRs unless the chosen matching criteria include a wide variety of confounding factors (Rodríguez-Monguió, Otero & Rovira 2003). During the design stage of the study reported in the chapter the risk factor analysis (2.3.11) had not yet been completed and it was difficult to know which criteria should be used to match cases and controls. Even if the outcome of the risk factor analysis had been known, vital clinical parameters were not available in a format that would easily allow them to be used as part of the matching process. Data such as underlying diagnosis, comorbidities, and type of procedure (for patients who had undergone a GA) had been collected in 'free text' format. It would have been very time consuming to standardise this information e.g. by using MedDRA terminology or International Classification of Diseases (ICD) coding. For many cases, it would have been difficult to find an appropriate matched control e.g. a post-operative cardiac patient with one or several ADRs may have an unexpectedly prolonged stay due to post-operative complications and it is hard to imagine that a patient with similar age, sex, and underlying diagnosis would have undergone a near similar procedure and developed the same complications. In addition, there was a significant number of patients with multiple ADRs in this study, which would have complicated the matching process further. In summary, a matched case control study was not deemed suitable for the given study design and study population.

3.2.1.2 Expected discharge versus actual discharge date

Upon admission to hospital, each patient receives a nursing assessment which is entered into the hospital database. Within this assessment, an expected discharge date is entered.

The feasibility of using these data to estimate excess bed days in all or perhaps at least a specific group of patients, e.g. patients admitted for well defined, elective procedures, was therefore explored through informal interviews with senior nursing staff with expertise in the care of orthopaedic, ENT, cardio-thoracic and general medical patients. For all patient groups, setting of the expected discharge date was based on the discretion and therefore individual experience of the nurse entering the patient data into the hospital database. There were no guidelines or protocols for this process and the approach was therefore also not deemed suitable for assessment of excess bed days.

3.2.1.3 Case-by-case assessment

Clearly, this approach had already been used for this study and potentially underestimated the burden. At the same time, it had been successfully used by Davies et al. in a similar sized single centre, adult, UK based study. Their assessment of whether an ADR directly led to an increase in duration of stay, was based on the clinical features of ADR and the underlying medical condition(s). The process included discussion with the medical team and the ward pharmacist and a review of relevant medical notes. Our methodology did not explicitly state how the duration of stay assessment should be made and there were no guidelines or requirements to discuss cases with the medical team or ward pharmacists. Although the medical notes had been reviewed for all ADR cases (2.3.7; Table 2.2), this might not have provided enough information for the researcher compiling the ADR case report to make the duration of stay assessment. Whereas in adult medicine patients are generally highlighted as 'ready for discharge' as soon as they judged to be medically fit for discharge, paediatric patients are generally considered ready for discharge when everyone involved in the child's care is happy for his/her discharge. For example, it is not unusual for a child to stay an additional night for parental reassurance. It was therefore possible that cases where the ADR did prolong the stay had been misclassified.

Due to the large number of different medical teams involved it would not have been feasible for this study to revisit each ADR and discuss each case with the clinical team involved. Bearing in mind that most ADRs are likely not to prolong duration of stay, the possibility of reducing the number of ADR cases that would have to be re-assessed by reliably excluding those ADRs that did not prolong the stay, was explored.

3.2.2 Stage 1b – Defining criteria for exclusion of ADRs that did not prolong duration of stay

3.2.2.1 Exclusion by end date of ADR

A pragmatic 'cut-off' of 2 days or more was chosen, as it is unlikely that an ADR ending 2 days before discharge or more, would prolong the hospital stay. For ADRs ending less than 2 days before discharge, there is a possibility that this might have influenced the hospital stay, e.g. for a child with vomiting, the clinical team and/or the parents might have wanted to have an additional period of observation to be certain that the child was tolerating diet and fluids prior to discharge.

3.2.2.2 Exclusion by reaction type

All reaction types were considered and only 3 reaction types were identified that very likely would not have prolonged the hospital stay:

- Topical candida infections
- Pruritus due to opioids
- Agitation/Tremor/Tachycardia due to salbutamol or aminophylline, drugs used in the treatment of acute exacerbations of asthma

Topical candida infections, as associated with use of systemic antibiotics, can be treated in the community and would not delay discharge. Duration of opioid treatment is dependent on other factors. Once opioids are discontinued the pruritus resolves and will therefore not delay patient discharge. Similarly, Asthma treatment is dependent on clinical factors. Once the patient improves clinically, the treatment can be reduced and the symptoms normalise or resolve and will therefore not delay patient discharge.

For all other reaction types, it could not be ruled out that the reaction did not delay discharge.

3.2.3 Stage 2 – Pilot of proposed methodology

The most time limiting factor for re-assessment was going to be access to the medical notes. These had to be requested through the hospital's medical records department, only a limited number of medical notes could be requested per day and clinical requests would of course always take priority. One aim of the pilot study was therefore to assess which data sources would be required to re-assess all ADR cases (Table 2.1) by estimating how many cases could be assessed by revisiting the database entry and/or electronically available data and for how many case reports medical notes would have to be requested.

First, the case summary stored the study database would be re-visited, then, if required, the hospital database and finally, if still more information was required to reach a decision, the medical notes would be reviewed. At each step the possible outcome for extended hospital stay was Yes/No/Unsure. Each assessor was asked to record where they found the relevant information (study database/hospital database/medical notes). A list of ADRs by ADR identification number (ADRID) was compiled. An ADRID was assigned automatically, by the study database, each time a new ADR case report is opened but ADRs had not necessarily been assessed in chronological order. At the time of this pilot study not all ADR assessments had been assigned a final causality outcome (2.3.7.1), therefore only those ADRs who had received their final causality outcome were included. Furthermore, the analysis plan had not yet been finalised and therefore ADRs with the outcome possible, probable and definite where included. After exclusion of those ADRs, deemed unlikely to have prolonged the hospital as per criteria above (3.2.2), every 3rd ADR was considered for assessment during the pilot study. ADRs that had already been identified as having prolonged the hospital stay using the original methodology, were not included. The following instructions for assessment were also given to the research team:

The aim of this assessment is to either confidently rule out that the ADR has caused prolonged the hospital stay or to highlight that the ADR has prolonged the stay and by how many days. There will be a considerable number of cases where we are unsure. For each level of assessment (i.e. 1. study database 2. hospital database 3. medical notes) please record one of the following 3 outcomes Yes/No/Unsure

- ***Yes:*** *If it is not possible to determine by how many days the stay was prolonged please make a note of 1) whether it would be possible to give a range e.g. 1-2 days 2) whether you think that we need to discuss this with a clinician to determine this.*

- ***No**: We can only confidently answer NO if there is indication that the patient needed to stay for other reasons. Therefore, please record the rationale behind making this decision. We need to be very careful in making this decision based on what information is available. It might well be that although a patient with constipation opened his/her bowels the day before discharge he/she was not discharged because of indirect reasons related to that (transport, time of day, take home medication, unavailability of medical staff to make decision etc.).*
- ***Unsure:** Record this outcome if you can't confidently say yes or no based on the information available. Continue with next level of assessment.*

3.2.4 Stage 3 – Re-assessment of contribution of ADRs to duration of stay

After assessing feasibility and time frame of the proposed methodology during the pilot study, a new section was added to the study database, allowing the team to record the outcome of the re-assessment(s). Only ADRs that did not meet exclusion criteria 3.2.2 were assessed. In addition, for ADRs that had been originally identified as prolonging stay the time scale were also re-assessed, as the original outcome had been recorded in a different section of the database. A rationale had to be recorded for each outcome decision, e.g. LOS prolonged? No - stayed to complete chemotherapy. Duration of stay was recorded in days. If the discharge was delayed but the patient was still discharged within the same 24hr period this was recorded as 0.5 days, but not included in the total cost calculation as bed day costs were calculated/24hr period.

To add validity, each case was assessed independently by either two research pharmacists, or one research pharmacist and a research nurse. If the two assessors agreed on the outcome this was considered the final outcome. In the case of discrepancy, the ADR was then reviewed again by a paediatrician (ST). Finally, any ADR cases where the outcome remained uncertain, were then referred for review by a panel of two senior investigators. All other aspects of the assessment were conducted as described for the pilot study. In line with the overall analysis plan, all ADRs that occurred prior to transfer to higher level of care were also assessed by a paediatrician (ST) and, if required, by a panel of two senior investigators (2.3.7.3). In line with the final study methodology, only the results for probable and definite ADRs were reported. Bed day costs were calculated using cost information (direct cost and overhead) for each ward for the year 2011 as supplied by the hospital information department.

3.3 Results

3.3.1 Results of Stage 2 - Pilot study

252 ADRs with the final causality outcome possible, probable and definite were identified at the beginning of the pilot study (Figure **3.1**). 205 ADRs were considered not to have prolonged the stay due to reaction type and 647 due to end date of ADR. Of the remaining 420, 15 had already been highlighted as having prolonged the hospital stay and were disregarded for purposes of the pilot study. Of the final 405 ADRs, every 3rd was re-assed (n=135). Results are summarised in

Table 3.1. 111/135 ADR cases (82.2%) could be assessed using electronic data sources (study and hospital database) alone. For 11 of the remaining 20 ADR cases (55%), a decision was reached after assessment of the medical notes. 6 cases were referred for next level review (paediatrician) and for 3 cases the medical notes were recorded as missing. In total 126 ADR were completed, of these, 6 ADRs (4.8%) were considered having prolonged the hospital stay by a total of 8.5 days (range less than 1 to 3 days).

