CLINICAL PHARMACOLOGY OF FIRST- AND SECOND-LINE TREATMENTS OF CONVULSIVE STATUS EPILEPTICUS

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DEDICATION

To my parents

for all their sacrifice and hard work

This achievement is as much theirs as mine.

ABSTRACT

Convulsive status epilepticus (SE) is a serious neurological disorder characterized by abnormally prolonged seizures or multiple discrete seizures lasting more than five minutes between which there is incomplete recovery of consciousness.[1,2] SE must be treated rapidly to avoid long term consequences such as neuronal loss and brain damage.[1,2] While benzodiazepines including lorazepam (LZP), midazolam (MDZ) and diazepam (DZP) are recommended as first-line treatments of SE and have been shown to be safe and effective[3–5], their failure rate ranges from 25-55%.[6–13] Fosphenytoin (FOS), levetiracetam (LEV) and valproic acid (VPA) are commonly used as second-line treatments; however, until recently there were no randomized controlled studies to guide the selection of one drug over the others. The Established Status Epilepticus Treatment Trial (ESETT) was a multicenter, blinded, randomized study to determine the most and/or the least effective therapy among FOS, LEV and VPA for the treatment of benzodiazepine-refractory generalized convulsive SE.[14] The primary outcome of ESETT was clinical cessation of seizures and improved responsiveness at 60 minutes after the start of study drug infusion without additional antiseizure medications.

The overarching objective of my dissertation work is to address two unmet needs related to better utilization of the available first- and second-line treatments of generalized convulsive SE using data collected as a part of ESETT. While evidence-based guidelines provide guidance on the use of benzodiazepines as initial therapy including which benzodiazepine to administer, time of administration relative to onset of SE, and the dose and route of administration based on the patient's weight; smaller studies have found that these guidelines are often not implemented in practice.[15–19] Further, underdosing is a

prognostic factor for refractoriness of SE and poor outcomes.[15–19] Specific aim one of my research was to perform a comprehensive characterization of benzodiazepine use as first-line treatment in a large cohort of adults and children with convulsive SE who failed treatment and progressed to established SE. I hypothesized that benzodiazepines as first-line treatment of SE are not appropriately used in practice despite the availability of evidence-based guidelines. The benzodiazepine dosing patterns were described using pre-enrollment data from 478 patients with convulsive SE enrolled at 57 centers across the United States in ESETT. The results showed that most of the DZP administrations were given by caregivers prior to emergency medical services (EMS) arrival by the rectal route and a majority of these met guideline-recommendations. On the other hand, > 85% of MDZ doses, mostly administered by EMS personnel using the intramuscular (IM) or intravenous (IV) routes, and > 75% of LZP doses, most of which were administered using the IV route in the emergency department (ED) setting, were lower than guideline recommendations. Following an interim analysis, additional training was provided to study teams at participating sites designed to increase the awareness of appropriate benzodiazepine dosing. However, these educational efforts did not improve the pattern of underdosing overall or specifically in the ED. Further, it took a median of 27 minutes to administer the first dose of benzodiazepine after the onset of SE and a median of six minutes more to receive the cumulative adequate dose of benzodiazepines after the first dose. Delays in treatment may be one of the reasons why these patients failed first-line therapy. Introduction of newer, easy-to-use benzodiazepine formulations like IN DZP (Valtoco®) and IN MDZ (Nayzilam®), updating EMS protocols to reflect the current guidelines, availability of rapidly interpreted electroencephalography in the EMS and ED settings,

regular training of EMS and ED personnel, and routine follow-up of practice patterns for quality assurance may improve the dosing patterns and, potentially, reduce benzodiazepine failure rates. Lastly, the association of benzodiazepine doses was tested with the ESETT primary outcome (clinical seizure cessation with improved responsiveness at 60 minutes after the administration of second-line treatment) using logistic regression. Higher cumulative doses of benzodiazepines were not significantly associated with higher likelihood of treatment success. Taken together, these results suggest that the recommended treatment must be administered as a single full dose as early as possible after the onset of SE.

Specific aim two was to evaluate whether fixed dosing affects efficacy of second-line therapies. Weight-based dosing capped at 75 kg was used in ESETT for FOS, LEV and VPA. While development of a drug exposure-response relationship is the approach that I intended to use, the study was stopped early because of futility. Consequently, only a small number of pharmacokinetic samples were collected, which was insufficient to develop the models needed to perform the analysis. As an alternative, the association of weight, weight-normalized dose, treatment, and sex with primary outcome success in ESETT was tested. I hypothesized that patients weighing > 75 kg who received the capped doses of ESETT drugs would have lower drug exposure and would be more likely to be non-responders as compared to those weighing ≤ 75 kg. However, capping the dose at 75 kg did not significantly affect the likelihood of primary outcome success in ESETT. However, future studies measuring drug concentrations and including heavier individuals with higher body mass index are needed to confirm these findings.

We were interested in measuring early exposure of the drug because rapid response desired with an emergent therapy is driven by the early exposure at the site of action. While a reliable estimate of early exposure is required to develop the exposure-response relationship to guide therapy, collecting early pharmacokinetic samples in an ED is challenging. Using ESETT and the ESETT drugs as a motivational example, I conducted a pharmacokinetic simulation study to evaluate the predictive performance of using two early plasma concentrations and a population pharmacokinetic modeling approach to estimate early drug exposure. The performance of the population pharmacokinetics approach was compared with standard non-compartmental analysis (NCA). I hypothesized that the estimates of early exposure obtained using the population pharmacokinetic approach will be closer to the true value as compared to those obtained using standard NCA. Using prior published pharmacokinetic models and two concentration measures in the first two hours post drug administration, I found that early drug exposure can be estimated with reasonable precision. Further, the performance of the population pharmacokinetic approach was found to be significantly superior as compared to a standard NCA approach. This approach can be applied in other emergent conditions or situations where early drug exposure may be of interest.

The results from ESETT showed that there was no difference in the efficacy of FOS, LEV and VPA for the treatment of benzodiazepine-refractory SE in adults and children. Based on these results, I hypothesized that LEV will most likely be the second-line treatment of choice as it is easier to administer, has a better overall safety profile with fewer side effects and is the most cost-effective drug among the three.[20] While the safety profiles for the three drugs were generally similar in ESETT, rate of endotracheal intubation with FOS was

significantly higher in children. Further, the response rates for second-line treatments in ESETT were approximately 50%, indicating that there is a clear unmet need in this area for better therapies. Among the available treatment options, ketamine, which is an N-methyl-D-aspartate (NMDA) receptor antagonist, might be a potential alternative. As a future direction, I designed a prospective, multicenter, randomized, double-blind study of ketamine for the treatment of convulsive SE in the ED.

Using data from ESETT, my research work suggests that the currently available therapies for initial treatment of convulsive SE can be better utilized to improve outcomes in patients. Further, my research indicates that that there is little reason to think that higher doses of FOS, LEV and VPA will result in greater response rates. Hence, the conduct of controlled clinical trials of other antiseizure medications for benzodiazepine-refractory SE is warranted. This work also provides valuable information to facilitate the design and conduct of a future study for the treatment of convulsive SE in an ED setting.

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PROLOGUE

In this dissertation, the clinical pharmacology of anti-seizure medications used for the treatment of generalized convulsive status epilepticus (SE) is described. The overall goal of my research is to improve the use of currently existing therapies for the management of generalized convulsive SE. This work investigates the first-line treatment of SE with benzodiazepines and second-line treatment with fosphenytoin (FOS), levetiracetam (LEV) and valproic acid (VPA) using data collected as part of the Established Status Epilepticus Treatment Trial (ESETT), a phase III, comparative-effectiveness study of FOS, LEV and VPA for the treatment of benzodiazepine-refractory generalized convulsive SE.

Chapter 1 describes the overarching hypothesis and specific aims of my research including the objectives and rationale of ESETT. Further, an overview of seizure emergencies focusing on convulsive SE, classification of SE and its treatment is provided. Lastly, the design of ESETT, study results and their implications on clinical care of patients with SE are presented in this chapter.

Chapter 2 contains systematic evaluation of benzodiazepine doses administered in adults and children as first-line treatment of convulsive SE prior to enrollment in ESETT. One of the inclusion criteria for ESETT was that patients must have received a cumulative adequate dose of benzodiazepines and yet continued to experience convulsive SE. Thus, data on timing, dose and route of administration for benzodiazepine doses administered by caregivers and/or medical personnel in the ambulance and/or in the emergency department

(ED) were recorded to determine eligibility for ESETT enrollment. The **objectives** of this opportunistic study were to:

1. Describe the patterns of benzodiazepine use as first-line treatment of SE and to compare the doses administered with guideline recommendations.
2. Test the association of benzodiazepine doses used in ESETT patients as initial therapy with ESETT outcomes assessed at 60 minutes after the administration of second-line treatment among FOS, LEV or VPA using logistic regression.

Chapter 3 presents the design of the ancillary pharmacokinetic study for the collection of blood samples from patients to measure the concentration of ESETT drugs post administration. This chapter describes a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay that was developed and validated for the simultaneous analysis of phenytoin (PHT, active form of FOS), LEV and VPA. The primary **objective** of this chapter was to report the total plasma concentrations of the three ESETT drugs and the plasma protein binding of PHT and VPA in young children enrolled in ESETT.

Chapter 4 presents a dose-response analysis to test the association of weight and weight-normalized dose, capped at a bodyweight of 75 kg for FOS, LEV and VPA with primary outcome success in ESETT using logistic regression.

Chapter 5 describes a pharmacokinetic simulation study inspired from ESETT. Estimating early drug exposure in emergent conditions like SE is challenging. Using ESETT as a motivational example, the primary **objective** of this simulation study was to evaluate the predictive performance of a population pharmacokinetic modeling approach using sparse

sampling data with two samples per patient to estimate early drug exposure and to compare these results with a standard non-compartmental analysis approach.

Chapter 6 is focused on my reflections from ESETT including the lessons learned during the conduct of the study and as a future direction, the design of a potential future study using ketamine for the treatment of convulsive SE in the ED is discussed.

CHAPTER 1

INTRODUCTION

1.1 Introduction

The following is a synopsis of convulsive SE followed by a brief description of ESETT to setup the overarching objectives of my dissertation work. Details about types of seizure emergencies and their treatments are provided in section 1.2, followed by the rationale and design of ESETT (section 1.3), its results (section 1.4) and their implications on patient care (section 1.5).

Convulsive SE is a serious neurological disorder characterized by abnormally prolonged or multiple discrete seizures lasting more than five minutes between which there is incomplete recovery of consciousness.[1,2] SE must be treated rapidly to avoid long term consequences like neuronal loss and brain damage.[1,2] In patients with epilepsy, SE can be precipitated due to withdrawal of antiseizure medications, intercurrent illness, or metabolic disturbance.[21] The mortality of SE has been estimated to be around 20%, but can be up to 40% in the elderly with acute symptomatic SE and comorbidities.[22–26] With the annual direct in-patient cost associated with SE estimated to be $4 billion in the United States[27], SE is also a severe economic burden on the healthcare system.

Appropriate and timely treatment of SE has been found to reduce the associated morbidity and mortality.[28] The goal of therapy of convulsive SE is rapid termination of clinical and electrical seizure activity.[5] Hence, typically the intravenous (IV) or intramuscular (IM) routes of administration are used for treatment. Several evidence-based guidelines have been published for the treatment of convulsive SE.[3–5] Benzodiazepines including lorazepam (LZP), midazolam (MDZ) and diazepam (DZP) are recommended as first-line treatment and have been shown to be safe and effective.[3–5] However, these treatments have only been found to be effective in terminating SE in 45% to 75% of the cases.[5]

Benzodiazepine-refractory SE is referred to as established SE. While FOS or its active form PHT, LEV, VPA or phenobarbital are commonly used as second-line agents for the treatment of established SE, there have been no randomized controlled studies to guide the selection of one drug over the other.[21] ESETT was a multicenter, blinded, randomized study to the determine the most and/or the least effective therapy among FOS, LEV and VPA for the treatment of benzodiazepine-refractory generalized convulsive SE.[14] The primary outcome of ESETT was clinical cessation of seizures and improved responsiveness at 60 minutes after the start of study drug infusion without additional antiseizure medication. Patients were enrolled at 57 EDs across the United States. The main objective of ESETT was to identify the best second-line treatment and guide clinical practice for the management of established SE.[14] Based on the package insert of FOS, the maximum amount that could be administered over 10 minutes was 1500 mg of phenytoin equivalents (PE) due to concerns about severe hypotension and cardiac arrythmia associated with faster administration.[29] As the recommended dose of FOS is 20 mg PE/kg, the 1500 mg PE corresponded with the therapeutic dose for a 75 kg patient. Hence, ESETT used weight-based dosing capped at 75 kg.[30,31]

Despite the recognition of SE as a critical care emergency, the goals of therapy and approaches to treatment vary dramatically. Often, inadequate treatment is administered due to the use of inefficient therapy such as sedatives or paralytics or administration of insufficient doses of anti-seizure medications (ASMs).[5] Underdosing could be due to lack of knowledge regarding what constitutes as adequate treatment with regards to the drug potency, safety, and preferred route of administration.

The overarching objective of my dissertation work is to address two unmet needs related to better utilization of first- and second-line treatments of generalized convulsive SE using data collected as a part of ESETT. As mentioned before, while benzodiazepines are effective as first-line treatment of convulsive SE, their failure rate ranges from 25-55%.[6–13] Evidence-based guidelines provide guidance on the use of benzodiazepines as initial therapy including the benzodiazepine to administer, time of administration and the dose and route of administration based on the patient's weight. However, smaller studies with three or fewer centers have found that these guidelines are often not implemented in practice.[15–19] A comprehensive characterization of benzodiazepine use as first-line treatment in a large cohort of adults and children with convulsive SE enrolled across multiple centers that failed this treatment and progressed to established SE has not been done previously. My research sought to address this unmet need using ESETT pre-enrollment data on benzodiazepine doses administered by caregivers and/or medical personnel for the initial treatment of 478 patients with convulsive SE enrolled at 57 centers across the United States. The primary goal was to assess whether guideline-recommendations were followed in practice, identify key areas of non-compliance, assess the benzodiazepine use in hospital and out-of-hospital, and to increase the awareness of appropriate benzodiazepines dosing as first-line treatment of SE in the clinical community. Further, the association of benzodiazepine doses used with the ESETT primary outcome measured after administration of the second-line treatment was also tested.

Specific Aim 1: To characterize the dosing patterns of benzodiazepines used for initial treatment of generalized convulsive SE in adults and children prior to enrollment in ESETT

and to test the association of benzodiazepine doses with ESETT primary outcome and clinical seizure cessation at 60 minutes after the administration of second-line treatment.

Hypothesis 1: Benzodiazepines as first-line treatment of SE are not appropriately used in practice despite the availability of evidence-based guidelines.

The results from ESETT would help identify the best and/or the least effective second-line treatment among FOS, LEV and VPA based on the clinical outcomes. Although weight-based dosing was used in ESETT, drug concentrations in patients receiving the same dose of the second-line treatment will vary. Plasma concentrations of PHT (after FOS administration), LEV and VPA, used in patients enrolled in ESETT, could explain the variability in treatment response for each drug and help separate the responders from non-responders. A sparse blood sampling approach was used in the ancillary pharmacokinetic study to measure concentration of the ESETT drugs within the first two hours after the start of study drug infusion. These concentrations were to be used to estimate drug exposure measured as partial area under the concentration-time curve till two hours. This exposure was to be correlated with treatment response to develop exposure-response relationships that can be used to guide future dosing of these drugs. Given the blinded nature of ESETT and limited blood volume that can be collected per sample, a LC-MS/MS method for the simultaneous detection and analysis of LEV, PHT and VPA was needed.

Specific Aim 2: To develop and validate a liquid chromatography-tandem mass spectrometry-based method for the simultaneous analysis of LEV, PHT and VPA in ESETT plasma samples and to test the association of dose or early exposure of LEV, PHT and VPA with treatment response in ESETT patients.

Hypothesis 2: As weight-based dosing capped at 75 kg was used in ESETT, patients weighing > 75 kg who received the capped doses of ESETT drugs will have lower drug exposure and are more likely to be non-responders as compared to those weighing ≤ 75 kg.

Estimating early drug exposure in emergent conditions like SE is a challenging. Using ESETT as a motivating example, a pharmacokinetic simulation study was performed to determine if two concentrations per patient in the first two hours post administration for PHT (after FOS administration), LEV and VPA and a population pharmacokinetic modeling approach can be used to estimate their early exposure reasonably well. The metric of early exposure used was partial area under the concentration-time curve.

Specific Aim 3: To evaluate the predictive performance of a population pharmacokinetic modeling approach using two samples per patient to estimate early exposure of PHT (after FOS administration), LEV and VPA and to compare these results with a standard non-compartmental analysis approach.

1.2 Epilepsy and Seizure Emergencies

A seizure is defined as a transient occurrence of signs and/or symptoms due to abnormal or excessive synchronous neuronal activity in the brain.[32] In 2014, a practical definition of epilepsy was established by the International League Against Epilepsy (ILAE) to aid in the diagnosis of the disease.[33] ILAE proposed that epilepsy be considered as a disease of the brain defined by any of the following conditions: 1) At least two unprovoked (reflex) seizures occurring > 24 hours apart; 2) one unprovoked (or reflex) seizure and the probability of further seizures similar to the general recurrence risk (at least 60%) after two

unprovoked seizures, occurring over the next 10 years; 3) diagnosis of an epilepsy syndrome.[33] As defined by the Epilepsy Foundation, a seizure is considered an emergency when it lasts a long time or when seizures occur close together and the person doesn't recover between seizures.[34] Seizure emergencies include prolonged episodes, seizure clusters and status epilepticus. A subgroup of patients with drug-resistant epilepsy may experience seizure clusters[35,36] whereas common causes of status epilepticus include cerebrovascular disorders, brain trauma, infections and lower than adequate levels of ASMs in patients with epilepsy.[37]

1.2.1 Seizure clusters

Seizure clusters are defined as a series of group seizures with short interictal periods. Various terminologies have been used to describe seizure clusters and include acute repetitive seizures or flurries[35], cyclical, serial, repetitive, crescendo and recurrent seizures.[38] Seizure clusters are not listed on the International League Against Epilepsy (ILAE) commission report on seizure terminologies and classification.[38,39] Further, there is lack of consensus on the number of seizures and the duration of interictal period. In what appears to be the first controlled trial investigating treatment of seizure clusters, investigators defined the condition as acute repetitive seizures which involved an episode of multiple complex partial or generalized seizures within a 24-hour period in adults or 12-hour period in children.[40] Several studies have defined seizure clusters as three or more seizures over a period of 24 hours (interictal period of 8 hours or less).[35,41] A randomized controlled trial of midazolam nasal spray for the treatment of patients with seizure clusters defined clusters as two or more seizures in a six-hour period.[42] Other definitions of seizure

clusters included two or more seizures in a period of 24 hours[43] or two to four seizures in less than 48 hours.[44]

Quantitatively defining seizure clusters is difficult as its prevalence varies by the criteria used to define them. For outpatient studies the prevalence is reported to be between 13 to 76% whereas for inpatient epilepsy monitoring unit studies reported prevalence ranges from 18 to 61%.[35] The high variability in the prevalence of seizure clusters in outpatient studies could be due to the highly individualized definition of seizure clusters as identified by caregivers/parents. The most significant risk factor for seizure clusters is found to be intractable epilepsy with high seizure frequency[41] (i.e., clustering of seizures could also be an indication of poorly controlled epilepsy)[45]. Various triggers for seizure clustering have been identified including sleep, waking, sleep-deprivation, stress, fever, illness, missing or changing medications, menstruation and alcohol.[43] Patients experiencing seizure clusters are at an increased risk of progressing to SE. A study of patients with intractable epilepsy found that SE had occurred in 44% of patients who typically had seizure clusters vs. 12.5% of patients without seizure clustering ($p< 0.002$).[36] Further, seizure clusters can result in increased emergency room visits or hospitalization.[35,41,46]

Benzodiazepines are the mainstay of treatment for seizure clusters. Although the IV route of administration is optimal and offers the fastest onset of action, this is usually not an option as most seizure clusters occur outside the hospital setting.[35,47] Several drugs other than the benzodiazepines MDZ, DZP, LZP and alprazolam, like allopregnanolone, brivaracetam and propofol are currently in use or are under development for rescue therapy.[47] Until recently, the standard of care for the treatment of seizure clusters was rectal DZP which had demonstrated a significantly higher response rate than placebo in

randomized controlled trials (RCTs).[40,48] Recommended dose of DZP rectal gel was based on age: 0.5 mg/kg for two-five years, 0.3 mg/kg for six to 11 years and 0.2 mg/kg for ≥12 years of age.[48] However, the rectal route of administration is socially objectionable. FDA recently approved intranasal (IN) formulations of DZP (Valtoco®) and MDZ (Nayzilam®) that will provide caregivers with an effective treatment option that is more convenient to administer than rectal DZP.[42,49]

1.2.2 Status Epilepticus

Previously, the commonly used definition of SE was seizure duration of greater than 30 minutes. This was based on a report by Meldrum et al. which suggested that seizure activity of 82 minutes or greater in baboons can lead to irreversible neuronal injury.[50] But given that the prognosis of SE worsens with increase in seizure duration[51], clinicians argued for a need to start treatment earlier. Lowenstein et al. addressed this issue and provided an operational definition of generalized convulsive SE as ≥ 5 mins of continuous seizure or two or more discrete seizures between which there is incomplete recovery of consciousness.[2] While this guided the treatment of generalized convulsive SE, other forms of seizures were not addressed. The Commission on Classification and Terminology and the Commission on Epidemiology of ILAE formed a task force to revise the concepts, definition and classification of SE. This task force defined SE as ‘a condition resulting from either failure of mechanisms responsible for seizure termination or initiation of mechanisms that lead to abnormally prolonged seizures (after time point t1). It is a condition that can have long-term consequences (after time point t2) including neuronal death, neuronal injury and alteration of neuronal networks, depending on the type and

duration of seizures'.[1] The division into two timepoints has clinical implications: time point t1 is when treatment should be considered or started whereas time point t2 determines how aggressively treatment should be implemented to prevent long term consequences. Based on the available data from animal experiments and clinical research, timepoint t1 was found to be five minutes for tonic-clonic SE, 10 minutes for focal SE with impaired consciousness and 10-15 minutes for absence SE. Timepoint t2 was 30 minutes for tonic-clonic SE and > 60 minutes for focal SE with impaired consciousness and was unknown for absence SE.[1]

1.2.3 Classification of SE

SE is classified based on the following axes[1]:

1. Semiology
2. Etiology
3. Electroencephalographic (EEG) correlates
4. Age

Axis 1: The semiology axis of classification refers to the clinical presentation of SE. The two main taxonomic criteria for this axis are the presence or absence of prominent motor symptoms and the degree of impaired consciousness. The SE's included in prominent motor symptoms group are tonic-clonic, tonic, myoclonic, focal and hyperkinetic whereas the without prominent motor symptoms includes nonconvulsive SE (NCSE) with or without coma. The without coma NCSE is further classified as generalized or focal. NCSE is also known as subtle SE and lacks overt motor manifestations but is associated with other observable features like coma, myoclonus, automatisms and nystagmus. NCSE is under-

recognized and under diagnosed as EEG data are essential to confirm definite NCSE.[52,53] Studies have reported persistent NCSE after the control of convulsive SE in a large proportion of patients.[54,55] Hence, rapid EEG is needed to confirm the termination of electrical seizure activity (i.e. lack of NCSE) along with clinical seizure cessation to fully evaluate treatment response in patients with convulsive SE.

Axis 2: This etiology axis of classification of SE refers to its etiology as known (i.e. symptomatic) and unknown (i.e. cryptogenic). The subgroups of the 'known etiology' category is based on temporal relationship of SE and is further classified as acute, remote and progressive.

Axis 3: In this axis, the classification is based on the EEG correlates, but currently there are no evidence-based EEG criteria for SE. The EEG patterns in SE can be described using location (generalized, lateralized, bilateral, etc.), name of the pattern (periodic discharges, rhythmic delta activity, etc.) , morphology (sharpness, number of phases, polarity, etc.), time-related features (prevalence, frequency, etc.), modulation (stimulus-induced vs. spontaneous) and effect of intervention on EEG.

Axis 4: This axis refers to the classification based on age and consists of subgroups as neonatal (0 to 30 days), infancy (one month to two years), childhood (> 2 to 12 years), adolescence and adulthood (>12 to 59 years) and elderly (≥ 60 years).[1]

SE is also classified based on the response to the treatment administered.[21] The first stage, also called as 'early SE', is defined as > 5 minutes of convulsive and > 10 minutes of focal non-convulsive seizure activity. If SE continues after administration of adequate dose of benzodiazepines, the second stage is termed as 'established SE'. The third stage is called

'refractory SE' and is reached when the SE fails to terminate after using another IV ASM (second-line agent) in addition to benzodiazepines. Lastly, if patients cannot be weaned off of their third-line agent (usually an anesthetizing agent) and/or if they have breakthrough seizures while being treated with a third-line agent, the SE is termed as 'super-refractory SE.[21]

1.2.4 Prevalence and etiology of SE

In 1996, a population-based study in Richmond, VA reported an incidence of SE at 41.3/100,000/year[56] whereas another study in 1998 from Rochester, MN reported a much lower incidence of SE at 18.3/100,000/year.[57] The higher incidence of SE in Richmond is attributed to the higher non-white population in Richmond, which has been reported to have a much higher incidence of SE.[56,58] A study of data from the US National Hospital Discharge Survey (NHDS) found that over a period of 31 years (from 1979-2010) there were a total of 760,117 discharges related to SE. Based on these data, the incidence of SE increased from 3.5/100,000/year in 1979 to 12.5/100,000/year in 2010 without a significant change in in-hospital mortality which remained at approximately 9.2%.[58] Looking at the age-specific incidence of SE, the study found that the incidence of SE was bimodal with high incidence in the first decade of life (14.3/100,000/year) and after the fifth decade of life (28.4/100,000/year). Similar results were reported by Betjemann et al. who conducted two retrospective serial cross-sectional studies using data from the Centers for Disease Control and Prevention and National Inpatient Sample between 1999 and 2010 with 408,304 SE related hospitalizations.[59] The study found a 56.4% increase in SE-related hospitalizations with incidence rate increasing from 8.86/100,000/year in 1999 to

13.86/100,000/year in 2010.[59] The increase in incidence of SE over the years has been attributed to several factors including changes in coding and billing practices for SE, increased diagnostic accuracy and wider availability and use of EEG in intensive care units (ICU).[58,59] A meta-analysis using data from 43 epidemiological studies conducted between 2000 and 2016 found a pooled crude annual incidence rate of 12.6/100,000 and pooled crude annual fatality rate of 14.9% (95% confidence interval of [11.7, 118.7]).[60] Stroke, non-adherence to antiseizure medications, trauma and central nervous system infections were found to be the most common causes of SE.[58,60]

1.2.5 Management of SE

Data on pharmacotherapy of SE, particularly for the later stages of SE (established, refractory and super-refractory), are mostly observational and heterogenous. Several RCTs for the early stages of SE have provided data to guide appropriate dosing.[8–11] However, evidence in the form of RCTs is scarce for later stages of SE with only one RCT conducted in super-refractory SE.[61] Several evidence-based guidelines have been published for the management of SE.[3–5] The most recent guidelines were published by the Guideline Committee of American Epilepsy Society in 2016.[5] The guideline focused on generalized convulsive SE as it is the most common type of SE and associated with substantial morbidity and mortality. Published articles that included RCTs, cohort studies, case-control studies, observational studies, meta-analysis or systematic reviews were used. In total, 38 relevant RCTs and four meta-analyses published in Cochrane library were identified. Further, additional information provided by pharmaceutical companies on three RCTs was used.[5] Relevant articles were evaluated for their class of evidence and ranged

from Class I (prospective, randomized controlled trials with masked outcome) to Class IV (evidence from uncontrolled studies, case series, case reports and expert opinion).[5] Using the scoring systems from the United States Agency for Health Care and Policy Research and the American Academy of Neurology[62], the collected evidence was translated to conclusions and recommendations. Figure 1.2.5-1 shows the proposed treatment algorithm for convulsive SE based on the evidence obtained.

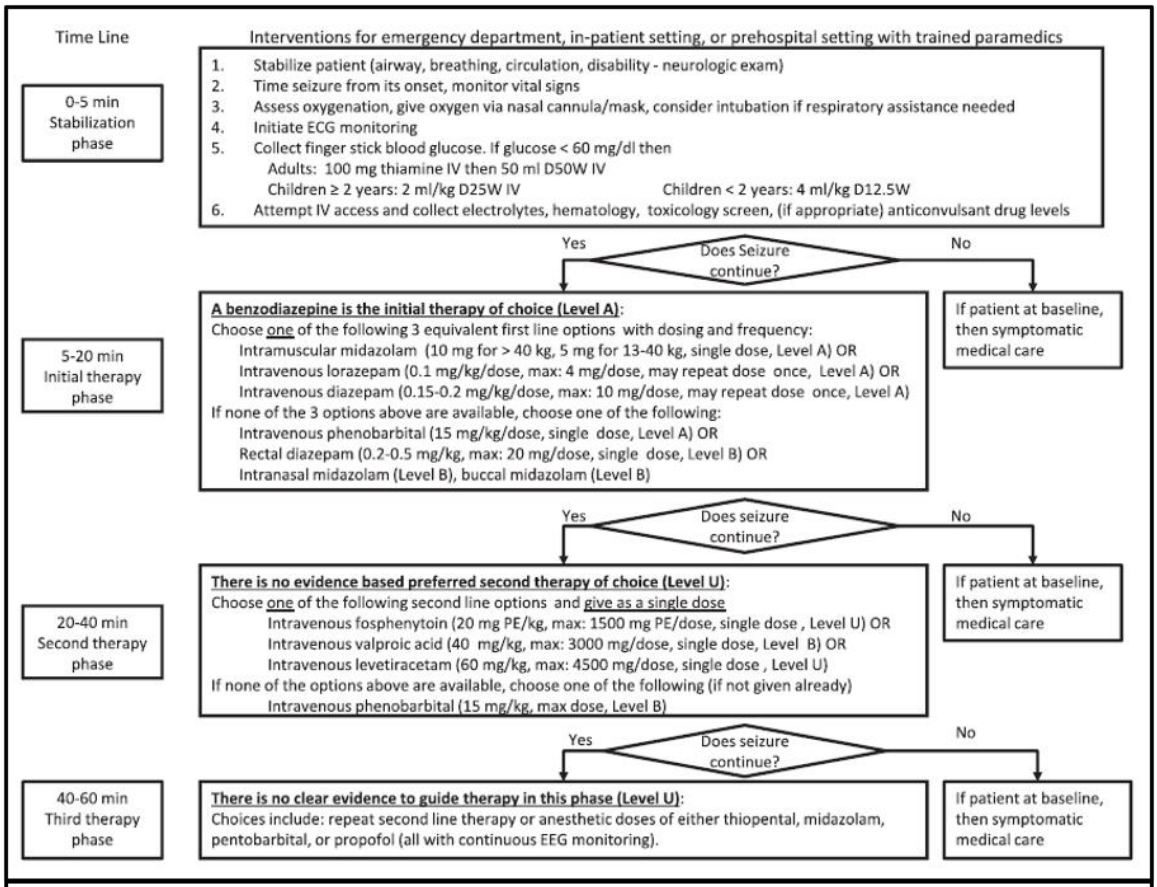

Figure 1.2.5-1: Algorithm for Treatment of Convulsive Status Epilepticus. Reproduced with permission from Glauser et al. 2016.

1.2.6 Early SE

Within the first five minutes of a generalized convulsive seizure the patient should be stabilized (airway, breathing, circulation), time for onset should be noted and vital signs monitored, blood glucose should be assessed, and IV access should be attempted to collect samples to measure electrolytes, hematology and toxicology screens and levels of ASMs if applicable. This phase is termed as the stabilization phase. If the seizure activity continues beyond five minutes, then a benzodiazepine should be administered as initial therapy. This phase is called the initial therapy phase and the goal of treatment is rapid termination of seizures and prevention of recurrent seizure activity. Based on evidence[8–11], the guideline-recommended benzodiazepines with routes of administration and weight-based doses are: IV LZP (0.1 mg/kg, max 4mg/dose, may repeat dose once), IV DZP (0.15-0.2 mg/kg, max 10mg/dose, may repeat dose once) or IM MDZ (10 mg for > 40 kg, 5 mg for 13-40 kg, single dose). If these 3 options are not available, the guidelines recommend using IV phenobarbital (15mg/kg/dose, single dose) which was found to have evidence level A[8] or rectal DZP (0.2-0.5 mg/kg, max 20mg/dose, single dose) or IN or buccal MDZ which have been found to have evidence level B.[63–67] While the available evidence for the use of rectal DZP is mainly for the acute treatment of prolonged seizures and seizure clusters, these findings and dose recommendations are extrapolated to convulsive SE as it falls in the same continuum of seizure emergencies.