Figure 3.1 Flow chart outlining the number of ADR cases included in the pilot study

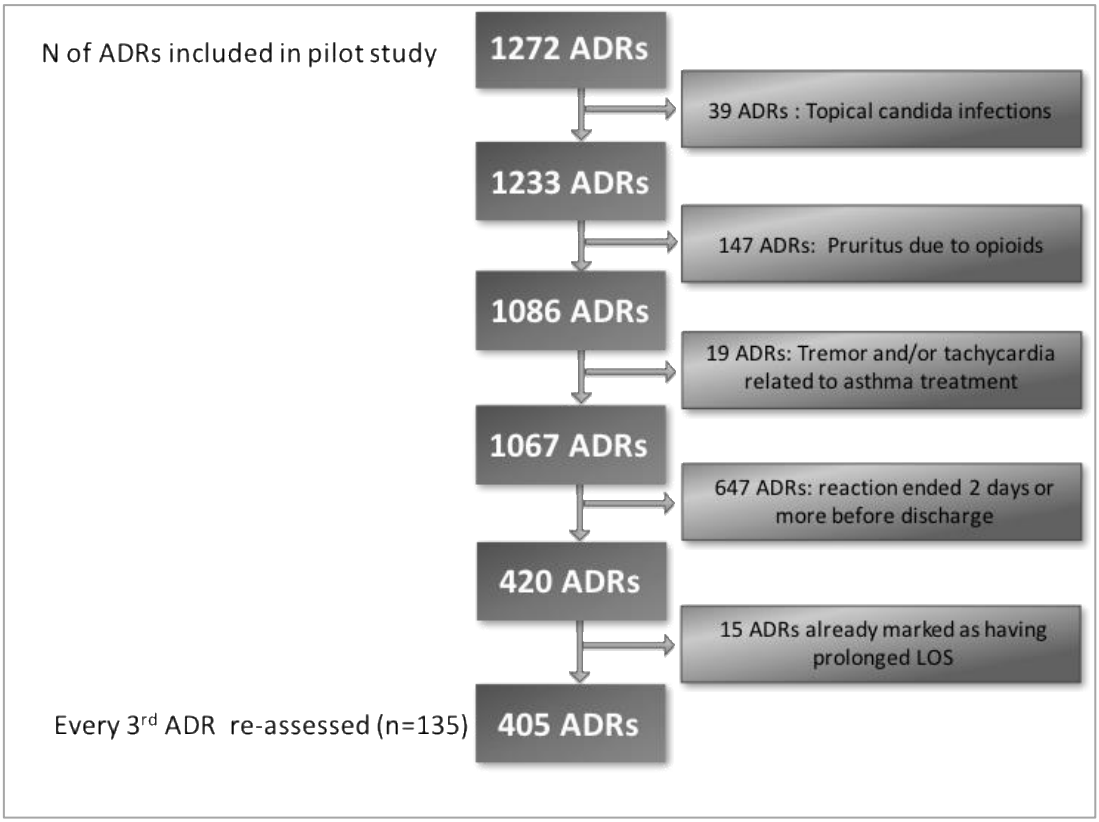

Table 3.1 Assessment of excess bed days due to ADRs - Results of pilot study (n=135)

Source	Total N of ADRs	Did ADR prolong stay?	N of ADRs for each outcome
Study database	135	Yes	0
		No	53
		Unsure	82
Hospital database	82	Yes	4
		No	58
		Unsure	20
Medical notes	20	Yes	2
		No	9
		Unsure	9*

*For 3 cases the medical notes were missing

3.3.2 Results of Stage 3 – Re-assessment of contribution of ADRs to duration of stay

Of 1446 ADRs included in the study analysis (2.4.2), 284 were considered not to have prolonged the stay due reaction type and 630 due to end date of ADR (Figure **3.2**). The remaining 532 ADRs were re-assessed.

27/1446 ADRs (1.9%) were identified as having prolonged the hospital stay by a total of 61 days (average 1.9 days; range 0.5 -12 days). Two patients experienced two ADRs that were both evaluated as having prolonged the stay by one day: A patient with dizziness post GA and PONV and an oncology patient with pain and oedema. As these ADRs occurred during the same admission, they were only counted once towards the total number of excess bed days. In 3 cases the excess bed days were censored as the patients died before discharge. Details of the reaction types and medicines involved are summarised in Table 3.2. The most common reaction type causing a prolonged stay was PONV (8/27; 29.6%) with up to 3 excess days per patient. The most common drug group associated with ADRs prolonging the stay were drugs used in GA (10/27 ADRs; 37.0%). Nearly one third of reactions occurred in oncology patients (8/27 ADRs; 29.6%) and accounted for 20/61 days (32.8%). The total cost of excess bed days due to ADRs for the study period was calculated as £34,126 as shown in Table 3.3.

Figure 3.2 Flow chart outlining the number of ADR cases re-assessed in a structured, case by case based process

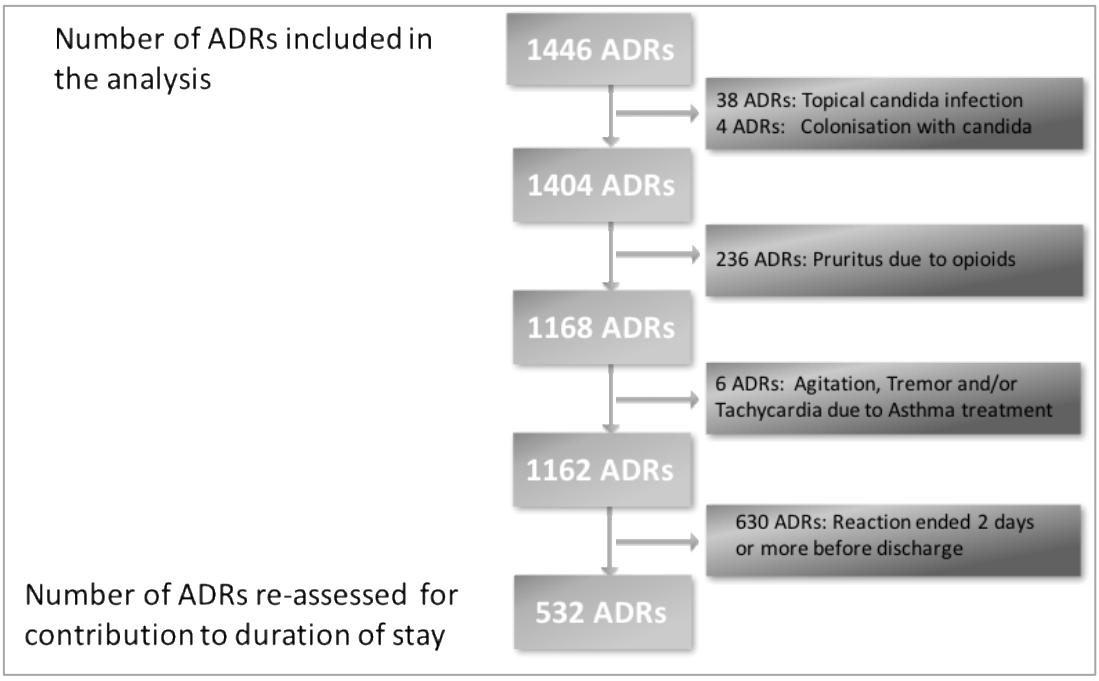

Table 3.2 ADRs that prolonged the hospital stay by ADR type, drugs implicated and number of additional bed days. ADR types listed in order of frequency

ADR type (N of same type if >1)	Drugs implicated (implicated in N of ADRs if > 1)	Total N of excess days caused by this ADR type (range; median)*
PONV (8)	Sevoflurane (7), nitrous oxide (7), morphine (5), propofol (4), codeine (4) , fentanyl (2), remifentanil (2), diclofenac, isoflurane, ketamine	14 ** (0.5 to 3; median 1)
Stomatitis (3)	Alemtuzumab (2), cyclophosphamide (2), melphalan, carboplatin, etoposide	6 (2 ADRs censored)
Vomiting (2)	Busulphan, melphalan, vincristine, etoposide, doxorubicin, ifosfamide	4 (1 + 3)
Constipation (2)	Fentanyl and codeine (2)	1.5 (1 + 0.5)
Cushing's syndrome	Methylprednisolone, prednisolone, hydrocortisone	10
Delayed recovery from GA	Midazolam, propofol	1
Diabetes mellitus	Dexamethasone	2
Diarrhoea	Daunorubicin, vincristine, rasburicase,	7
Dizziness (post GA)	Morphine , codeine, sevoflurane, nitrous oxide, propofol	1**
Sedation withdrawal	Fentanyl, chloral hydrate	1
Hyponatraemia	Furosemide, spironolactone	2
Oedema	Aldesleukin, Anti-G2 chimeric antibody	1**
Pain	Anti-G2 chimeric antibody	1**
Adrenocortical insufficiency	Prednisolone, hydrocortisone	1
Cardiac failure	Bisoprolol	12
Clostridium difficile colitis	Meropenem, gentamicin	Censored

* If the discharge was delayed, but the patient was still discharged within the same 24hr period this was recorded as 0.5 days. Patients who died before discharge were censored. ** Two patients experienced two ADRs which were both considered to have prolonged the stay independently by one day each. These ADRs were only counted once towards the total number of excess bed days.

Table 3.3 Calculation of excess bed day costs by ward type

ADR type	Excess bed days	Ward	Cost by ward type in £ bed/day	total stay
Cushing's syndrome	10	Medical 1	354	3540
Clostridium difficile colitis	Censored*	Oncology and PICU		Censored*
Cardiac failure	12	HDU	837	10,044
Constipation	1	Cardiology	387	387
Delayed recovery from GA	1	Cardiology	387	387
Diabetes mellitus	2	OUA	766	1,532
Constipation	0.5	Cardiology	387	N/A
Diarrhoea	7	OUA	766	5,362
Sedation withdrawal syndrome	1	Cardiology	387	387
Hyponatraemia	2	Cardiology	387	774
Oedema and Pain	1**	Oncology	766	766
Dizziness and PONV	1**	Surgical 3	299	299
PONV	0.5	Surgical 1	303	N/A
PONV	1	Surgical 2	368	368
PONV	1	Surgical 2	368	368
PONV	0.5	Neurosurgery	459	N/A
PONV	3	Surgical 3	299	897
PONV	1	Surgical 4	316	316
PONV	2	Surgical 2	368	736
Adrenocortical insufficiency	1	Surgical 1	303	303
Stomatitis	Censored*	Oncology and PICU		Censored*
Stomatitis	Censored*	Oncology and PICU		Censored*
Stomatitis	6	Oncology	766	4,596
Vomiting	1	Oncology	766	766
Vomiting	3	Oncology	766	2,298
		Sum of excess bed day costs in £		34,126

* Cost for was not calculated for patients whose discharge was delayed less than one day and patients who were censored. ** These patients experienced two ADRs which both prolonged the same admission by one day and were therefore only counted once towards the total cost of extended duration of stay.

3.4 Discussion

Our data suggest that only a small number of ADRs (27/1446; 1.9%) in hospitalised children prolong the overall hospital stay. Given that less than 1% of ADRs were considered severe (2.4.2), these numbers are perhaps not surprising. Weiss et al. reported 7.8% of ADRs in their study had prolonged duration of stay (Weiss et al. 2002). However, their population was much smaller (n=214) and the study was conducted in a very specific setting, a 10-bed isolation ward. They identified 68 ADRs in 46 patients and antibiotics were implicated in 50% of ADRs vs 10.6% in this study (Table 2.10).