The high level of evidence in benzodiazepine therapy is based on their safety, efficacy and tolerability as seen in four Class I RCTs[8–11], one Class II study[7] and several open-label Class III studies.[5] The first RCT was conducted in 384 patients with overt generalized SE and 134 with subtle SE enrolled at 16 Veterans Affairs Medical Centers and six affiliated

university hospitals over five years.[8] The study compared four IV treatment regimens: DZP (0.15 mg/kg) followed by PHT (18 mg/kg), LZP (0.1 mg/kg), phenobarbital (15 mg/kg) and PHT alone (18 mg/kg). Success was defined as cessation of all motor and EEG seizure activity within 20 minutes without return of seizure activity within the next 40 minutes. The response rates for the overt generalized convulsive SE treatment were 64.9% for LZP, 58.2% for phenobarbital, 55.8% for DZP+PHT and 43.6% for PHT alone.[8] LZP was found to be significantly superior to PHT in a pairwise comparison. The response rates for subtle SE ranged from 7.7 to 24.2% with no significant difference in the regimens. The second RCT compared IV LZP (2 mg), IV DZP (5 mg) and placebo for the out-of-hospital treatment of prolonged (≥ 5 minutes) or repetitive generalized convulsive seizures in adults.[9] The primary outcome was termination of SE by the time of arrival at the ED as determined by lack of clinical and electrical seizure activity and regained consciousness in the patient. Of the 205 patients enrolled in the study, 66 received LZP, 68 received DZP and 71 received placebo. The response rate for LZP (69.1%) or DZP (42.6%) was significantly higher than placebo (21.1%).[9] The third RCT called the Rapid Anticonvulsant Medication Prior to Arrival Trial (RAMPART) compared IM MDZ to IV LZP for termination of seizures in the pre-hospital setting prior to ED arrival, without additional rescue therapy and found that time to treatment has an impact on the outcome.[10] The study used a double-dummy design and enrolled a total of 893 subjects of which 448 received IM MDZ and 445 received IV LZP. Obtaining IV access in patients undergoing convulsive SE is difficult and often requires longer than 10 minutes even for expert paramedics. In fact, 31 (7%) patients randomized to IV LZP in the RAMPART study never received the medication because of failure to establish an IV line. The study reported a significant

difference in the response rates (73% in IM MDZ group vs 63% in IV LZP group) and IM MDZ was found to be superior to IV LZP.[10] While the time to IM drug administration was shorter as compared to IV LZP, it was offset by the faster onset of action after IV administration which resulted in similar latency until seizure termination for the two groups. The study showed that while the IV route is optimal to attain effective drug concentrations rapidly, establishing an IV access is challenging in an out-of-hospital setting even for a trained technician and that the IM route is a safe and effective alternative in this situation. The fourth Class I RCT compared 0.2 mg/kg IV DZP vs 0.1 mg/kg IV LZP for the treatment pediatric patients aged 3 months to 18 years with convulsive SE.[11] A total of 140 patients were enrolled in the IV DZP group vs. 133 in the IV LZP group. There was no difference in the efficacy (72.1% in DZP vs. 72.9% in LZP) or safety between the two treatments.[11] Looking across the four Class I RCTs, in 43-73% of the cases the seizures were terminated within 20 minutes whereas 11-39% of the cases had reoccurrence of seizures within the study period. Common side-effects associated with the use of benzodiazepines are cognitive impairment that includes anterograde amnesia, psychomotor slowing, sedation, drowsiness, motor impairment, inattentiveness and ataxia.[68,69]

1.2.7 Established SE

If seizure activity continues beyond the initial therapy even after administration of adequate dose of benzodiazepines, the second therapy phase should be initiated. There is no evidence-based second therapy of choice. The guidelines recommend giving a single dose of one of the following: IV FOS (20 mg PHT equivalents/kg, max 1500 mg PE), IV VPA (40 mg/kg, max 3000 mg) or IV LEV (60 mg/kg, max 4500 mg). If none of the options are

available, the guidelines recommend using IV phenobarbital (15 mg/kg) if not given already.

1.2.7.1 FOS (Fosphenytoin)

FOS is a prodrug of PHT and has to undergo dephosphorylation before it can enter the brain.[70] A survey of fifteen American academic medical centers found that FOS is the most commonly recommended drug for the treatment of established SE[71]. Similar results were reported in an international survey of 50 neurologists and epileptologists.[72] FOS is generally considered as the standard of care for established SE. It is highly bound to plasma proteins (93-98%). Following intravenous administrations, FOS is rapidly and completely converted to PHT by endogenous phosphatases with a conversion half-life of 7-15 minutes.[70] While the anticonvulsant effect of FOS is attributed to its active form, PHT, FOS is considered to be superior to PHT mainly because of the following: (1) safe rapid IV administration of FOS is possible (maximum rate of 150 mg/min for FOS vs. 50 mg/min for PHT), (2) FOS has better physicochemical properties than PHT (greater water solubility, less alkaline pH of 8.8 vs. 12 for PHT), (3) FOS has low potential for adverse reactions like pain, burning and phlebitis at the site of injection than PHT and (4) it can be safely administered IM unlike PHT which may lead to severe pain and possible tissue damage if used IM.[70] Cellular mechanisms of action thought to be responsible for the anticonvulsant activity of PHT include modulation of voltage-gated sodium channel of neurons, inhibition of calcium flux across neuronal membranes, modulation of voltage-dependent calcium channels of neurons, and enhancement of the sodium-potassium ATPase activity of neurons and glial cells.[29] Modulation of sodium channels is considered

as the primary mechanism of anticonvulsant effect as this property is shared with other anticonvulsants.

Based on the FOS package insert[29], FOS can be administered at a maximum rate of 150 mg PE per minute. This limits the maximum dose of FOS that can be administered safely over 10 minutes to 1500 mg PE. The recommended loading dose of FOS is 18-20 mg/kg. In the plasma, PHT is extensively bound to albumin and in the absence of FOS, the free fraction of PHT in plasma is approximately 12%. However, FOS displaces PHT from its binding site and the free fraction of PHT in the presence of FOS may increase up to 30%.[29] PHT is extensively metabolized by CYP2C9 and CYP2C19 in the liver and excreted in the urine. The hepatic metabolism of PHT is saturable and mean total half-life of PHT after administration of FOS has been reported to be between 12-29 hours.[29]

FOS and PHT are not effective in termination of benzodiazepine refractory SE in animal models.[73–75] In open-label clinical studies for initial treatment of SE, the efficacy of FOS/PHT in terminating SE was found to be 44-90%.[76]

1.2.7.2 LEV (levetiracetam)

LEV is recommended as an alternative to FOS by guidelines[3–5] and is the second most commonly used drug for the treatment of established SE in neurological ICUs[71,72] after FOS. LEV binds to the synaptic vesicle protein SV2A thought to be involved in the regulation of vesicle exocytosis in the brain. This mechanism of action of LEV is likely responsible for its anticonvulsant effect by inhibition of glutamatergic transmission in the brain.[77]

While the recommended oral dose of LEV in children weighing up to 40 kg is 20-60 mg/kg per day[77], higher doses are commonly used in adults. Doses of 4000 mg administered IV over 15 minutes appear to be safe in adults.[78] LEV is almost completely absorbed after oral administration and tablets/injection are bioequivalent.[77] LEV has linear pharmacokinetics at therapeutic doses. Plasma protein binding of LEV is low (< 10%) and 66% of the dose is renally excreted unchanged. The major metabolic pathway is enzymatic hydrolysis of the acetamide group and accounts for 24% of the dose. This process is independent of the cytochrome P450 enzymes in the liver. All metabolites are inactive and are excreted renally. Terminal half-life of LEV has been reported to be 6-8 hours. Dose adjustment is needed in patients with renal impairment.[77]

LEV has been shown to be effective in terminating behavioral and/or EEG seizures in animal models of established SE.[79,80] An analysis of open case series and reports of use of LEV in 707 patients (adults and children) with various forms of SE reported a success rate of 70%.[81]

1.2.7.3 VPA (valproic acid)

Like FOS and LEV, VPA has been recommended by guidelines for the treatment SE[3–5] and surveys show that it is the third or fourth most commonly used drug for the management of benzodiazepine-refractory SE.[71,72] The exact mechanism of action by which VPA exerts its therapeutic effects is unknown. The anticonvulsant effect of VPA is thought to be mainly due to blockade of voltage-gated sodium channels and increased brain

concentrations of gamma-aminobutyric acid (GABA) which is an inhibitory neurotransmitter.[82]

Recommended doses of VPA range from 15-45 mg/kg/day. VPA has been administered at rates of up to 11 mg/kg/min for dose up to 40 mg/kg[83–85]. Plasma protein binding of VPA is concentration dependent and has been found to increase from approximately 10% at 40 µg/mL to 18.5% at 130 µg/mL.[82] While glucuronide conjugation and excretion in the urine accounts for 30-50% of the VPA dose, mitochondrial β-oxidation is responsible for 40% of the dose. Remaining drug is eliminated mostly by other oxidative mechanisms with < 3% of the dose eliminated unchanged in the urine.[82]

Analysis of retrospective and prospective case series and two randomized open label studies found that in 693 cases of adults or children with SE, the response rate for VPA ranged from 60-83%.[76]

1.2.7.4 Phenobarbital

The mechanism of action responsible for the anticonvulsant effect of the barbiturate phenobarbital is thought to be similar to that of benzodiazepines by binding to an allosteric site on the GABA-A receptor which leads to increased influx of chloride and inhibition of action potentials.[86] However, unlike the benzodiazepines, at high anesthetic doses barbiturates directly activate the chloride channel even in the absence of GABA.[86] Further, while benzodiazepines produce a limited increase in chloride currents, barbiturates increase the current many fold. Doses used in adults typically range from 10-20 mg/kg administered at a rate of 100 mg/min up to a total amount of 700 mg in seven minutes.[21] Respiration and

blood pressure should be monitored with patients under clinical observation. The primary drawback of phenobarbital as second-line therapy is its central nervous system depressant effect, particularly after benzodiazepine use, which leads to sedation, respiratory depression and hypotension.[21]

In an RCT of convulsive SE, IV phenobarbital was found to be at least as effective as a combination of DZP and PHT.[87] Further, in the Veterans Affairs- study phenobarbital was found to be non-inferior to LZP for the initial treatment of SE.[8]

1.2.8 Refractory SE and Super-refractory SE

If SE is not terminated by 40 minutes after the administration of adequate doses of first- and second-line agents, the third phase of therapy should be initiated. There is no clear evidence from controlled trials to guide therapy in this phase because of the ethical challenges in randomization in the ICU setting. The typical choices recommended by the guidelines include either repeating a second-line therapy or administering anesthetic doses of thiopental, MDZ, phenobarbital or propofol along with continuous EEG monitoring.[5] Studies have reported that between 31-43% of patients enter the refractory stage.[88,89] Two systematic reviews and one small study comparing propofol and barbiturates found that none of the treatments currently used as third-line therapy were superior to another.[90–92] Among the third-line agents, MDZ continuous IV infusion was found to have the lowest rate of metabolic and cardiovascular complications which leads to severe immunosuppression and infections in compromised patients.[90,91] One study compared continuous IV MDZ infusion with IV DZP infusion in children with refractory SE and

found that both treatments were equally effective for termination of SE (86% for MDZ vs. 89% for DZP), but MDZ was associated with higher seizure recurrence rate and mortality.[93] A small study auditing the clinical experience of using high dose IV MDZ for the treatment of 17 patients with refractory SE, found that while MDZ terminated seizures in 13 (76%) of the patients, breakthrough seizures occurred in eight (47%) patients.[94] Based on the pathophysiology of convulsive SE, while GABA-A receptors internalize during SE, N-methyl-D-aspartate (NMDA) receptors accumulate in the synapse.[95] Hence, NMDA receptors are a potential treatment target for refractory SE. Propofol has been found to have NMDA antagonist activity invitro[96], but has a shorter duration of action and lower accumulation tendency than barbiturates. A systematic review that evaluated the outcomes of patients with refractory and super-refractory SE treated with anesthetics (thiopental/pentobarbital, n=192, midazolam, n= 585 and propofol, n=143)[91] found that while barbiturates achieved seizure control in 64% of the cases as compared to 78% for MDZ and 68% for propofol, the death rate during treatment was highest with barbiturates at 19% vs. 2% in MDZ and 8% in propofol.[91] The authors concluded that barbiturates should be restricted only for the most severe forms of SE. While the evidence for the use of other drugs like isoflurane (inhalation) and IV etomidate is limited and based on small case series, cardiovascular complications are common with both drugs.[21]

When seizure activity continues for more than 24 hours after treatment with an IV anesthetic, the condition is termed as super-refractory SE. Like refractory SE, there is insufficient evidence to guide therapy. This is mainly due to the rarity of the condition and difficulty in interpreting the findings from studies due to the complex interaction of multiple drugs used in parallel and the comorbidities seen in the later stages of SE. Several

treatment options have been evaluated such as allopregnanolone[61,97–99], perampanel[100,101], ketamine[102–104], stiripentol[105,106], rufinamide[107], corticosteroids[21], cannabidiol oil[108], magnesium sulphate[21] and IV immunoglobulins.[21] The generic name of allopregnanolone provided by the United States Adopted Names Council is brexanolone. While most of the other studies were smaller, often retrospective, single center, open label in nature, a phase III placebo-controlled trial of brexanolone in 132 adults and pediatric with super-refractory SE was conducted. The study found that brexanolone was not significantly different than placebo in meeting the primary endpoint of weaning patients from third-line agents without re-initiation of other third-line agents during brexanolone administration. Magnesium sulphate is used a drug of choice for the treatment of seizures occurring in eclampsia.[109] However, evidence of its use in patients with SE is extremely limited. Other interventions that have been used for the treatment of super-refractory SE include electro-convulsive therapy[110], thalamic deep brain stimulation[111], therapeutic hypothermia[112] and ketogenic diet.[113–115] Consensus protocol for the treatment of super-refractory SE based on discussion of literature-evidence by intensive care and neurology experts found that anesthetics including ketamine, propofol, thiopental and MDZ had the highest level of evidence.[112] While the level of evidence for ASMs was found to be lower, the protocol recommended using LEV, topiramate, pregabalin, lacosamide, VPA, PHT and perampanel in combination with the anaesthetics.[112] One retrospective case series of ketamine with 60 episodes of refractory SE in 58 patients found that in 32% of the cases (19 of 60) permanent control of SE could be potentially attributed to use of ketamine.[103] Another study of ketamine use in nine children with refractory SE (eight with super-refractory SE with seizures persisting > 24 hours) found that ketamine was associated with resolution of SE

in six patients.[104] Enteral topiramate has been used to treat refractory SE in 95 adults and children and has been found to terminate seizures in 65% (62/95) of the patients.[21] IV lacosamide has been available since 2008 and using data from 19 studies (10 single case reports and 9 case series) with 136 episodes of refractory SE an overall success rate of 56% (76/136) was reported.[116] Adverse events were reported in 25% of the cases and included mild sedation, hypotension, allergic skin reaction, angioedema pruritis.[116]

As described in section 1.2.7, while several drugs are commonly used as second-line treatment of convulsive SE, there is no evidence from an RCT to guide the selection. ESETT sought to address this unmet need and guide clinical practice.

1.3 ESETT

ESETT was a multicenter, randomized, blinded, comparative effectiveness study of FOS, LEV and VPA in the ED treatment of patients with benzodiazepine-refractory SE. The primary objective of the study was to determine the best and/or the least effective treatment of benzodiazepine-refractory SE in patients older than two years. Patients were enrolled at 57 hospital EDs across the US. Sites included academic medical centers and community hospitals. Twenty-six sites only enrolled adults, 18 only enrolled children and 13 enrolled both adults and children.

1.3.1 Rationale

Benzodiazepines are used as first-line treatment of SE and several studies have demonstrated their safety and efficacy in both adults and children.[6–13] Benzodiazepines are also recommended for initial therapy of SE by evidence-based guidelines.[3–5] However, approximately 35-45% of the patients are refractory to treatment with benzodiazepines. SE refractory to benzodiazepines is called established SE. Benzodiazepines act on the GABA-A receptor and enhance the inhibitory synaptic transmission.[117] Biochemical studies have revealed a decrease in the number of functional receptors on the post synaptic neurons and a decrease in the inhibitory synaptic transmission mediated by GABA-A in the hippocampi of animals in established SE.[118–121] Thus, prolonged seizures modify GABA-A receptors and could explain the reduced efficacy of benzodiazepines in terminating seizures in less than 25% of patients as the duration of SE increases.[8]

FOS, LEV and VPA were selected for ESETT based on recommendations of clinical groups, current clinical practice and, safety and efficacy data. As discussed above, the evidence-based guidelines for the treatment of SE published by the Neurocritical Care Society and the American Epilepsy Society reported all three drugs to have a level of evidence of Class IIa or IIb i.e. weight of evidence/opinion is in favor of usefulness as second-line treatment.[4,5] Results of a survey conducted in neurocritical care units to study the patterns of antiseizure medication use for the management of benzodiazepine-refractory SE found that FOS was the most commonly used second-line treatment followed by LEV and then VPA.[71] Another survey of 50 neurologists and epileptologists found that 80% chose FOS, 6% chose LEV and 2% VPA for the treatment of established SE.[72] Similar results were obtained from a survey of second-line treatment preference of 21 pediatric

emergency department directors. Further, these drugs have diverse mechanisms of actions focusing on targets other than GABA-A. Literature evidence for the use of FOS, LEV and VPA as second-line treatment of SE and their pharmacology has been described in detail in section 1.2.2.3 (Management of SE). Other agents like phenobarbital and lacosamide were not considered for ESETT due to known respiratory depression and sedation effect of phenobarbital in patients treated with benzodiazepines and lack of safety data after rapid IV administration in children for lacosamide at the time.

1.3.2 Systematic comparison of FOS, LEV and VPA

A retrospective analysis of protocol-driven treatment of established SE found that for 75 cases treated with VPA, failure rate was 25.4%, for 59 cases treated with PHT failure rate was 41.9% and for 52 cases treated with LEV 48.3% were failures.[122] After post-hoc adjustment for etiology and severity, LEV was found to have a significantly higher failure rate than VPA whereas the response rate for PHT was not significantly different than the other drugs.[122] A prospective randomized open label study comparing VPA and PHT for the treatment of convulsive SE reported that SE was aborted in 23 of the 35 patients randomized to VPA and 14 of the 33 patients randomized to PHT.[123] A second open label randomized prospective study of 100 patients with established SE found no significant difference in the response rate for PHT (81%) and VPA (88%).[124] In another study, patients were randomized to IV LEV or IV VPA after failing DZP treatment of SE and reported no significant difference in response rates.[125] While FOS/PHT has been the standard of care as second-line treatment of SE it suffers from limitations like modest efficacy, infusion rate toxicities (mainly cardiovascular)[29], has a narrow therapeutic window, and is an inducer of

cytochrome P450 enzymes and thus has a strong potential for drug-drug interactions.[126] Thus, based on the available information, LEV and VPA have the potential to be as effective or better than FOS.

1.3.3 Dosing

Based on the package insert for FOS, a maximum dose of 1500 mg PE can be administered over 10 minutes due to increased risk of severe hypotension and cardiac arrhythmia at higher rates.[29] The recommended dose of 20 mg/kg of FOS was thus capped at 75 kg in ESETT (1500 mg PE) and patients weighing < 75 kg received a 20 mg PE/kg loading dose over 10 minutes whereas those weighing 75 kg or more received a dose of 1500 mg PE over 10 minutes. The accepted therapeutic range of unbound plasma concentration of PHT is 1-2 mg/L.[127] Based on the pharmacokinetics of FOS[70], patients receiving a single dose of FOS (1500 mg) are expected to maintain a free PHT concentration above 2 mg/L for 90-120 minutes. To maintain the blind in ESETT, doses for LEV and VPA were also capped at 75 kg so that the volume administered for each drug will be the same.

Given the available information on safety and efficacy of LEV, a 60 mg/kg loading dose was administered over 10 minutes in ESETT patients weighing up to 75 kg and for those weighing > 75 kg a fixed dose of 4500 mg was used. The therapeutic concentration of LEV is considered to be 12-46 mg/L[127] and based on the pharmacokinetics of LEV[128], patients are likely to have therapeutic concentrations of drug for 8-10 hours after the start of infusion. For VPA, based on its safety and efficacy considerations and the pharmacokinetics of IV VPA[129–133], a 40 mg/kg loading dose was administered over 10

minutes for patients weighing up to 75 kg. For those weighing > 75 kg, a fixed dose of VPA of 3000 mg was used.

1.3.4 Outcomes

Primary outcome of ESETT was clinical cessation of SE determined by the absence of clinically apparent seizures and improving responsiveness at 60 minutes after the start of study drug infusion, without the use of additional anti-seizure medication. Assessment of treatment effect was done at 20 minutes and 60 minutes. Clinically apparent seizure is defined as obvious focal or generalized tonic-clonic movements, nystagmoid or rhythmic eye movements, or generalized or segmental myoclonus at the time of assessment. Improvements in responsiveness were characterized by purposeful responses to noxious stimuli, the ability to follow commands or verbalization.

The primary safety outcome was absence of life-threatening hypotension and cardiac arrythmia within 60 minutes after the start of the study drug infusion. Life-threatening hypotension was defined as systolic blood pressure remaining below the age-specified thresholds for more than 10 minutes after the reduction of the rate (or termination) of study drug infusion and a fluid challenge. Life-threatening cardiac arrythmia is defined as any arrythmia that persists despite reducing the rate of study drug infusion and that requires termination with chest compressions, pacing, defibrillation, or use of an anti-arrhythmic agent or procedure.

Additional predefined safety outcomes included mortality, need for endotracheal intubation within 60 minutes of start of study drug infusion, Richmond agitation and

sedation score at 60 minutes, acute seizure recurrence within 12 hours and acute anaphylaxis. Secondary efficacy outcomes included time to termination of seizures, admission to ICU, and the length of ICU and hospital stays.

1.3.5 Study design

ESETT was a multicenter, randomized, Bayesian response-adaptive comparative effectiveness trial of three active treatments of patients with SE who have failed initial treatment with benzodiazepines. Figure 1.3.5-1 shows the design used in ESETT. The patients were to be initially randomized in a 1:1:1 ratio across the three treatments. After randomizing the first 300 patients, response-adaptive randomization was utilized with the goal of maximizing the likelihood of identifying the most effective treatment. Interim analyses were planned after 400, 500, 600 and 700 patients were enrolled (i.e. after every 100 patients starting at 400). At each interim analysis, the randomization probabilities were to be updated based on the treatment response.[134] The trial had the provision to stop early for success or futility if any one of the following criteria was met at any interim analysis:

1. Probability of a treatment being the best was > 97.5%

   At each interim analysis the probability that each treatment offers the highest response rate is calculated. Trial was to be terminated for success if the most effective treatment is identified with a probability > 97.5%

2. Probability of determining a winner or a loser was < 5%

   If the predictive probability of identifying the most effective or least effective treatment at the maximum sample size was < 5%, the trial was unlikely to achieve

its primary objective of determining the best and/or the least effective treatment and was to be terminated for futility.

3. <u>Probability of response rate being ≥ 25% was < 5% for all 3 drugs</u>

   Each treatment was to be monitored independently and to be terminated in case of clinically unacceptable response rates. If the probability that a treatment offers at least a 25% response rate was < 5% then the treatment arm was to be terminated. If that happened for all three arms, then the trial was to be stopped for futility.

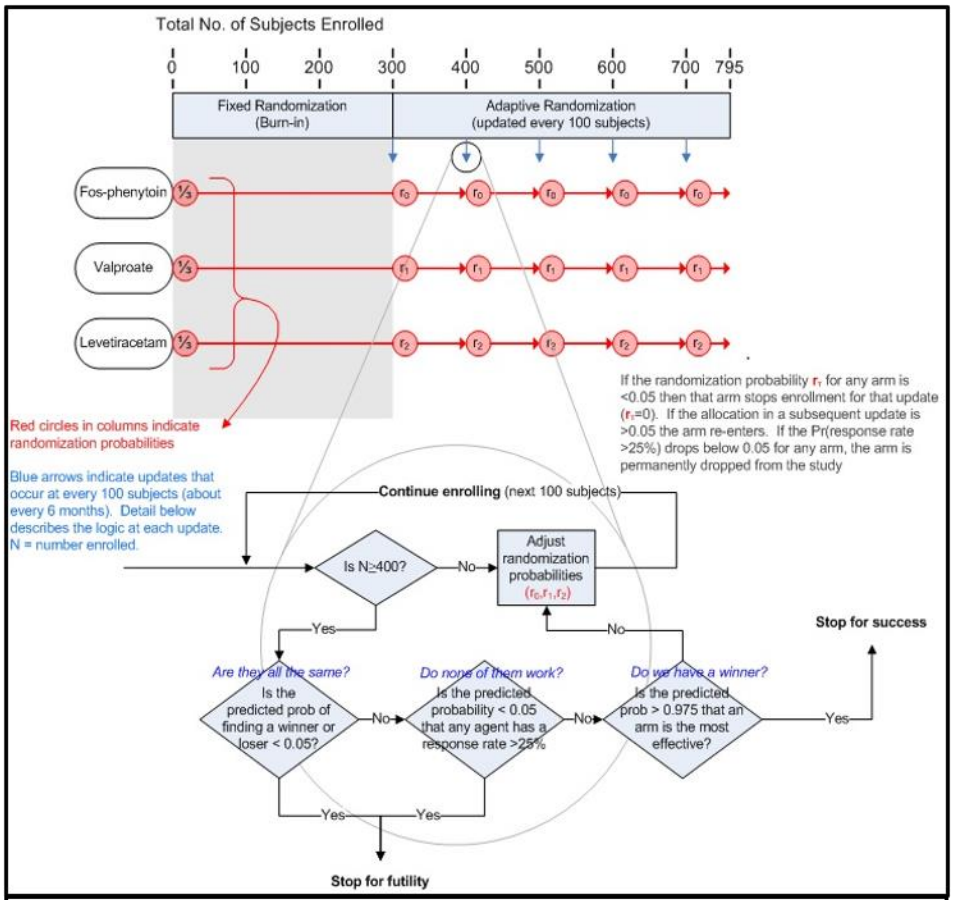

Figure 1.3.5-1: Schematic of ESETT study design. Adapted from the ESETT study protocol[133]

Before the assessment of the study results, all three drugs were considered to be equally likely to be the most effective or least effective treatment. Response rate in each of the treatment arm was modeled independently using Bayesian analysis. Starting with a uniform (0,1) prior probability distribution, the percentage of patients with treatment success in each arm was calculated. The uniform prior distribution allows treatment success to take any values from 0 to 100%. This was updated based on the observed binomial data using the conjugate beta-binomial model. From the posterior distributions, the probability that each treatment was the most or least effective was calculated.[134]

1.3.5.1 Sample size calculation

A maximum sample size of 720 unique patients provides 90% power to identify a single treatment as best if it had a response rate at least 15% greater than the other two treatments. The expected baseline response rate was at least 25%. The primary analysis was intent-to-treat and included all patients as they were randomized regardless of the treatment actually received or missing outcome. In case of re-enrollment of the same patient over the course of the trial, data from only the first enrollment was to be used. Thus, assuming a five percent re-enrollment rate and accounting for treatment crossovers and missing data, the maximum sample size was inflated from 720 to 795.[134]

1.3.5.2 Operating characteristics for Bayesian adaptive design

Closed-form solutions for trial operating characteristics such as power, type I error, sample size distribution and proportion of patients expected to be randomized to each treatment do

not exist and therefore these key trial characteristics were calculated using simulations (1000 simulations per scenario). During the design stage, these operating characteristics were compared between various competing designs (for example more frequent interim looks, fixed randomization, and starting response adaptive randomization at 200 patients vs. 300 patients).[134] Six scenarios were considered to explore a broad range of potential treatment effects (Figure 1.3.5-1). Scenario one, five and six included all the three treatments as equal but at varying levels of response rate: 50%, 25% and 10%, respectively. In scenario two, one treatment arm was better than the other two arms, in scenario three, two treatment arms were equally better than the third, and in scenario four, the response rates decreased across arms: 65%, 57.5% and 50%.[134]

Scenario (response rates)	Probability identify				
	t_{max} Early	t_{max} Max N	t_{max}	t_{min}	t_{max} or t_{min}
Null (0.50–0.50–0.50)	0.012	0.001	0.013	0.018	0.031
One Good (0.50–0.50–0.65)	0.879	0.013	**0.892**	0.033	0.902
Two Good (0.50–0.65–0.65)	0.115	0.003	0.118	**0.672**	0.763
One Middle, One Good (0.50–0.575–0.65)	0.481	0.022	**0.503**	**0.245**	0.682
All Bad (0.25–0.25–0.25)	0.016	0.001	0.017	0.030	0.044
All Really Bad (0.10–0.10–0.10)	0.006	0.000	0.006	0.000	0.006

t_{max} Early and t_{max} Max N show the proportion of trials in which an arm is identified as the best treatment at an interim analysis and at the end of the trial, respectively. t_{max} is the total probability of identifying a superior treatment. t_{min} is the probability of identifying the least effective treatment at the end of the trial. t_{max} or t_{min} is the probability of identifying either (or both). Values are bolded denoting true positives under t_{max} and t_{min}.

Figure 1.3.5-2: Results of 1000 simulated trials for the six response-rate scenarios.[134] Reproduced with permission from Connor et al. 2013.

These results show that the trial would be able to identify the most/least effective treatment if one exists (scenarios two, three and four) with high probability ($\geq$ 0.682). The false positive rate is the probability of declaring a treatment to be the most or least effective when in truth there is no difference between the treatment arms. This rate is demonstrated

in scenarios one, five and six and the maximum false positive rate is < 0.044 seen for scenario five. Further, the simulation results showed that compared to fixed randomization, when treatment effect differences truly existed (scenarios two, three and four), power was maintained generally with a lower sample size while a higher proportion of patients were randomized to superior treatment.[134]

The final study design characteristics were chosen via a tradeoff between logistical practicality and the operating characteristics they produced. For example, more frequent updating of randomization probability like every 20 patients vs. every 100 patients as was used in the study, would have slightly improved the operating characteristics. But given that sites would have to order new boxes at the time of update of allocation, with more frequent updates it would be burdensome, wasteful and expensive and thus increase the probability of human error.[134] Another example is the criteria for determining the most or least effective treatment was a probability > 97.5%. This threshold was chosen by convention as it was analogous to alpha of 0.025 in a one-sided comparison and because the simulation study showed that the type I error rate was controlled with this threshold.

1.3.5.3 Inclusion criteria

Patients aged two years or older who had been treated for generalized convulsive seizure of greater than five minutes duration with adequate doses of benzodiazepines and who continued to have persistent or recurrent convulsions in the ED at least five minutes and no more than 30 minutes after the last dose of benzodiazepines. Benzodiazepine doses based on evidence-based guidelines that were determined to be adequate for inclusion in

this study were: DZP 10 mg IV, LZP 4 mg IV or MDZ 10 mg IV or IM for those ≥ 32 kg. For children weighing < 32 kg, adequate doses were: DZP 0.3 mg/kg IV, LZP 0.1 mg/kg IV or MDZ 0.3 mg/kg IM or 0.2 mg/kg IV. While the required doses for DZP and LZP matched with guideline-recommendations, the MDZ dose in kids was slightly higher (0.3 mg/kg IM) than that recommended by Neurocritical Care Society's guidelines (0.2 mg/kg)[4] because the investigators wanted patients to receive more than one dose of benzodiazepines before getting enrolled in ESETT. The weight cut-off used was 32 kg instead of the 40 kg recommended by the guidelines because at 0.3 mg/kg the MDZ dose required for ESETT enrollment for kids weighing 32 to 40 kg would have been higher than those weighing > 40 kg. The last dose of benzodiazepines must have been given at least five minutes prior to study drug initiation to provide this dose enough time to act. The last dose of benzodiazepine must have been given within 30 minutes prior to study drug initiation to avoid enrolling patients in whom re-dosing of benzodiazepines may be more appropriate. Transmucosal benzodiazepines doses like rectal DZP or buccal MDZ may be included in the calculation of cumulative adequate dose, but at least one dose of benzodiazepines must also have been given by emergency medical services (EMS) personnel or in the ED between five to 30 minutes prior to study drug initiation.

1.3.5.4 Exclusion criteria

The purpose of exclusion criteria was to prevent enrollment for three narrow categories of patients in ESETT: those who can or should not be enrolled under exemption from informed consent (EFIC), those who have already received confounding therapy, and those with medical contraindications to the study drugs or an indication for alternative treatment.

The exclusion criteria and specific contraindications have been explained in the Tables 1.3.5-1 and 1.3.5-2).

Table 1.3.5-1: ESETT Exclusion Criteria and Rationale (Part 1)

Criteria	Rationale
Known pregnancy	Pregnant women are excluded from studies performed under exception from informed consent (EFIC)
Prisoner	Prisoners are excluded from studies performed under EFIC
Opt-out identification	Provides a mechanism for those who wish to prospectively opt-out of EFIC or identify and exclude cooperative potential re-enrollers.
Treatment with a second-line agent (FOS, PHT, VPA, LEV or phenobarbital or other agents defined in the MoP) for this episode of SE	Use of second-line agents prior to enrollment would confound the effects of the study drug
Treatment with sedatives with anticonvulsant properties other than benzodiazepines (ketamine, etomidate, propofol or other agents defined in the MoP)	Anticonvulsant sedative drugs are used for sedation and rapid sequence intubation. These drugs could terminate SE and would confound the primary efficacy outcome.
Endotracheal intubation	Prevents evaluation of patients for responsiveness
Acute traumatic brain injury	Other care, often requiring sedation, takes precedence.
Known metabolic disorder	Patients with known metabolic disorder are at a risk of liver failure when treated with VPA[81]
Known liver disease	Patients with liver disease are at a risk of liver failure when treated with VPA[81]

Table 1.3.5-2: ESETT Exclusion Criteria and Rationale (Part 2)	
Criteria	Rationale
Known severe renal impairment	Severe renal impairment has been associated with reduced plasma protein binding of fosphenytoin and valproic acid, resulting in substantially elevated free concentrations of these drugs.
Known allergy or other known contraindication to FOS, PHT, LEV or VPA	As per package inserts for FOS, LEV and VPA
Hypoglycemia < 50 mg/dL	SE due to hypoglycemia is first treated by giving glucose and correcting it.
Hyperglycemia > 400 mg/dL	SE due to hyperglycemia is first treated by reducing glucose and correcting it
Cardiac arrest/ post-anoxic seizures	Prognosis of SE due to anoxia and cardiac arrest is uniformly poor and distinct from other causes.

1.3.6 Consenting

As patients in generalized convulsive SE are unconscious, an informed consent process was not possible as patients cannot say whether or not they would want to participate in the research. The alternative process of identifying a legally authorized representative is not practicable in SE because the emergency treatment being studied must be initiated as quickly as possible as patient morbidity and mortality increases with increased seizure duration. Identification of the optimal emergency care for those in SE in this trial was conducted with exception from informed consent (EFIC) for emergency research in compliance with the FDA regulations for EFIC found at 21 CFR 50.24.

Per the FDA guidance on EFIC[135], community consultation was done prior to initiation of research. With guidance from each site's investigational review board (IRB), the community was asked to give their opinions of the research and included mechanisms like community meetings, town hall meetings, focus groups, meetings with established community advisory boards, in-person surveys and random-digit dialing surveys. The specific type of community consultation was determined by each site's IRB and reporting of results was standardized across all the ESETT sites.

Public disclosure was initiated prior to approval of the trial, continued during the enrollment process and was concluded with the dissemination of study results after the trial was concluded. IRB for each site was to determine the type and form of local public disclosure. Reporting of public disclosure efforts was standardized. Summaries of public disclosures was reported to each IRB and composite reports of local and national disclosures at the trial-level was provided to the FDA docket.

1.3.7 Manufacturing of study drug formulations

While commercial IV formulations with assured quality are generally available, they tend to be more expensive and may require prior approval from the drug company for use in a clinical study.[30] Purchasing the active pharmaceutical ingredient (API) is generally less expensive but can be difficult to source and due diligence is needed to ensure quality as evidenced by a certificate of analysis and track record of manufacturing API for products used in humans. Further, given the blinded nature of the study, the appearance, volume and rate of infusion of the three ESETT drugs had to be identical. Hence, it was decided to

manufacture the formulations. There were several challenges encountered during this process. For example, for FOS, an API manufacturer provided a fake certificate of analysis claiming high purity levels of FOS but up-on testing the sample, it failed to meet the USP standards. Further, use of API resulted in need for additional regulatory documentation including manufacturing process and quality control.

The drug formulations were manufactured using API for LEV and commercially available formulations were appropriately diluted using 0.9% saline for VPA and FOS so as to have the same appearance, formulation, packaging and administration (including volume and rate of infusion). LEV powder was purchased from Manus Akkteva Biopharm LLP located in Ahmedabad, Gujrat, India, valproate sodium injectable USP solution was procured from Abbvie located in North Chicago, IL, and fosphenytoin sodium injectable USP solution was procured from Pfizer located in New York, NY. Diluent (Sodium chloride (0.9%) injection United States Pharmacopeia (USP)) was obtained from the Pharmacy at University of California, Davis and stored in the Good Manufacturing Practices (GMP) facility at UC Davis, at controlled room temperature.

The concentration of study drug used in the individual formulation was 16.7 mg/mL of PHT equivalents for FOS, 33.3 mg/mL for VPA and 50 mg/mL for LEV. The dose of each drug used for ESETT was 20 mg PHT equivalents/kg, max 1500 mg PHT equivalents for FOS, 40 mg/kg, max 3000 mg for VPA and 60 mg/kg, max 4500 mg for LEV. As described before, the doses were capped at 75 kg due to safety considerations with rapid administration of FOS and to maintain the blind.[30,31] The three formulations were manufactured and filled in 100 mL vials which were placed in study boxes. The study

boxes were labelled to clearly identify them as ESETT medication for the three age groups: 2-18, 18-65, and 66 years and older.

1.3.7.1 Quality Control

The formulations were produced, packaged and labelled by UC Davis GMP facility and shipped to the study sites. Sterility (<USP 71> or an equivalent method), endotoxin (Endosafe, FDA approved LAL test), mycoplasma (polymerase chain rection (PCR)), osmolarity (<USP785)>), pH, drug concentration (high performance liquid chromatography (HPLC)) and particulate matter (<USP788>)[136] testing were completed before release of the final vials. Of these the sterility, mycoplasma, osmolarity and endotoxin tests were performed in the UC Davis GMP Quality Control laboratory. Particulate matter, pH, and drug concentration measurements were performed by ARL Biopharma Inc, located in Oklahoma City, OK. Each formulation was subject to quality control and stability testing and were not released for clinical use until they met the criteria preset for each of the drug formulations. All three study drugs were stored at 4-8°C, under controlled conditions in the UC Davis GMP facility.

Stability testing was carried out under refrigerated and accelerated conditions to ensure that the drug formulations met the preset criteria for quality. HPLC or gas chromatography method developed and validated for purity testing was used by ARL Biopharma to quantify the drug concentration for stability testing. Testing for particulate matter was done as per <USP 788> method[136] initially using light scattering and in the event that a vial failed to meet specifications, microscopy testing was carried out. Long-term stability studies at 5°C at times zero, one month, three months, six months, nine months and 12 months after the date of manufacture were performed for all three drug products. Stability studies were also

conducted under accelerated conditions (25°C/60% relative humidity) at times one month, three months and six months from the date of manufacture. Stability testing was extended out to 24 months (eg.18 months and 24 months) when needed. Three vials of each product were assayed at each time point. Twelve vials of each drug were kept in reserve in case further testing was required.

Based on <USP788>[136] criteria, there must be ≤ 6000 particles with diameter of 10 µm and ≤ 600 particles with a diameter of 25 µm in each vial. The light scattering method was used for this test. At the 12 months timepoint for FOS lot#1, one of the three tested vials was reported to have 6607 particles of 10 µm (7647 particles upon rerun) and failed to meet the preset particulate count standards. While some of the earlier timepoints for FOS lot#1 had five vials (one vial each at time zero, three months and six months and two vials at one month) with > 4500 particles of 10 µm diameter, this was the first instance when a vial had failed the criterion. When similar results with the 10 µm particulate count of > 5000 particles were reported in one of the vials tested at time zero for the newly manufactured FOS lot#2, it triggered a thorough investigation of the entire manufacturing and filling process to identify the cause of the high particulate count. As part of this investigation, ten vials of FOS lot#1 were randomly sampled from the inventory and tested for particulate matter. Neither of them failed the test. In fact, the 10 µm particulate count was < 500 in all vials. Further, to test if the glass vials were contributing to the particulate count, they were filled with just the sterile water for injection using the same process as used for the manufacturing and particulate count testing was done first using light scattering and later, using microscopy <USP788> for confirmation. This analysis found that microscopic bubbles present in the formulation during the filling process, were in fact detected as

particulates using the light scattering method; but it was possible to identify them as bubbles (and not particles) using microscopic testing method. Based on these findings, the chemistry manufacturing and controls (CMC) section of the investigational new drug (IND) application was updated to include microscopy testing for vials that failed to meet the preset particulate count criterion using the light scattering method.

1.3.7.2 Expiration dating and trends analysis

The initial expiration date was set at 12 months after the date of manufacture. Results of stability testing in the refrigerated and accelerated conditions were used to extend the expiration date using trends analysis. These shelf-life extension analyses were carried out as specified in the International Council on Harmonization (ICH) guidelines[137,138] using linear regression of all concentration data. Extrapolation was used to extend the shelf-life of the drugs products beyond the period covered by long term stability data mainly because no significant change was observed in the drug products under accelerated condition. Per the ICH guidance, the proposed shelf-life could be twice as long but should not be more than 12 months beyond the period covered by the long-term stability data.

The relationship between % drug concentration (y-axis) and time in months (x-axis) was best represented by a linear function. Hence, linear regression was used for this analysis. Since drug concentration is an attribute that can either decrease with time due to degradation of the drug or increase with time due to evaporation of the solvent, its direction of change is unknown. Hence, based on the guidance, two-sided 95% confidence limits for the mean were calculated and compared to the upper and lower acceptance criteria (Figure

1.3.7-1).[138] The drug concentration/quantity criteria to meet the quality control for the study was 90-110% of the specified value. Hence, for the trends analysis 110% and 90% concentration were used as the upper and lower acceptance criteria, respectively.

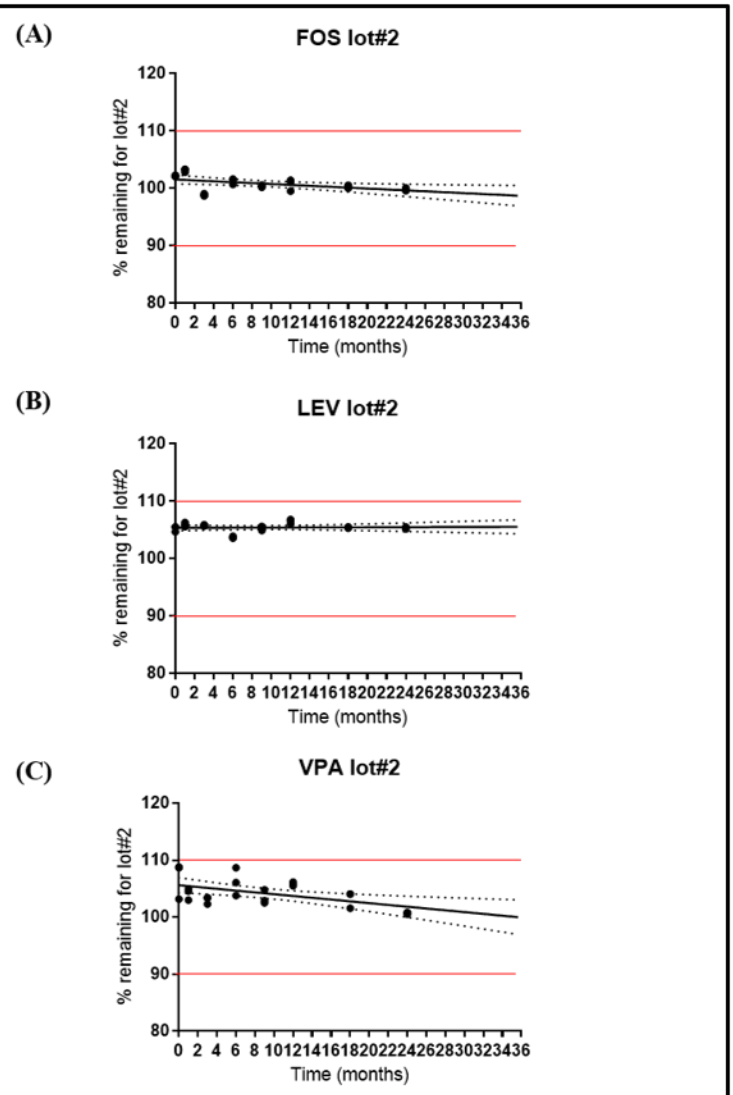

Figure 1.3.7-1: Trends analysis to predict shelf-life for the second lot for FOS (panel A), LEV (panel B) and VPA (panel C). Solid black lines indicate the mean regression line and dotted lines indicate the 2.5th and 97.5th percentile. Red lines indicate the upper (110%) and lower (90%) acceptance criteria.

As seen in figure 1.3.7-1, the % of drug remaining in the drug products over 24 months is well within the acceptance criteria for all three drugs. This shows that the drug formulations are stable over prolonged periods of time under refrigerated conditions with little to no degradation of the active ingredient. Trends analysis was done using GraphPad Prism (version 6, GraphPad Software Inc., CA, USA).

1.3.8 Study Enrollment Procedure

All patients presenting with a diagnosis of seizures were evaluated for participation in ESETT. Figure 1.3.8-1 shows the steps involved in enrollment of a new patient.

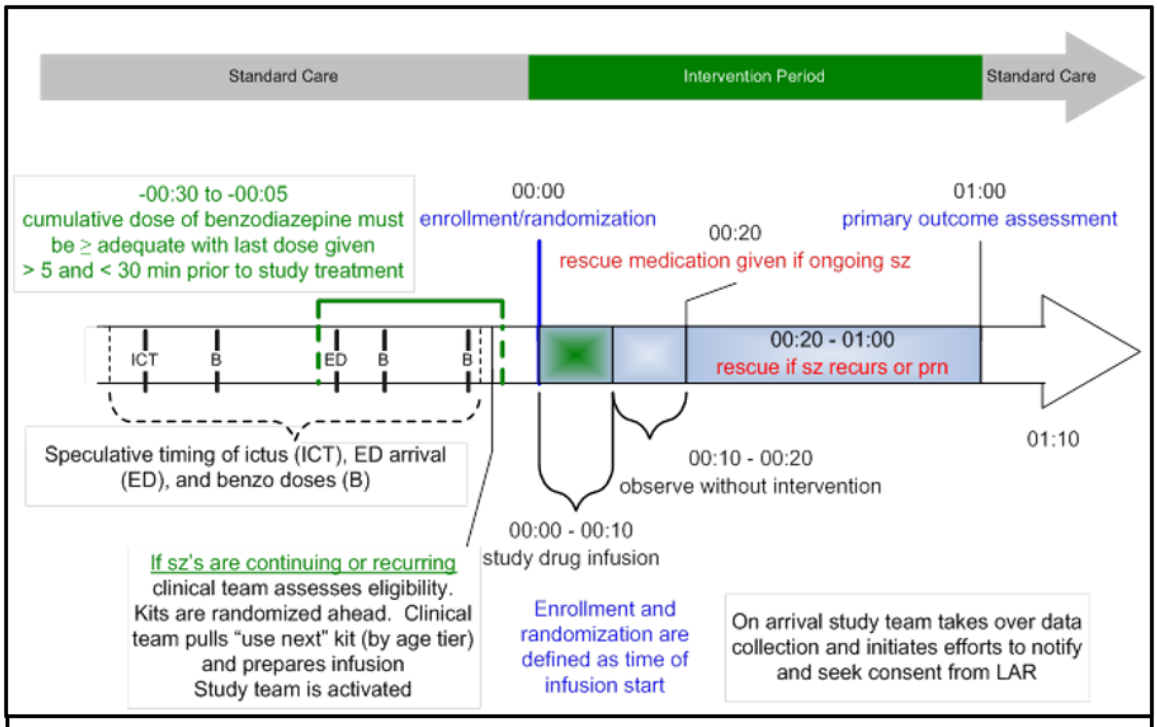

Figure 1.3.8-1: ESETT enrollment flow diagram. Adapted from the ESETT study protocol[133]

When an eligible patient presented to the ED, the clinical team accessed the age appropriate ESETT 'use next' box, which was most often located in the secured ED medication dispensing system or ED pharmacy. Upon opening the 'use next' box, the weight-based infusion rate was determined from dose administration chart (Table 1.3.8-1), the infusion pump was programmed with the determined rate and the infusion was started. Study team was alerted at this time if not already done.

Table 1.3.8-1: Dose administration chart. Adapted from the ESETT protocol[133]

Subject Weight (kg)	Infusion volume (mL)	Infusion Rate (mL/min) over 10 min	FOS dose (mg)	VPA dose (mg)	LEV dose (mg)
7.5	9	0.9	150	300	450
10	12	1.2	200	400	600
12.5	15	1.5	250	500	750
15	18	1.8	300	600	900
20	24	2.4	400	800	1200
25	30	3.0	500	1000	1500
30	36	3.6	600	1200	1800
35	42	4.2	700	1400	2100
40	48	4.8	800	1600	2400
50	60	6.0	1000	2000	3000
60	72	7.2	1200	2400	3600
70	84	8.4	1400	2800	4200
75	90	9.0	1500	3000	4500
>75	90	9.0	1500	3000	4500

Study drug was administered by pump for 10 minutes. The infusion was then discontinued and discarded. The patient was then observed for 10 more minutes, until 20 minutes after the start of study drug infusion. Rescue therapy was to be administered at 20 minutes by the care team for persistent or recurrent seizures. Upon arrival, the study team assisted the clinical team in completing the protocol and seek legally authorized representative to notify of the patient's participation and to determine consent to continue. The study team completed the required case report forms, collected the spent 'use next' box and placed a new 'use next' box in its stead.

1.3.9 Protection and Safety of Human Subjects

Protection of human subjects was of primary importance in this study. Clinical treatment teams were to rapidly assess and treat patients with SE. The trial was designed to avoid delays in treatment because longer delays may increase morbidity or mortality.

1.3.9.1 Institutional Review Board (IRB)

The trial protocol, informed consent document and any subsequent modifications were reviewed and approved by the IRB for each site participating in the study.

1.3.9.2 Data Safety and Monitoring Board (DSMB)

A data safety and monitoring board (DSMB) appointed by the National Institutes of Neurological Disorders and Stroke (NINDS) provided ongoing evaluation of safety data as

well as the overall conduct of the trial. The DSMB was formed by NINDS as per institute guidelines. The statisticians from the Statistical and Data Management Center generated DSMB reports semi-annually or more frequently as needed to aid in identifying any safety issues.

1.3.9.3 Subject Confidentiality

All data (case report forms, recordings, laboratory specimens and other records) kept at sites were physically and electronically secured to maintain subject confidentiality. Paper records and computers with data from trial patients were kept in a locked office or cabinet. The database used for the study was maintained behind a secure firewall, was password protected and used Secure Socket Layer Encryption for data entry and access. Clinical information was not released without written permission of the subject except when necessary for monitoring by the sponsor or other regulatory authorities/government agencies.

1.3.9.4 Study Modification/Discontinuation

If needed, the study had the provision to be modified or discontinued by the IRB, NINDS, the sponsor, FDA or other government agencies as a part of their duties to ensure that research subjects are protected.

1.4 ESETT Results

ESETT started enrolling patients on November 3, 2015. By October 31, 2017, enrollment of the first 400 patients was completed followed by the first interim analysis.[139] Sixteen of these 400 patients were enrolled twice and only their first enrollment was considered for the intention-to-treat analysis. Five of these 16 received LEV, seven received FOS and four received VPA. Of the 384 unique patients, 145 were assigned to LEV, 118 were assigned to FOS and 121 were assigned to VPA. In November 2017, enrollment was discontinued at the recommendation of the DSMB after the study had met one of the futility criteria (criterion# 2 listed in section 1.3.5) during the interim analysis. The three treatments had very similar response rates and there was a 1% chance of being able to identify the most or least effective treatment if the trial were to continue to the maximum sample size of 795. A predefined analysis did not exclude an interaction with age. Hence, it was decided that enrollment will continue in a pediatric sub-cohort to enrich a planned secondary subgroup analysis based on age.

The final cohort was enriched with an additional 78 patients (76 children and two adults) recruited between Nov 2017 and December 2018 after a predefined futility stopping boundary specific to the pediatric cohort was crossed.[140] The stopping boundary was assessed at an interim analysis that was originally planned at 500 patients but was completed at 478 patients as a part of a response to an adverse event. Using just the first enrollments for the 16 patients that were enrolled twice, the final cohort had 462 patients that included 225 children, 186 adults and 51 older adults.

1.4.1 Demographics

Kapur et al. reported the results for the first 400 patients.[139] The baseline characteristics of patients enrolled in ESETT shown in Figure 1.4-1 were similar across the three treatments.

Characteristic	Levetiracetam (N=145)	Fosphenytoin (N=118)	Valproate (N=121)
Age — yr			
Mean	33.3±26.0	32.8±25.4	32.2±25.4
Range	1–94	1–84	1–85
Age group — no. (%)			
0–5 Yr	30 (20.7)	24 (20.3)	28 (23.1)
6–10 Yr	17 (11.7)	15 (12.7)	7 (5.8)
11–20 Yr	9 (6.2)	10 (8.5)	18 (14.9)
21–40 Yr	31 (21.4)	20 (16.9)	19 (15.7)
41–60 Yr	34 (23.4)	26 (22.0)	26 (21.5)
≥61 Yr	24 (16.6)	23 (19.5)	23 (19.0)
Male sex — no. (%)	77 (53.1)	71 (60.2)	65 (53.7)
Race — no. (%)†			
Black	62 (42.8)	49 (41.5)	54 (44.6)
White	62 (42.8)	49 (41.5)	49 (40.5)
Other, more than one, or unknown	21 (14.5)	20 (16.9)	18 (14.9)
Hispanic ethnic group — no. (%)†	23 (15.9)	18 (15.3)	22 (18.2)
History of epilepsy — no. (%)‡	97 (66.9)	80 (67.8)	83 (68.6)
Final diagnosis — no. (%)‡			
Seizure or status epilepticus	128 (88.3)	104 (88.1)	102 (84.3)
Nonepileptic spell	13 (9.0)	11 (9.3)	13 (10.7)
Unable to adjudicate	4 (2.8)	3 (2.5)	6 (5.0)
Median lorazepam dose equivalents (IQR)§			
Dose for patients weighing ≥32 kg — mg	4.7 (4.0–6.0)	4.9 (4.0–6.0)	5.0 (4.0–7.0)
Dose for patients weighing <32 kg — mg/kg	0.2 (0.2–0.3)	0.2 (0.1–0.3)	0.2 (0.1–0.2)
Median duration of seizure at enrollment (IQR) — min‡¶	62.0 (43.0–85.0)	59.0 (43.0–94.0)	61.5 (38.5–86.5)
Benzodiazepines given before arrival — no. (%)	89 (61.4)	68 (57.6)	62 (51.2)

* Plus–minus values are means ±SD. Percentages may not total 100 because of rounding. IQR denotes interquartile range.
† Information on race and ethnic group was obtained from the medical record.
‡ The determination of this characteristic was based on adjudication.
§ Data include all 400 enrollments (i.e., including the patients who enrolled more than once).
¶ The duration of seizure at enrollment could not be determined for 39 enrollments.

Figure 1.4-1 Baseline characteristics of ESETT patients. Reproduced with permission from Kapur et al. 2019, Copyright Massachusetts Medical Society.

A total of 55% of the enrolled patients were male. With regards to race, 42% were white and 43% were black with the remaining categorized as other, more than one or unknown. Thirty-nine percent of the patients were children (up to 17 years of age), 48% were younger adults (18-65 years of age) and remaining 13% were older adults (> 65 years of age). For the enriched cohort, baseline characteristics were similar between the treatment groups within the three age groups.

Given the emergency setting of the trial, deviations from eligibility criteria occurred in 108 enrollments (27%). Most of the deviations were related to either the timing of last benzodiazepine dose or enrollment prior to administration of cumulative adequate benzodiazepine dose. Unblinding of investigators and treating clinicians to the assigned drug occurred in 200 of the 400 in which it was considered necessary for patient care. Of these, in 46 cases the unblinding occurred before 60 minutes, but only after a criterion for failure with regard to the primary outcome had been met.

1.4.2 Efficacy analysis

In the intention-to-treat analysis, the primary outcome of absence of seizures and improvement in responsiveness without additional antiseizure medications at 60 minutes was met in 68 of 145 (47%, 95% credible interval 39% to 55%) patients in the LEV group, 53 of 118 (45%, 95% credible interval 36% to 54%) in the FOS group and 56 of 121 (46%, 95% credible interval 38% to 55%) in the VPA group.[139] Figure 1.4-2 shows the distribution of posterior probabilities of primary outcome success for the three ESETT treatments. The posterior probability that LEV was better than FOS and VPA was 0.41, the

probability FOS was better than the other two drugs was 0.24 and the probability that VPA was better than the other two drugs was 0.35. There was no interaction between treatment group and age group or any other interaction with baseline covariates. In patients with treatment success, post-hoc analysis found no significant difference in seizure cessation at 20 minutes after the start of study drug infusion between the three treatments.

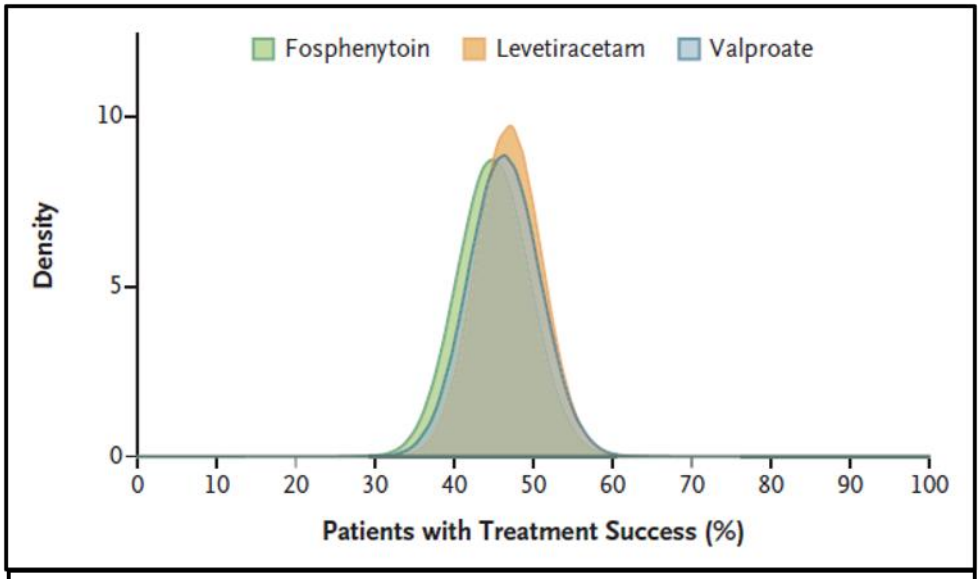

Figure 1.4-2: Posterior probability of success by treatment group for primary outcome ESETT at 60 minutes. Reproduced with permission from Kapur et al. 2019, Copyright Massachusetts Medical Society.

In the subgroup analysis based on age in the enriched cohort with 478 patients, no difference was seen in the primary outcome between treatment groups within each age group (≤ 18 years and > 18 years; p=0.93).[140] As seen in Figure 1.4-3 and 1.4-4, although children overall had numerically higher response rates, the probability of treatment success did not vary as a function of age in years (p=0.69 for the main effect of age and p=0.88 for

interaction of age [in years] by treatment). Figure 1.4-3 is similar to figure 1.4-2 and shows the distribution of posterior probabilities of primary outcome success for the three ESETT treatments stratified by age group. Results of the per protocol analysis were similar to those of the intention-to-treat analysis.

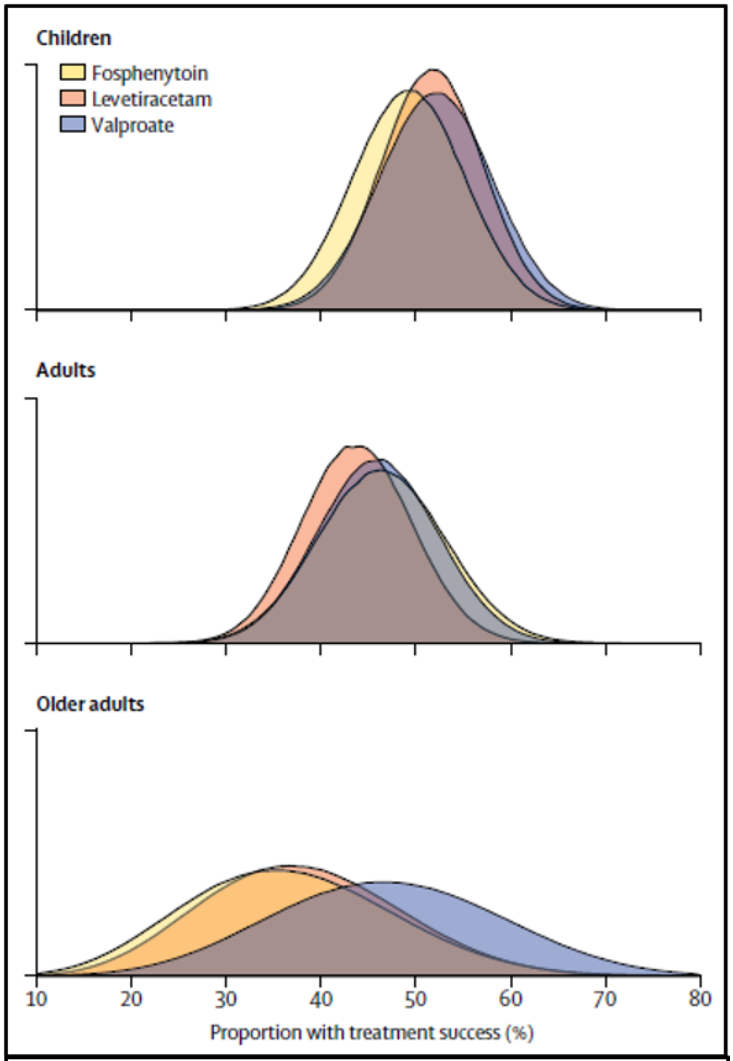

Figure 1.4-3: Posterior probabilities of success by age and treatment groups for the primary outcome. Reproduced with permission from Chamberlain et al. 2020. Like figure 1.4-2, y-axis denotes density of the distribution.

	Children (aged <18 years; n=225)	Adults (aged 18–65 years; n=186)	Older adults (aged >65 years; n=51)
Levetiracetam	85	71	19
Primary outcome	44 (52%; 41–62)	31 (44%; 33–55)	7 (37%; 19–59)
Probability treatment is the most effective	0·37	0·22	0·22
Probability treatment is the least effective	0·27	0·44	0·40
Fosphenytoin	71	54	17
Primary outcome	35 (49%; 38–61)	25 (46%; 34–59)	6 (35%; 17–59)
Probability treatment is the most effective	0·22	0·41	0·19
Probability treatment is the least effective	0·47	0·27	0·46
Valproate	69	61	15
Primary outcome	36 (52%; 41–63)	28 (46%; 34–58)	7 (47%; 25–70)
Probability treatment is the most effective	0·41	0·37	0·59
Probability treatment is the least effective	0·26	0·29	0·14

Data are n, n (%; 95% credible interval), or probability.

Figure 1.4-4: Efficacy analysis of ESETT enriched cohort by age group. Reproduced with permission from Chamberlain et al. 2020

1.4.3 Safety analysis

The frequency of life-threatening hypotension was 0.7% in LEV, 3.2% in FOS and 1.6% in VPA group and of endotracheal intubation was 20% in the LEV, 26.4% in FOS and 16.8% in VPA group.[139] Arrythmia was seen in 0.7% of LEV cases but in none of the FOS or VPA cases. Other safety outcomes did not differ significantly between the three treatment groups. There was no significant difference in the frequency of composite safety outcome of life-threatening hypotension or cardiac arrhythmia between the three

treatments. A total of 248 serious adverse events occurred in 42% of the patients and most frequently were convulsions after 60 minutes, reduced level of consciousness and respiratory distress.[139] For the subgroup analysis in the enriched cohort, the primary safety outcome of life-threatening hypotension or cardiac arrhythmia did not differ by treatment group in any age group. The rate of endotracheal intubation of children was significantly higher in the FOS group (33%) as compared to the LEV (8%) and VPA (11%) groups (Fisher's exact test p=0.0001) but did not differ by treatment in the adult age group. There was no detectable difference in the other safety outcomes.[140]

1.5 Implications of ESETT Results

The response rates seen in ESETT contrasted with previously published reports, mostly from observational studies.[122,124,125,141–144] A retrospective review of 187 episodes of SE in 159 adult patients treated over four years at a single center found that the percentage of patients in whom seizures (both clinical and electrographic) were stopped for more than 48 hours was 51.7% for LEV, 74.6% for VPA and 59.6% for PHT.[122] A meta-analysis of 22 studies using data from patients treated for convulsive benzodiazepine-resistant SE found that the response rate for LEV was 68.5% (95% CI: 56.2-78.7%), PHT was 50.2% (95 % CI: 34.2-66.1%) and VPA was 75.7% (95 % CI: 63.7-84.8%).[142] While the response rate for PHT reported in this study was in agreement with the ESETT results, the LEV and VPA response rates were much higher than those seen in ESETT. An open label randomized study of second-line treatment of convulsive status epilepticus (EcLiPSE) that compared LEV with PHT in 286 patients aged six months to 18 years enrolled at 30 emergency departments in the UK found that the response rate for LEV was 70% as compared to 64

% for the PHT group.[143] While, the response rates for the two drugs reported in this study are not significantly different, they are much higher than those seen in ESETT. Further, the study concluded that given the study results, the safety profile and the comparative ease of administration of LEV, it would be an appropriate alternative to PHT as the first-choice second-line treatment of convulsive SE in children.[143] A similar study (ConSEPT) undertaken at 13 EDs in Australia and New Zealand enrolled 233 children aged three months to 16 years found cessation of clinical seizure activity five minutes post-infusion (response) in 60% of the patients in the PHT group vs. 50% in the LEV group.[144] Another meta-analysis and cost-effectiveness study of second-line treatment of convulsive SE used data from 24 studies and 1185 SE episodes to compare effectiveness of five drugs FOS/PHT, LEV, VPA, phenobarbital and lacosamide.[20] The study found that phenobarbital was the most effective with a probability of seizure cessation of 0.8 whereas FOS/PHT was least effective with a probability of seizure cessation of 0.53.[20] A limitation of this analysis was that only three of the 24 studies included phenobarbital and only two studies included lacosamide. Further, the cost-effectiveness, calculated as cost per seizure stopped in this study, was the highest for LEV with FOS/PHT being consistently less cost effective than LEV, VPA and phenobarbital.[20] There are several possible reasons for this variability in response rates including different inclusion/exclusion criteria, time to treatment after the onset of SE, underlying genetic differences in these geographically diverse population, underlying etiology of SE, dose and rate of drug administration, criteria used to define a successful treatment outcome, and the time of outcome assessment relative to the start of drug administration. It is this variability in the response rates that poses a great challenge to appropriately design clinical trials for the treatment of SE.

Following publication of the ESETT results, there is a debate in the emergency medicine and neurology communities regarding the best second-line treatment among FOS, LEV or VPA. FOS/PHT is the only FDA-approved treatment among the three and is generally considered as the standard of care for the second-line treatment of convulsive SE. Further, FOS/PHT is the most commonly used second-line treatment as demonstrated by results from several expert surveys.[71,72] Another study comparing the SE treatment pathways at 10 pediatric hospitals in the United States found that nine of these pathways recommended FOS as the second-line treatment.[145] However, FOS/PHT suffers from several limitations including the risk of cardio-respiratory complications with rapid administrations requiring close monitoring, has a narrow therapeutic index, is prone to drug-drug interactions due to its CYP induction potential and is found to be the least cost-effective treatment.[20,29,146,147] LEV is easier to administer (can be given as a bolus vs. infusion), has a much better safety profile with fewer side effects and has been found to be the most cost-effective second-line treatment of SE.[20,143,144,146] However, an RCT evaluating prehospital treatment of SE in 107 adults found that administering 2500 mg of IV LEV with IV clonazepam terminated seizures in 74% of the cases as compared to 84% of the cases in the clonazepam and placebo group.[148] The authors concluded that the addition of LEV to pre-hospital treatment with clonazepam presented no advantage over treatment with clonazepam alone. This lack of efficacy with LEV may be due to the slow penetration of LEV into the brain.[149] Pharmacokinetic studies in rats have shown that LEV concentrations in the cerebrospinal fluid peaked at one hour after IV administration[150], comparatively rat brain levels of PHT peaked at 15 minutes after IV infusion[151] whereas for DZP, after IV administration, brain penetration is suggested to peak within seconds in rats[152] and in about 10 minutes in

humans.[153] However, the dose of LEV used in this study was much lower than that used in ESETT (60 mg/kg, 4500 mg maximum) and may have a played a role. VPA has a broad-spectrum of activity, is easy to administer and has been found to be effective in terminating SE refractory to benzodiazepines.[20,142,146] However, VPA use in pregnancy has been associated with several congenital malformations[82,154] and hence should be used with caution in women of child-bearing age. Further, in utero exposure to > 800 mg dose of VPA during pregnancy has been associated with lower IQ in the offspring.[155] Additionally, hepatoxicity, particularly in patients with mitochondrial disorders and children under the age of two years, pancreatitis including fatal hemorrhagic cases, hyperammonemia, thrombocytopenia and other forms of coagulopathy have been associated with use of VPA.[82,149] However, while most of these problems have been seen with chronic use of VPA, patients known to be pregnant, younger than two years and with known/suspected metabolic or liver disorders were considered to be at risk for VPA toxicity and were excluded from ESETT. In case of a pregnant patient not identified as pregnant, the ESETT investigators determined that benefit outweighed the risk of VPA use.