The resulting financial impact of prolonged duration of stay in our study amounts to approximately £35,000 per year for a 300-bed hospital (of these, 22 PICU beds). Comparable adult studies have estimated costs for prolonged stay caused by ADRs to be more than 40 times higher (£2 million for a 400-bed hospital) (Davies et al. 2009). This discrepancy may be partly explained by the different spectrum of ADRs and medicines implicated. To give just two examples, the most commonly implicated group of medicines in Davies' study were diuretics (20.6%), whereas these featured low in this study (1.36%). The ADRs associated with diuretics included renal failure, which is much rarer in children and was indeed not observed in this study. Penicillin, cefalosporins, and macrolides together, were implicated in 24.9% of ADRs in Davies' study but all antibiotic groups together were implicated in only 10.6% in this study. ADRs associated with antibiotics included Clostridium difficile infection (CDI) which is frequently associated with prolonged hospital stay and occurs more commonly in adults than in children: Studies from the US report 8.2 vs 3.1 cases of CDI per 1000 discharges in adult and paediatric inpatients respectively, and higher mortality rates in adults (7.2%) compared to children (1.7%)(Deshpande et al. 2013; Reveles et al. 2014). In this study only 7 patients developed clostridium difficile colitis which in one patient lead to an extended duration of stay.

Studies assessing the financial burden of ADRs in the paediatric population remain scarce. In a recent study of 697 PICU admission, Du et al reported that the duration of stay for patients with ADRs was 3.8 times longer and the total ICU cost was 3.5 time higher (Du et al. 2013). They had observed an ADR incidence of 13.1% and used a matched case-control approach to assess excess bed days, adjusting for clinical and demographic factors. In contrast to this study, 72.2% of observed ADRs were electrolyte imbalances and 69.1% of ADRs were associated with diuretics.

Using a national US database and code identified ADRs, Tundia et al. estimated the mean excess bed days for AE (excluding accidental poisoning, but including medication errors) to be 1.5 days

and the mean excess cost to be $3842. However, the significant differences with regards to study design (retrospective, case-match control, age 0-20) and methods (code identification of ADRs and diagnoses, ADR definition included medication errors, direct cost estimation using charge data) make a direct comparison of the financial impact with this study again problematic. For example, ADE (that comprises AEs but also cases of accidental poisoning) were identified in only 0.9% of all patients, which suggests that not all ADRs that occurred in hospital were coded. It is likely, that ADRs that were considered minor and did not prolong the stay and hence would not count towards the overall charge of the hospital stay, are underrepresented when considering coding data only. Assuming a similar ADR incidence rate as in our study (17.7%) and excluding medication errors, accidental poisoning and patients older than 16 (32.5% of all AEs identified in Tunida et al.'s study occurred in 16-20-year-old patients), the percentage of ADRs leading to an extended duration of stay would perhaps be closer to 2-3%, making it comparable to our findings.

3.4.1 Limitations

With the methodology used, ADRs with an indirect effect on the duration of stay would not have been captured. For example, a child who developed chemotherapy related diarrhoea and vomiting and required total parental nutrition (TPN), could have developed TPN related hepatitis which might have prolonged the hospital stay. As reactions to TPN were not included in the study methodology this would have been missed. In addition, if the first ADR, diarrhoea and vomiting, ended more than 2 days before discharge, this ADR would not have been assessed for duration of stay. An admission based assessment could capture these indirect effects of ADRs but would not have been a feasible approach given the large number of ADRs in this study.

Furthermore, intermittently occurring ADRs, that prolonged the hospital stay could have also been missed as in accordance with the study methodology this study, only the first ADR was counted (2.3.11). For example, Children who received pulsed high-dose intravenous methylprednisolone, might have developed hypertension that initially did not require treatment, but worsened throughout the course and required treatment towards the end, which then may have prolonged the hospital stay. If the episode of hypertension ended more than 2 days before discharge, this would not have been investigated and therefore would have been missed.

In some cases, it might be difficult to quantify the contribution of the ADR to the overall duration of stay. For example, a child with PONV might have stayed an additional night for parental reassurance and it would have been difficult to quantify the contribution of the ADR to parental

anxiety. It is likely that such ADRs would have not been highlighted as having contributed to the duration of stay.

Some ADRs with significant cost implications would not necessarily lead to an extended duration of stay and would therefore not have been captured at all. For example, the lifetime cost of a single case of severe hearing loss in an infant has been estimated to be more than $ 1 million (Shield 2006).

Toxicity from treatment of acute asthma with Salbutamol and Aminophylline, include agitation, tremor, tachycardia and vomiting. However, tachycardia and agitation, may also be caused by hypoxia due to acute asthma and it can be challenging to distinguish between these two. On reflection, it is quite possible that an ADR in this patient group was mistaken for poor response to treatment and treatment was prolonged unnecessarily. This is turn could have led to a longer stay than clinically necessary and thus could have contributed to the total number excess bed days due to ADRs. These reactions should have therefore been included into the re-assessment.

In line with the analysis of the main study, only ADRs with the causality outcome probable or definite were considered in this study. ADRs with the outcome possible might well have contributed to excess bed days, but were not included in this analysis.

The author of this thesis has no experience in the field of health economics and involving a health economist would have added validity to this study. In addition, a sensitivity analysis, for example re-assessment of a random sample of excluded ADR cases (Figure 3.2) would have strengthened the analysis.

3.4.2 Conclusion

Only a small number of ADRs in hospitalised children lead to prolonged hospital stay and the resulting costs from excess bed days are relatively low compared to adult studies. However, indirect costs, for example the financial burden for the family arising from an extended hospital stay were not considered. This burden might be considerable as it could involve one parent losing a job or having to give up a job, to care for the child.

To estimate the economic burden of paediatric ADRs, more, carefully designed studies are required, including community settings and considering costs arising after discharge. However, to fully investigate and understand the burden of ADRs to the child, patient/carer and the health system, studies need to look beyond these aspects. Assessing patient reported outcome measures (PROMs) with a specific focus on ADRs would perhaps be a good starting point.

3.5 Acknowledgements

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4 Thiopurine-S-Methyltransferase (TPMT) and Cathecol-O-Methyltransferase (COMT) genetic variants in paediatric cancer patients with cisplatin-induced ototoxicity

4.1 Introduction

As highlighted in the previous chapter, some ADRs may have significant socio-economic implications. The lifetime cost of a single case of severe hearing loss in an infant has been estimated to be more than $ 1 million (Shield 2006). In addition, permanent hearing loss is a devastating prospect for any child as it affects crucial areas of development, such as speech and language, communication and social skills, and overall academic skills. For example, children with minimal degrees of sensorineural hearing loss (SNHL) may experience significant speech recognition difficulties in the presence of background noise, for example in a normal classroom environment (Crandell 1993). Even minimal SNHL can thus affect both academic ability (37% failing at least one school grade) and overall level of function (behaviour, energy, stress, social support, and self-esteem) (Bess, Dodd-Murphy & Parker 1998).

A drug well known to cause permanent hearing loss in a large proportion of children exposed to it is cisplatin, a chemotherapeutic agent used to treat solid malignancies in childhood, both within the central nervous system (CNS) (e.g. medulloblastoma) and in the rest of the body (e.g. osteosarcoma, neuroblastoma, hepatoblastoma). Cisplatin is highly effective and hearing loss might thus seem “a small price to pay” for children undergoing lifesaving treatment. However, due to life changing effect of permanent hearing loss, children undergoing cisplatin treatment are proactively and tightly monitored for ototoxicity. Once significant hearing loss is confirmed, treatment will often be changed to alternative treatment with carboplatin. However, ototoxicity is irreversible and it would be desirable to be able to identify children at high(er) risk of developing cisplatin induced hearing loss before treatment is commenced, thus allowing clinicians to discuss treatment options with the parents and or the child.

4.1.1 Cisplatin induced hearing loss

Cisplatin induced hearing loss occurs typically as bilateral, irreversible, high-frequency (HF; > 4000 Hz) sensorineural hearing loss (McHaney et al. 1983). With increasing severity, the hearing loss can also affect lower frequencies.

HF hearing loss impairs the recognition of several consonants, which are critical for speech comprehension, in particular s, f, th, sh, h, k and t (Stelmachowicz et al. 2004).

4.1.2 Incidence of cisplatin-induced hearing loss

Reported incidence rates for cisplatin-induced hearing loss in children range between 42 and 88% (Brock et al. 1991; Brock et al. 2012; Coradini et al. 2007; Li, Womer & Silber 2004; McHaney et al. 1983; Paulino et al. 2010; Ross et al. 2009; Skinner et al. 1990). The wide difference is unsurprising, considering that study populations have often been small (median study size 36.5 patients; range 22-162), and mostly heterogeneous with regard to diagnoses (single tumour type - varying types), median age (2 years 2 months – 13 years), age range (youngest included patient 1 months – 10.4 years; oldest included patient 8 years - 23 years), dosages (mean cumulative cisplatin dose 397-540 mg/m^2), treatment schedules, record of exposure to other ototoxic agents (4.1.3) and concomitant radiation (none - 100%). In addition, there is no consensus about how to define and grade cisplatin related hearing loss, which leads to significant variability in the assessment of ototoxicity. An overview of studies investigating cisplatin-induced hearing loss is given in Table 4.1.

4.1.3 Risk factors

Established risk factors for the development of hearing loss in children receiving cisplatin therapy include increased cumulative dose of cisplatin (Brock et al. 1991; Coradini et al. 2007; Li, Womer & Silber 2004; McHaney et al. 1983; Schell et al. 1989), younger age (Brock et al. 1991; Coradini et al. 2007; Li, Womer & Silber 2004; McHaney et al. 1983; Schell et al. 1989), cranial irradiation (Hua et al. 2008; Paulino et al. 2010; Schell et al. 1989) and carboplatin in myeloablative doses (Parsons et al. 1998; Punnett et al. 2004). The role of exposure to other potentially ototoxic medication is less clear, largely owing to the significant variability of the medications investigated (treatment protocols differ between cancer types and risk groups) (Table 4.2). One early study did not investigate this aspect (McHaney et al. 1983) while two more recent studies did not comment on exposure to other medications (Li, Womer & Silber 2004; Paulino et al. 2010). Knight et al. concluded that concurrent administration of long-term intravenous gentamicin had likely contributed to increased hearing loss in two patients (Knight, Kraemer & Neuwelt 2005). In contrast, three studies did not find an increased risk of hearing loss with various concomitant potentially ototoxic medications (Brock et al. 1991; Coradini et al. 2007; Skinner et al. 1990)(Table 4.2).

4.1.5 Pharmacogenetics of cisplatin-induced hearing loss

There appears to be significant inter-individual variability in predisposition to cisplatin-induced hearing loss (Brock et al. 1991; Skinner et al. 1990). The observed differences in toxicity are greater than the variability in pharmacokinetics, despite equivalent doses. Irreversible hearing loss can occur after a single dose of cisplatin whereas some children do not develop hearing loss even after multiple and high doses of cisplatin (Brock et al. 1991). It is therefore likely that genetic factors influence cisplatin-induced hearing loss (Skinner et al. 1990). Several studies have identified potential predisposing genetic variants, but the evidence for most of these remains controversial.

Table 4.1 Studies investigating cisplatin-induced hearing loss. Comparison of size, mean age, age range, diagnoses, dosage, exposure to cranial irradiation, percentage and definition of hearing loss

Author , year	Size (n)	Age mean (range)	Diagnoses [a)]	Cumulative dose cisplatin mg/m² median (range)	% cranial irradiation	Ototoxicity grading	% hearing loss [b)]
McHaney, 1983 (McHaney et al. 1983)	24	median 7 years (3.5 years, 17.5 years)	neuroblastoma (66.5%) or other solid tumours	540 (90, 1350)	none	> 25db at 4 kHz and above	88.0%
Skinner, 1990 (Skinner et al. 1990)	22	13 years (7 years, 19 years)	osteo 50%, PNET 22.7%, rhabdo 13.6%, other (non CNS) 13.6%	542 (312, 1072)	9.0%	Brock	73.0%
Brock, 1991 (Brock et al. 1991)	29	median 2 years 2 months (1 month, 13.5 years)	neuroblastoma 62.0%, GCT 27.3%, other (non CNS) 10.3%	540 (120, 1680)	none	Brock	47.5%
Li, 2004 (Li, Womer & Silber 2004)	153	no mean/median (6 months, 18 years)	hepatoblastoma, GCT, neuroblastoma, osteo	397 (120, 1213)	none	Brock	52.6%
Knight, 2005 (Knight, Kraemer & Neuwelt 2005)	67	9.65 years (8 months, 23 years)	varying, including CNS	mean 493[c)]	34.3%	Brock/ASHA[f)]/CTCAE	41.8%
Coradini 2007 (Coradini et al. 2007)	23	12.3 years (10.4 years, 16.1 years)	osteo (61%), GCT, hepatic tumor,	406 (317, 575)	none	>20db HL 4 , 8 kHz	52.0%
Ross 2009 (Ross et al. 2009)	162	cases: 6 years controls: 9 years (0, 19 years)	varying, including CNS	400 (100, 720)	18.5%	CTCAE	65.4%
Paulino 2010 (Paulino et al. 2010)	44	median 9 years (33 months, 8 years)	medulloblastoma	300[d)] and 450[e)] (75, 562.5)	100.0%	POG[g)]	75.0%

a) GCT = germ cell tumor; PNET = primitiv neuroectodermal tumour; osteo = osteosarcoma, rhabdo = rhabdomyosarcoma;
b) for patients graded according to any hearing scale hearing loss was defined as > grade 0; c) median and range not given d) patients with grade 0-3 ototoxicity; e) patients with grade 4 ototoxicity f) ASHA = American Speech-Language-Hearing Association g) POG=Paediatric oncology group

Table 4.2 Studies investigating cisplatin-induced hearing loss. Comparison of size and concomitant exposure to potentially ototoxic medication

Author , year	Size (n)	Exposure to potentially ototoxic medication
Author , year	24	none - patients with exposure were excluded
McHaney, 1983 (McHaney et al. 1983)	22	gentamicin, vancomycin, netilmicin, amphotericin B, bleomycin
Skinner, 1990 (Skinner et al. 1990)	29	gentamicin, furosemide, bleomycin
Brock, 1991 (Brock et al. 1991)	153	no data
Li, 2004 (Li, Womer & Silber 2004)	67	gentamicin, carboplatin
Knight, 2005 (Knight, Kraemer & Neuwelt 2005)	23	aminoglycosides, amitriptyline, ampicillin, amphotericin B, atropine, carboplatin, cephalexin, clarithromycin, furosemide, ibuprofen, mannitol, metronidazole, naproxen, and vinblastine.
Coradini 2007 (Coradini et al. 2007)	162	gentamicin, vancomycin, tobramycin, vincristine
Ross 2009 (Ross et al. 2009)	44	none - use of proposed ototprotective agent amifostine

4.1.5.1 Megalin

In a cohort of 50 children on cisplatin treatment (25 with hearing loss, 25 controls), the A-allele of the Megalin SNP rs2075252 was observed more frequently in the group with hearing impairment ($p<0.016$, OR 3.45; 95% CI 1.11-11.2) (Riedemann et al. 2008). In a Canadian study of 162 paediatric patients, an association between the A-Allele and cisplatin related hearing loss could not be demonstrated (Ross et al. 2009).

4.1.5.2 Glutathion-S-transferases (GSTs)

Peters et al. found that a GSTM3 allele was associated with protection against hearing loss in 39 children and young adults treated with cisplatin ($p=0.02$, OR and 95% CI not given). The group did not find an association between cisplatin-induced hearing loss and GSTT1, GSTM1 or GSTP1 polymorphisms. In an adult population of 173 testicular cancer survivors, Oldenburg et al. found that a GSTP1 variant protected against cisplatin-induced hearing loss ($p < 0.001$, OR 4.21; 95% CI 1.99-8.88) and presence of a GTSM1 variant carried an increased risk of hearing loss ($p=0.022$, OR 2.36; 95% CI 1.13-4.93) (Oldenburg et al. 2007b). The same group described the association of the GTSM1 variant with hearing loss again in an extended population of 238 adult testicular cancer survivors ($p=0.025$, OR 1.81; 95% CI 1.08-3.3). Furthermore, they found a GSTP1 variant to be protective against tinnitus ($p=0.008$, OR 0.33; 95% CI 0.44 -0.74) but not hearing loss ($p= 0.553$; OR=0.81; 95% CI not given) (Oldenburg et al. 2007a). Barahmani et al. did not find an association with GSTT1, GSTM1 or GTSTM1T1 combined (Barahmani et al. 2009) and Ross et al. did not find an association with GSTP1 or GSTM1 and hearing loss (Ross et al. 2009).

4.1.5.3 TPMT and COMT

In a Canadian cohort study of 162 paediatric patients receiving cisplatin therapy, of which 106 (65%) had hearing loss, Ross et al. identified an association with genetic variants in *TPMT* and *COMT* (Ross et al. 2009). Based upon these findings, the U.S. Food and Drug administration (FDA) changed the cisplatin label in 2011 to indicate the association with TPMT (*U.S. Food and Drug Administration*):

*"Certain genetic variants in the thiopurine S-Methyltransferase gene (e.g., TPMT*3B and TPMT*3C) are associated with an increased risk of ototoxicity in children administered conventional doses of cisplatin […] Twenty-six of the 162 patients had one or more TPMT gene variants. Of these 26 patients, 25 had severe ototoxicity (96%). For Caucasians and*

African Americans, approximately 11% of the population inherit one or more of these variants."

A study investigating the economic impact of genetic testing for this, based on Ross et al.'s findings, estimated that by administering the test, potentially $ 19.6 million could be saved (Dionne et al. 2011). Furthermore, it was estimated, that of about one third of children expected to test positive, serious ototoxicity could be prevented depending on the availability of alternative treatment providing the same rate of cure. However, about 50% of those testing negative would still develop serious hearing impairment.

4.2 Aim

The aim of this study was to test for an association between the described variants in the *TPMT* and *COMT* genes and cisplatin-induced hearing loss, in a carefully phenotyped UK paediatric cohort.

4.3 Materials and Methods

This study was a sub-study of the Molecular Genetics of Adverse Drug Reactions in Children (MAGIC) study, which is a retrospective, multicentre case-control study, focusing on the pharmacogenomic analysis of adverse drug reactions in children and has a generic study protocol and generic CRF. However, each MAGIC sub-study requires additional study specific information which must be recorded on additional pages added to the CRF and more importantly also requires specific instructions explaining the scientific background, how patients are identified, how the phenotype is defined etc. This information is similar to that generally described in a study protocol, however given that a generic study protocol exists and has ethical approval for the MAGIC study, the study specific details for this study were comprised in a cisplatin-ototoxicity study guide

The author of this thesis planned this sub-study with regards to patient identification at Alder Hey, development of a study guide and additional pages for the CRF. She recruited patients, collected DNA samples, extracted data from clinical notes, helped to develop the database, entered data into the database, performed data control (CRF and database). She assessed and graded all audiograms (4.3.10).

Details of the author's contribution to the laboratory work and analysis of this study are outlined below.

4.3.1 Study design

Participants were recruited to the MAGIC study. For this sub-study, children with cancer were recruited from six UK paediatric oncology centres: Alder Hey Children's NHS Foundation Trust, Liverpool; Royal Manchester Children's Hospital, Manchester; Royal Victoria Infirmary, Newcastle; Leeds General Infirmary, Leeds; Great Ormond Street Hospital, London; Nottingham Children's Hospital, Nottingham.

The author of this thesis recruited patients at Alder Hey Children's centre

4.3.2 Inclusion criteria:

For inclusion into the study patients needed to have

1) started cisplatin on or after 1st January 2001, and,

2) had at least one evaluable audiogram following the last dose of cisplatin (post treatment audiogram). To be considered evaluable, the audiogram had to fulfil the following criteria:

Either pure tone audiogram (PTA) or visual response audiogram (VRA) in decibel hearing level (db HL) and tested at 1, 2 and 4 kilo Hertz (kHz) and either 6 or 8 kHz.

4.3.3 Exclusion criteria:

The exclusion criteria were as follows:

1) Parent/guardian unwilling to take part (if participant <16 years at time of recruitment).

2) Participant unwilling to consent (if >16 years at time of recruitment).

3) Competent participant unwilling to assent (competence assessed on a case by case basis).

4) Hearing impairment prior to cisplatin treatment.

5) No evaluable post treatment audiogram.

6) Patient was, in the opinion of the investigator or the clinical team, not suitable to participate in the study for other reasons.

4.3.4 Recruitment

With permission of the patient's named paediatric oncology consultant, patients and/or parents/guardians were approached. Patients or parents/guardians were provided with verbal information about the study by a study team member, usually a research nurse, and given information leaflets, including age appropriate leaflets for patients under 16 years. Informed written consent was then sought once the patient or parent/guardian had had time to read and understand the information leaflet (at least 24 hours after receiving the information).

4.3.5 Consent

All patients aged > 16 years at the time of recruitment, possessing the capacity to consent, were required to give written informed consent. For patients < 16 years of age or patients aged > 16 years, who lacked capacity to consent, written informed consent from a parent or guardian was required. Assent was sought from those able to understand (assessed on a case by case basis).

4.3.6 Ethical considerations

This study was part of the MAGIC study and had ethical approval. Research Ethics No: 10/H1002/57.