Another factor that governs the choice of therapy is the availability of drugs. In countries outside North America where FOS is not available, LEV is commonly favored as second-line treatment of SE.[149] Other commonly used drugs in the absence of FOS include PHT and phenobarbital. A nationwide observational study in Japan evaluating the changes in real-world practice patterns of drugs used for SE treatment found that there was a significant increase in the use of IV LEV as second-line treatment of SE in 2016 and 2017 after IV LEV was introduced in the Japanese market in December 2015.[156] A significant decrease in the use of IV PHT as second-line treatment was also seen during this phase.[156]

The general consensus among experts is that the choice of drug should be based on patient-related factors like etiology of SE, prior treatments, age, sex, hypersensitivity reactions, etc. and these factors should also be incorporated into treatment guidelines.[146,149]

1.6 Newer Treatment Options

Based on response rates for FOS, LEV and VPA as seen in ESETT, there is a clear unmet need for a more effective and safe treatment option for benzodiazepine-refractory convulsive SE as the current best second-line treatments work only in about 50% of the cases. Several newer treatment options are being evaluated for the management of SE. This section provides currently available evidence on some of the newer treatments that have shown promising results. However, conclusive evidence of their efficacy in the form of prospective RCTs is lacking in most cases. The current evidence is based mostly on case reports, case series and retrospective observational studies. Further, most of the currently available data for these treatments is in the management of later stages of SE (refractory and super-refractory).

1.6.1 Brivaracetam (BRV)

BRV is a synaptic vesicle 2A protein ligand and has greater affinity for this target than LEV. BRV has been tested primarily in the refractory SE and super-refractory SE and based on two retrospective chart reviews of 11 and seven patients, the response rate was found to be 27% and 57%, respectively.[157] BRV has higher permeability than LEV.[150] Permeability of BRV estimated using Caco-2 cells has been found to be close to DZP and

PHT which are currently used for the treatment of SE.[150] Pharmacokinetic studies in rats have shown that BRV concentrations in the cerebrospinal fluid peak at about 15 minutes as compared to one hour for LEV.[150] Further, positron emission tomography (PET) studies in humans have shown that the drug entry half-times in the brain for BRV are between three and seven minutes which are seven-fold faster than LEV.[150] These properties of high permeability, rapid onset of action and high potency for its target, synaptic vesicle 2A protein, make BRV an attractive alternative for the treatment of SE. Additional studies to ensure its safety and efficacy for the early treatment of SE are needed.

1.6.2 Neurosteroids like allopregnanolone, tetrahydrodeoxycorticosterone and androsteindiol

Neurosteroids like allopregnanolone (generic name brexanolone) interact with both synaptic and extrasynaptic GABA-A receptors unlike the benzodiazepines that act only on the synaptic GABA-A receptors.[158] With increase in the duration of convulsive SE synaptic GABA-A receptors are internalized reducing the efficacy of benzodiazepines.[95] The high affinity of the neurosteroids towards the extra-synaptic GABA-A receptors is an advantage that makes them potential candidates for SE treatment. A small study showed that the levels of allopregnanolone in the cerebrospinal fluid samples of patients with SE obtained during an episode of SE, were significantly lower as compared to patients with non-epileptic neurologic disorders which supports the use of allopregnanolone as a potential treatment of SE.[159] Brexanolone has been studied as potential therapy of super-refractory SE. Encouraging results were seen for brexanolone in two case-series reports and a phase I/phase II open label multicenter study.[97–99] The inclusion criteria for the phase I/phase II

study were patients who failed to respond to at least one first-line agent, one second-line agent and who were unable to be weaned off of third-line agents after ≥ 24 hours or had breakthrough seizures after six hours of initiation of burst suppression using third-line therapies. Response was defined as patients who were successfully weaned from third-line agents without re-initiation of other third-line agents during brexanolone therapy. Brexanolone was found to be well tolerated with a response rate of 77% (17/22) and improved overall functional status measured at follow-up day 29. The phase III trial that followed was a randomized, double-blind, placebo-controlled trial designed to evaluate safety and efficacy of brexanolone as continuous IV infusion to subjects in super refractory SE and enrolled 132 patients.[61] The inclusion criteria for the phase III study required patients to have failed a qualifying wean. Hence, the patients included were ones who had failed at least one wean prior to screening. The primary outcome was number of participants able to be weaned off all third-line agents prior to the end of infusion of brexanolone/placebo and remain off all third-line agents for ≥ 24 hours after the end of brexanolone/placebo infusion. In contrast to the Phase I/Phase II study, the response was defined with a stricter criterion i.e. no re-initiation of third-line agents within 24 hours and brexanolone infusion was also designed to last an additional day.[61] Further, there was an open-label high dose arm if the patient failed the primary outcome. Brexanolone failed to show any difference in the primary outcome of weaning from third-line agents as compared to the standard of care.[61] There are several possible explanations for this lack of effect of brexanolone in the phase III study. The study design and inclusion criteria were different between the successful phase I/II studies versus the phase III study i.e. the patients enrolled in phase III may have been harder to treat. Lack of control group in the phase III study

made it difficult to estimate placebo response. Also, nearly 40% of the legally authorized representatives of the study participants opted into the open-label high dose arm of allopregnanolone increasing the chance that clinicians may have declared an early failure in order to receive the high-dose of the 'active' treatment. And lastly, the target concentration used for the phase III study was based on the highest observed concentration in healthy, pregnant women (150 nM, ~ 50 ng/ml) and may not have been high enough to treat super-refractory SE. A pharmacokinetic-pharmacodynamic model relating allopregnanolone plasma concentrations after IV and IM administration to EEG effects in dogs found that the beta frequency band of EEG was most sensitive to allopregnanolone concentrations and had an EC50 of ~ 270 ng/mL.[160] The EC50 values for effects on other frequency bands were ~500-700 ng/mL which indicates that much higher concentrations than those used in the phase III study will be needed to elicit response. While allopregnanolone has a high potential to be an effective treatment of early SE, additional studies are needed to test its safety and efficacy as first- or second-line treatment of convulsive SE. Ganaxolone is another neuroactive steroid being studied for the treatment of multiple different types of seizures.[158] While allopregnanolone has the potential to be converted back to its precursor, progesterone, and may lead to hormonal side-effects, ganaxolone has an added methyl group, which prevents back conversion to an active steroid. IM ganaxolone and allopregnanolone are effective for the treatment of SE in a mouse model where SE was induced using tetramethylenedisulfotetramine.[161] Further, ganaxolone is effective for the treatment of DZP-resistant seizures in lithium chloride-pilocarpine model of SE in rats.[162] Marinus Pharmaceuticals, Inc. (Radnor, PA) is developing ganaxolone for the treatment of seizures, anxiety and depression. A phase III

study for the treatment of children and young adults with CDKL5 Deficiency Disorder (CDD), a rare form of pediatric epilepsy with no current approved treatment, with oral ganaxolone is currently underway.

1.6.3 Ketamine

Ketamine is an NMDA antagonist and is a promising option for the treatment of SE. After the onset of SE there is a progressive decrease in the number of functionally active GABA-A receptors on the post-synaptic membrane whereas the number of functionally active NMDA glutamate receptors progressively increases.[95] These changes could explain the pharmaco-resistance to GABA-A receptor modulators such as benzodiazepines as SE progresses versus an increase in the sensitivity to NMDA antagonists like ketamine.[95,96,158,163–165] Ketamine has neither cardiac or respiratory depressant properties, which is an advantage as its use does not routinely require endotracheal intubation that is considered as a negative prognostic factor of increased morbidity and mortality in critically ill patients.[166] While the use of ketamine for anesthesia has been studied extensively, no RCTs with ketamine have been conducted in SE. Like other newer treatments, current use of ketamine has been limited to refractory and super-refractory SE treatment. A review of clinical studies using ketamine for the treatment of refractory SE included 244 cases (207 in adults and 37 in children) from 26 studies (18 single case reports and 8 case series) and found that the response rate was 74% for adults and 73% for children.[165] Another systematic review of published case reports and case series that included a total of 248 SE patients (219 adults and 29 children) reported similar results. Additionally, this study found that regardless of the type of SE, ketamine was twice as effective if administered early

(64% efficacy rate in refractory SE lasting 3 days vs. 32% when the mean duration of refractory SE was 27 days).[166] These results are encouraging and indicate that ketamine has a high potential if used early- either alone or in combination with other second-line treatments- as suggested by several experts.[167,168] Ketamine has also been shown to have neuroprotective activity in preclinical rodent models of chemically-induced SE which further warrants its use earlier in the treatment cascade to prevent widespread neuronal necrosis, as seen in rats after 60 minutes of SE.[168] An open-label, randomized, controlled multicenter study to the test efficacy of ketamine in refractory SE in patients aged one month to 18 years is currently underway.[169] The ketamine dosing used for this study is two IV bolus doses of 2-3 mg/kg of ketamine administered five minutes apart followed by 5-10 µg/kg/min of continuous IV infusion of ketamine which can be gradually increased to a maximum of 60 µg/kg/min for a maximum duration of seven days.[169] MDZ, thiopental and propofol are used as active comparators. The primary outcome of the study is resolution of refractory convulsive SE.[169] Another randomized, single-blind pilot study to test the efficacy of ketamine infusion compared with traditional ASMs (benzodiazepines, barbiturates and propofol) in refractory SE is currently recruiting patients.[170] In this study, a loading dose of ketamine of 2.5 mg/kg is followed by a continuous infusion of three mg/kg/hour with a one mg/kg/hour increment until burst suppression or a maximum dose of 10 mg/kg/hour is reached.[170] Primary outcome is time taken for burst suppression or time taken for termination of seizures. The results from these studies will provide valuable information to plan and execute future studies of ketamine for the treatment of earlier stages of SE.

1.6.4 Topiramate

Topiramate is known to have multiple mechanisms of action including enhanced GABA-mediated inhibition, enhanced potassium channel conduction, inhibition of sodium channels and L-type calcium channels, decreased glutamatergic transmission and inhibition of carbonic anhydrase.[158] Based on its pathophysiology, as SE progresses there is increased glutamatergic transmission and internalization of GABA-A receptors. Hence, potentially the most relevant mechanisms of topiramate for second-line treatment of SE is the inhibition of sodium-channels and decreased glutamatergic transmission. Reports from small case series that used topiramate for the treatment of refractory and super-refractory SE suggest that it may be useful for the management of SE.[21,158,171,172] As described in section 1.2.8, enteral topiramate has been used to treat refractory SE in 95 adults and children and has been found to terminate seizures in 65% (62/95) of the patients.[21] A prospective, open-label study of use of topiramate as third/fourth line treatment of 20 adults with refractory SE has been conducted.[171] Response was defined as the termination of SE and was categorized as successful (termination within 24 hours of introduction of topiramate), possible (termination with concomitant use of other medications) or unsuccessful. Topiramate was found to be successful in five patients (25%), possibly successful in 11 patients (55%) and unsuccessful in four patients.[171] Another systematic review of the treatment of generalized convulsive SE in 35 adults in whom topiramate was used as third-line agent found that topiramate was the last drug administered in 20 of the 35 patients, leading to seizure control in 14/20 (70%) cases.[172] Though limited in sample size, these results are encouraging. However, currently topiramate is only available enterally which limits its use in acute situations. An IV formulation has been developed by

Ligand Pharmaceuticals Inc. (La Jolla, CA)[173] and has been licensed to CURx Pharmaceuticals (San Diego, CA) for development as replacement therapy for epilepsy patients on oral therapy. Topiramate has been known to cause metabolic acidosis.[158] Additional studies are needed to test the safety and efficacy of topiramate for SE treatment.

1.6.5 Perampanel

Based on the pathophysiology of convulsive SE, another potential target for the treatment of refractory SE is the α-amino-3-hyrdoxy-5-methyl-4-oxazolepropionicacid (AMPA) receptor.[95] In a rodent model of SE, benzodiazepine resistant convulsive SE was successfully terminated using a non-competitive AMPA receptor antagonist GYKI-52466.[174] Perampanel is a selective, non-competitive antagonist of AMPA receptors and has been shown to be effective for the treatment of SE in preclinical animal models.[158] It has been primarily used for the treatment of refractory and super refractory SE. A summary of clinical case reports where perampanel was used for the treatment of established, refractory or super-refractory SE at five European hospitals identified 52 patients treated with perampanel.[175] Patients had failed benzodiazepines and a median of five other ASMs and the median latency from onset of SE to perampanel initiation was 10 days. Median doses used were 6 mg/day titrated up to 10 mg/day administered orally or via the nasogastric tube in patients unable to swallow. Perampanel was the last drug added in 32/52 patients with response attributed to perampanel in 19 of the 52 patients (36.5%).[175] A systematic review that included 10 articles with 69 episodes of SE in 68 patients aged 18 to 91 years found that the proportion of patients achieving clinical seizure cessation varied from 17% to 100%.[100] Between one to nine drugs were used prior to perampanel and initial

perampanel doses ranged from two to 32 mg administered mainly via the nasogastric tube. Further, the time to seizure cessation after administration of perampanel ranged from one hour to four weeks.[100] Lack of conclusive results for the use of perampanel could be due to its late use in the course of treatment of refractory SE, wide variability in the latency between onset of SE and treatment with perampanel, difference in demographics and underlying SE etiology of the tested population and a range of doses used for treatment.[158] However, currently perampanel is available only as tablets and oral suspension in the United States. An IV formulation of perampanel is currently under development at Eisai (Bunkyo City, Tokya, Japan). A phase I study to evaluate bioavailability of single 12 mg dose of perampanel administered using three different infusion durations relative to a single 12 mg oral tablet in 48 healthy volunteers was completed (NCT03376997) but the results are yet to be reported. While perampanel appears to be a promising option, an IV formulation and results of its safety and efficacy from additional studies are needed to confirm its utility for SE treatment.

1.6.6 Lacosamide

The mechanism of action of lacosamide is suppressing neuronal hyperactivity through selective enhancement of the slow inactivation of voltage-gated sodium channels. Lacosamide is not FDA approved for SE treatment but has been used for the treatment of SE as shown by several case reports, case series and retrospective reviews.[157] The largest investigation of lacosamide was a retrospective evaluation of 165 SE patients.[176] Eighty-seven of the 165 had NCSE. The median IV loading dose of lacosamide was 400 mg (range 100-600 mg) and weight adjusted median dose was 5 mg/kg (3-6 mg/kg). In 46.1% of these

patients lacosamide was administered as a third-line treatment with seizure-control reported in 105 patients (64%) and seizure cessation occurred within 12 hours of drug administration for 91 (55%) of these 105 patients.[176] A systematic review of use of IV lacosamide for the treatment of 522 SE episodes in 486 adults and 36 children and adolescents found the response rate of lacosamide to be 57%[177] which was in agreement with the 56% response rate reported previously[116]. This response rate is based on pooled efficacy from wide-range of studies that included refractory and non-refractory SE, used different criteria and methods to diagnose SE and to evaluate drug efficacy.[177] Typical doses of IV lacosamide used ranged from 200 mg to 400 mg. Main adverse events during the treatment of SE were found to be dizziness, abnormal vision, diplopia and ataxia.[177] A small dose-related mean increase in PR interval with lacosamide was observed and first degree AV block was reported as a non-serious adverse event.[178] While second or higher degree AV block was not reported, monitoring of electrocardiography (ECG) will have to be considered in patients with known conduction problems.[177] Based on literature evidence, lacosamide seems to be a promising therapeutic option for focal or partial types of SE than for refractory or nonconvulsive SE.[157] While earlier administration and higher doses were reported to confer higher efficacy rates, neither of the strategies have yet been compared in a prospective manner or in a homogenous cohort of SE patients with currently recommended therapies.[157]

1.6.7 Other treatment options

Drugs of interest with anticonvulsant activity, that may have shown some efficacy in animal models of SE, but have very little or no clinical evidence (≤ 2 studies) include

marketed drugs like lidocaine, felbamate, pregabalin, gabapentin, stiripentol, rufinamide and zonisamide.[157,158] Valnoctamide is an amide derivative of VPA shown to have less adverse effects than VPA in animal models whereas sec-butylpropylacetamide is a more potent homologue of valnoctamide.[158] However, no human studies have been done with either of the two drugs. Additional studies are needed to confirm the safety and efficacy of these drugs when used for the treatment of SE.

1.6.8 Early polytherapy for the treatment of SE

Based on the pathophysiology of SE, early polytherapy using a combination of ASMs promptly at the onset of seizures may be most effective and has been suggested by experts.[95] Polytherapy can be employed at all stages of treatment of SE starting at the onset. Several factors will need to be considered in the design of an effective combination therapy for each stage of SE including the mechanism of action of drugs, onset and duration of action, available formulations and routes of administration, pharmacokinetic-pharmacodynamic and physicochemical properties of the drugs. Various combinations, based on data obtained in animals models of SE, have been suggested for early polytherapy that include DZP-ketamine, MDZ-ketamine, DZP-LEV, DZP-BRV, DZP-perampanel, atropine-ketamine and benzodiazepine-ketamine-VPA/BRV.[158] Well designed, controlled clinical trials are needed to confirm the utility of these combinations.

CHAPTER 2

FIRST-LINE TREATMENT OF CONVULSIVE STATUS EPILEPTICUS WITH BENZODIAZEPINES

Benzodiazepines are used as the first-line treatment of generalized convulsive SE as described in section 1.2.5. Evidence-based guidelines for the treatment of SE, provide information on the choice of benzodiazepine, the dose, and route of administration based on a patient's bodyweight.[3–5] Previous reports from small, retrospective cohort studies with up to three centers have found that these guideline-recommendations are often not followed in practice.[16–19,179] However, there are no studies comprehensively characterizing benzodiazepine use as first-line treatment in a large cohort of adults and children with convulsive SE who failed initial treatment with benzodiazepines and progressed to established SE. My research addresses this unmet need using pre-enrollment benzodiazepine dosing data for 478 patients with convulsive SE enrolled in ESETT at 57 centers across the United States.

This chapter is a compilation of two manuscripts. The **primary objective** of the first manuscript was to describe the dosing patterns of benzodiazepines as initial treatment of convulsive SE based on the choice of benzodiazepine, dose, route of administration and the patients' age-group in the first 207 ESETT patients (cohort 1) and compare these doses with recommendations from evidence-based guidelines.[180]

Hypothesis 1: Despite the availability of evidence-based guidelines, benzodiazepines as first-line treatment of SE are not appropriately used in practice.

Based on the underdosing of benzodiazepines seen in cohort 1[180], the ESETT team implemented additional trainings for the study teams at participating sites and at investigator meetings to increase the awareness of appropriate benzodiazepine dosing for SE treatment. The **primary objective** of the second manuscript was to report benzodiazepine dosing patterns in the entire ESETT population of 478 patients and to

compare the dosing patterns seen in cohort 1 (first 207 patients) with the remaining subset of ESETT patients (271 patients, cohort 2) to evaluate the impact of the educational activities on benzodiazepine use. A **secondary objective** of the second manuscript was to test the association of benzodiazepine doses with the ESETT primary outcome and clinical seizure cessation assessed at 60 minutes after the second-line treatment administration using logistic regression.

Hypothesis 2: Higher cumulative doses of benzodiazepines are significantly associated with an increase in the likelihood of ESETT primary outcome success.

Given that the introduction, methods and discussion sections for the two manuscripts were largely similar, these sections were consolidated whereas the results sections from the two manuscripts are presented separately.

2.1 Introduction

The European Federation of Neurological Societies (EFNS), the Neurocritical Care Society (NCS) and the American Epilepsy Society (AES) have published evidence-based guidelines for the treatment of convulsive SE.[3–5] These national and international guidelines recommend the use of benzodiazepines as initial therapy and include routes of administration and doses for DZP, MDZ and LZP based on the patient's weight. The guidelines emphasize the administration of benzodiazepines as a first-line treatment of SE as early as possible, as a single full dose, and by an appropriate route.[3–5] A number of trials have demonstrated the safety and efficacy of benzodiazepines for the treatment of SE;

however, the failure rate ranges from 25-55%.[3–5] This may be attributable, in part, to sub-optimal benzodiazepine dosing, and timing and route of administration used.

Based on these guidelines, initial benzodiazepine treatment should consist of either a 10 mg dose of IM MDZ for patients weighing > 40 kg or 5 mg for those 13-40 kg; or IV LZP 0.1 mg/kg/dose (maximum 4 mg/dose) or IV DZP 0.15-0.2 mg/kg/dose (maximum 10 mg/dose).[3–5] The LZP and DZP doses can be repeated if the initial dose fails to stop the seizure. As the guidelines were obligated to use published results, IM MDZ is listed based on results from RAMPART[10] but IV MDZ is not listed. However, pharmacokinetic studies of MDZ have demonstrated that IV MDZ produces exposures comparable to IM but with a faster time to peak concentrations.[181,182] Hence although not included in the guidelines, based on MDZ pharmacokinetics, 10 mg IV MDZ dose was also considered adequate therapy.

ESETT offered an opportunity to characterize patterns of benzodiazepine use prior to enrollment in the trial. Benzodiazepine dosing pattern results in the first 207 patients enrolled in ESETT are reported in section 2.3.1[180] whereas, results for the entire ESETT cohort are reported in section 2.3.2.

2.2 Methods

Using pre-enrollment data from ESETT subjects, we describe benzodiazepine treatment with respect to: 1) drug choice, dose, and route of administration, 2) timing and setting in which the drugs were administered, and 3) patient weight (< or ≥ 40 kg for LZP, ≤ or > 40 kg for MDZ, and < or ≥ 66.7 kg for DZP). EFNS, NCS and AES guidelines were used to

define underdosing for our analyses.[3–5] The settings in which benzodiazepines were administered were categorized as: 1) Prior to EMS arrival, 2) EMS, and 3) ED.

Because patients could receive more than one benzodiazepine, the cumulative dose was determined using LZP equivalents to account for differences in drug potencies. For patients weighing ≥ 32 kg, 10 mg MDZ or DZP were considered equal to 4 mg LZP[3–5]. For patients weighing < 32 kg, 0.3 mg/kg of DZP IV or 0.2 mg/kg of MDZ IV or 0.3 mg/kg of MDZ IM were considered equal to 0.1 mg/kg LZP IV.[3–5] Transmucosal benzodiazepines, e.g. rectal DZP or intranasal/buccal MDZ, given prior to EMS arrival are included in the calculation of cumulative benzodiazepine dose.

The ESETT eligibility criteria required those enrolled into the study to have been administered an adequate minimum dose of benzodiazepines (Table 2.2-1) without termination of SE, but there was no upper limit on the benzodiazepine dose allowed prior to enrollment. The minimum cumulative adequate dose for enrollment (4 mg LZP equivalents for those ≥ 32 kg and 0.1 mg/kg LZP equivalents for < 32 kg)[180], was intended to represent the lowest doses that might be given in standard practice. As described in section 1.3.5.3 focused on the ESETT inclusion criteria, a weight cut-off of 32 kg was used instead of the 40 kg recommended by guidelines. The study protocol did not stipulate dose or instructions on the rate and frequency of dosing because benzodiazepine dosing occurred prior to study enrollment. ESETT sites dosed benzodiazepines as per their local standards of care and established clinical guidelines. Although benzodiazepine dosing was not dictated by the study protocol, best practices based on the guidelines were part of the study training, in part because of pre-existing published data about underdosing in clinical practice.

Table 2.2-1. Guideline Recommended Doses and ESETT Protocol Eligibility Criteria[142]

Drug	Route	Guideline Recommended Doses per Administration [11,13]	ESETT Eligibility Criteria for Minimally Adequate Cumulative Dose*	
			Dose for ≥ 32 kg Patients (mg)	Dose for < 32 kg Patients (mg/kg)
Diazepam	IV Rectal	0.15-0.2 mg/kg/dose, max: 10 mg/dose, may repeat dose once If IV route not available, then 0.2-0.5 mg/kg/dose, max: 20 mg/dose	10	0.3
Lorazepam	IV	0.1 mg/kg/dose, max: 4 mg/dose, may repeat dose once	4	0.1
Midazolam	IV IM IN/Buccal	IM Dosing: 10 mg for > 40 kg, 5 mg for 13-40 kg Dosing not specified	10 10	0.2 0.3

*Cut-off criteria for the transmucosal routes were the same as those for the intravenous route

2.2.1 Using data from first 207 ESETT patients (cohort 1)

As a component of ongoing quality improvements, ESETT investigators performed a preliminary analysis of pre-enrollment benzodiazepine dosing after completing the first year of enrollment. Based on the results from this preliminary work, I undertook a

comprehensive analysis of benzodiazepine dosing patterns in the first 207 patients.[180] Data collected from patients enrolled at 41 US academic and community hospitals was used. For this analysis, the ESETT database was frozen on December 12, 2016. I categorized the timing of drug administration as: 1) the first administration of the first benzodiazepine and 2) all administrations of all benzodiazepines prior to enrollment including the first administration. Individual doses were compared with the guidelines to identify those doses that met recommendations.

2.2.2 Using data from the entire ESETT population of 478 patients

Last ESETT patient was enrolled in December 2018 and the database was locked for analysis in April 2019. The method used to describe dosing patterns in the entire ESETT population was identical to that used for cohort 1 as described in section 2.2.1. In addition to comparing the administered doses with guideline recommendations, to account for approximation of patient weight and dose rounding at the time of administration, individual doses were also compared with 80% and 90% of the guideline-recommended doses. Data were collected from 478 enrollments in ESETT at 57 US academic and community hospitals. Among these, 16 patients (12 adults, 4 children) were enrolled more than once (7 in cohort 1 and 9 in cohort 2), but only the data from their first enrollments were used for these analyses. Of the remaining 462 patients, the first DZP dose administered in two patients was unknown and these patients were excluded. Thus, data from 460 patients formed the basis for these analyses. Data were analyzed using R (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria), RStudio (version 1.2.5001,

Delaware Public Benefit Corporation) and SAS (version 9.4, SAS Institute Inc., Cary, NC) to compute descriptive statistics.

2.2.3 Time from onset of SE to first benzodiazepine dose and time from first dose to cumulative adequate dose

Adjudicated time for the onset of SE to the ESETT study drug administration[139] and the recorded time from the first dose of the first benzodiazepine to ESETT study drug administration were used to calculate the time, in minutes, from onset of SE to the first dose of benzodiazepine. In cases where the time for SE onset was adjudicated to be a range, the mean value was used. On one occasion, when the time from SE onset to benzodiazepine dose was negative, the upper limit of the range was used. The time to cumulative adequate dose after the first dose was calculated using the recorded time for the first benzodiazepine administration and the administration to reach the cumulative adequate dose per the ESETT enrollment criteria.

2.2.4 Comparison of cohort 1 vs. cohort 2

Dosing patterns for the first dose of the first benzodiazepine administered were compared between the first set of published data (n=200 patients, cohort 1) versus the subsequent enrollments (n=260 patients, cohort 2). I also performed time series trend analyses.

2.2.5 Association of benzodiazepine doses with response to subsequent second-line medications

Logistic regression modeling was used to test the association of benzodiazepine doses with ESETT primary outcome and clinical seizure cessation. The dependent variables included:

1. ESETT primary outcome was defined as cessation of SE at 60 minutes after the start of study drug infusion without use of additional anti-seizure medication, as determined by absence of clinically apparent seizures and improved responsiveness (1=treatment success, 0= treatment failure).
2. Clinical seizure-cessation at 60 minutes after the start of study drug infusion without additional use of additional anti-seizure medication and with/without improved responsiveness (1= success, 0= failure)

The following benzodiazepine doses were used as primary predictors:

1. First dose of first benzodiazepine
2. Cumulative dose of benzodiazepines prior to enrollment

To allow adults and children to be included in the same model, both the first dose and the cumulative dose were calculated as mg/kg of LZP equivalents (continuous variable). Four logistic models were used to test the association of first dose of first benzodiazepine and cumulative dose of benzodiazepines with each of the two dependent variables of primary outcome and clinical seizure cessation. Other predictors included in all four models were age ($<$ or $\geq$ 18 years), etiology (1= acute brain pathology including acute stroke/hemorrhage, central nervous system (CNS) tumor or CNS infection, 0= no acute brain pathology), and ESETT study drug (FOS, LEV and VPA).

2.2.6 Data availability

ESETT data will be available through the NINDS repository of Archived Clinical Research Datasets which is found at https://www.ninds.nih.gov/ Current-Research/Research-Funded-NINDS/ClinicalResearch/Archived-Clinical-Research-Datasets. Trial results will also be posted to clinicaltrials.gov. NINDS requires all investigators seeking access to data from archived NINDS-supported trials to agree to certain terms and conditions. To request a dataset, please complete the NINDS Data Request Form and send it to the NINDS Clinical Research Liaison at CRLiaison@ninds.nih.gov

2.3 Results

2.3.1 Results for cohort 1 (first 207 ESETT patients)[180]

This analysis included 207 ESETT subjects: 88 children, 95 adults aged 18-65, and 24 older adults aged ≥ 66 enrolled at 41 US academic and community hospitals (Table 2.3.1-1). There were 511 administrations with (mean ± standard deviation) 2.47 ± 1.04 doses per subject. LZP comprised of 61% of doses, followed by MDZ (31%), and DZP (8%). Most DZP doses (65%) were given prior to EMS arrival, whereas 68% of MDZ doses were given by EMS personnel, and 94% of LZP doses were administered in the ED. A comparison of routes of administration reveals that 95% of LZP doses were administered IV, while 5% (N=17) were by IM, IN, or buccal routes. With regards to MDZ, 41% of doses were given IM, 45% were by the IV route and the remaining 14% by IN or buccal routes. The rectal

route was used for 69% of DZP administrations. Of these, 78% and 96% were in patients younger than 12 and 18 years, respectively.

Table 2.3.1-1: Distribution of Benzodiazepine Doses by Route of Administration, Setting and Age Group (N=511 administrations in 207 patients)

	Lorazepam		Midazolam		Diazepam		Total	
	N= 312		N= 159		N= 40		N=511	
	n	%	n	%	n	%	n	%
Route of administration								
Intravenous	295	95%	72	45%	12	31%	379	74%
Intramuscular	15	5%	65	41%	0	0%	80	16%
Transmucosal*	2	1%	22	14%	27	69%	51	10%
Setting								
Prior to EMS	4	1%	9	6%	26	65%	39	8%
EMS	14	5%	108	68%	9	23%	131	26%
ED	294	94%	42	26%	5	13%	341	67%
Age group								
Pediatric**	97	31%	66	42%	27	68%	190	37%
Adult	215	69%	93	58%	12	30%	320	63%

EMS- Emergency Medical Services; ED- Emergency Department

*Transmucosal administration for diazepam was per rectum, while intranasal or buccal routes were used for lorazepam and midazolam. **The pediatric group includes ages less than or equal to 17, the adult group includes those greater than 17. Administration information for one case was missing due to unknown dose and route.

2.3.1.1 First Dose of First Benzodiazepine

Among the 207 patients, 105 received their first dose of any benzodiazepine prior to arrival in the ED whereas the remaining 102 patients received it in the ED. Overall, 29.8% of first doses met minimum recommendations per guidelines. Of these, 86.7% of DZP, 14.5% of MDZ and 23.2% of LZP administrations met the minimum dose recommendations. Figure 2.3.1-1 shows that for subjects < 40 kg the guideline recommended LZP (≥ 0.1 mg/kg) or

MDZ (≥ 5 mg) dose was administered as a first dose in 41.9% and 12.5% of the cases, respectively. In contrast, for those weighing ≥ 40 kg the recommended LZP (≥ 4 mg) or MDZ recommended (≥ 10 mg) dose was administered in 14.7% and 15.4% of the subjects, respectively. A DZP dose ≥ 10 mg was administered in 60% of the subjects ≥ 66.7 kg, while 96% of DZP administrations were ≥ 0.15 mg/kg in those < 66.7 kg.