4.3.8 Patient enrolment, data collection and storage

Patients were enrolled by the local study team member, usually a research nurse, at the participating centre. Data on date of birth (Dob), gender, ethnicity (White, black African, Indian, black Caribbean, black other, Bangladeshi, Pakistani, Chinese, other), diagnosis, treatment details (cisplatin doses, date of administration, concomitant vincristine use, cranial radiotherapy) and hearing tests, were collected retrospectively from medical case notes, prescription charts and audiology records, using a specifically designed case record form (CRF). Anonymised paper copies of audiograms and tympanograms were attached to the CRFs and both were forwarded to the lead study team in Liverpool where data were subsequently entered onto the study database. Patient age in this study was defined as age at start of cisplatin treatment, and was calculated using the following formula:

Age = Dob – date of first dose of cisplatin.

4.3.9 Genetic samples and genotyping

Unless stated otherwise, all chemicals were obtained from Sigma-Aldrich, www.sigmaaldrich.com.

4.3.9.1 DNA collection and extraction and purification

Patient samples were collected as whole blood ethylenediaminetetraacetic acid (EDTA) samples or saliva. The preferred sampling method was whole blood and a minimum of 2.6 ml per sample was required. Whole blood samples were stored at -80°C. DNA from saliva was captured and stabilised using the OrageneTM DNA collection Kit (OG-575) for assisted collection (DNA Genotek Inc. Ontario, Canada; www.dnagenotek.com). Following collection of the sample, and mixing with the Oragene DNA preserving solution, samples were stored at -80°C. DNA extraction and archiving was performed at the Wolfson Centre for Personalised Medicine, Department of Pharmacology, University of Liverpool.

The author of this thesis performed the extraction of DNA from saliva and whole blood.

4.3.9.2 DNA extraction and purification from saliva

For genomic DNA extraction and purification from saliva samples, the Oragene DNA kit (DNA Genotek Inc. Ontario, Canada; www.dnagenotek.com) was used. Samples were defrosted for 1 hr in at room temperature (RT) and then incubated in a water incubator at 50°C for a minimum of 1 hour. After that, Oragene DNA Purifier was added 1:25 to each sample, e.g.

160 µl purifier was added to 4ml saliva, and mixed by vortexing for a few seconds. Samples were then incubated on ice for 10 minutes and following this centrifuged at 4600 x g for 10 minutes. An equal volume of 95% ethanol was added to each resulting supernatant and the pellet discarded. After that, samples were mixed 10 times by inversion and left to stand at RT for 10 minutes. Then, samples were centrifuged at 4600 x g for 10 minutes. The resulting supernatant of each sample was discarded and the DNA pellet rinsed with 1ml 70% ethanol and then left to stand at RT for 1 min. Samples were rehydrated by addition of 500 µl Tris-EDTA (TE) buffer and mixed by vortexing for 30 seconds. Following this, samples were stored at -20°C and rehydration assessed after 7 days. If rehydration was incomplete, additional cold storage was undertaken for another 7 days.

4.3.9.3 DNA extraction from whole blood

EDTA blood samples were defrosted at RT for 30 min. Genomic DNA was extracted using the Chemagen whole-blood DNA extraction kit on the Chemagic Magnetic Separation Module I, according to the manufacturer's protocol (PerkinElmer Chemagen Technologie GmbH, Baesweiler, Germany; www.chemagen.com).

4.3.9.4 Quantification and normalisation of DNA

Quantification of DNA was carried out using a NanoDrop 8000 spectrophotometer (Thermo Fisher scientific, Waltham, USA; www.thermofisher.com). DNA was normalised to 20 ng/µl on a liquid handler Biomek NXP, using Biomek software (Beckman Coulter (UK) Ltd; www.beckmancoulter.com).

The author of this thesis carried out quantification using Nanodrop and prepared the samples for normalisation. The liquid handler was operated by Dr Eunice Zhang (EZ).

4.3.9.5 Genotyping

Genotyping was undertaken for three *TPMT* variants (rs12201199, rs1142345 and rs1800460) and two *COMT* variants (rs4646316 and rs9332377) as described by Ross et al. (Ross et al. 2009), using an ABI 7900HT Real time PCR System, Taqman chemistry and SDS plate utility software version 2.3 (all Thermo Fisher Scientific, Waltham, USA; www.thermofisher.com). PCR was carried out using Taqman Drug metabolism genotyping Assays C__19567_20, C__31923406_10, C__30634116_20, C__29193982_10, C__29614343_10 and C__11643398_10. A reaction volume of 6 µl contained 10 ng DNA, 1X Taqman master mix and 1X Taqman drug metabolising genotyping assay mix. To minimise

cross contamination of samples, a dry-down DNA method was performed according to the manufacturer's protocol. The PCR conditions were as follows: activation of AmpliTaq Gold DNA polymerase at 95°C for 10 min, followed by 40 cycles of (1) denaturing at 95°C for 15 sec and (2) extension at 60°C for 90 sec. Alleles were clustered using fluorescent signals (VIC and FAM). As part of quality control, 10% of samples were analysed as duplicates and one negative control was included per 96 samples. To avoid bias, the phenotypical classification was carried out without knowledge of genotype.

The author of this thesis assisted in the preparation of the genotyping assay and mixed the reagents. The assay was carried out by EZ in the presence of the author of this thesis

4.3.10 Phenotyping

To replicate the originally described cohort as closely as possible, all audiograms were graded according to CTCAE (National Cancer 2010; Ross et al. 2009) (Table 4.3). 6 kHz test results were accepted when 8 kHz results were not available. If several post treatment audiograms were provided, the audiogram showing the worst sensorineural hearing loss was used for grading. For patients with asymmetric hearing loss, both ears were graded separately and the results of both grades analysed as outlined below. All audiograms were assessed and graded centrally by the author of this thesis. Ambiguous cases were discussed with a second reviewer, a Consultant Paediatric Oncologist (BP) with expertise in cancer treatment related ototoxicity. Patients with audiograms that did not meet the inclusion criteria (4.3.2.) were withdrawn from the study at this stage.

Table 4.3 CTCAE ototoxicity grading criteria as used by Ross et al. (Ross et al. 2009)

Grade	**CTCAE criteria**
0	< 20 db at all frequencies
1	> 20 db at 8 kHz*
2	> 25 db at 4 kHz and above
3	> 25 dB at 2 kHz and above
4	> 40 dB at 1 kHz and above

*6 kHz test results were accepted for grading where 8 kHz results were not available.

4.3.11 Statistical Analysis

The analysis was planned and conducted by Dr Peng Yin and Dr Andrea Jorgensen. The author of this thesis worked with the team of statisticians to provide the clinical context. She also

summarised the data for patient demographics and compared allele frequencies with population data from the 1000 genomes project (Table 4.5)

Quality control procedures were applied to the genotype data and individuals or SNPs included in the analysis, based on the following criteria: sample call rate (samples missing 2 or more SNPs were excluded), SNP call rate (only SNPs with a call rate > 95% were included), minor allele frequency (MAF) (only SNPs with a MAF > 0.01 were included) and Hardy-Weinberg (HW) test (only SNPs with a HW test p-value > 0.05 were included). An additive mode of inheritance was assumed with SNPs coded 0, 1 or 2 to represent wild-type homozygotes, heterozygotes and mutant-type homozygotes respectively.

Univariate and multivariate analyses were undertaken using 'R' version 3.2.0. First, a univariate multinomial logistic regression model was fitted to each non-genetic factor in turn. Next, multivariable multinomial logistic regression models were fitted to each SNP in turn. For each SNP two models were fitted. The first model included covariates to represent all non-genetic factors with $p<0.25$ univariately. Backward stepwise variable selection was applied to this baseline model, to remove any covariates no longer significant in the multivariable model, with an inclusion p-value=0.25 and an exclusion p-value=0.10. The final model was called the 'baseline model'. The second model was the same as the baseline model but also included a covariate to represent the SNP and was called the 'genetic model'. The likelihood ratio test was applied to compare the two models and thus assess for statistical significance of the SNP. P-values were adjusted using Bonferroni correction for multiple testing, adjusting for 5 tests (5 SNPs). In cases of asymmetric hearing loss, the worse ear grade was used as final ototoxicity grade.

To avoid bias arising from this approach, a sensitivity analysis was carried out, using the ototoxicity grade of the better ear as final grade. Further sensitivity analyses of ototoxicity grades were performed by dichotomising outcomes in three different ways: CTCAE grade 0 vs. 1-4; CTCAE grade 0 vs. 2-4; CTCAE grade 0 vs. 3-4. The approach to the sensitivity analyses was the same as for the ordinal outcome but logistic regression models were used, instead of multinomial logistic regression models.

4.4 Results

149 patients were enrolled in the study and included in the genotyping assay but data was not available for six. 23 patients did not have the required audiograms and were therefore also withdrawn. For the 10 of these, post treatment audiograms did not include frequencies > 4kHz and for 13, no post treatment audiology records were available: one child died, one moved out of the region, two children had their audiograms performed at other hospitals, one child relapsed and required further treatment, for one child all audiology records were lost and for 7 children the reason was unclear.

Audiograms from the remaining 120 patients were evaluated according to CTCAE criteria.

4.4.1 Genomic quality controls

Four patients were removed from the analysis after quality control as two or more SNPs were missing. All variants had a MAF > 5% and all passed the HW test (p-values > 0.05).

4.4.2 Patient demographics

The distribution of tumour types was: medulloblastoma 30.2% (35/116), other CNS tumours 14.7% (17/116), osteosarcoma 24.1% (28/116), hepatoblastoma 12.9% (15/116), neuroblastoma 12.9% (15/116) and other non-CNS tumours 5.2% (6/116). 12 patients only experienced hearing loss in one ear. Considering the CTCAE grade of the worse ear, 90/116 patients (77.6%) experienced hearing loss vs 78/116 patients (67.2%) when considering the better ear (CTCAE grade 0).

Patients were between 7 months and 18.6 years old. The median age was 7.1 years and 92% were younger than 16 years, when they started cisplatin treatment. For two children, the age at start of treatment could not be calculated (4.3.7) due to a CRF completion error: DoB given was chronologically later than date of first dose of cisplatin given. The self-reported ethnicity for 89.4% (101/113) patients was Caucasian, for 5% (6/116) Asian, for 2.5% African (3/116) and for three children unknown.

18.1% (21/116) of patients received cisplatin and carboplatin as part of the same treatment protocol. Of the remaining patients 56.9% (66/116) were not exposed to carboplatin at all, one patient was exposed to carboplatin before cisplatin treatment was started, and 22.4% (26/116) where exposed to carboplatin after having completed cisplatin treatment. Patients in this last group may have been changed from cisplatin to carboplatin therapy due to

nephrotoxicity or ototoxicity. Further characteristics are shown in Table 4.4 Patient characteristics and results of the univariate analysis using the worse ear grade in cases of asymmetric hearing loss.

4.4.3 Results of univariate analysis

In the univariate analysis ototoxicity was shown to be significantly ($p < 0.05$) associated with age, gender, cumulative dose of cisplatin, cranial irradiation and concomitant exposure to vincristine (Table 4.4). Ethnicity and concomitant use of carboplatin was not associated with an increased risk of hearing loss.

4.4.4 Analysis of COMT and TPMT risk genotype association

Clinical factors included in the multivariable model ($p<0.25$) were patient age at diagnosis, gender, cranial irradiation, cumulative dose of cisplatin, exposure to vincristine and carboplatin (Table 4.4). On applying variable selection to the model including all these factors, vincristine was removed due to correlation with cranial irradiation ($r = 0.52$). The allele frequency in this population was compared to population data from the 1000 genomes project (Auton et al. 2015) and showed similar distribution (Table 4.5). None of the 5 SNPs was significantly associated with hearing loss, with none of the corrected p-values reaching the significance threshold of $p < 0.05$. In sensitivity analyses, using 1) better ear and ordinal outcomes (Table 4.6), 2) worse ear and binary outcomes (Table 4.7) and 3) better ear and binary outcomes (Table 4.8), there was still no significant association for any of the variants.

Table 4.4 Patient characteristics and results of the univariate analysis using the worse ear grade in cases of asymmetric hearing loss.

CTCAE	Grade 0 (n=26)	Grade 1 (n=8)	Grade 2 (n=41)	Grade 3 (n=35)	Grade 4 (n=6)	P-value
Age, years (median(min, max))	7.73 (0.59, 17.67)	12.78 (0.83, 18.60)	8.80 (0.80, 18.18)	4.94 (0.62, 17.15)	3.95 (1.17, 10.05)	0.010
Ethnicity						
White	23 (22.8%)	7 (6.9%)	33 (32.7%)	33 (32.7%)	5 (5.0%)	0.45
Non-white	3 (25.0%)	0 (0.0%)	6 (50.0%)	2 (16.7%)	1 (8.3%)	
Gender						
Male	17 (23.0%)	6 (8.1%)	32 (43.2%)	15 (20.3%)	4 (5.4%)	0.030
Female	9 (21.4%)	2 (4.8%)	9 (21.4%)	20 (47.6%)	2 (4.8%)	
Cumulative cisplatin dose (mg/m^2) (median (min, max))	344 (60, 600)	317.0 (240, 560)	480 (208, 560)	320 (180, 560)	260 (100, 800)	0.023
Cranial irradiation						
YES	3 (7.5%)	2 (5.0%)	16 (40.0%)	17 (42.5%)	2 (5.0%)	0.028
NO	23 (30.3%)	6 (7.9%)	25 (32.9%)	18 (23.7%)	4 (5.3%)	
Vincristine						
YES	9 (14.3%)	2 (3.17%)	22 (34.9%)	25 (39.7%)	5 (7.94%)	0.0091
NO	17 (32.1%)	6 (11.3%)	19 (35.8%)	10 (18.9%)	1 (1.89%)	
Carboplatin + Cisplatin*						
YES	2 (9.52%)	0 (0%)	8 (38.1%)	10 (47.6%)	1 (4.76%)	0.097
NO	24 (25.3%)	8 (8.42%)	33 (34.7%)	25 (26.3%)	5 (5.26%)	

Total number of patients = 116. Data are presented as number (%) of patients unless otherwise indicated. *YES refers to patients who received cisplatin and carboplatin as part of the same treatment protocol. NO refers to patients who were not exposed to carboplatin whilst they were also treated with cisplatin. Patients in the latter group may have been changed from cisplatin to carboplatin

Table 4.5 Allele frequency for the five SNPs and comparison to population data from the 1000 genomes project (Auton et al. 2015)

SNP	Allele	Frequency in the study population; N (%)	Frequency in 1000 genomes	
			all	European
COMT_rs9332377	C	192 (82.7%)	82.8%	84.9%
	T	40 (17.3%)	17.2%	15.1%
COMT_rs4646316	C	184 (79.3%)	78.2%	75.3%
	T	48 (20.7%)	21.8%	24.7%
TPMT_rs12201199	A	209 (90.1%)	83.7%	95.1%
	T	23 (9.9%)	16.3%	4.9%
TPMT_rs1142345	T	216 (93.1%)	96.1%	97.1%
	C	16 (6.9%)	3.9%	2.1%
TPMT_rs1800460	C	221 (95.3%)	98.7%	97.2%
	T	11 (4.7%)	1.3%	2.8%

Table 4.6 *COMT and TPMT* genetic variants association using CTCAE grading and ordinal outcomes; results of multivariable ordinal logistic regression analysis

		Worse ear				Better ear			
Gene	SNP (reference)	Estimate (SE)	p-value	Overall p-value	Adjusted p- value*	Estimate (SE)	p-value	Overall p-value	Adjusted p- value*
COMT	rs9332377 (CC)								
	Grade 1 vs Grade 0:	1.03 (0.72)	0.15	0.30	1.00	-1.14 (1.49)	0.44	0.40	1.00
	Grade 2 vs Grade 0:	0.19 (0.53)	0.72			-0.37 (0.45)	0.42		
	Grade 3 vs Grade 0:	-0.54 (0.60)	0.37			-1.16 (0.65)	0.074		
	Grade 4 vs Grade 0:	0.47 (0.76)	0.54			-0.16 (1.62)	0.92		
	rs4646316 (CC)								
		0.52 (0.77)	0.50	0.93	1.00	0.62 (1.13)	0.58	0.86	1.00
		-0.10 (0.56)	0.85			0.38 (0.49)	0.43		
		0.079 (0.59)	0.89			0.0093 (0.58)	0.87		
		-0.24 (0.94)	0.80			1.30 (1.62)	0.42		
TPMT	rs12201199 (AA)								
		-1.07 (1.12)	0.34	0.068	0.34	-16.8 (4860)	1.00	0.07	0.35
		-0.92 (0.65)	0.16			-0.40 (0.58)	0.48		
		-1.66 (0.78)	0.033			-2.11 (0.94)	0.024		
		-17.6 (2940)	0.99			-17.5 (4700)	1.00		
	rs1142345 (TT)								
		-0.47 (1.10)	0.67	0.55	1.00	-16.7 (6060)	1.00	0.24	1.00
		-0.43 (0.70)	0.53			0.058 (0.62)	0.93		
		-0.72 (0.83)	0.39			-1.90 (1.19)	0.11		
		-16.9 (3450)	1.00			-15.7 (6340)	1.00		
	rs1800460 (CC)								
		-0.041 (1.10)	0.97	0.83	1.00	-16.2 (8070)	1.00	0.13	0.65
		-0.13 (0.78)	0.87			0.36 (0.68)	0.60		
		-0.23 (0.96)	0.81			-19.1 (7230)	1.00		
		-16.4 (3990)	1.00			-15.7 (4970)	1.00		

*Determined using Bonferroni-corrected p-values, comparing to a significance threshold of $p < 0.05$. SE=Standard error

Table 4.7 *COMT and TPMT* genetic variants association using worse ear grade and dichotomised outcomes; results of the multivariable logistic regression analysis

		CTCAE grade 0 vs. 1-4			CTCAE grade 0 vs. 2-4			CTCAE grade 0 vs. 3+4		
Gene	SNP (reference)	Estimate(SE)	P-value[a]	Adjusted p-value*	Estimate (SE)	P-value	Adjusted p-value*	Estimate (SE)	P-value	Adjusted p-value*
COMT	rs9332377 (CC)	0.061 (0.44)	0.89	1.00	-0.19 (0.47)	0.69	1.00	-0.21 (0.57)	0.71	1.00
	rs4646316 (CC)	0.058 (0.48)	0.90	1.00	0.012 (0.49)	0.98	1.00	-0.35 (0.62)	0.58	1.00
TPMT	rs12201199 (AA)	-1.41 (0.60)	0.020	0.10	-1.54 (0.66)	0.019	0.095	-1.85 (0.84)	0.028	0.14
	rs1142345 (TT)	-0.73 (0.63)	0.25	1.00	-0.85 (0.68)	0.21	1.00	-1.13 (0.90)	0.21	1.00
	rs1800460 (CC)	-0.28 (0.69)	0.68	1.00	-0.41 (0.73)	0.58	1.00	0.38 (1.01)	0.71	1.00

*Determined using Bonferroni-corrected p-values, comparing to a significance threshold of $p < 0.05$. SE=Standard error.

Table 4.8 *COMT and TPMT* genetic variants association using better ear grade and dichotomised outcomes; results of the multivariable logistic regression analysis

		CTCAE grade 0 vs. 1-4			CTCAE grade 0 vs. 2-4			CTCAE grade 0 vs. 3+4		
Gene	SNP (reference)	Estimate(SE)	P-value[a]	Adjusted p-value*	Estimate (SE)	P-value	Adjusted p-value*	Estimate (SE)	P-value	Adjusted p-value*
COMT	rs9332377 (CC)	-0.55 (0.40)	0.17	0.85	-0.58 (0.41)	0.16	0.80	-1.20 (0.70)	0.088	0.44
	rs4646316 (CC)	0.27 (0.44)	0.54	1.00	0.31 (0.44)	0.49	1.00	0.16 (0.62)	0.80	1.00
TPMT	rs12201199 (AA)	-0.94 (0.56)	0.097	0.49	-0.88 (0.57)	0.12	0.60	-1.91 (1.04)	0.067	0.34
	rs1142345 (TT)	-0.49 (0.61)	0.42	1.00	-0.43 (0.61)	0.48	1.00	-1.61 (1.31)	0.22	1.00
	rs1800460 (CC)	-0.21 (0.67)	0.75	1.00	-0.16 (0.67)	0.82	1.00	-15.1 (1.68)	0.99	1.00

*Determined using Bonferroni-corrected p-values, comparing to a significance threshold of $p < 0.05$. SE=Standard error

4.5 Discussion

This study was planned in 2010. Whilst most patients from Alder Hey were recruited by 2012, there was considerable delay in recruitment of patients and extraction of clinical data from other centres. Once genotyping and phenotyping data were available for patients included in this study, there was further delay with regards to the generic database, which in turn delayed the analysis. By the time this study was concluded, several other studies had been published which had also attempted replication of Ross et el.'s findings. In addition, further genes for cisplatin induced ototoxicity have been proposed since, namely ABCC3 and ACYP2 which are discussed below.

This study did not replicate *TPMT* and *COMT* as risk factors for cisplatin related hearing loss in paediatric cancer patients. Comparing our study population to that of Ross et al., gender distribution and age range were similar (Ross et al. 2009)(Table 4.4). The median cumulative dose of cisplatin in our study was lower (350 mg/m^2 vs 400 mg/m^2) and more children experienced hearing loss (77.6% vs 65.4%). The distribution of tumour types differed, in that twice as many children in this study had brain tumours (44.8% vs 20%), with nearly twice as many being exposed to cranial radiotherapy (34.5 % vs 18.5%) and ten times as many patients receiving vincristine (54.3% vs 5.5%). Ross et al. did not include carboplatin in the list of concomitant medications.