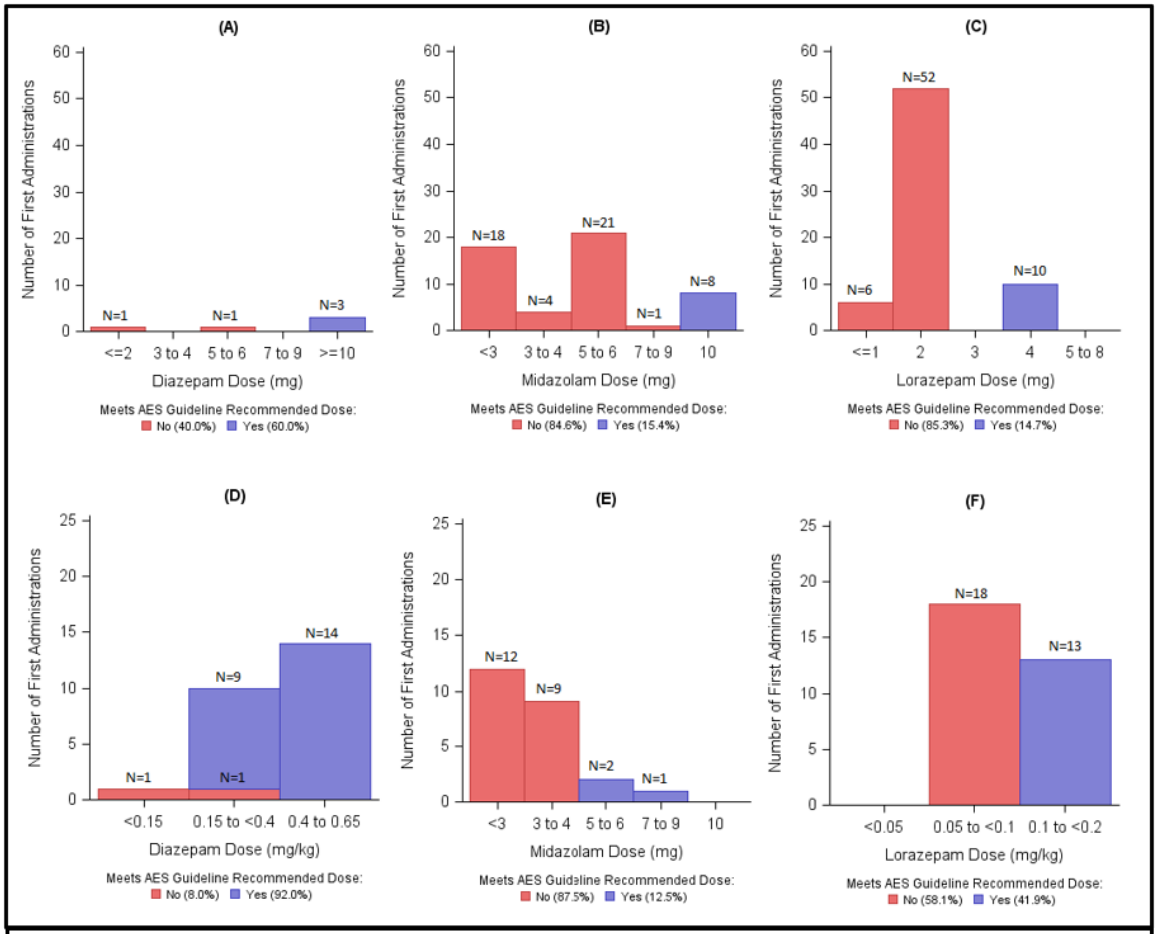

Figure 2.3.1-1: Distribution of first dose of the first administered benzodiazepine (DZP, MDZ or LZP) as actual doses. Top panel: fixed dosing, bottom panel: weight-based dosing. A:DZP doses for those ≥ 66.7kg (IV) or ≥ 50 kg (rectal); B: MDZ doses for those > 40 kg; C: LZP doses for those ≥ 40 kg; D: DZP doses for those < 66.7 kg (IV) or < 50 kg (rectal); E: MDZ doses for those ≤ 40 kg; F: LZP doses for those < 40 kg . Categorized as met (blue) or did not meet (red) guidelines.

2.3.1.2 Dose per Administration

Seventy-seven percent of DZP, 10.7% of MDZ and 21.8% of LZP doses administered were at or above the recommendations (Figure 2.3.1-2). Prior to EMS, most administrations of DZP (25/37) were given at or above the minimum recommended doses, whereas in both the EMS and ED settings, most of the administered benzodiazepine doses were below recommendations.

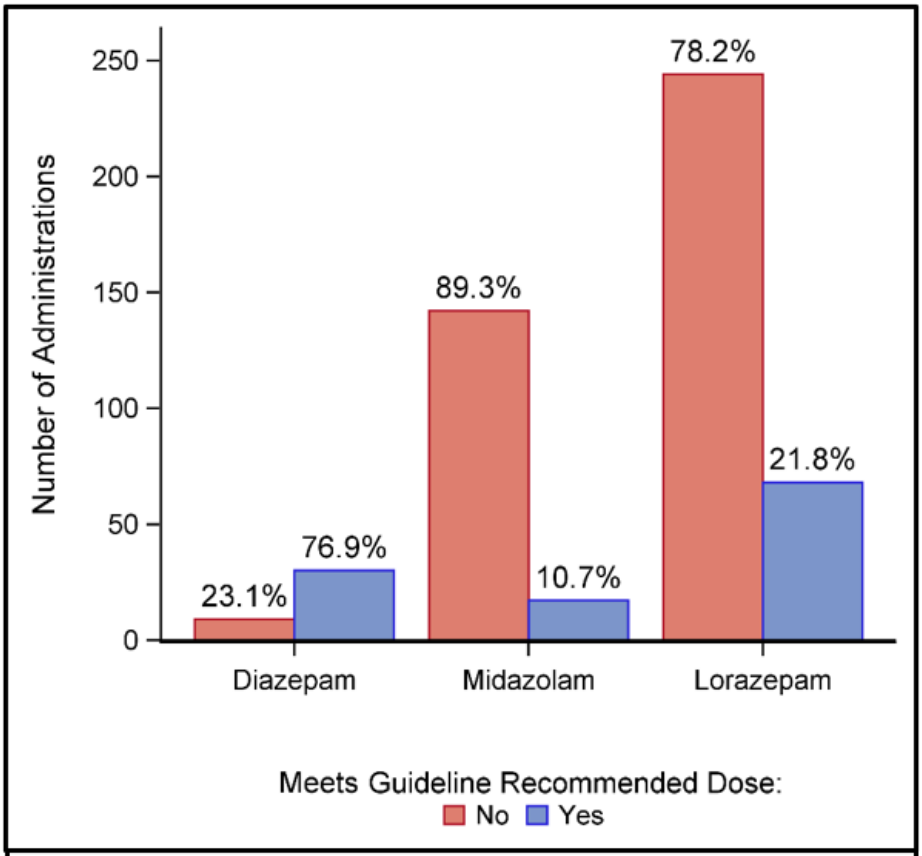

Figure 2.3.1-2: Total number of administrations that met (blue) and did not meet (red) guideline recommendations for DZP, MDZ and LZP (N=511) *(Numbers on top of the bars represent % administrations for each drug)*

2.3.1.3 Cumulative Benzodiazepine Doses

Cumulative dosing patterns were examined using LZP equivalents (Figure 2.3.1-3). Among 138 adults and older children weighing ≥ 32 kg, the cumulative dose in LZP equivalents was < 4 mg in 9%, 4 mg in 42%, 5-6 mg in 25% and > 7 mg in 24%. In 68 children weighing < 32 kg, the cumulative dose was < 0.1 mg/kg in 18%, 0.1 to < 0.2 mg/kg in 44%, 0.2 to < 0.3 mg/kg in 28% and > 0.3 mg/kg in 10% of subjects.

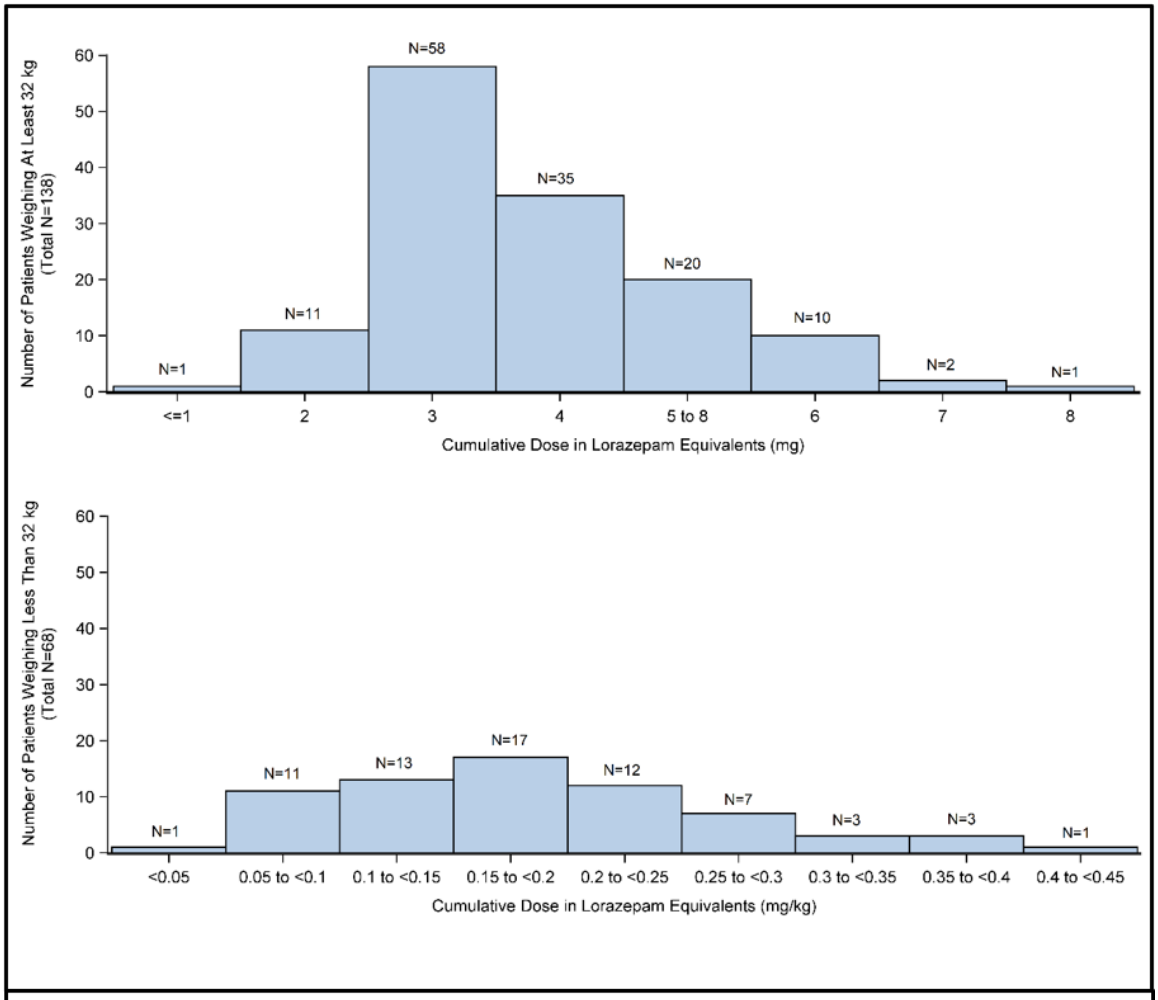

Figure 2.3.1-3: Distribution of the cumulative benzodiazepine dose in lorazepam equivalents for subjects weighing ≥ 32 kg (top panel) and < 32 kg (bottom panel)

2.3.2 Results for the entire ESETT cohort

These results include 1170 benzodiazepines doses administered to 460 ESETT patients. Of these, 539 doses were administered to 224 children while 631 doses were given to 236 adults. Although the number of benzodiazepine administrations given to patients prior to ESETT enrollment ranged from one to nine, most patients (74.2%) received 2-3 doses each (Figure 2.3.2-1).

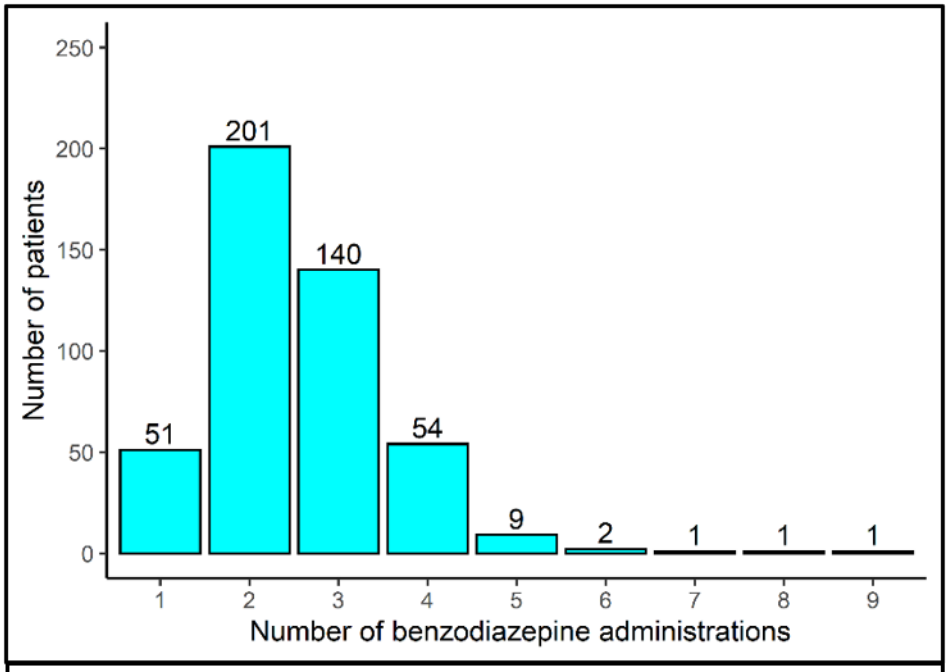

Figure 2.3.2-1: Distribution of ESETT subjects by the number of benzodiazepine doses received prior to enrollment (n=460)

The distribution of the administrations by age group, drug, route and setting are summarized in Table 2.3.2-1. Of all DZP administrations (n=103), 73 (70.9%) were given

Table 2.3.2-1: Distribution of all benzodiazepine doses by route of administration, setting and age group (N=1170 in 460 patients)

	Lorazepam		Midazolam		Diazepam		Total	
	N= 669		N= 398		N= 103		N=1170	
	n	%	n	%	n	%	n	%
Route of administration								
Intravenous	638	95%	159	40%	21	20%	818	70%
Intramuscular	25	4%	137	34%	4	4%	166	14%
Transmucosal*	5	1%	96	24%	76	74%	177	15%
Unknown	1	-	6	2%	2	2%	9	1%
Setting								
Prior to EMS	16	2%	20	5%	73	71%	109	9%
EMS	47	7%	269	68%	22	21%	338	29%
ED	606	91%	109	27%	8	8%	728	62%
Age group								
Children**	269	40%	196	49%	80	78%	545	47%
Adult	400	60%	202	51%	23	22%	625	53%

EMS- Emergency Medical Services; ED- Emergency Department
*Transmucosal administration for diazepam was per rectum, while intranasal or buccal routes were used for lorazepam and midazolam.
**The children group includes ages less than or equal to 17 years, the adult group includes those greater than 17.

prior to EMS, of which 68 (91.9% of 73) were by the rectal route. Of the 398 MDZ administrations, 269 (67.7%) were given by the EMS personnel mainly by IM (n=109, 40.4% of 269) or IV (n=95, 35.2% of 269) routes. In contrast 606 (90.6%) of the 669 LZP administrations were in the ED, primarily intravenously (n=589, 97.2% of 606). Twenty-five LZP administrations were via the IM route; and on four occasions LZP was given by intranasal or buccal routes.

2.3.2.1 First dose of first benzodiazepine

Among all first doses, 32.4% met the minimum recommendations per guidelines. Figure 2.3.2-2 shows the distribution of first doses for each drug. For all patients, the percent of first doses that met guideline recommendations were 85.9% for DZP, 18.9% for MDZ and 24.1% for LZP.

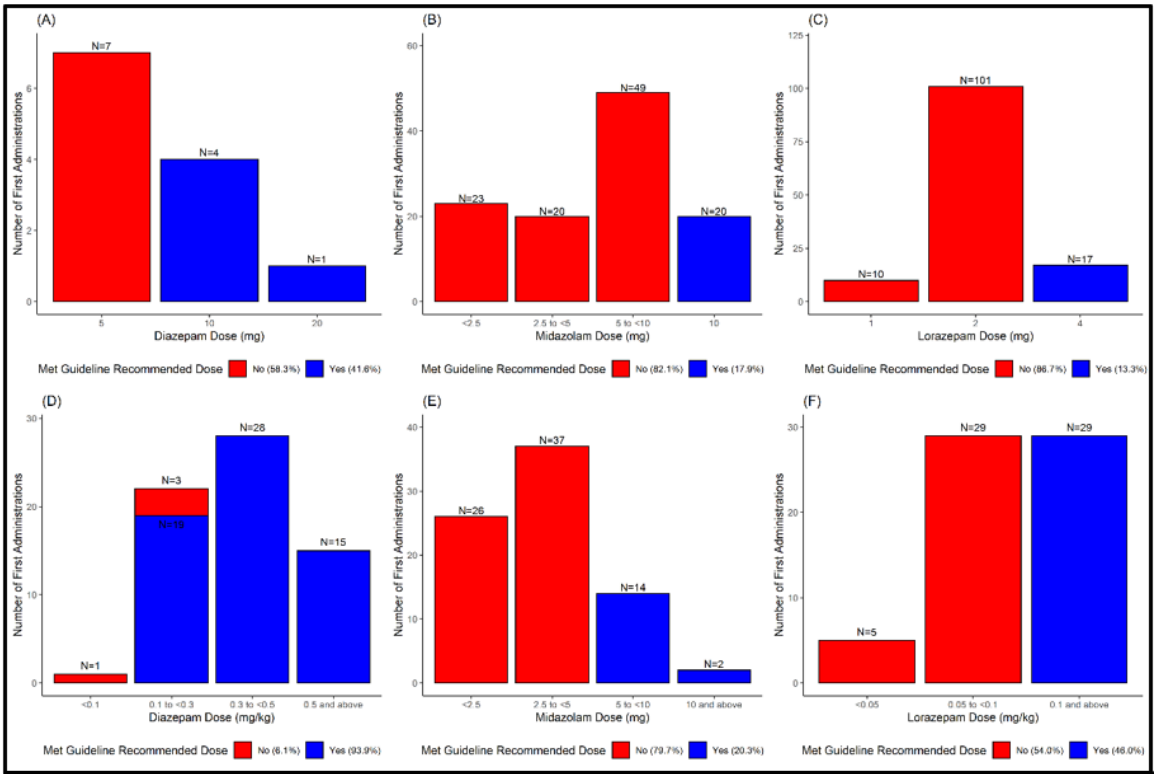

Figure 2.3.2-2: Distribution of the first dose of the first administered benzodiazepine (n=460).

Panel (A), (B) and (C): fixed dosing, Panel (D), (E) and (F): weight-based dosing. A: Diazepam doses for those ≥ 66.7kg (IV) or ≥ 50 kg (rectal); B: Midazolam doses for those > 40 kg; C: Lorazepam doses for those ≥ 40 kg; D: Diazepam doses for those < 66.7 kg (IV) or < 50 kg (rectal); E: Midazolam doses for those ≤ 40 kg; F: Lorazepam doses for those < 40 kg . Categorized as met (blue) or did not meet (red) guideline recommendations.

For those weighing > 40 kg, the guideline-recommended MDZ dose of ≥ 10 mg was administered in 17.9% of the cases and LZP dose of ≥ 4 mg was administered in 13.3% of

the cases. For patients weighing ≤ 40 kg, the recommended MDZ dose of ≥ 5 mg was used in 20.3% of the cases and LZP dose of ≥ 0.1 mg/kg was used in 46% of the cases. For DZP, patients weighing ≥ 66.7 kg received the recommended dose of ≥ 10 mg in 41.6% cases, and 93.9% of those weighing < 66.7 kg received the recommended DZP dose of ≥ 0.15 mg/kg. To account for approximation of patient weight and dose rounding, the first doses were also compared with 80% and 90% of the guideline recommended doses. Eighty-nine percent of DZP, 23% of MDZ and 33% of LZP doses were ≥ 80% of the guideline-recommended dose; whereas, 86% percent of DZP, 19% of MDZ and 31% of LZP doses were ≥ 90% of guideline-recommended dose.

2.3.2.2 Doses of all benzodiazepine administrations

When including all benzodiazepine administrations (N=1170), 78.6% of DZP, 14.3% of MDZ and 23.9% of LZP doses met guideline recommendations (Figure 2.3.2-3).

2.3.2.3 Cumulative benzodiazepine doses

Distribution of cumulative benzodiazepines doses received prior to ESETT enrollment calculated in lorazepam equivalents shows that patients weighing ≥ 32 kg received a cumulative dose (median, interquartile range (IQR)) of 4.8 mg (4, 6.5 mg), whereas those < 32kg received 0.19 mg/kg (IQR: 0.14, 0.24 mg/kg) (Figure 2.3.2-4).

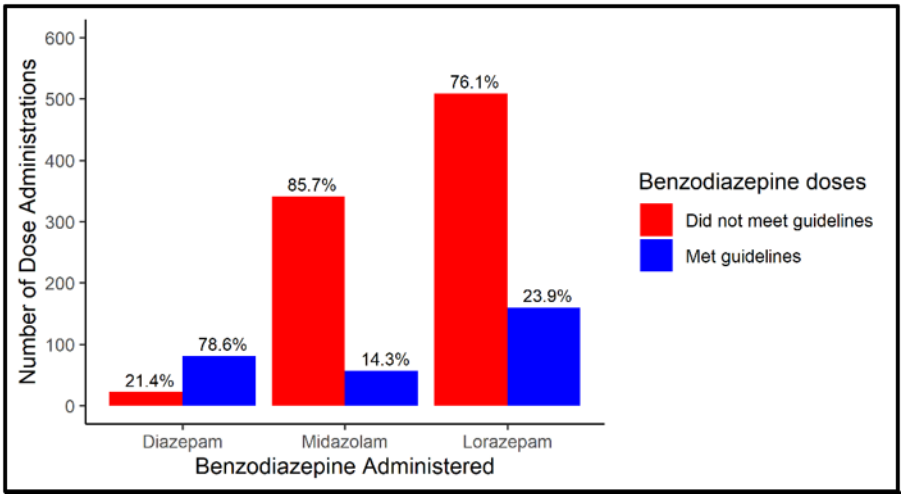

Figure 2.3.2-3: Distribution of all doses of diazepam, midazolam and lorazepam (n=1170). Administrations that met (blue) and did not meet (red) guideline recommendations.

2.3.2.4 Time from onset of SE to the first dose of benzodiazepine

The median estimated delay from the onset of SE to the first dose of the first benzodiazepine was 27 minutes (IQR: 11, 49 minutes) (N=354).

2.3.2.5 Time to cumulative adequate benzodiazepine dose after the first dose

The median delay from the first dose of benzodiazepine to adequate cumulative dose (N=423) was six minutes (IQR: 0, 20 minutes). When analyzed by weight group, the median delay was eight minutes (IQR: 1, 21 minutes) in those weighing ≥ 32 kg (N=258), which was longer and more variable than two minutes (IQR: 0, 18 minutes) in subjects weighing < 32 kg (N=165).

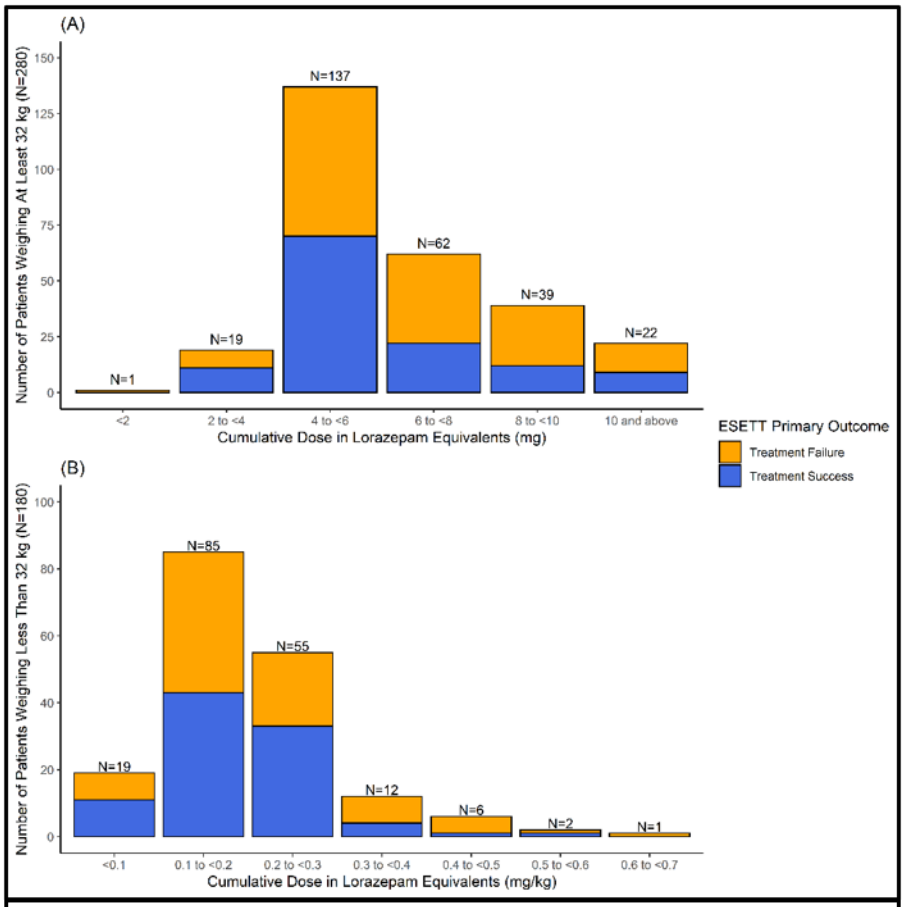

Figure 2.3.2-4: Distribution of the cumulative benzodiazepine dose in lorazepam equivalents.
For subjects weighing ≥ 32 kg (panel A) and < 32 kg (panel B) and the response to the second-line treatment as treatment success (blue) or treatment failure (orange)

2.3.2.6 Time trend of first doses

Figure 2.3.2-5 shows the proportion of first doses overall and in the ED given to adults and children that met guideline recommendations during the course of ESETT. The proportion of first doses meeting guidelines was approximately 50% for children both overall and in the ED. In adults, early in the study approximately 10% of the first doses overall and 20%

of first doses in the ED met guidelines. By the end of enrollment, the percent of first doses meeting guidelines was approximately 15% both overall and in the ED.

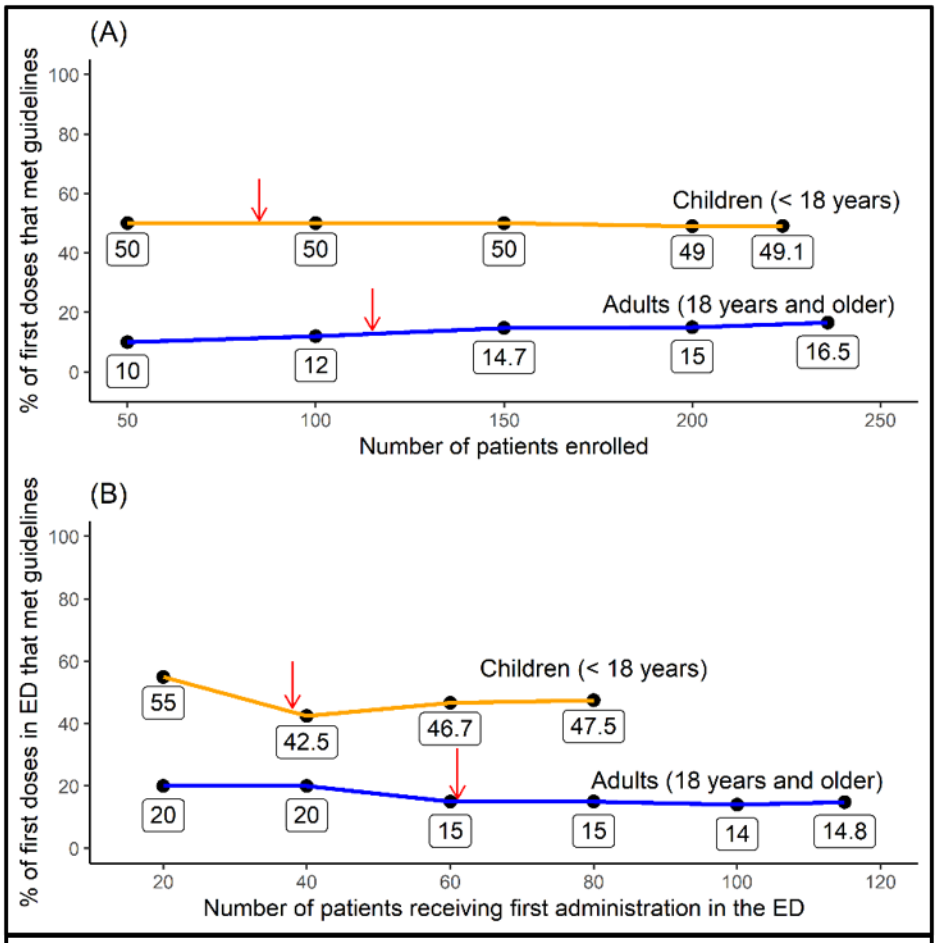

Figure 2.3.2-5: Time trend of the proportion of first doses that met guidelines over the course of ESETT overall and in the emergency department.
Panel (A): All first doses (n=460) and Panel (B): First doses administered in the emergency department (ED) (n=195) for children (orange) and adults (blue). The red arrows indicate the total number of adult and children included (115 adults, 85 children) in the first cohort (panel A) and number of first doses administered in the ED (61 in adults, 38 in children) in the first cohort (panel B).

2.3.2.7 Comparing cohort 1 and cohort 2

Table 2.3.2-2 shows the distribution of first doses of DZP, MDZ and LZP in adults and children across the two cohorts. The percent of first doses meeting guidelines did not change in adults and children except the proportion of MDZ first doses which increased from 11.1% in cohort 1 to 23.6% in cohort 2 in adults.

Table 2.3.2-2: Comparison of first doses based on patient's age and drug administered between the 2 cohorts

Drug Used	**% of first doses that met guideline recommendations (n=number of patients)**			
	Cohort 1 (n=200)		**Cohort 2 (n=260)**	
	Adults (n=115)	Children (n=85)	Adults (n=130)	Children (n=141)
Diazepam	33.3 (n=3)	96 (n=25)	54.5 (n=11)	92.3 (n=39)
Midazolam	11.1 (n=45)	16.1 (n=31)	23.6 (n=55)	21.7 (n=60)
Lorazepam	12.1 (n=66)	43.3 (n=30)	10.7 (n=56)	48.7 (n=39)

2.3.2.8 Association of benzodiazepine doses and other predictors with ESETT outcomes

Higher first or cumulative benzodiazepine doses were not associated with better outcomes or seizure cessation after subsequent second line medications in these benzodiazepine-refractory seizures.

1. First dose of the first benzodiazepine

There was no association of a 0.1 mg/kg increase in first dose of the first benzodiazepine with the primary outcome (adjusted odds ratio (aOR) 1.07, 95 % confidence interval (CI) [0.63, 1.85]) or clinical seizure cessation (aOR 1.05 [0.61, 1.85]).

2. Cumulative benzodiazepine dose

In this cohort of patients with benzodiazepine-refractory SE, not achieving the primary outcome of clinical seizure cessation and improved responsiveness after treatment with levetiracetam, fosphenytoin, or valproic acid was associated with higher cumulative doses of benzodiazepines given prior to the second-line drug. A 0.1 mg/kg increase in the cumulative benzodiazepine dose was associated with a decrease in the likelihood of treatment success for the primary outcome (aOR 0.71 [0.53, 0.92]). However, there was no association of cumulative benzodiazepine dose with response to second-line antiseizure drugs as determined by clinical seizure cessation alone (aOR 0.8 [0.62, 1.04]).

3. Interaction of benzodiazepine dose with other predictors

The association of age and etiology with primary outcome was significant and was unaffected by corrections for cumulative benzodiazepine dose. Age ≥ 18 years was associated with a decrease in the likelihood of treatment success (aOR 0.54 [0.33, 0.87]) after adjusting for cumulative benzodiazepine dose, etiology and ESETT study drug. Etiology of acute stroke/hemorrhage, CNS tumor or CNS infection was associated with a

decrease in the likelihood of treatment success (aOR 0.42 [0.18, 0.92]) after adjusting for cumulative benzodiazepine dose, age and ESETT study drug.

2.4 Discussion

The results of the analyses of the first 207 patients (includes seven patients who were enrolled twice) showed that majority of patients with SE who failed benzodiazepine treatment did not receive the recommended initial doses of benzodiazepines.[180] The observed practice was not consistent with published evidence-based guidelines which stipulate that the initial treatment of SE begin with a benzodiazepine administered as early as possible, as a single full dose, and by an appropriate route.[3–5] In contrast, we found a pattern of administering multiple, small doses with approximately 70% of patients receiving a lower than guideline-recommended first dose of the first drug. If, however, rectal DZP was excluded, the first doses of MDZ and LZP, mostly administered by EMS and/or ED personnel, were below guideline recommendations 80% of the time. Administration of subsequent doses continued the pattern of underdosing. Regardless of the number of administrations, approximately 12% of patients never received the required cumulative dose needed to meet ESETT eligibility criteria.

Based on the analyses of the full cohort (460 patients after excluding those who were enrolled twice) enrolled in ESETT, our results show that the first dose of the first benzodiazepine as first-line treatment of SE was lower than guideline recommendations in > 80% of all MDZ cases and > 75% of all LZP cases. Reducing the guideline recommended doses by 20% to account for approximation of patient weight and rounding did not

meaningfully change the pattern of underdosing. These findings support my first hypothesis that guideline-recommended doses of benzodiazepines as first-line treatment of convulsive SE were not followed in practice. Although underdosing was pervasive across the age spectrum, children received recommended doses more often than adults. The exception was DZP, mainly administered rectally to children by caregivers prior to EMS, for which most doses met guidelines. The high rates of guideline adherence for DZP may be due to the availability of a pre-packaged, easy-to-use product with preset doses based on weight and age. Similar results were obtained when all benzodiazepine doses were evaluated. Our results from 57 ED's across the United States confirm and extend the findings from our previous analysis of the first 200 unique enrollments (after excluding the second enrollment for seven patients) and are consistent with other reports.[16–19,180,183] Like the first 200 patients, when analyzing results from the entire ESETT population of 460 patients, we observed a pattern of administering multiple, smaller than recommended benzodiazepine doses in the EMS and ED settings. MDZ was primarily administered as IM or IV by EMS personnel, while LZP was almost exclusively dosed IV in the ED. These patterns of route of administration and settings are in general agreement with previously published literature.[4,5,9,10]

Following our preliminary analyses of the first 200 patients, increased educational efforts were implemented at investigator meetings and participating clinical sites. Despite these efforts, the proportion of first doses meeting guideline recommendations remained similar throughout the course of study in both adults and children. While the proportion of MDZ first doses in adults that met guidelines showed a modest improvement, the overall trend

of underdosing continued through the course of the study with only a small fraction of MDZ and LZP first doses meeting guideline recommendations.