4.5.1 Non-genetic influencing factors

This study confirms known risk factors for cisplatin therapy induced ototoxicity in children such as increasing cumulative dose of cisplatin (Brock et al. 1991; Coradini et al. 2007; Li, Womer & Silber 2004; McHaney et al. 1983; Yancey et al. 2012), younger age (Coradini et al. 2007; Li, Womer & Silber 2004; Yang et al. 2013), cranial radiotherapy (Hua et al. 2008; Paulino et al. 2010; Schell et al. 1989), male gender (Yancey et al. 2012; Yang et al. 2013) and concomitant exposure to vincristine (Pussegoda et al. 2013). Ethnic origin as a risk factor for hearing loss in patients receiving cisplatin therapy, was not detected. However, with nearly 90% of the study population being Caucasian, the study did not have enough power to detect an effect of ethnicity.

Patients in this study who received carboplatin and cisplatin combined were not at a higher risk of experiencing hearing loss. This is not unexpected. Most children who experience hearing loss after carboplatin therapy, have also received cisplatin and/or have received high-dose carboplatin regimens prior to stem cell transplant (Landier et al. 2014; Parsons et

al. 1998; Punnett et al. 2004). Children who receive standard dose carboplatin alone experience no or only mild hearing loss (Bertolini et al. 2004), indeed carboplatin is often used as an alternative to cisplatin, once significant ototoxicity has been confirmed. One of the limitations of this study is that the retrospective study design did not make it feasible to collect detailed dosage data of concomitant medications. Of the 95 children in this study who did not receive combined cisplatin and carboplatin therapy, 26 received carboplatin after cisplatin therapy, likely as alternative therapy due to cisplatin-induced nephro- or ototoxicity. 24/26 children in this group also had grade 2-4 hearing loss. Of the remaining two children, one had grade 2 hearing loss in the worse ear and the other grade 1 hearing loss. It is therefore possible that any effect from the combined exposure to cisplatin and carboplatin could have been underestimated. In addition, the median cumulative dose in patients who received carboplatin and cisplatin was lower (320 mg/m2) compared to those who didn't (360 mg/m2).

For pragmatic reasons, exposure to aminoglycosides or furosemide was not investigated in this study: gentamicin is commonly used in the treatment of febrile neutropenia in the UK. Due to the setup of paediatric cancer treatment in the UK, many children in our cohort will have received any treatment for febrile neutropenia in shared care units and it would therefore not have been feasible to the relevant collect treatment data. The same applies to the use of furosemide, e.g. in conjunction with transfusion of blood products which is also frequently done in shared care units. Furthermore, although ototoxicity is listed in the adverse drug reaction profile in the SmPC for furosemide as well as aminoglycosides (DataPharm Communications Ltd. 2010), several studies that did include these medicines, did not find an association in patients with cisplatin-induced hearing loss (Punnett et al. 2004; Pussegoda et al. 2013; Ross et al. 2009; Yancey et al. 2012). In contrast, an increased risk of hearing loss was observed in high-risk neuroblastoma patients admitted to hospital for infection during the induction phase of their treatment (Landier et al. 2014) and the authors of the study suggested this was a surrogate for additional ototoxic exposure, such as aminoglycoside use. Finally, aminoglycoside induced ototoxicity has been linked to mutations of the mitochondrial *MT-RNR1* Gene (Usami et al. 1998). Given a population prevalence in Caucasian children of approx. 1:500 (Bitner-Glindzicz et al. 2009), it is unlikely that more than one child in our cohort was a mutation carrier and this would therefore not have biased the results.

Association of TMPT and COMT variants in other studies

To date, the evidence for the association of variants in the *TPMT* and *COMT* genes with cisplatin-induced hearing loss remains controversial. Pussegoda et al. confirmed the association with *TPMT* but not *COMT* in a cohort of 155 patients (Pussegoda et al. 2013) , but Yang et al. did not find an association with any of the variants in either gene in a study of 213 paediatric medulloblastoma patients (Yang et al. 2013). Hagleitner et al. investigated the proposed variants in a Dutch cohort of 110, and a Spanish cohort of 38 patients and again demonstrated a lack of association (Hagleitner et al. 2014). However, Hagleitner et al. also performed a meta-analysis including all the above studies and found that the rs4646316 variant in the *COMT* gene was significantly associated with ototoxicity. Since then, Lanvers-Kaminsky et al. did not find an association any of the described variants in their cohort of 63 children (Lanvers-Kaminsky et al. 2014). Moreover, the FDA approved drug label for cisplatin was amended in 2015 to reflect this uncertainty (*U.S. Food and Drug Administration*):

"Genetic factors (e.g. variants in the thiopurine S-Methyltransferase [TPMT] gene) may contribute to cisplatin-induced ototoxicity; although this association has not been consistent across populations and study designs."

4.5.2 Implications of results

It is interesting that the only two studies demonstrating an association with any of the described *TPMT* and *COMT* variants were much larger, i.e. the meta-analysis by Hagleitner et al. (Hagleitner et al. 2014) and/or very closely resembled the study population and methods of the original study, i.e Pussegoda et al. (Pussegoda et al. 2013). The most likely reasons for the lack of association in this study as well as in other studies is probably due to the heterogeneity between study populations as outlined above (4.1.2). This has already been discussed by Hagleitner, Yang and Carleton, all highlighting critical differences between their respective studies that might mask genetic susceptibility findings (Carleton et al. 2014; Hagleitner et al. 2014; Yang et al. 2013). Association studies of SNPs with adverse drug effects are known to be at risk of producing false positive or false negative results, especially if the study population is small and the association is examined in stratified groups of the population (Royal Society Working Group 2005). The aim of additional sensitivity analysis in this study was to address these issues, but the results remained the same.

A factor that has not been regularly discussed, but may have a significant impact on any study investigating cisplatin-induced ototoxicity, is the grading of asymmetric hearing loss, i.e.

worse ear vs. better ear. Whilst it is clinical practice in the UK to use Brock ototoxicity grading and to use the better ear to assign the overall grade, other classifications, such as Chang, CTCAE and the new SIOP Boston scale (Brock et al. 2012) do not stipulate how to proceed in such cases (Gurney & Bass 2012). Yang et al. used the worse ear to grade ototoxicity but few other authors have described how they proceeded in their study population. In this study, the number of patients with different grades of hearing loss in both ears was 10.3% (12/116). Assuming a similar percentage of patients with asymmetric hearing loss in other study populations, this could have led to a significant number of misclassifications and may have added to the difficulties in trying to replicate the association.

As already discussed by Yang et al. the association between *COMT, TPMT* and cisplatin induced hearing loss was not expected (Yang et al. 2013). *TPMT* metabolises thiopurines, such as the immunosuppressant and antineoplastic 6-MP. *TPMT* deficiency has significant implications for treatment with thiopurines, which has already been highlighted at the beginning of this thesis (1.6.5). *COMT* interacts with S-adenosylmethionine (SAM) and although *COMT* has been has been linked to sensorineural hearing loss in mice and humans (Du et al. 2008), it is predominantly linked with psychiatric disorders such as schizophrenia (McKusick 2007) and the biological mechanism for cisplatin induced hearing loss remains unclear.

Since Ross et al. first published their findings, 2 other genetic variants have been proposed. ABCC3 SNP rs1051640 (Pussegoda et al. 2013) and ACYP2 SNP rs1872328 (Xu et al. 2015). The same SNP for ABCC3 had already been investigated by Ross et al. in 2009, but no significant association had been found in the original cohort (Ross et al. 2009). The ACYP2 variant was first was identified using a genome-wide association study in 238 children with brain tumours (Xu et al. 2015), which was replicated within the original study. An additional study has also recently replicated the association with ACYP2 in patients (n=156) with osteosarcoma (Vos et al. 2016). Furthermore, association between the ACYP2 polymorphism and cisplatin induced hearing loss has also been replicated in the cohort that was used for this study and was confirmed in a meta-analysis(Thiesen et al. 2017)

4.6 Conclusion

This study did not confirm an association with TPMT and COMT and cisplatin-induced hearing loss. Cisplatin is used in a wide variety of tumours, and patient heterogeneity is thus likely to be a confounding factor. Meta-analyses have since shown an association with COMT

rs464316 SNP. Further studies in larger populations and including the more recently described association with ACYP2 SNP rs1872328, would still be beneficial in order to define factors that modulate this association. We also need to understand the biological basis of the genetic associations

4.7 Acknowledgements

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The following UK paediatric oncology centres recruited to the study (listed by name of centre/hospital and name of principal investigator): Alder Hey Children's Hospital, Dr Daniel Hawcutt (DH); Great Ormond Street Hospital, Dr Penelope Brock; The Great North Hospital, Prof Simon Bailey; Royal Manchester Children's Hospital, Dr Guy Makin; Leeds Teaching Hospitals, Dr Sue Picton; Nottingham Children's Hospital, Prof David Walker and Prof Vyas. Patient recruitment, DNA and data collection at Alder Hey was carried out by ST, DH and designated research nurses. At all other centres patient recruitment, DNA and data collection was carried out by designated research nurses and the principal investigators.

Mr Lawrence McEvoy taught the author of this thesis DNA extraction, purification and quantification, which the author then carried out independently. Overall, Dr Eunice Zhang supervised the laboratory work, operated the liquid handler and carried out the final genotyping assays as outlined above.

The analysis plan for the cisplatin-ototoxicity study was designed by Dr Peng Yin and Dr Andrea Jorgensen and the analysis was carried out by Dr Peng Yin.

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5 Final Discussion

5.1 ADRs in hospitalised children - summary of findings, interpretation and implications for research

In this thesis, adverse drug reactions occurring in children after admission to hospital were investigated. It is the largest prospective inpatient study so far. The study included general paediatric patients, as well as a large variety of patients from different paediatric surgical and medical specialities. ADRs were characterised in terms of type of reaction, causality and medications suspected to have caused the reaction. Incidence rates were calculated and factors associated with an increased risk of experiencing an ADR were identified. Considering the first admission only, incidence of ADRs was 43.7% in the Oncology population and 14.0% in the non-Oncology population. Opiate analgesics and drugs used during GA were the most commonly implicated medications. Less than 1% of ADRs lead to permanent harm or resulted in patient transfer to higher level of care and no ADR was associated with death.