Our findings are in agreement with several published reports, mostly from smaller, observational studies, and showed that underdosing of benzodiazepines is pervasive in this population.[16–19,179] A retrospective analysis of treatment of 100 adult patients with initial presentation of SE (convulsive SE or NCSE with or without coma) at a single site found that benzodiazepines were not administered as first line therapy in seven percent of the patients and adequate dose of benzodiazepines calculated in LZP equivalents was administered in only 31% of the patients.[19] Further, among patients who progressed to refractory SE and those who developed NCSE with coma, > 75% had not received an adequate dose of benzodiazepines.[19] Another retrospective observational study of 170 adults and children treated for generalized convulsive SE found that of the 146 patients that received a benzodiazepine in the ED, only 17 (11%), all children, received the guideline-recommended dose, thus indicating that a majority of doses administered in the ED were lower than guideline recommendations.[16] A prospective observational study assessed the use of clonazepam, LZP and MDZ as first-line treatment of SE in 177 patients enrolled at four tertiary care centers and found that among the 82 patients who received LZP, ≥ 84% of the doses were lower than guideline recommendations.[17] One more retrospective study of 44 adult patients with generalized convulsive SE found that of all the benzodiazepine doses (n=108) administered by the EMS personnel (n=39) and in the ED (n=69) only a single dose met guideline-recommendations while the remaining 107 administrations were half of the recommended dose.[18]

Delay in administering appropriate therapy is thought to place patients at risk for longer seizures and poor outcomes.[15,184–192] A multicenter, observational, prospective cohort study of 218 pediatric patients with refractory convulsive SE admitted in 11 tertiary pediatric hospitals in the United States with a median (IQR) age of 4 years (1.2-9.6 years) evaluated whether delay in administering first-line benzodiazepines treatment is associated with unfavorable short-term outcomes.[186] The study found that a delay of > 10 minutes between the onset of seizure and the administration of benzodiazepine treatment was significantly associated with increased odds of death, longer duration of convulsive seizures and more frequent hypotension.[186] If an initial benzodiazepine dose does not terminate a prolonged seizure, preclinical evidence suggests that higher subsequent doses may be required.[73,117–121] This could be due to changes in benzodiazepine pharmacodynamics. Prolonged seizures result in enhanced endocytosis of synaptic GABA-A receptors, thus reducing benzodiazepine potency.[117–119,121] This internalization is associated with decreased effectiveness of DZP and, presumably, other benzodiazepines.[73,120] For example, in a rat model of SE, the DZP ED50 for terminating seizures was 10-fold higher, 40 mg/kg vs. 4.2 mg/kg, when administered after 45 minutes of continuous seizures as compared to 10 minutes (Figure 2.4-1).[120] Further, rapid receptor plasticity has been attributed to activation of some secondary messengers during prolonged seizures.[193] As SE continues, the activity and number of N-methyl-D-aspartate (NMDA) receptors and excitatory amino-acid synaptic concentrations increase. Hence, administration of an adequate dose of benzodiazepines as soon as possible is recommended to terminate SE and avoid established SE.[194,195]. In our study, attainment of adequate cumulative benzodiazepine dose after the first administration took a median of 6 minutes. Combining this time interval with the

elapsed time from seizure onset to the first benzodiazepine dose (median 27 minutes, IQR 11 to 49 minutes) gives a total median time of 33 minutes from the onset of SE to attainment of adequate cumulative benzodiazepine dose. This potentially reduced the response to benzodiazepines and highlights the importance of administering an adequate dose as soon as possible following the onset of SE.

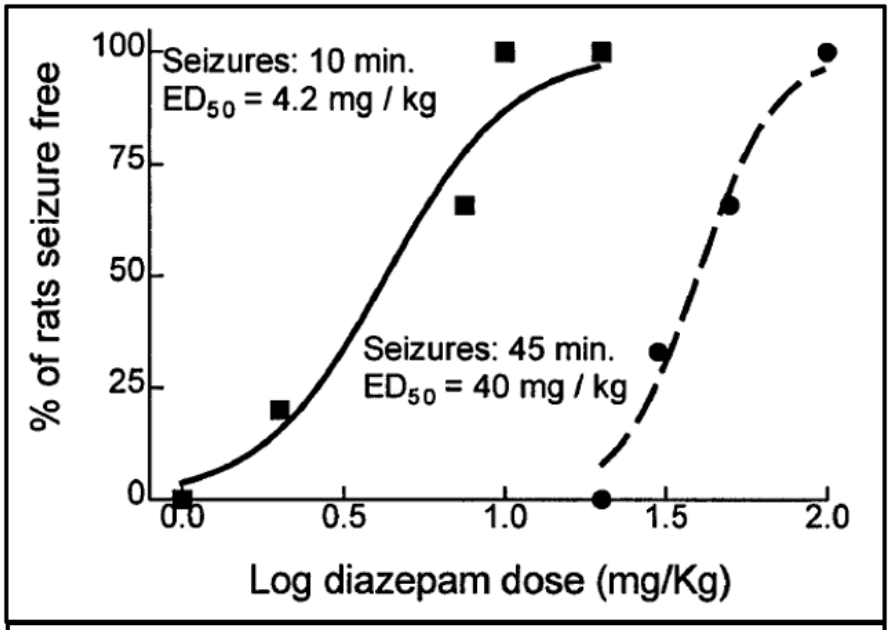

Figure 2.4-1: Efficacy of DZP in controlling brief seizures in rats induced by intraperitoneal administration of lithium chloride and pilocarpine. Reproduced with permission from Kapur et al.[119] Copyright [1997] Society for Neuroscience.

It is not surprising, in this cohort, that responsiveness of seizures to second-line agents was not enhanced by higher doses of benzodiazepines given earlier, since the study population was, by design, those whose SE was benzodiazepine refractory. The ESETT primary outcome was a composite, which consisted of clinical seizure cessation and improved

responsiveness at 60 minutes after the start of study drug infusion. We found that even after taking into account the underlying brain pathology responsible for SE, higher cumulative benzodiazepine doses were significantly associated with decreased odds of primary outcome success, but not with clinical seizure cessation. These findings do not agree with my second hypothesis that higher cumulative doses of benzodiazepines will significantly increase the likelihood of primary outcome success in ESETT. One of the potential explanations for this association is that higher doses of benzodiazepines may be used in patients with more severe or persistent seizures. Alternatively, the lack of association with clinical seizure cessation suggests that those with higher cumulative doses failed the primary outcome due to lack of improved responsiveness. This could be due to either the patients remaining unconscious after the dose of second-line treatment or due to underlying subtle SE or NCSE. As discussed in section 1.2.3, EEG data is needed to confirm the diagnosis of NCSE. Further, studies have reported persistent NCSE in patients after the convulsive SE appears to have been resolved.[54,55] It is likely that many patients who were given higher doses of benzodiazepines did respond better to those higher doses, and thus were excluded from enrollment in ESETT and this analysis. Participants in this study were those whose seizures were truly benzodiazepine refractory. Expectedly, they did not have more seizure cessation from the benzodiazepines but did have more sedation after their seizures were terminated with the second-line medication, thus they likely woke up slower and did not achieve the primary outcome as often. Furthermore, there may be confounding factors that influence this finding including the elapsed time over which the cumulative doses were administered.

There are several possible reasons for this wide-spread underdosing. Betjemann et al. found that many of the EMS protocols in California did not follow evidence-based guidelines and MDZ initial dose was lower than guideline-recommendations in most protocols.[196] Further, this problem of benzodiazepine underdosing in SE may also be attributable to the perceived risk of cardio-respiratory compromise associated with benzodiazepines.[9] However, Alldredge et al. showed that the rate of respiratory or circulatory complications was nearly doubled (p=0.08) in untreated SE patients versus those treated with DZP or LZP when administered by EMS personnel.[9] As another explanation for underdosing, ED physicians may feel compelled to start treating seizure emergencies with lower doses of benzodiazepines because at presentation in the ED, they may not have yet established whether the patient is in SE or has not yet recovered from a recent seizure. It is difficult to generalize our results with patterns in all patients given first-line therapy for SE. ESETT only examined benzodiazepine dosing in patients who ultimately had SE so it was not possible to compare the dosing or outcome in patients whose seizures stopped to determine if higher benzodiazepine doses would have been helpful. Nevertheless, once the diagnosis of SE is established, randomized clinical trials make clear what dosing of benzodiazepines should be and ED physicians should move with haste to administer adequate doses.

Other deviations from best guideline-recommended practices were also observed. On 29 occasions LZP was administered by IM, IN, or buccal routes. Physicochemical properties of the drug affect its rate of absorption across biological membranes and typically, higher the lipid solubility of a drug, faster is its absorption rate.[197] Lipid solubility of LZP is 4-6 times lower than that of MDZ or DZP.[197,198] Hence, when administered by extravascular route, the absorption rate for LZP is significantly lower as compared to MDZ or DZP due

to its low lipid solubility. The pharmacokinetics of LZP following administration by these routes do not support rapid absorption and, therefore are not appropriate for SE therapy.[199–202] Given the high rate of appropriate dosing of rectal DZP by caregivers, the recently approved IN DZP and IN MDZ products intended for treatment of seizure emergencies may facilitate early administration of recommended benzodiazepine doses, particularly in adults.[47,49] While these therapies have been approved for the treatment of seizure clusters, as described in section 1.2, seizure clusters lie on the same continuum of seizure emergencies as SE. So even if the formulations are used for the approved indication, it will likely prevent the evolution of SE. Further, just like rectal DZP which is approved for acute repetitive seizures but is recommended and commonly used for the treatment of SE, these newer formulations will likely be beneficial for SE treatment.

There are several limitations that affect generalizing our findings to a broader population. Our analysis is limited to SE patients who continued to have seizures despite benzodiazepine treatment. Since initial benzodiazepine underdosing is associated with treatment failure, our study population may overestimate the rate of underdosing among all patients for SE. Additionally, we were not able to precisely determine the latency between the onset of SE and initial benzodiazepine dose, a factor that affects treatment response rates. It may be that the latency for the ESETT patients was different from those who respond to initial benzodiazepine treatment. Gaínza-Lein et al. summarized results from 15 studies that reported time to treatment in SE and found the variability was high with the median time for EMS arrival of 22 minutes (range: 0-875 minutes) and median time to hospital arrival of 56 minutes (range: 0-16660 minutes).[184] Nonetheless, benzodiazepine underdosing is particularly important in this subpopulation in whom

seizures continue and may progress to refractory SE with attendant high rates of morbidity and mortality. Conversely, our analysis may underestimate the rate of underdosing because only those given an adequate cumulative benzodiazepine dose were eligible for ESETT enrollment. It is possible that eagerness to enroll subjects could bias toward lower cumulative benzodiazepine doses, but that would not be expected to impact the first dose. However, in this scenario, ESETT participating EDs would be more likely to administer larger individual doses in order to meet the minimum adequate dose sooner and should not affect EMS practice. Another minor limitation is that different routes of administration of benzodiazepines were assumed to be equivalent for the calculation of cumulative dose in LZP equivalents.

Benzodiazepines as first-line therapy for SE in patients who failed this treatment and progressed to established SE appear to be routinely underdosed in the EMS and ED settings regardless of drug, route of administration, or patient weight. The frequency of underdosing was far greater in adults than children. Further, on average it took 33 minutes from the onset of SE to receive a cumulative adequate dose of benzodiazepines. This pattern may contribute to poorer responses. This broad and generalizable cohort confirms prior single site reports that underdosing is both pervasive and difficult to remediate.

Although there are published guidelines for the initial treatment of status epilepticus, additional efforts are needed to change clinical practice. On-going and future efforts and new developments in the field that may help facilitate appropriate dosing of benzodiazepines as initial therapy for convulsive SE are discussed below.

2.4.1 Availability of newer, easy-to-administer formulations of benzodiazepines

Greater than 78% of all DZP doses used in ESETT met guideline recommendations and a majority of these were administered by caregivers using the rectal route prior to EMS arrival. It is surprising that most of the benzodiazepine doses that did not meet the guideline recommendations were administered by trained medical professionals while caregivers with little or no medical training in the majority of cases administered appropriate doses of rectal DZP. A possible explanation for this high rate of DZP doses meeting guideline recommendations is the availability of an easy-to-use rectal DZP gel product (DIASTAT®) that is pre-filled with unit doses of either 2.5 mg, 5 mg, 10 mg or 20 mg. The FDA has recently approved benzodiazepine formulations for IN delivery of DZP (Valtoco®) and MDZ (Nayzilam®) for the treatment of seizure clusters.[49] Valtoco® (Valium+tocopherol) is a DZP nasal spray developed by Neurelis Inc. (San Diego, CA) available in 5 mg, 10 mg, 15 mg and 20 mg doses to allow for individualized dosing based on age and weight. Valtoco® is approved for use in patients aged six years or older. Each device of Valtoco® contains 5 mg or 10 mg dose and is for single use only i.e. the device must be discarded after use. Based on the dose needed, each pack may contain one or two devices. Valtoco® uses Intravail® which is a class of transmucosal delivery enhancement agents that are highly effective and non-irritating enhancers of transmucosal absorption.[203] Absolute bioavailability of Valtoco® has been found to be 97%.[204] A phase III study was not required for Valtoco® as rectal DZP is approved for use and the exposure of Valtoco® has been shown to be comparable to rectal DZP. Nayzilam® is an MDZ nasal spray approved for the treatment of seizure clusters in patients 12 years and older developed by UCB (Brussels, Belgium). A randomized, double-blind, placebo-controlled trial of 201 patients

for the treatment of single seizure cluster episode in an outpatient setting with either a blinded dose of 5 mg of Nayzilam® (n=134 patients) or placebo (n=67) was conducted.[205] The primary efficacy endpoint was termination of seizures within 10 minutes and the response rate for Nayzilam® was significantly higher at 53.7% vs. 34.3% in the placebo group.[205] Nayzilam® is also a single-dose nasal spray unit containing 5 mg of MDZ.[205] While the absolute bioavailability of Nayzilam® was found to be 44%, the median time to maximum plasma concentration was 17 minutes.[205] Like rectal DZP, which is only approved for the treatment of seizure clusters, but is commonly used for the management of convulsive SE and is recommended by evidence-based guidelines for SE treatment[5], it is likely that these newer intranasal formulations with preset doses of benzodiazepines will also be used for prolonged seizures and SE treatment and will help mediate the problem of underdosing to some extent.

Another newer benzodiazepine formulation is being developed by Engage Therapeutics (Summit, NJ) which is subsidiary of UCB (Brussels, Belgium) called Staccato® Alprazolam for intra-pulmonary delivery of alprazolam for rapid termination of epileptic seizures. The Staccato® system consists of a small portable device with three components: a heating substrate, a thin film of the drug coated on the substrate and an airway through which the patient orally inhales. Placing the Staccato® device between the lips and taking a normal breath rapidly vaporizes the drug to form small particle aerosol which is drawn into the patient's lungs where it diffuses into circulation and has a rapid systemic effect. A phase IIa study in 15 adults with photosensitive sensitive seizures found that 0.5 mg, 1 mg and 2 mg of Staccato® Alprazolam suppressed epileptiform activity in all patients.[206] In a phase IIb, Staccato® Alprazolam was found to be effective and generally well tolerated in

all seizure types and had a fast onset of action with an average time of 30 seconds to seizure cessation. Currently, alprazolam is not commonly used for SE treatment in the USA, but given the rapid onset of action seen with Staccato® Alprazolam, it has a high potential of being used for early treatment of convulsive SE. A phase III study will be needed for the approval of Staccato® Alprazolam as rescue therapy for the treatment of seizure clusters. Based on the estimated response rate of Staccato® Alprazolam, the study can be designed either as superiority or non-inferiority compared with rectal DZP which is the current standard of care. Further, a double dummy design will be needed given the different modes of administration of the standard of care and test. Another consideration for this study is the setting. A pre-hospital setting where the drugs are administered by paramedics or by caregivers may be appropriate for this study.

The use of IM route of administration for rescue therapy in the prehospital setting is rapidly increasing mainly due to the results of RAMPART where IM MDZ was found to be superior to IV LZP for prehospital treatment of patients with convulsive SE.[10] Meridian Medical Technologies (a Pfizer company) is developing an autoinjector for IM MDZ administration.[47] This autoinjector was used as an investigational device in RAMPART. Majority of the MDZ doses in ESETT patients were administered by the EMS personnel in the ambulance and > 80% of these doses were lower than guideline-recommendations. An IM autoinjector device with prefilled MDZ doses, once available, will facilitate rapid administration of appropriate MDZ doses in SE patients based on their body weight.

2.4.2 Updating EMS protocols for the treatment of convulsive SE

Betjemann et al. reviewed protocols for the management of generalized convulsive SE in adults from 33 EMS systems covering 58 counties in California.[196] The study found that only 17 (51.5%) of the protocols are updated after the evidence-based guidelines for the treatment of SE were published in 2016.[5,196] While 32 protocols included IM MDZ for initial treatment of SE, only two protocols recommended a dose of 10 mg in accordance with the guidelines whereas 30 recommended a lower initial dose.[196] While this analysis was only limited to one state (CA), it provides a potential explanation for the extensive underdosing of MDZ seen in ESETT. Although the protocols may not necessarily reflect what the EMS techs do in practice, a nationwide analysis and followed by an update of EMS protocols to reflect the current guidelines may help reduce benzodiazepine underdosing in the EMS setting as seen in ESETT.

2.4.3 Availability of rapid EEG

One of the challenges associated with administering early, appropriate treatment for convulsive SE is identification of cases with the underlying electrical seizure activity (NCSE) even after the termination of clinical seizures. Availability of rapid EEG in the EMS and the ED would not only allow medical personnel to recognize subtle SE but also help identify if an unconscious patient is currently in SE or is simply postictal from a previous seizure based on their electrographic measures. It will then be possible to rapidly administer appropriate treatment as needed.

2.4.4 Additional training to increase awareness of appropriate benzodiazepine dosing

Based on the results of the benzodiazepine dosing pattern assessment in the first 207 ESETT patients that found pervasive underdosing of benzodiazepines particularly in the EMS and ED setting, additional trainings for study teams to emphasize appropriate benzodiazepine dosing were implemented during investigator meetings. However, the results of the full cohort of 478 patients showed that the training did not have any impact on the dosing patterns of benzodiazepines in the ED setting. The ESETT cohort enrolled patients at 57 different sites across the US which indicates that this culture of underdosing benzodiazepines is not limited to a site or region but is in fact a systematic issue. Rigorous efforts in the form of regular trainings for EMS and ED personnel and routine follow-ups for quality assessment are needed to overcome this problem and change clinical practice. Future studies for the treatment of established or refractory SE should consider incorporating intermittent trainings to periodically reinforce the appropriate dosing of benzodiazepines as first-line treatment of SE.

CHAPTER 3

EARLY DRUG CONCENTRATIONS OF SECOND-LINE TREATMENT FOR CONVULSIVE STATUS EPILEPTICUS

The goal of ESETT as described in chapter one was to identify the best and/or the worst second-line treatment of convulsive SE from among FOS, LEV and VPA. An ancillary study was designed to understand the pharmacokinetics of these drugs when used for the treatment of benzodiazepine-refractory SE. The goal was to assess if, within each treatment group, drug concentrations can help explain response to the treatment administered. The **specific aim** of this ancillary pharmacokinetic study was to develop exposure-response relationships by relating early exposure of PHT, LEV and VPA with the ESETT primary outcome and key secondary outcomes in patients with benzodiazepine-refractory convulsive SE. As discussed in section 1.3.4, the primary outcome for ESETT was clinical cessation of SE and improved responsiveness at 60 minutes after the start of study drug infusion without additional antiseizure medications. As it is the early drug concentrations that will drive the response to treatment assessed at 60 minutes, the ancillary pharmacokinetic study sought to measure drug concentrations in the first 2 hours post-infusion. The metric of early drug exposure was partial area under the drug concentration-time curve from 0-2 hours (pAUC) after the start of study drug infusion. Value of pAUC for each subject will be derived using established pharmacokinetic models for FOS, LEV and VPA and the individual subject's drug concentration data. The effect of covariates such as age, sex, weight/body mass index (BMI) and seizure etiology on these exposure-response relationships will be explored. The hypotheses for this work were:

Hypothesis 1: Patients with higher drug exposures (pAUC based on plasma concentrations of PHT, LEV and VPA) will more likely respond to treatment.

Hypothesis 2: Patients with higher drug exposures will have a higher incidence of serious adverse events commonly associated with the study drugs.

Hypothesis 3: The relationship between exposure and response will differ by weight/BMI, sex and age group.

The following section is based on a manuscript that describes the novel bioanalytical method used to measure concentrations of PHT, LEV and VPA and reports their plasma concentrations in ESETT patients. Further, the challenges encountered in collecting blood samples in emergent conditions like SE particularly in pediatric patients are also discussed in this manuscript.

3.1 Introduction

As described in chapter 2, benzodiazepines are used as first-line treatment of convulsive SE but fail to terminate seizures in approximately 10-55% of the cases.[3–5] For patients who fail the first-line treatment, PHT or FOS are used as the standard of care for second-line treatment as shown by the multiple expert surveys.[71,72] While LEV and VPA are potentially useful[122,124,125], prior to ESETT no randomized, controlled study was done to demonstrate their efficacy. Furthermore, limited information was available on their plasma exposures after a high IV dose, particularly in children with SE.[132,207–209] Most of the currently available pharmacokinetic information in children at the time ESETT was designed came from the use of these drugs as oral maintenance therapy.

As discussed in section 1.3.4, because the primary outcome of ESETT of clinical cessation of SE and improved responsiveness without additional antiseizure medication was to be determined at 60 minutes after the start of study drug infusion, we were interested in measuring early drug concentrations, as it is early exposure that will drive the response.

ESETT provided an opportunity to investigate PHT (after FOS administration), LEV and VPA concentrations within the first two hours after the start of infusion in young children. Given the challenges in obtaining blood samples from children in the ED, a sparse sampling approach was employed in which two blood samples were to be collected per patient, one between 20-50 minutes and the second between 60-120 minutes after the start of study drug infusion to measure drug concentrations.

Patients enrolled in ESETT included young children. Hence, blood volumes were limited to less than three milliliters. Further, study drug assignment was blinded, so the identity of the drug in each sample was unknown to the investigators which precluded the ability to conduct separate analyses for each drug. Also, since PHT and VPA are highly plasma protein bound[210] and it is the unbound concentration that is available to cross the blood brain barrier and elicit a pharmacological response, it was necessary to quantitate both the total plasma concentrations for the three drugs and the unbound concentrations for PHT and LEV. Additionally, it was possible that patients enrolled in ESETT may have been taking one or more of the study drugs as maintenance therapy. Thus, a bioanalytical method capable of measuring total concentrations of PHT, LEV and VPA and unbound concentrations of PHT and VPA was needed.

The widely differing physicochemical and protein binding properties of LEV, PHT and VPA make simultaneous extraction and analysis challenging. For example, LEV is hydrophilic with a logP of -0.6 and pKa of less than -2. On the other hand, PHT and VPA are both lipophilic with logPs of 2.47 and 2.7, respectively, and are acidic in nature with pKas of 8.3 and 4.8, respectively[211]. As a result, the extraction and separation of these drugs using a single method is difficult. In addition, since PHT and VPA are highly bound to

plasma proteins we expected the unbound concentrations to be a small fraction of the total concentrations. Hence, the method should be capable of measuring concentrations over a wide range (> 100-fold) using a small sample volume.

The **primary objective** of this work was to report the concentrations of LEV, PHT and VPA and plasma protein binding of PHT and VPA in young children with benzodiazepine-refractory SE enrolled in ESETT.

3.2 Methods

As discussed in section 1.3, ESETT was conducted under the exception from informed-consent requirements for emergency research (FDA regulation 21 CFR 50.2412). The institutional review boards for all participating institutions approved the protocol after consultation with the local community and public disclosure.[139] Patients or their legally authorized representatives were notified about enrollment in the trial by the research team as soon as possible, usually while the patient was still in the emergency department and were asked to provide written informed consent for continued data collection through the end of the trial.

As described by the inclusion criteria in section 1.3.5.3, study participants were patients ≥ 2 years, witnessed to have clinically apparent seizures after having received an adequate dose of benzodiazepines. After the participants were randomized, study drug was intravenously infused over 10 minutes. They were then observed for 20 minutes after the start of study drug infusion and, if clinically apparent seizures continued, rescue medication was administered.

As described in section 1.4, the dates for ESETT enrollment were November 2015 through December 2018. The ancillary pharmacokinetic study began enrollment in November 2017 and sites were initiated on a rolling basis. However, the adult arm of ESETT was terminated for futility prior to the start of the ancillary pharmacokinetic study[139]; hence, blood samples could only be collected from pediatric patients (2 to < 18 years) that were enrolled in ESETT as a part of the enrichment cohort for the secondary subgroup analysis based on age.

3.2.1 Blood sample collection

A sparse sampling approach for pharmacokinetic assessment was used to minimize blood draws as convulsive SE is an emergent condition. Two blood samples (total blood volume of 6-8 mL) were collected into two EDTA vacutainer tubes from each subject. One between 20-50 minutes (3-5 mL) and another one between 60 to 120 minutes (~3 mL) after the start of study drug infusion either via a venipuncture or from an indwelling catheter not used for drug infusion. The exact time of sampling was recorded. Upon collection, tubes were inverted several times to ensure mixing with EDTA. The blood samples were refrigerated/stored on ice and, within two hours of collection, were centrifuged at 1000-2000 RCF for 10-20 minutes to separate plasma. Plasma was aliquoted into labelled cryovials and stored at -80^{0}C prior to and after shipment to the Center for Orphan Drug Research for LC-MS/MS analysis.

3.2.2 Development and validation of LC-MS/MS method for simultaneous detection of PHT, LEV and VPA

3.2.2.1 Sample preparation

LEV, PHT, and VPA (USP grade) were purchased from Sigma Aldrich (Missouri, USA). Deuterated (stable labeled) internal standards for the 3 drugs were purchased from Cerillant (Texas, USA). Acetonitrile (LCMS grade) was purchased from Burdick and Jackson (Michigan, USA). Ammonium acetate was purchased from Fisher Scientific (Georgia, USA) and acetic acid from Fluka Chemika (Honeywell Fluka, USA). Water was prepared in the laboratory with a MilliQ® Advantage ultrapure water system (Millipore, MA, USA). Acrodisc syringe filters were purchased from Pall Corporation (New York, USA).

Standard stock solutions of LEV and PHT were individually prepared at 10 mg/mL in methanol. Neat stock solution of VPA (908 mg/mL) and the internal standard stock solutions of LEV-d6 (1 mg/mL), PHT-d10 (0.1 mg/mL) and VPA-d6 (1 mg/mL) were purchased from Sigma. Appropriate dilutions of the stock solutions were made with methanol to obtain mixed standard working solutions ranging from 18.75 to 3000 µg/mL for LEV and VPA, and 6.25 to 500 µg/mL for PHT. A mixed internal standard working solution at 500 µg/mL for VPA-d6 and LEV-d6 and 100 µg/mL for PHT-d10 was prepared. Calibration standards were freshly prepared by spiking 20 µL of mixed standard working solutions into 100 µL of blank human plasma to yield final concentrations ranging from 3.1 to 600 µg/mL for LEV and VPA and 0.625 to 100 µg/mL for PHT. Separate concentrated, quality control (QC) stock solutions were prepared in methanol to avoid addition of a large volume of methanol to plasma. For LEV and VPA the QC stock solutions were 25 mg /mL each and for PHT it was 10 mg/mL. Three levels of QC samples

(low, mid and high) were prepared for each drug by addition of appropriate volumes of stock solutions to 25 mL of blank plasma to obtain final QC concentrations of 60 µg/mL, 125 µg/mL and 250 µg/mL for LEV, 75 µg/mL,150 µg/mL and 300 µg/mL for VPA, and 10 µg/mL, 25 µg/mL and 50 µg/mL for PHT.

For the preparation of unbound samples, an ultrafiltration method was used to separate the protein from the plasma matrix. One mL of plasma was pipetted into Centrifree® ultrafiltration (Merck Millipore, Ireland) devices with a molecular weight cutoff of 30,000 Da. The samples were centrifuged for 30-60 min at 2500 x g at 37 ^{0}C. The filtrate was then used for the measurement of unbound drug concentrations and treated in the same manner as other plasma samples. Twenty-five microliters of stable-labeled internal standard working solution was added to 100 µL of each of the patient plasma samples. Two mL of acetonitrile was added for protein precipitation and samples were vortexed for several seconds. All samples were then centrifuged for 10 min at 2500 rpm, supernatant was collected in clean glass tubes and evaporated at 35 ^{0}C using nitrogen. Samples were reconstituted in 150 µL of mobile phase and filtered using 13 mm Pall Acrodisc® (Pall Corporation, MI, USA) PTFE syringe filters (0.2 micron) for LC-MS/MS analysis.

3.2.2.2 Assay conditions

The samples were analyzed using a TSQ Quantum Access triple quad mass spectrometer (Thermo Scientific, CA, USA) with electrospray ionization and a Dionex Ultimate 3000 HPLC system (California, USA) that included an autosampler and pump. Reverse-phase chromatographic separation was performed using an InfinityLab Poroshell 120 EC-C18

(Agilent, California, USA) column (2.1×100 mm, 2.7 µm). The analytes were separated using isocratic mobile phase with a composition of 25% 10 mM ammonium acetate and 75% acetonitrile at a flow rate of 0.15 mL/min and run time of 5 min. Excalibur software (Thermo Scientific, CA, USA) was used for data acquisition and analysis. The conditions for LC-MS/MS included heated electrospray ionization (HESI) with a vaporizing temperature of 300 ^{0}C and capillary temperature of 350 ^{0}C. The spray voltage was set at 3500 V and collision gas pressure of 1.5 mTorr for the multiple reaction monitoring (MRM). The detection of VPA and PHT was done using negative polarity, whereas positive polarity was used for LEV. The m/z ratios for parent and product ions used for the MRM method were 171 and 126 for LEV, 251 and 102 for PHT, and 143 and 143 for VPA.

3.2.2.3 Assay validation and data analysis

The three study drugs were separated within 3 min with LEV eluting at 1.7 min, PHT at 2 min and VPA at 2.5 min with a total run time of 5 min (Figure 3.2-1). There were no interfering peaks observed for any of the drugs at their individual retention times. The drug concentrations were calculated using the linear equation with (1/x) weighting for LEV and VPA and uniform weighting for PHT due to wide calibration concentration range. The standard curves for the 3 drugs were linear over a wide range of concentrations (> 150-fold) with r^2 values > 0.99 (Figure 3.2-2)**.**

The lower limit of quantitation was 3 µg/mL for LEV and VPA and 0.6 µg/mL for PHT. The limit of detection for LEV, PHT and VPA was 2, 0.5, and 3 ng/mL, respectively. The

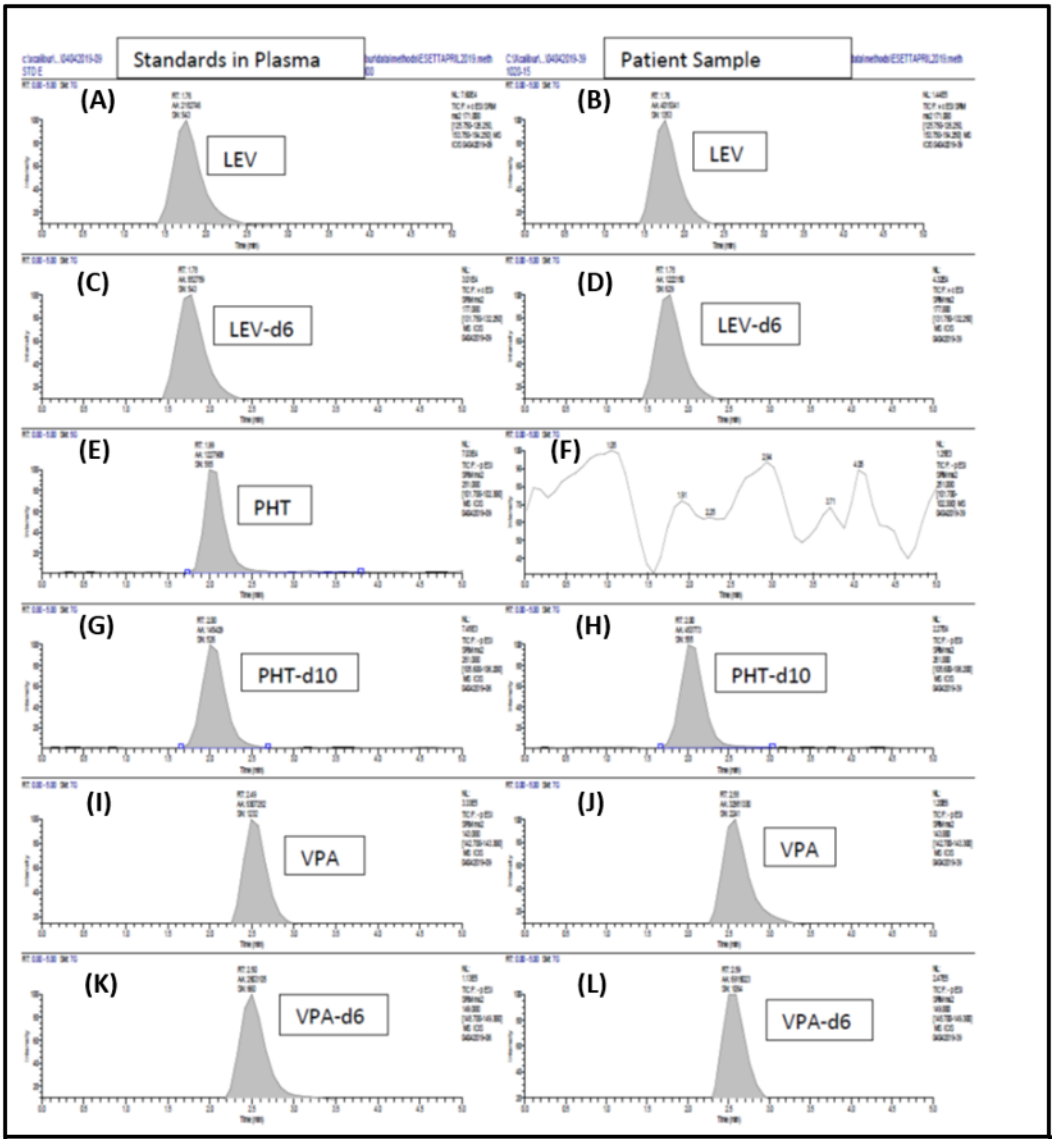

Figure 3.2-1: Sample chromatograms of the three ESETT drugs and the stable-labeled internal standards from plasma standard and patient sample
Panels A, E and I are for LEV, PHT, and VPA, respectively detected in a plasma standard with the corresponding stable-labeled internal standards LEV-d6 (panel C), PHT-d10 (panel G) and VPA-d6 (panel K). Panel B and J are for LEV and VPA, respectively detected in a patient plasma sample with corresponding internal standards LEV-d6 (panel D) and VPA-d6 (panel L). PHT concentration was below the limit of detection in the patient sample (panel F) but the corresponding internal standard PHT-d10 can be seen in panel H.

method was validated according to FDA guidelines for bioanalytical assays.[212] The variability measured as % coefficient of variation (CV) for the calibration standards was < 10%, < 15%, and < 9% for LEV, PHT and VPA, respectively. The calibration standard

accuracy was 82-115% for LEV, 97-105% for PHT and 90-107% for VPA. The variability (%CV) in intra- and inter-day precision values for quality control samples were < 11% and < 16%, respectively. The intra- and inter-day accuracy for quality control samples ranged from 94-104% and 92-114% respectively, which are within the acceptable limit of ± 15% of the target concentration (Table 3.2-1 and 3.2-2)

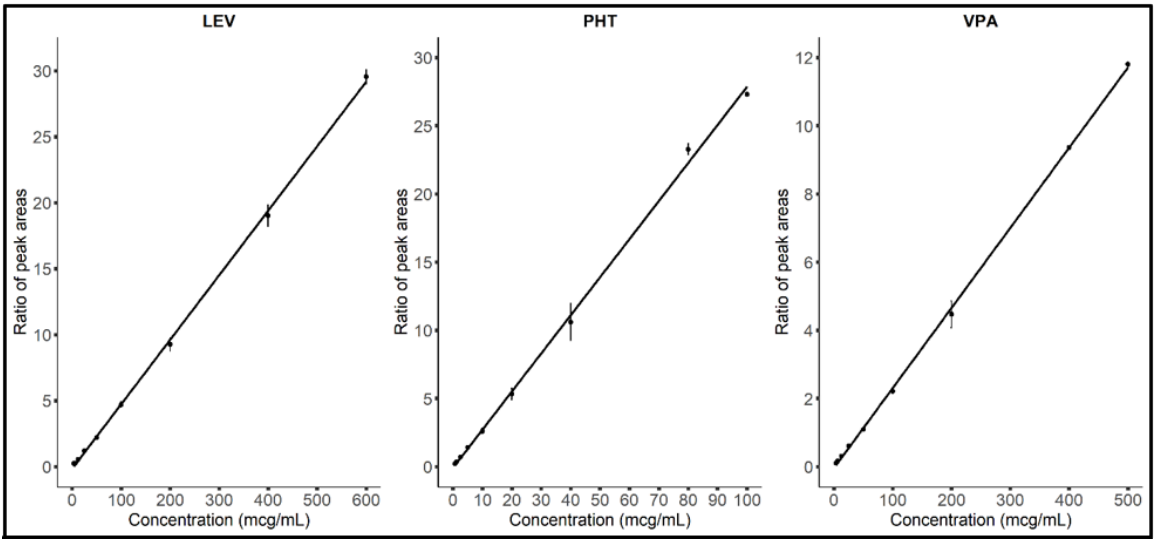

Figure 3.2-2: Performance of calibration standards for LEV (left), PHT (middle), VPA (right). R^2 was > 0.99 for all 3 drugs.

Table 3.2-1: Accuracy and Precision of Calibration Standards in Plasma (n=3 per day over 5 days)

LEV			PHT			VPA		
Target Conc. µg/mL	**Accuracy (%)**	**Precision (%CV)**	**Target Conc. µg/mL**	**Accuracy (%)**	**Precision (%CV)**	**Target Conc. (µg/mL)**	**Accuracy (%)**	**Precision (%CV)**
3.125	115.2	8.0	0.625	105.4	12.6	3.125	89.6	6.5
6.25	81.8	9.4	1.25	97.7	15.4	6.25	95.6	8.6
12.5	97.6	6.9	2.5	99.2	12.9	12.5	98.8	7.6
25	95.6	10.3	5	102.2	6.9	25	105.0	3.1
50	95.3	9.5	10	100.3	5.1	50	101.1	5.5
100	100.6	7.2	20	98.3	7.1	100	104.0	4.3
200	101.0	6.7	40	94.4	7.7	200	106.5	7.0
400	99.9	5.7	80	104.8	8.1	400	107.3	2.5
600	100.4	7.2	100	96.9	4.8	600	92.1	2.9

Table 3.2-2: Precision and Accuracy of Quality Control Samples (n=3 per day over 5 days)

QC samples	Target Concentration	Precision		Accuracy	
		Intra-day (% CV)	Inter-day (% CV)	Intra-day (%)	Inter-day (%)
LEV Low	60	5.7	7	94	99
LEV Mid	125	3.4	14	97	105
LEV High	250	11.3	13	104	104
PHT Low	10	8.5	12	111	102
PHT Mid	25	8.3	16	103	109
PHT High	50	2.4	8	99	93
VPA Low	75	4.9	5	98	92
VPA Mid	150	4.2	12	105	114
VPA High	300	2.7	5	106	102

To assess the extraction efficiency of the method, the recovery of five replicates of three QC samples 12, 24 and 40 µg/mL for PHT, and 60, 120 and 200 µg/mL for LEV and VPA prepared in filtered plasma was measured using standards prepared in regular plasma. Three replicates of the standard curve with all three drugs were used for this and these standards were prepared in regular plasma as described above. The QC samples were prepared using the same procedure as described above except filtered human plasma was used instead of regular plasma. During the analysis, the QC samples were bracketed between the three standard curves and analyzed using the method described above. The average accuracy of all QC's using five replicates each was 99.3% for LEV, 85.3% for PHT and 95.9% for VPA whereas the average precision was 3.9% CV for LEV, 9.5% CV for PHT and 2.4% CV for VPA. These results showed that the extraction efficiency of the method was close to a 100%. Hence, standards made in regular plasma were used for the analysis of both the total and unbound concentrations in the patient plasma samples.

3.3 Results

Blood samples were collected from 27 of the 78 pediatric patients enrolled in ESETT as a part of the enrichment cohort, with a median (interquartile range) age of 4 years (2.5, 6.5 years) and weight of 17 kg (15.7, 20.9 kg). Eighteen patients were primary outcome successes and nine failed the primary outcome. Twenty-one of the 27 children had epilepsy and the remaining six had neither a known diagnosis of epilepsy nor were taking any anti-seizure medications. Five of the 21 epilepsy patients were taking one anti-seizure medication while the remaining 16 were taking at least two anti-seizure medications as oral maintenance therapy. Fourteen (67%) of the 21 epilepsy patients were taking one or two of the ESETT study drugs as chronic therapy prior to enrollment. Nine patients were on LEV (three of whom were randomized to LEV), three on VPA (one was randomized to VPA) and two patients were receiving both LEV and VPA (both randomized to FOS). Maintenance doses ranged from 400-2250 mg/day for LEV and 200-1050 mg/day for VPA. Other co-administered drugs that may have potential drug-drug interaction with one or more of the study drugs included oxcarbazepine, diazepam, ibuprofen, zonisamide, clonazepam, gabapentin, carbamazepine, phenobarbital and clobazam.

A total of 44 blood samples were collected from 27 pediatric patients (two blood samples each from 17 patients and one sample each from 10 patients). Of these, 11 patients (40.7%) were randomized to FOS, nine (33.3%) to VPA and seven (25.9%) to LEV. Nine patients had measurable concentrations of two of these drugs in their plasma (15 samples), whereas one patient had measurable concentrations of all three drugs. The remaining four patients were randomized to the same drug that they were taking on a chronic basis. Protein binding

could not be measured in three plasma samples due to a limited volume. Unbound VPA concentrations were below the limit of detection for two samples.

The total concentrations ranged from 69-151.3 µg/mL for LEV, 11.3-26.7 µg/mL for PHT and 126-223 µg/mL for VPA. Figure 3.3-1 shows concentrations and their corresponding treatment response. The unbound concentrations ranged from 1-2.8 µg/mL for PHT and

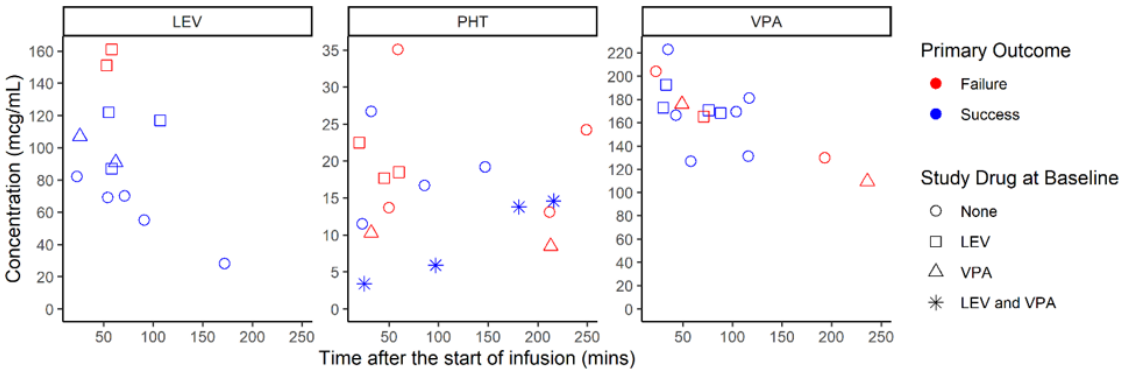

Figure 3.3-1: Total concentrations (µg/mL) of LEV (left), PHT (middle) and VPA (right) versus the time of blood collection after the start of study drug infusion (minutes) overlaid with primary outcome results (red= failure, blue= success)

31-114 µg/mL for VPA (Figure 3.3-2). The free fraction ranged from 4-19% for PHT and 17-51% for VPA. Correlation between the unbound and total concentrations for PHT and VPA measured using Spearman's rho was R^2=0.616 and R^2=0.797, respectively. As expected with increasing concentrations, VPA shows a trend towards saturable plasma protein binding. Two patients with measurable VPA concentrations had the highest free fraction of PHT following IV infusion.

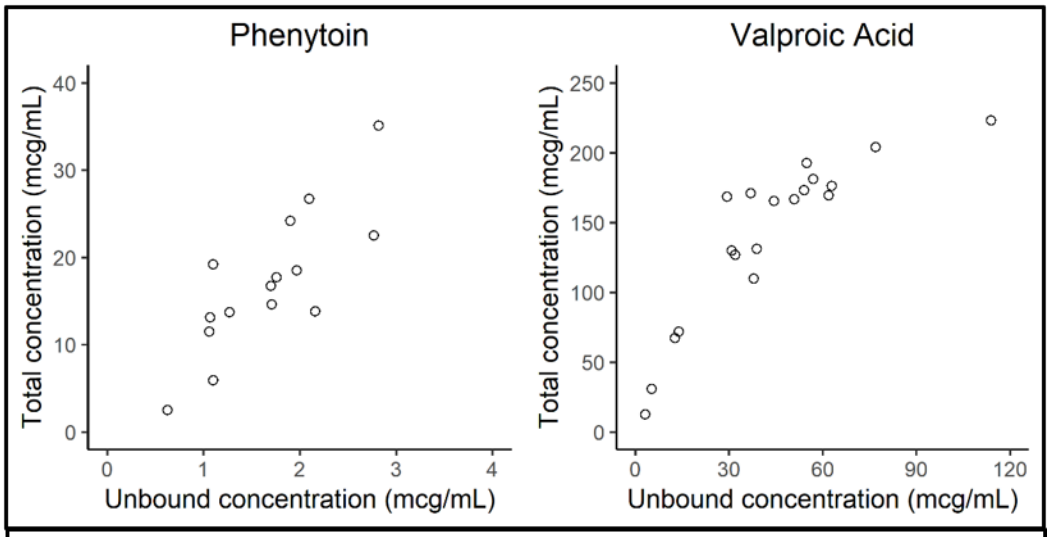

Figure 3.3-2: Total vs. unbound concentration (µg/mL) for PHT (left) and VPA (right)

3.4 Discussion

We developed and validated an LC-MS/MS method that simultaneously quantitated LEV, PHT and VPA concentrations in blinded samples after IV administration in young children with benzodiazepine-refractory SE. Unblinding confirmed that the measured concentrations corresponded to the intended randomized drug. Additional anti-seizure drugs reported to have been used prior to or after the randomized therapy were also measurable in the plasma samples. While drug concentrations were generally in the therapeutic ranges (PHT 10-20 µg/ml, LEV 12-46 µg/ml, and VPA 50-150 µg/ml)[127,131], a wide range of concentrations were observed despite mg/kg dosing.

This is the first report of LEV concentrations in young children with generalized convulsive SE. Two previous studies reported LEV concentrations in older children, although neither of these were in patients with generalized convulsive SE. Abend et al. measured LEV concentrations (8-41 µg/ml) within 60 minutes post-administration in 10 critically ill

children who received doses 6.5-31 mg/kg for conditions including non-convulsive SE or acute repetitive seizures.[208] Wheless et al. reported LEV concentrations (64-180 µg/ml) in 15 adults and children with epilepsy following a mean dose of 47 mg/kg, which generally agrees with the concentrations obtained in our study after accounting for differences in dosing and timing of sample collection.[207] It is worth noting, that the concentrations in three of the seven patients randomized to LEV in our study may have been influenced by use of LEV as chronic oral therapy. There have been two reports of VPA concentrations in children with SE, one case report[209] and one in 11 children with SE or acute repetitive seizures dosed at 15-20 mg/kg.[132] When concentrations (median concentration at 30 minutes 99 µg/mL, range 67-161 µg/mL) are dose-normalized, they agreed with our results following 40 mg/kg doses.[132] Similarly, PHT concentrations, both unbound and total, were consistent with previously published reports.[213–217] While we have limited information, it still represents the largest cohort of pediatric SE patients with drug concentrations and treatment response. Based on visual observation in this limited sample size, there was no apparent signal that drug concentration was a driver of responsiveness. This observation was consistent with a previous report of 29 SE patients treated with LEV that found no significant difference in LEV exposure between responders and non-responders after a median loading dose of 28 mg/kg.[218] Supporting these observations, we found no significant association of weight-normalized dose and treatment response in ESETT adult patients, although pharmacokinetic samples were not collected in adults.[31]

Given that PHT and VPA are highly plasma protein-bound, total drug concentrations may not be a good surrogate for concentrations at the site of action because only the unbound drug can cross the blood-brain barrier. Hence, plasma protein binding of PHT and VPA

was assessed. While we found a moderate correlation between the total and unbound concentrations for PHT and VPA, we found non-linearity in VPA plasma protein binding, which is in agreement with previously published reports[219]. For example, in our study children with total VPA concentration of approximately 175 µg/ml had unbound drug concentrations ranging from 37 to 63 µg/ml. Furthermore, the use of a loading dose of PHT or VPA could have led to drug-drug interactions, particularly in patients routinely taking VPA or other highly protein-bound anti-seizure medications. For example, we found decreased plasma protein binding of PHT in the presence of VPA for two patients as suggested by prior reports.[220,221] Plasma protein binding for PHT was much less variable than VPA and in the range consistent with literature.[222,223] For VPA, the unbound fraction was higher than what has been previously reported following oral administration[219,224] most likely due to higher VPA concentrations in our study. Our results are in agreement with the fraction unbound reported after IV administration in adult epilepsy patients taking antiseizure medications including oxcarbazepine, carbamazepine and phenobarbital.[225]

Our results are limited by the number of patients randomized to each drug from whom plasma samples could be collected. This was mainly due to the timing of the start of the ancillary pharmacokinetic study and the early termination of the parent ESETT. Enrollment for pharmacokinetic assessment was delayed as blood collection was not a part of the parent study. Thus, funding needed to be secured and site IRB approvals were required prior to study initiation. Further, a Bayesian adaptive study was used in ESETT, with early stopping criteria for success/futility. Unexpectedly, the stopping criteria for those ≥ 18 years were met at the first interim analysis and the adult arm of the study was terminated. Then an incidence of overdose of FOS triggered an early interim analysis in children

resulting in termination due to futility. We also found that obtaining plasma samples in young children within ESETT was challenging as a second IV line or multiple venous punctures were required.[30] Hence, many sites required consent for blood sample collection in children as it was not considered to be a part of exception from informed consent (EFIC).[135] Most sites followed a procedure where after 60 minutes, blood could be collected without consent if the child was unconscious or had a second IV line. If neither of these conditions were met, then consent was obtained prior to blood collection. The use of a device (e.g., PIVO) that allows blood collection from the same IV line used for drug infusion, could greatly increase the number of children participating in pharmacokinetic studies as well as the number of samples collected per patient.[226,227]

Given the difficult setting to collect blood samples, we utilized blood sampling windows instead of discrete sampling times, which allows for more flexibility in sample collection. Even so, we had instances of blood collection beyond the sampling window and, in approximately one third of the patients, only 1 sample could be collected. These limitations can be resolved using population pharmacokinetic techniques which allow for the utilization of all samples regardless of the collection time and number of samples per patient. Additionally, information regarding time of the last dose of oral VPA and/or LEV in specific patients was not known.

3.5 Conclusions

A robust LC-MS/MS method was developed and validated using a small plasma volume for simultaneous analysis of LEV, PHT and VPA with a wide dynamic range and a short

run time. This is the first report of LEV concentrations in young children with convulsive SE as well as VPA concentrations after a 40 mg/kg dose. The measured concentrations were in the expected range for the doses used in the ESETT study. Consistent with previous reports, interactions between PHT and FOS and/or VPA were found to affect the unbound fraction following large IV doses. The small number of patients precluded analysis of exposure-response which warrants a further study with a larger sample size. However, the exposure measures reported will provide clinicians with some perspective on drug concentrations after high dose intravenous administration. Further, these data show that the relatively high plasma concentrations of PHT, LEV and VPA in young children post-administration of ESETT doses were found to be safe and tolerable. The challenges encountered in this study could help better plan and execute studies in emergent conditions in the future.

CHAPTER 4

DOSE-RESPONSE ANALYSIS FOR SECOND-LINE TREATMENT OF CONVULSIVE STATUS EPILEPTICUS

4.1 Introduction

As described in chapter one, ESETT was a multi-center, randomized, double-blind study to determine the best or worst second-line treatment among FOS, LEV and VPA in patients with benzodiazepine-refractory generalized convulsive SE.[139] To maintain the blind, the three drugs, FOS, LEV, and VPA, had to be administered in the same volume and at the same infusion rate even though the drugs had different mg/kg doses as discussed in section 1.3.3.[30] The FOS product label recommends a maximum dose of FOS (prodrug of PHT) of 20 mg PE/kg and a rate of intravenous administration not to exceed 150 mg PE per minute due to cardiovascular risks associated with rapid injection.[29] Given that the infusion time was fixed to 10 minutes, dosing was capped at 1500 mg PE. As a result, all patients weighing ≥ 75 kg received the same capped dose of FOS (20 mg/kg, maximum 1500 mg PE). Similarly, weight-based dosing was also capped at 75 kg for LEV (60 mg/kg, maximum 4500 mg) and VPA (40 mg/kg, maximum 3000 mg).

As discussed in chapter three, drug concentrations could only be measured in a small number of pediatric patients enrolled in ESETT. Hence, it was not possible to conduct an exposure-response analysis and evaluate if lower drug exposures were significantly associated with higher treatment failure rates of the second-line treatments used in ESETT. As an alternative, a dose-response analysis was conducted. The **hypothesis** for this work was that patients weighing > 75 kg who received the capped doses of ESETT drugs will have lower drug exposure given the pharmacokinetic properties of these drugs and thus were more likely to be non-responders as compared to those weighing ≤ 75 kg. The **primary objective** of this analysis was to assess whether the odds of primary outcome success were lower in patients weighing more than 75 kg as compared to those up to 75 kg

using logistic regression. As described in section 1.3.5, a primary outcome failure could be a result of one or more of the following: (1) need for an additional anti-seizure medication before 60 minutes, (2) clinically apparent seizures at 60 minutes or (3) lack of improvement in consciousness and response at 60 minutes. Hence as a **secondary objective**, I also evaluated the association of weight and other predictors with clinical seizure cessation alone at 60 minutes.

4.2 Methods

ESETT was approved by institutional review boards for all participating institutions.[139] Of the 478 patients enrolled in ESETT, 48.2% of adults and 0.9 % of children weighed > 75 kg. Because of the low number of children receiving a fixed dose and the possibility of differing response rates within children and adults, the analyses were limited to those ≥ 18 years (n=249). Two patients were excluded because the study drug volume administered could not be determined. Among the 247 enrollments, 12 patients were enrolled more than once; but only the data from their first enrollments were used. Among the 235 unique adult patients, 132 (56.2%) failed the ESETT primary outcome. Of the 132 failures, 87 (65.9% of failures) failed because they needed an additional anti-seizure medication prior to 60 minutes, 10 (7.6%) failed due to clinically apparent seizures at 60 minutes, and 35 (26.5%) failed because they did not show an improvement in responsiveness at 60 minutes despite clinical seizure cessation.

4.2.1 ESETT primary outcome as the dependent variable

The ESETT primary outcome was expressed as binary (0=treatment failure, 1=treatment success) and used as the dependent variable for the following logistic regression models:

4.2.1.1 Association of weight with primary outcome

Two logistic regression models were used to test the association of weight, as a binary predictor, with primary outcome using weight cut-offs of 75 kg and 90 kg. A 90 kg cut-off was chosen to examine the association for higher weight individuals more rigorously. A third logistic regression model tested the association of interactions of weight, sex and treatment with the primary outcome. The model included treatment group (FOS, LEV or VPA), sex (male or female) and weight as binary ($\leq$ or > 75kg) with all the interaction terms (weight x treatment group x sex) as predictors of the primary outcome.

4.2.1.2 Association of weight-normalized dose and sex with primary outcome

Separate logistic regression models were built for FOS, LEV and VPA to test the association of weight-normalized dose in mg/kg as a continuous variable, sex (male or female) and the interaction of dose and sex with the ESETT primary outcome.

4.2.2 Clinical seizure cessation without additional anti-seizure medication as the dependent variable

Clinical seizure cessation, as binary (1=success, 0=failure), was used as the dependent variable for this analysis. A logistic regression model was used to test the association of weight and other predictors with clinical seizure cessation without additional antiseizure

medication. Adult ESETT patients whose seizures were terminated but failed the primary outcome due to lack of improved responsiveness at 60 minutes (n=35) were treated as successes. A logistic model with weight (≤ or > 75kg), sex (male or female) and treatment group (FOS, LEV or VPA) with all interactions (weight x treatment group x sex) as predictors was used to test their association with clinical seizure cessation.

Significance was determined as an alpha level < 0.05. All the analyses were conducted using R (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria), RStudio (version 1.2.5001, Delaware Public Benefit Corporation) and SAS (version 9.4, SAS, Cary, NC).

4.3 Results

4.3.1. Distribution of weights

ESETT patients ≥ 18 years weighed from 36-157 kg and were approximately normally distributed with a mean of 76.7 kg and standard deviation of 18.9 kg (Figure 4.3-1). Of the 235 patients, 113 (48.1%) weighed > 75 kg and received the maximum doses. The overall success rate for the primary outcome was 45.1% in those ≤ 75 kg vs. 42.5% in those > 75 kg. Baseline characteristics of the adult population by weight group (Table 4.3-1) show that male patients were more likely to weigh > 75 kg (50% vs. 66.4%), but all other baseline characteristics were evenly distributed between the ≤ 75 kg and > 75 kg groups.

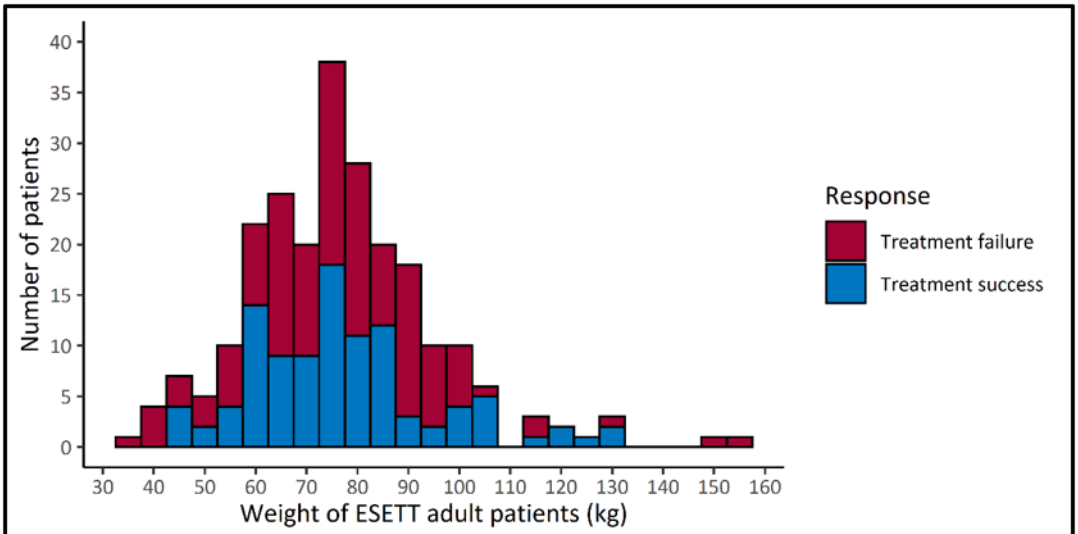

Figure 4.3-1: Distribution of ESETT adult patient weights and the response to the treatment administered as treatment success (blue) or treatment failure (red)

Table 4.3-1: Baseline characteristics of the adult population enrolled in ESETT by weight group

Characteristic	**Weight ≤ 75 kg (N=122)**	**Weight > 75 kg (N=113)**
Age- mean (standard deviation; range)	49.6 (20; 18-94)	49.3 (16.3; 18-86)
Sex- n male (%)	61 (50%)	75 (66.4%)
Treatment allocation-n (%)		
Fosphenytoin	31 (25.4%)	38 (33.6%)
Levetiracetam	48 (39.3%)	42 (37.2%)
Valproic acid	43 (35.3%)	33 (29.2%)
Race- n (%)		
Black	60 (49.2%)	57 (50.4%)
White	43 (35.3%)	48 (42.5%)
Other, > 1 race, or unknown	19 (15.6%)	8 (7.1%)
Hispanic Ethnicity- n (%)	17 (13.9%)	9 (7.9%)

4.3.2 Comparison of response rates between the weight-based dosing group and fixed dose group

The difference [95% confidence interval] in the response rates for those ≤ 75 kg vs. those > 75 kg were 3.1% [-20.5%, 26.6%] for FOS, -1.2% [-21.6%, 19.3%] for LEV and 6.4% [-16.1%, 28.9%] for VPA. None of the differences were statistically significant since the 95% confidence intervals included 0 for each drug.

4.3.3 Association of weight and other predictors with primary outcome

Primary outcome vs. weight: The odds of success were 10.1% lower (odds ratio of 0.9 (95% confidence interval of 0.54, 1.51)) for those > 75 kg compared to those ≤ 75 kg and 15.4% lower (odds ratio of 0.85 (95% confidence interval of 0.42, 1.66)) for those > 90 kg compared to those ≤ 90 kg. These differences were not significant since the 95% confidence intervals for the odds ratios included 1. Similarly, there was no statistically significant association with treatment success when sex, treatment group and interaction of weight with sex and treatment group were included as predictors in the model (Table 4.3-2).

Primary outcome vs. sex and weight-normalized dose: When each drug was modeled separately, the weight-normalized dose was not associated with success, nor was sex or the interaction of dose and sex (Table 4.3-3).

Table 4.3-2: Logistic regression models of the probability of success for those > 75 kg using weight, treatment and sex with all interactions (weight ≤ 75 kg as reference group) (n=235)

Treatment	**Sex**	***Using primary outcome as dependent variable*** **Adjusted Odds Ratio (95% confidence interval)**	***Using clinical seizure cessation without additional anti-seizure medication as dependent variable*** **Adjusted Odds Ratio (95% confidence interval)**
Fosphenytoin	Male	0.71 (0.20, 2.55)	0.56 (0.14, 2.26)
Fosphenytoin	Female	1.13 (0.26, 4.94)	1.16 (0.27, 5.05)
Levetiracetam	Male	0.82 (0.26, 2.56)	0.47 (0.15, 1.49)
Levetiracetam	Female	1.70 (0.45, 6.44)	1.08 (0.29, 4.08)
Valproic acid	Male	0.83 (0.26, 2.66)	0.72 (0.22, 2.40)
Valproic acid	Female	0.64 (0.14, 2.91)	0.49 (0.11, 2.20)

Table 4.3-3: Logistic regression model results of the probability of primary outcome success by treatment group

	Adjusted Odds Ratio (95% confidence interval)
Fosphenytoin (n=69)	
Every 1 mg/kg increase in weight-normalized dose of fosphenytoin for males	1.01 (0.86, 1.19)
Every 1 mg/kg increase in weight-normalized dose of fosphenytoin for females	1.10 (0.84, 1.42)
Levetiracetam (n=90)	
Every 1 mg/kg increase in weight-normalized dose of levetiracetam for males	1.01 (0.93, 1.10)
Every 1 mg/kg increase in weight-normalized dose of levetiracetam for females	0.99 (0.94, 1.05)
Valproic acid (n=76)	
Every 1 mg/kg increase in weight-normalized dose of valproic acid for males	1.06 (0.91, 1.23)
Every 1 mg/kg increase in weight-normalized dose of valproic acid for females	0.93 (0.82, 1.07)

4.3.4 Association of weight, sex and treatment with clinical seizure cessation without additional anti-seizure medication

A total of 138 (59%) patients had no clinical apparent seizures at 60 minutes and didn't receive additional anti-seizure medication (regardless of whether they were responsive to verbal commands or noxious stimuli). As seen from Table 4.3-2, the association of weight (≤ or > 75 kg), sex (male or female), treatment group (FOS, LEV or VPA), and all the interaction terms (weight x sex x treatment group) with clinical seizure cessation was not significant.

4.4 Discussion

The results of these secondary analyses demonstrate that the differences in response rates between the fixed dosing regimen (above 75 kg) and weight-based regimen (up to 75 kg) were not significant when the study drugs were grouped together or analyzed separately. The results of logistic regression models using the ESETT primary outcome and clinical seizure cessation without additional anti-seizure medication as dependent variables also showed no significant associations with weight, treatment, sex or weight-normalized dose.

Fixed dosing, which is commonly used in adults, results in lower doses per body weight in heavier individuals and potentially lower drug concentrations for many drugs. Furthermore, if drug concentrations fall in the non-saturated portion of the dose-response curve, lower drug concentrations may result in reduced efficacy. In this study, while approximately half of the ESETT adult patients received the maximum dose, the response rates between weight-based and fixed dosing regimen were similar. It is possible that

weight or weight-normalized dose did not affect the primary outcome or clinical seizure cessation because the doses used in the trial resulted in drug concentrations above those needed for therapeutic outcome even in patients above 75 kg. While this may be true, other predictors, such as drug concentration, would have been a better metric to evaluate the differences between responders and non-responders. We know that drug concentrations can be variable in individuals receiving an identical dose.[216,228,229] There is also evidence that the pharmacokinetics of FOS, LEV and VPA are altered in overweight and obese patients.[230–233] In particular, patients with higher body fat will likely have greater volume of distribution. However, we were not able to investigate the effect of drug concentrations or BMI as sufficient information was not available. Furthermore, only 18 (7.7%) patients weighed above 100 kg. Thus, differences in pharmacokinetics, if any, may not have been large enough to impact the outcome.

The ESETT primary outcome was a composite that included absence of clinically apparent seizures and improved responsiveness at 60 minutes. It is possible that those who had higher doses were more likely to stop seizing but also more likely to have no improvement in responsiveness. To evaluate the association of weight and other variables with clinical seizure cessation alone, we included those who failed the primary outcome only due to the lack of improved responsiveness at 60 minutes as successes but found no significant differences between fixed and weight-based dosing. Future studies of status epilepticus will likely include EEG as a part of outcome and allow us to better understand this subgroup.

A limitation of these analyses is the small number of patients in each treatment group (~40/drug) weighing above 75 kg. The wide confidence intervals for the difference in

response rates suggest that a larger sample size would be needed to confirm these findings. While these were secondary analyses, the adaptive study design was in fact powered adequately for the primary outcomes.

The results from this analysis can be used as preliminary data to calculate the sample size that will be needed to detect a significant difference in response rates between those weighing ≤ 75 kg and those > 75 kg (should one truly exist) with 90% power. For this sample size calculation exercise, I used the following formula:

$$N\ per\ group = \frac{\left[Constant\ (\alpha)\sqrt{2p(1-p)} + Constant\ (\beta)\left(\sqrt{p_c(1-p_c)+p_t(1-p_t)}\right)\right]^2}{(p_c-p_t)^2} \qquad \text{Eq.4.4.1}$$

where α and β are type I and type II error rates, p_c is the response rate for the control group (≤ 75 kg in our case), p_t is the response rate in the test group (> 75 kg in our case) and p is calculated as follows:

$$p = \frac{(p_c+p_t)}{2} \qquad \text{Eq. 4.4.2}$$

I will use the response rate and variability seen for VPA (section 4.3.2) as an example and calculate the sample size. For VPA, of the 43 adults weighing ≤ 75 kg, 21 were primary outcome success (response rate (p_c): 21/43 for ≤ 75 kg). Similarly, among 33 patients weighing > 75 kg that were randomized to VPA, 14 were found to be primary outcome success (response rate (p_t): 14/33 for those > 75 kg).