Comparison of ADR incidence rates between this study and other recent adult and paediatric studies is problematic due to differences in methodology, specifically the use of other causality assessment tools and inclusion or exclusion of 'possible' ADRs. Risk factors identified in this study confirmed established risk factors such as the use of more than one medication and oncology treatment. 'Undergoing a procedure under GA', was a new and significant risk factor identified in this study and more than a third of patients who had undergone a GA and developed at least one ADR had experienced PONV. New consensus guidelines regarding the management and prevention of PONV have been published after this study was conducted (Gan et al. 2014; Martin et al. 2016). It will be important to investigate whether the overall risk of ADRs in children undergoing a GA has decreased since the implementation of these guidelines.

There is good evidence from other areas of paediatric medicine that even common, benign and short lasting events such as febrile seizures, may be perceived as extremely frightening by parents (Baumer et al. 1981), and give rise to anxiety and disruption of family life well beyond the actual event (Wirrell & Turner 2001). Wirrel and Turner concluded that "the extreme fear felt by most parents is in sharp contrast to the physician's perception that most febrile seizures are benign and are associated with a minimal risk of brain injury, death or subsequent epilepsy (Wirrell & Turner 2001)".

Parents' experience of ADRs was investigated as part of the ADRIC programme (Arnott et al. 2012; Smyth et al. 2014) and the outcome suggests a similar discordance between clinicians' and parents' views: "Parents of children included in this study reported that suspected ADRs cause them concern irrespective of the 'medical' severity of the suspected reaction". Importantly, Arnott et al. observed that parents of children receiving cancer treatment viewed the communication they received about ADRs as positive, whereas most other parents were dissatisfied with the communication about ADRs (Arnott et al. 2012; Smyth et al. 2014). They highlighted a case of a child with pruritus due to morphine, which clinically presented as agitation. This is a common dilemma in children who are unable to verbalise their itchiness or indicate it non-verbally e.g. by scratching. The parent, who was unaware that agitation could be a result of pruritus, interpreted the agitation as uncontrolled pain and continued to give booster doses of morphine which worsened the agitation. Scenarios like this are preventable by better communication about possible side effects of medication before they are given and perhaps this should form part of the routine pre-operative assessment.

However, there is a paucity of studies investigating patients', especially children's, experience of ADRs. One adult study found that patients who reported possible ADRs experienced a reduction in health related quality of life. (Rolfes et al. 2016). Guo et al. reported that ADRs that required discontinuation of treatment or additional treatment led to reduction in health-related quality of life with particular negative impact on mental health (Guo et al. 2010). These aspects need to be investigated in the paediatric population

The study did not assess avoidability of each ADR, due to the lack of a suitable assessment tool. A novel ADR assessment tool (AAT) developed specifically with the paediatric setting in mind, has been proposed since the study was conducted, but has so far shown mixed inter-rater reliability (Bracken et al. 2013). This is most likely a reflection of the complexity of the influencing factors. Determining the avoidability of ADRs would be a vital step towards the translation of research findings, such as those described in this thesis, into improved medicines safety for children. It would allow intervention efforts to be concentrated on those ADRs that are likely to be avoidable and help health care professionals when communicating about ADRs.

Spontaneous reporting systems such as the UK yellow card scheme remain an important source of ADR signal detection. However, studies such as the retrospective study by Maitre et al. investigating adverse neurodevelopmental outcomes after exposure to antiepileptic

drugs (Maitre et al. 2013) highlight the ongoing importance of formal pharmacoepidemiological studies. This is not only because underreporting is a common problem of spontaneous reporting systems (Hawcutt et al. 2011), but also because late effects may be difficult to capture with this approach. More long term follow-up studies designed to monitor the effect of medicines on growth and (neuro-) development are required and projects like the Attention Deficit Hyperactivity Drug Use Chronic Effect (ADDUCE) project, investigating the long-term adverse effects of methylphenidate (The ADDUCE Consortium 2012), are aiming to address this.

A recent systematic review of pharmacoepidemiology safety studies in children since 1979 reported a significant imbalance with regards to the variety of drug classes investigated and safety outcomes considered (Osokogu et al. 2016). Furthermore, although the overall number of studies are increasing, the authors found a distinct lack of studies including neonates and suggested that some of the specific challenges such as small sample size and bias towards developed countries, may be overcome by international collaborations such as the Global Research in Paediatrics (GRiP) project (The GRiP consortium). In addition, a large amount of data is now being collected on electronic health records and data from electronic prescribing systems, thereby carrying an enormous potential for future pharmacovigilance and pharmacoepidemiological studies.

Prospective review of potential ADRs, as conducted in this study, is a powerful tool but is resource-intensive. This approach is probably best used in clinical trials or targeted disease settings with a high burden of potentially preventable ADRs.

5.2 Financial burden of ADRs in children - summary of findings interpretation and implications for research

This thesis also investigated the economic impact of ADRs in hospitalised children by assessing excess bed days caused by ADRs. Compared to adult studies, the financial impact in terms of hospital bed days was small. This might reflect the different spectrum of ADR types in children, the reduced number of comorbidities in children (and hence reduced number of drugs), their generally better health and shorter recovery times. Aside from limitations of the methodology which were discussed in chapter 3, determining excess LOS might not be the best proxy parameter to assess the financial impact of ADRs in children. ADRs with major clinical and personal impact, such as hearing loss, severe cardiomyopathy or neurodevelopmental deficits, can also be expected to have a significant financial impact. The cost of severe hearing loss has been highlighted in chapter 4, the cost of a heart transplant has been estimated to be $1 million (United Network for Organ Sharing (UNOS) 2016), lifetime cost for an individual with an autistic spectrum disorder and a learning disability is estimated to be £ 4.7 million (Foundation for People with Learning Disabilities 2007). To assess the financial impact of ADRs children it might therefore be conducive to focus on reaction types with an expected financial impact and to conduct detailed case based cost analyses.

In adult populations ADRs have been shown to be one of the most important contributors to non-compliance with medication (Leporini, De Sarro & Russo 2014). This in turn frequently leads to preventable hospital admissions and preventable progression of chronic disease with significant health care cost implications (Iuga & McGuire 2014). It would be interesting to investigate the burden of this in the paediatric population.

5.3 Cisplatin-induced ototoxicity in paediatric cancer patients - summary of findings, interpretation and implications for research

The association between variants in the TMPT and COMT genes and cisplatin-induced ototoxicity in form of hearing loss, was tested in a UK cohort of children. In this cohort, none of the TPMT and COMT polymorphisms was associated with hearing loss. However, combining data of this study with those of previous study the pooled odds ratio was statistically significant for the associations with the COMT SNP rs464316 (odds ration 1.53, 95% CI:1.7-2.00, I^2:5%)(Thiesen et al. 2017). This supports the findings from a previous meta-analysis (Hagleitner et al. 2014), discussed in chapter 4. There remains considerable uncertainty about whether TMPT, COMT and other previously described genetic polymorphisms represent genuine risk factors for cisplatin-induced hearing loss. Heterogeneity between study populations remains a challenge for pharmacogenomic replication studies, notably in the paediatric population. Adequately powered paediatric studies can be achieved through collaboration projects and could reduce the potential for bias in this aspect (Maagdenberg et al. 2016). Whilst good quality evidence is of course the prerequisite for any advancement towards personalised medicine, translating this genetic evidence into clinical practice remains the foremost challenge (Maroñas et al. 2016). For example, aminoglycoside-induced hearing loss has been associated with several mitochondrial mutations. Mitochondrial inheritance had first suspected in 1991 (Hu et al. 1991) clinical testing is now widely available for the most common mutation, A1555G. However routine pre-treatment screening has frequently been evaluated over recent years. In a detailed analysis by Veestra et al. 'the potential clinical, patient and economic outcomes associated with the use of A1555G testing in a cystic fibrosis population', i.e. a high risk population, were evaluated and the authors concluded that testing could not be recommended, potentially lead to worse patient outcomes by increasing the mortality risk from pseudomomas infections and might not even be cost-effective (Veenstra et al. 2007).

For cisplatin induced ototoxicity, an additional genetic variant ACYP2 (rs1872328) has recently been identified by GWAS in a cohort of 238 children with brain tumours (Xu et al. 2015). All patients who carried the variant in one or both alleles developed hearing loss, and none of the patients without hearing loss carried the variant. However, only 12.4 % of children with hearing loss also carried the risk allele (Xu et al. 2015). The findings have been replicated in two subsequent studies (Thiesen et al. 2017; Vos et al. 2016). Combining the data of all three studies in a meta-analysis the pooled odds ration remained significant (odds

ratio 5.91, 95% CI: 1.51-23.16). These results are promising. Indirectly, they also highlight the importance of accurate phenotyping. All three studies classified the ear with the worst hearing loss according to Chang criteria, which Chang developed specifically for the evaluation of cisplatin induced hearing loss in children (Chang & Chinosornvatana 2010). As discussed in chapter 4, most studies investigating cisplatin-induced ototoxicity in children did not report on asymmetric hearing loss which may have led to a significant degree of misclassification. However, further studies need to confirm the association between ACYP2 and cisplatin induce hearing-loss and further work is required to understand the association between risk genotypes and cisplatin-induced ototoxicity.

Currently, the only way of preventing cisplatin-induced ototoxicity is by avoiding cisplatin treatment. The clinical utility of identifying genetic risk genotypes proactively is thus dependant on the availability of alternative treatment options. Currently, the alternative treatment offered to patients with established hearing loss is carboplatin. However, the effectiveness of carboplatin treatment is not as well established as for cisplatin and there is still a risk of severe hearing loss (Boddy 2013). It is therefore unlikely that parents of a child with cancer would choose anything but the treatment proven to most likely to safe their child's life.

Although progress has been made in uncovering the underlying biological mechanisms leading to cisplatin induced ototoxicity, crucial steps, such as how cisplatin/cisplatin metabolites enter the sensory hair cells, are still unclear (Callejo et al. 2015). Without this knowledge, successful translation into oto-protective treatment will remain a challenge. Whilst a number of oto-protective agents have been shown to be effective in pre-clinical experiments, many of them also reduce the therapeutic efficacy of cisplatin, which renders them unprofitable in clinical practice (Callejo et al. 2015). A recent Cochrane review update of 'medical interventions for the prevention of platinum-induced hearing loss in children with cancer' concluded that there is not enough evidence to give recommendations for clinical practice and that 'more high quality research is needed' (van As, van den Berg & van Dalen 2016).

5.4 Conclusion

ADRs remain a significant cause of morbidity and mortality worldwide. ADRs occurring in children admitted to hospital are frequent, but rarely associated with permanent harm or death. Few studies to date have investigated the financial burden of ADRs in paediatric populations. The significant inter-individual differences in cisplatin-induced ototoxicity are far from being fully understood and evidence for genetic variants has been mostly controversial.

Overall, increasing use of electronic clinical databases, electronic prescribing technology, national and international collaborations and advances in genomic technology, are providing vast opportunities for future research projects aiming to optimise treatment and improve medication safety for children.

6 References

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