Thus, $p = \frac{\left(\frac{21}{43}\right)+\left(\frac{14}{33}\right)}{2}$

= 0.456

Power is calculated as a (1-β). So, assuming a 10% type II error rate translates to 90% power of being able to detect a difference between the two groups if a difference truly exists. The value of the constant (α) term in equation 4.4.1 assuming a type I error rate of 5% is 1.96 and the value of constant (β) for type II error rate of 10% is 1.28. Thus, to detect a 6.4% difference in response rates as currently seen with VPA we get

$(p_c\text{-}p_t)^2 = (0.064)^2 = 0.004$ between patients weighing ≤ 75 kg and those weighing > 75 kg. The number of patients needed per group can be calculated using equation 4.4.1 as:

$$N\ per\ group$$

$$= \frac{\left[1.96 * \sqrt{2 * 0.456(1 - 0.456)} + 1.28\left(\sqrt{0.488(1 - 0.488) + 0.424(1 - 0.424)}\right)\right]^2}{0.004}$$

$= (1.381 + 0.9)^2 / 0.004$

$= 1300$

Thus, at least 1300 patients must be recruited in each group ≤ 75 kg and > 75 kg (total 2600 patients at least) to be able to detect a 6.4% difference in response rate for VPA with 90% power. If the expected difference in response rates is higher, for example 10%, then at least 520 patients will be needed in each group (1040 total) to retain 90% power. On the other hand, a lower number of patients per group will be required (389 per group, 778 total) to be able to detect a 10% difference in response rates if the power was dropped to 80%. Thus, a very large sample size (at least 2600) will be needed to detect if the effect of dose capping on the primary outcome is significant for VPA. Among the three drugs, VPA had the highest difference in response rates between the weight-based dose group and capped dose group. Using this method, the minimum sample size required for FOS and LEV to detect

a significant difference in the response rates for two weight groups can be calculated as described above for VPA. Assuming similar variability of the difference in response rates of the two weight groups and given the lower point estimate of difference in response rates, the sample size required for FOS and LEV will be even higher.

4.5 Conclusions

Weight-based dosing used in ESETT with a 75 kg cut-off did not appear to have an impact on the primary outcome or clinical seizure cessation. Given the wide-confidence intervals for the difference in response rates, a very large sample size (highly impractical) will be required to detect a significant effect of dose capping on outcome, if one truly exists. It is possible that the concentrations attained with the capped doses were above those needed for therapeutic outcome. However, studies with larger sample size and additional data (drug concentrations, BMI, etc.) are required to confirm our findings. Future studies which measure drug concentrations would allow exploration of exposure-response instead of dose-response relationships.

These results show that dose capping at 75 kg to ensure safety did not have a significant impact on the drug efficacy assessed using the results of the ESETT primary outcome. The point estimates for the difference in response rates between the weight-based and capped dose groups for the three drugs were quite small (3.1% for FOS, -1.2% for LEV and 6.4% for VPA) and likely clinically insignificant. Characterization of exposure-response relationship, if one exists, may help optimize dosing further and potentially justify capping

the dose at an even lower weight. Alternatively, it may reveal patient specific covariates that explain the variability in pharmacokinetics and guide dosing.

CHAPTER 5

EVALUATING THE PREDICTIVE PERFORMANCE OF A POPULATION PHARMACOKINETICS APPROACH USING SPARSE SAMPLING DATA TO ESTIMATE EARLY DRUG EXPOSURE

This chapter is based on a manuscript focused on the evaluation of an alternate method to estimate early drug exposure using a pharmacokinetic simulation study. This simulation study was inspired from ESETT as estimating early drug exposure in emergent conditions like SE with blood sampling limitations is very challenging.

5.1 Introduction

Treatments used for the management of emergent conditions are administered by a suitable route (intravenously in most cases) to rapidly achieve therapeutic drug concentrations at the site of action as it is these early concentrations that will drive the response to treatment. Plasma concentrations are often used as a surrogate to reflect the concentrations at the site of action. While extensive blood sampling to measure drug exposure is considered as the gold standard for pharmacokinetic (PK) modeling using traditional methods like non-compartmental analysis (NCA), rich blood sampling is challenging when conducting studies in emergent conditions and in young children. Often, study designs with sparse sampling (one-four samples) at opportunistic schedules are implemented while studying special populations that are difficult to study. Population PK modeling is better suited to utilize sparse PK samples than traditional approaches like NCA or individual compartmental modeling as information from each individual can be utilized, even if it is a single concentration, in the estimation of population PK parameters. Further, models can quantitate between subject variability allowing for the prediction of an individual's early drug exposure.

The challenge of estimating early drug exposures was encountered during the conduct of the ESETT. As described in chapter 3, the objective of the ancillary PK study was to measure early exposure of the study drugs (within the first two hours post-administration) and relate it to the treatment response. Two pharmacokinetic samples, one within the time window of 20 and 50 min (W1) and second between 60 and 120 min (W2) following the start of the 10-minute drug infusion were chosen to measure early drug concentrations.

Using ESETT as a motivating example, we were interested in testing if using two blood samples, like those collected in the specified time-windows in ESETT, would give a reliable estimate of early drug exposure. Testing this in a real-world setting was not reasonable because extensive early blood sampling is not feasible during the treatment of an emergent condition like convulsive SE as the therapy includes stabilizing the patient (airway, breathing and circulation), initiating electrocardiogram and electroencephalographic monitoring, treating the underlying cause of seizures and attempting IV access to administer pharmacotherapy quickly.[5] As an alternative, we chose to conduct a pharmacokinetic simulation study. The metric of exposure used was partial area under the drug concentration-time curve (pAUC) till 2 hours after the start of drug infusion.

The objective of this work was to evaluate the predictive performance of using two concentration measures and prior information about the drug, for estimating early drug exposure using a population pharmacokinetics modeling approach. Further, the predictive performance of the population pharmacokinetics approach for estimating early drug exposure was compared with the standard NCA approach. The drugs used in ESETT (FOS, LEV and VPA) were chosen as examples drugs for this simulation study.

5.2 Methods

Published population pharmacokinetic models used for this work

Literature-based population pharmacokinetic models for FOS (prodrug of phenytoin (PHT)[70]), LEV and VPA were used for this simulation work.[216,228,229] The models selected were developed using concentration-time data collected after intravenous (IV) administration of the drug in healthy volunteers or patients who were not on chronic therapy of that drug. For the FOS/PHT model, data from 24 healthy adults, and seven adult and 20 pediatric patients that included those with SE, acute repetitive seizures or neurosurgical patients in whom FOS was administered to prevent seizures, were used. For the LEV model, data from 11 adult patients treated with IV LEV for SE (10 with convulsive and one with non-convulsive SE) were used for model building[228]; whereas for VPA, peak and trough concentrations after IV administration in 102 newly diagnosed treatment-naïve patients with epilepsy were used.[229]

The value of residual unexplained variability (RUV) was not reported for the LEV model.[228] For the FOS/PHT model, an RUV value (σ^2 exponential error 0.00148 and σ^2 additive error 0.317) was reported[216] which was much lower than other FOS/PHT pharmacokinetic models.[234–236] To reflect the unexplained variability typically seen in a clinical study, particularly in an emergency department, a proportional RUV with 20% coefficient of variation (CV) was modeled as shown in Eq. 1 was used for the LEV and FOS/PHT models.

$$C_{ij} = Pred_{ij} * (1 + \varepsilon_{ij}) \quad \text{Eq.1}$$

where C_{ij}, is the measured plasma concentration for subject i at time j, $Pred_{ij}$ is the corresponding model predicted concentration for subject i at time j and ε is residual error term distributed normally with mean 0 and variance of σ^2. All models included allometric scaling using a median weight of 75 kg and an exponent of 0.75 for all clearance terms (total body clearance and intercompartmental clearance) and 1 for all volume terms (central and peripheral).

Doses and weights used for simulations

Table 5.2-1 provides the model structure and parameter values used for this work. The doses and body weights used were from ESETT. The doses used, 20 mg/kg for FOS, 40 mg/kg for VPA and 60 mg/kg for LEV, were weight-based till 75 kg and capped thereafter.[31] The proportion of adults:children in the simulated patients was 60:40. Adult weights were sampled from a normal distribution with mean 75 kg and standard deviation (SD) 19.3 kg, whereas weights for children were sampled from normal distribution with mean 20 kg and SD 7.3 kg. The lowest weight of a child enrolled in ESETT was 9 kg. Hence, the lower end of the simulated weights was capped at 9 kg.

Simulation and modeling

The metric of early exposure chosen for this work was pAUC 0-2 hours after the start of drug infusion. Given the relatively long terminal half-life of LEV (6-8 hours[77]), PHT (12-29 hours[29]) and VPA (16 hours[82]), we assumed that the early exposure of these drugs will be mainly driven by the volume of distribution.

Table 5.2-1 Selected population pharmacokinetic model used for the simulation work

	PHT	LEV	VPA
REFERENCE	Tanaka et. al. 2013[174]	Uges et al. 2009[186]	Park et al. 2002[187]
POPULATION	24 healthy volunteers + 14 adult patients + 33 pediatric patients Mean age 19.6 (± 15.6) Mein weight 43.8 (± 21.3)	≥ 18 Years (mean age 64 years; mean weight 64 kg)	≥ 16 Years (mean age 45 years; mean weight 60 kg)
PK MODEL	Two-compartment	Two-compartment	One-compartment
CL (L/HR/60KG)	1.61 (44% CV)	2.86 (30.9% CV)	0.849 (32% CV)
V (L/60 KG)	20.8 (40.1% CV)	27.0 (18.7% CV)	15.1 (18% CV)
$V_{(peripheral)}$ (L/60KG)	26 (20.7% CV)	-	-
Q	53 (52.1% CV)	-	-
K_{12} (/HR)	-	0.24 (50% CV)	-
K_{21} (/HR)	-	0.70 (31.4% CV)	-
RUV reported	3.85% CV (exponential) 0.56 SD (additive)	Not Reported	26.7% CV (Proportional)
RUV used	20% CV (proportional)	20% CV (proportional)	26.7% CV (Proportional)
DOSE	20 mg/kg for ≤ 75 kg; 1500 mg for > 75 kg	60 mg/kg for ≤ 75 kg; 4500 mg for > 75 kg	40 mg/kg for ≤ 75 kg; 3000 mg for > 75 kg

Figure 5.2-1 shows a schematic of the methodology used. The steps used were as follows:

Step 1: *Simulating concentration time profiles with and without the RUV*

Using the selected population pharmacokinetic models, rich concentration-time profiles (from zero-two hours after the start of study-drug infusion) including the RUV (to generate random-noise corrupted concentrations) and without the RUV (true concentrations) were

simulated for 100 individuals for PHT (active form of FOS), LEV and VPA. Simulated profiles were generated using R (version 3.6.1), RStudio (version 1.2.5001) and mrgsolve package.

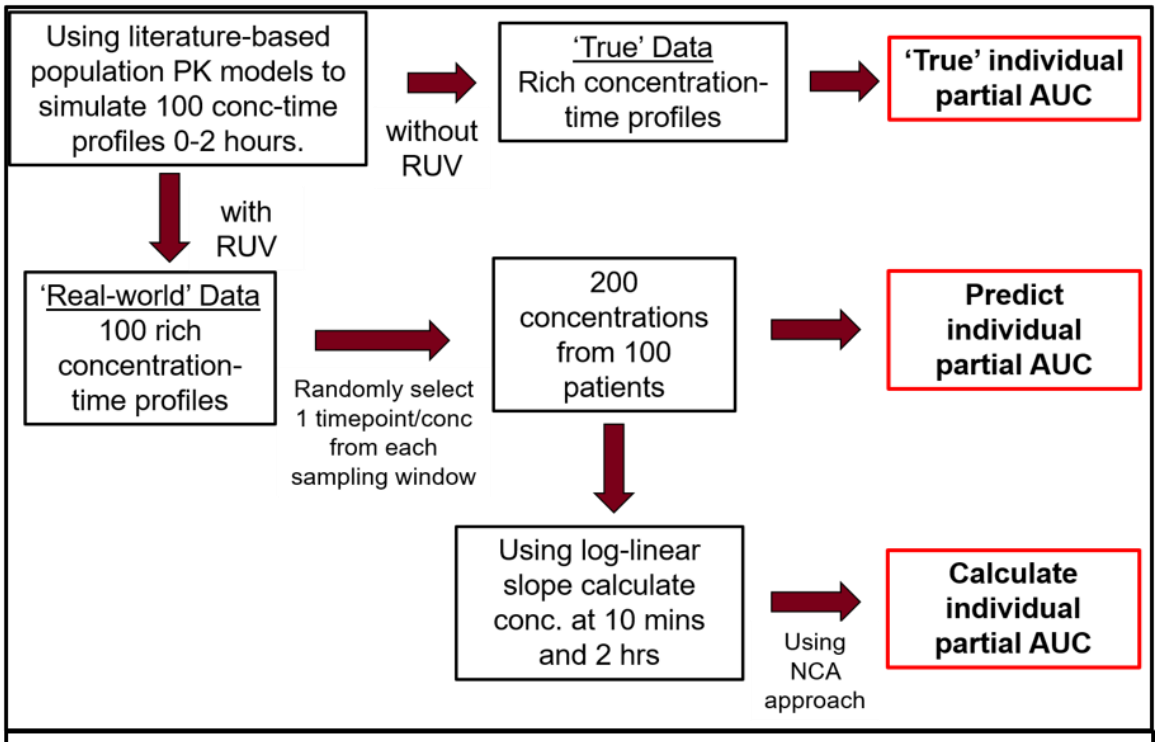

Figure 5.2-1: Methodology used for the simulation work

Step 2: *Calculation of true pAUC*

The profiles without the RUV were used to calculate the 'true' pAUC for each individual by integrating the concentrations in the central compartment over two hours after the start of study drug infusion. This 'true' pAUC is used as a reference for comparison.

Step 3: pAUC calculation using a population pharmacokinetic analysis

Using the rich concentration-time profiles with the RUV, one data point in W1 from among five possible data points at 24, 30, 36, 42 and 48 minutes and another in W2 from among 10 possible data points at 66, 72, 78, 84, 90, 96, 102, 108, 114 and 120 minutes, were randomly selected for each patient to generate a sparse sampled dataset of 200 concentrations from 100 simulated patients (R version 3.6.1, RStudio version 1.2.5001). These data were analyzed under a population pharmacokinetic model (NONMEM® version 7.3, ICON Development Solutions, Hanover, MD, USA) using the first order conditional estimation method (ADVAN13). Due to the sparseness of data, the population-level clearance, peripheral volume of distribution and inter-compartmental clearance terms were fixed to literature values with the between subject variability set to zero. The population-level central volume of distribution was estimated along with its variability. The RUV was also estimated. This was called the PopPK approach and the individual two-hour pAUCs called the 'PopPK pAUCs' were calculated by integration of the concentration in the central compartment from time zero to time two hours.

Step 4: pAUC estimation using NCA

As a comparator for the population pharmacokinetics approach, pAUC estimates were also generated using NCA. Using the two data points per patient in the sparse sampled dataset of 100 simulated patients and a mono-exponential assumption, concentrations at 10 minutes after the start of study drug infusion were calculated using back-extrapolation. Similarly, concentrations at two hours were calculated using forward-extrapolation. Using

a linear trapezoidal rule (PKPDmisc package, R version 3.6.1, RStudio version 1.2.5001), the 'NCA pAUC' from zero to two hours for each simulated patient was then calculated with these four data points. This was called the NCA approach.

Step 5: Evaluation of predictive performance

The PopPK pAUC and NCA pAUC were compared with the true pAUC. The percent prediction error (PPE) was calculated as a test statistic and shown in Eq.2

$$PPE = \{ \frac{sparse\ pAUC - true\ pAUC}{true\ pAUC} \} * 100 \qquad \text{Eq.2}$$

where sparse pAUC is PopPK pAUC or NCA pAUC

It isn't critical that a predicted pAUC exactly match the true pAUC and we set a threshold for an acceptable prediction. Success in estimating early exposure for each simulated patient had a value of 1 if the PPE for pAUC was within ± 20%, else it was 0.

Step 6: Generating a distribution of the success statistic across 100 different datasets

Steps 1-5 were repeated 100 times with 100 different datasets obtained using different seed values. The number of simulated individuals with success value of 1 (i.e. individuals with PPE within ± 20%) per dataset was obtained and a distribution of the success statistic was generated for PHT, LEV and VPA for both the PopPK and NCA approaches.

Step 7: Comparing the predictive performance of PopPK and NCA

As the success statistic obtained in step 6 for the PopPK and NCA approaches may not be normally distributed, a two-sample t-test may not be appropriate. Hence, its non-parametric counterpart, the Mann-Whitney U test, was used to test if the distribution of the success statistic was significantly different between PopPK and NCA approaches.

Plots were generated using R (version 3.6.1), RStudio (version 1.2.5001) and ggplot2 package.

5.3 Results

Distribution of the success statistic

Figure 5.3-1, figure 5.3-2 and figure 5.3-3 show the distribution of the success statistic for PHT, LEV and VPA respectively, across 100 datasets obtained using the NCA and the PopPK approach. Median (5^{th}, 95^{th} percentile) value of the success statistic for PHT was 72% (65%, 79%) for the NCA approach and 81% (73%, 88%) for the PopPK, for LEV it was 80% (73%, 87%) for the NCA approach and 92% (87%, 96%) for the PopPK approach, and for VPA it was 67% (58%, 73%) for the NCA approach and 88% (82%,93%) for the PopPK approach.

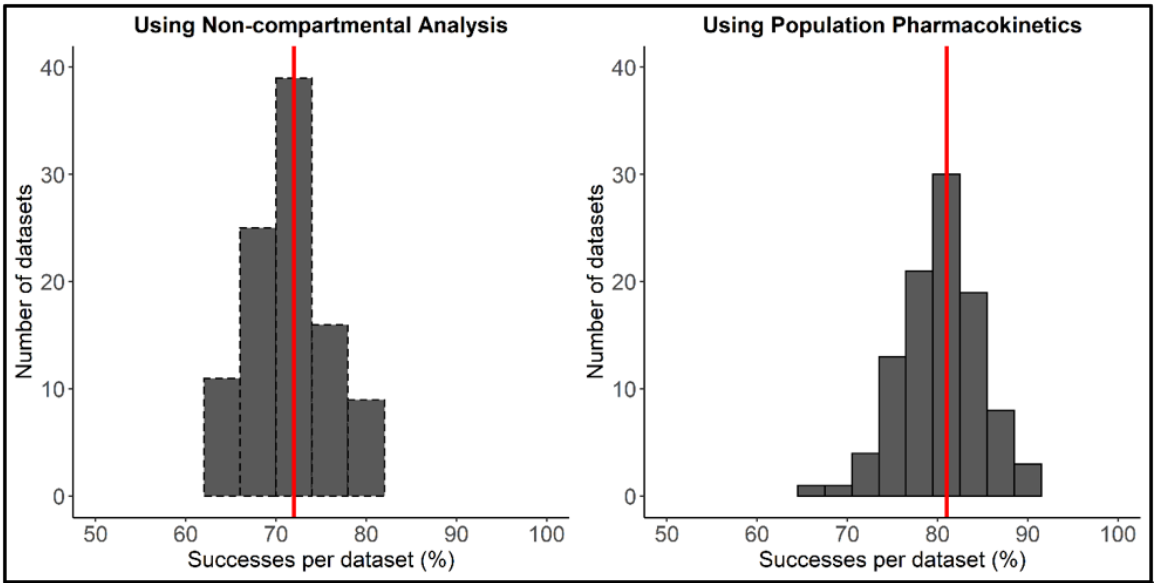

Figure 5.3-1: Distribution of percent of successes per dataset for phenytoin using non-compartmental analysis (left) and population pharmacokinetic analysis (right). The red line indicates the median percent of successes per dataset

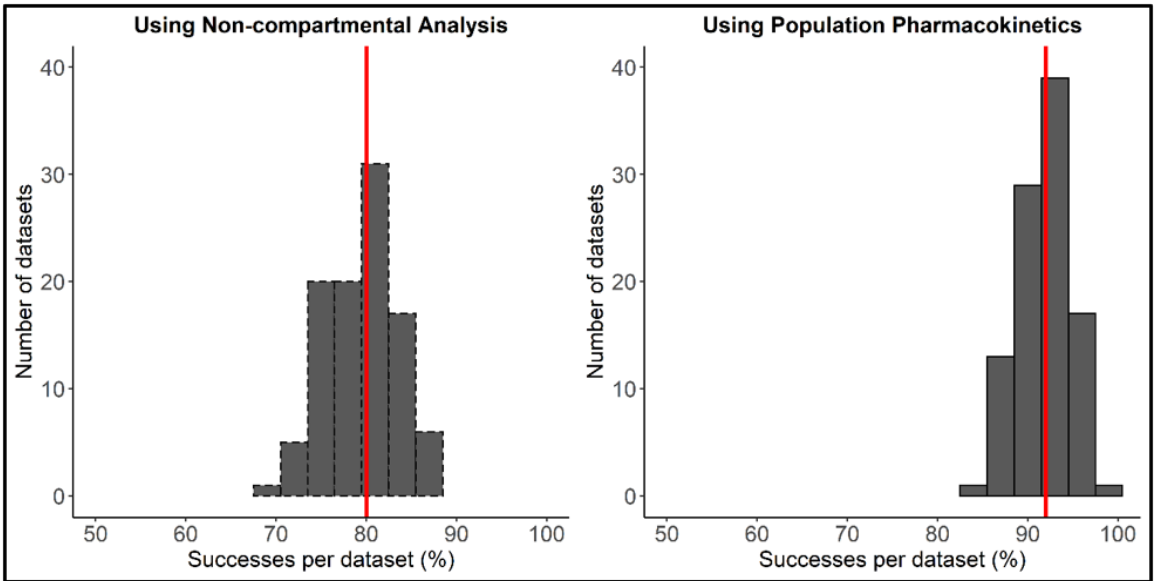

Figure 5.3-2**:** Distribution of percent of successes per dataset for levetiracetam using non-compartmental analysis (left) and population pharmacokinetic analysis (right). The red line indicates the median percent of successes per dataset

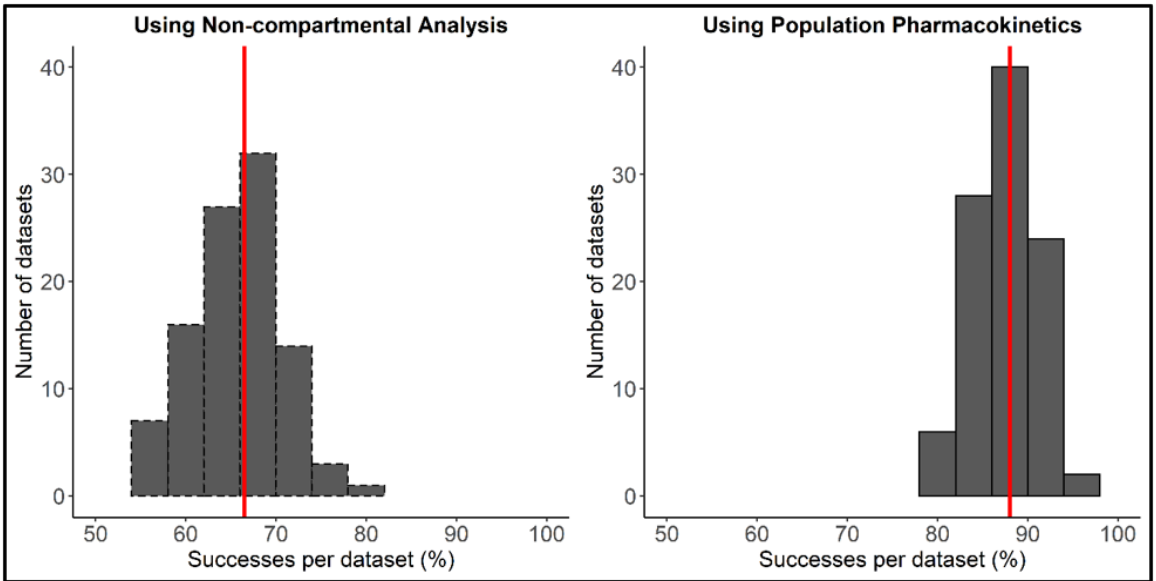

Figure 5.3-3: Distribution of percent of successes per dataset for valproic acid using non-compartmental analysis (left) and population pharmacokinetic analysis (right). The red line indicates the median percent of successes per dataset.

Comparing the predictive performance of PopPK and NCA

Table 5.3-1 shows the results of the Mann-Whitney U test comparing the distribution of success statistic between the PopPK and NCA approaches. The difference in the location of the median for the success statistic distributions obtained using the two approaches was highly significant ($p < 2.2e-16$) for all three drugs and showed that the PopPK approach performed significantly better than a standard NCA approach.

Table 5.3-1**:** Results of Mann-Whitney U test comparing distribution of success statistic between the PopPK approach and NCA approach

Drug	**W-statistic**	**Difference in location of the median of success statistic between PopPK and NCA (95 % confidence interval)**	**Associated p-value**
PHT	792	9 (7, 10)	< 2.2e-16
LEV	51.5	12 (11, 13)	< 2.2e-16
VPA	2	22 (21, 23)	< 2.2e-16

5.4 Discussion

Our results from this pharmacokinetic simulation study using FOS, LEV and VPA as example drugs show that using data from two blood samples collected from each patient over the first two hours after acute drug administration and prior drug information, it is possible to estimate early drug exposure reasonably well using the PopPK approach. Further, a significantly higher number of simulated patients had their pAUC estimate closer to the true value (within ± 20% PPE) with the PopPK approach as compared to the standard NCA approach. These results demonstrate the superiority of the PopPK approach over the standard NCA approach to estimate early drug exposure under these conditions.

While much of the literature on PK sampling limitations is focused on pediatric studies[237–239], similar challenges are also encountered in emergent conditions. This is further limited by the potential need to measure early drug exposure. Under these conditions, it is often impractical to collect multiple blood samples without interfering with patient care and consenting can delay sample collection.[239] Hence, wider sampling windows and flexibility in timing is essential. Based on a PubMed search, we found no published reports focusing on estimation of early drug exposures using sparse (less than three) samples in emergent conditions. There are several reports of population pharmacokinetic models developed using concentration-time data collected within 48 hours after acute administration of rescue therapies.[240–242] Most of these models rely on more extensive blood sampling and did not use prior information about the drugs. Our approach is novel and focused on estimating early drug exposure with reasonable precision using very sparse data (two concentrations

per patient) in the first two hours post drug administration and a population pharmacokinetic modeling approach.

Often, a single blood sample drawn per patient is used as the measure of drug exposure.[207,214,217,218] A single sample may not be appropriate as it is an exposure measure at one instant in time and can be highly variable and hence is always likely to be imprecise. Further, a rapidly changing concentration-time profile will increase this imprecision. In this simulation study, two concentration measures within the first two hours post drug administration and a population pharmacokinetics approach was utilized to estimate early drug exposures. As an alternative, we also tested a standard NCA approach which has fewer assumptions.[243] While NCA assumes that there is no error in drug concentrations, a PopPK approach imposes a pharmaco-statistical model on the data and the error in the form of RUV is estimated during the model fitting process. This could explain why the pAUC estimates generated using the PopPK approach were closer to the true value than those generated by the NCA approach.

Our approach is driven by prior information available for FOS/PHT, LEV and VPA. While the RUV value used for the VPA model was same as that reported in the published model[229], a proportional RUV with 20% CV was used for FOS and LEV models to reflect the variability seen in a clinical setting and thus increase the generalizability of our results. While it is possible to reduce analytical error, several other sources of errors, including but not limited to incorrect time record of sample collection, dosing errors like administration of incorrect dose volume, time of dose administration, etc. that occur in a clinical setting, may contribute to the RUV. The probabilities of these errors are likely to be increased in an ED setting.

Our analysis has several limitations. It was assumed that the subjects in the simulation study were similar to the literature population and that the exact times of dosing and sampling are known. Concentrations are changing rapidly during early exposures and may be sensitive to model misspecification which was not incorporated into this analysis. The same structural model was used for the simulations and for fitting the sparse dataset using the PopPK approach.

Using a population pharmacokinetic modeling analysis with three antiseizure medications as example drugs, we demonstrate that early drug exposures can be estimated adequately utilizing prior information on the drug and sparse concentration data collected early after drug administration. The PopPK approach was found to be significantly better at estimating early drug exposure as compared to a standard NCA approach. Like convulsive SE, this approach can be used in other emergent conditions or situations where early drug exposure is of interest. These results encourage further investigation of pharmacometric models of drug exposure which can then be related to factors affecting outcomes in difficult emergent settings.

CHAPTER 6

SUMMARY OF ESETT

6.1 My Reflections from ESETT

I experienced first-hand the detailed planning and successful execution of a large, multicenter, double blind study for an emergent condition. The conduct of large phase III studies like ESETT is challenging and requires effective teamwork and coordination between experts from various fields including emergency medicine physicians, neurologists, epileptologists, statisticians, clinical pharmacologists, study coordinators and administrative staff. I learned that even after having an intricate plan in place to address issues that may occur during the conduct of the study, several unforeseen problems will arise and will have to be addressed at the time keeping patient safety as the highest priority. Routine follow-ups with sites were often needed to address issues like low enrollment rates and several protocol violations to ensure that the trial was conducted as per the protocol without compromising patient safety. A large database to record study data which includes tracking enrollments, patient and treatment information in clinical case report forms, vial numbers to facilitate emergency unblinding, and tracking information for shipments of drug formulations and study samples had to be routinely maintained by a central facility. Further, various safety measures had to be put in place to protect patient data and accidental unblinding, including regulating access to the database. As a graduate student, I was fortunate to have had the opportunity to experience the conduct of a study of this magnitude so early in my career.

I was involved in ESETT as a part of the Pharmacology Core, which was responsible for managing the drug manufacturing, safety assessment and the clinical pharmacology aspects of the study. Further, I was an integral part of the ancillary pharmacokinetic study described in chapter 3 and was involved with the planning and execution of the study,

including preparing study kits for blood sample collection, storage and shipment and assisting with site trainings. As discussed in section 1.3.7, very high counts of particles (10 µm) detected in FOS lot#1 vials were ultimately found to be caused by the presence of microscopic bubbles in the formulation. Standard operating procedures (SOP) that address what to do when the quality or stability results for a particular test fail to meet specifications was essential and should be written prior to initiating the manufacturing. Such an SOP should also include clear steps to take if certain aberrant situations like temperature excursions occurred during shipment or handling of the drug products.

In some cases, the investigational pharmacies at the clinical sites enrolled in ESETT needed additional information on the expiration dating of drug formulations to satisfy local regulations. However, blinding precluded inclusion of expiration dates on individual vials. Further, expiration dates were extended through the course of the study based on the trends analysis work that I did using the stability testing data as described in section 1.3.7.2. While the most updated expiration date for each drug product was maintained in the ESETT database, procedures on how to access these expiration dates were needed for clinical sites. Further, timelines and procedures that will be used to update expiration dates should be presented in the manual of procedures and emphasized in site trainings for future studies.

As described in chapter 1, the doses of FOS, LEV and VPA used in ESETT were capped at 75 kg. While each vial was filled with 100 mL of the drug solution, the maximum dose of the drug (for patients weighing ≥ 75 kg) was attained in a 90 mL volume, the additional 10 mL was to account for overages and loss during dispensing/administration. Dosing in children is more complicated because of the wide range of weights in this age group. Further, prior reports have shown that medication errors in the ED are three times as likely

in children as compared to adults.[244] Hence, the ESETT study drug boxes provided to the sites included weight-based dosing charts (Table 1.3.8-1). Even so, there were two potentially serious medication errors, both involving FOS in young children. In both cases, the entire vial (100 mL) of the drug was administered to the child instead of the weight-appropriate dose leading to a several-fold overdose resulting in an adverse event. After the second overdose event, the study enrollment was paused, an early interim analysis was conducted, and enrichment cohort was terminated for futility. Several approaches to avoid this type of problem are available and should be considered when designing such studies. Prior studies have successfully used other approaches like prefilled syringes and/or category-based dosing (e.g. separate adult and child doses)[10] to avoid these dosing errors. These approaches were not feasible for ESETT as a Bayesian adaptive design with randomization stratified by age-group was used for this study. The randomization probabilities were updated at every interim analysis based on the response rates for the three drugs at which time new 'use-next' boxes were shipped to each study site. If these approaches like prefilled syringes or category-based dosing were attempted in ESETT, it would have significantly increased the manufacturing costs, logistical challenges and chance of human errors. Another potential solution that has been used to avoid medication errors, is to limit enrollment of children to sites with dedicated pediatric EDs in which staff are accustomed to weight-based dosing.[30] Such sites have high volumes of pediatric patients and teams are more accustomed to treating critically ill pediatric patients. While the limitation of this approach is reduced generalizability and potentially slower recruitment, it will provide additional safety protections, which are critical in studies like ESETT.

Lastly as described in section 3.4, we found that obtaining pharmacokinetic samples during ESETT was challenging. Due to the delay in the approval and starting of the ancillary pharmacokinetic study and early termination of the adult arm of ESETT, blood samples could only be collected in a small subset (all children) of the entire cohort. Including blood sampling as a part of the study protocol at sites, after the parent ESETT study had been underway for two years, was challenging. As a means of overcoming this problem, future studies should incorporate a pharmacokinetic-pharmacodynamic component from the beginning. This will facilitate the inclusion of blood collection with other study procedures in the manual of procedures and site trainings. While micro sampling approaches like dried blood spot or finger prick for blood sample collection are possible, experts consider these techniques to be equally painful and challenging as a venous blood draw, particularly in the ED setting. Implementing the use of newer devices like PIVO™ (VelanoVascular, Inc., San Francisco, CA) will allow collection of blood samples from the same IV line used for dosing. PIVO™ is a needle-free, single use sterile device that is temporarily attached to a peripheral IV catheter and a flexible, internal flow tube is advanced through the IV line, beyond the catheter tip, into the blood vessel to collect a blood sample.[245] Use of PIVO™ will potentially improve the number of patients recruited in the pharmacokinetic study and the number of samples that can be collected per patient.[226,227]

6.2 Future Directions

ESETT results showed that the response rate for FOS, LEV and VPA was around 50% and there was no significant difference in the safety and efficacy of the three drugs. Given the safety concerns and limitations associated with the use of FOS and VPA as described

before, it is likely that their use for second-line treatment of SE will be limited. I hypothesize that given its safety profile, ease of administration and cost-effectiveness, LEV will be the second-line treatment of choice for benzodiazepine-refractory SE in the future.[20] The poor response rates of the currently used second-line therapies is a clear unmet need in the treatment of established SE. As described in section 1.6, several effective alternatives are available and need to be tested either alone or in combination with currently used second-line agents. The section below describes the considerations for the design and conduct of a potential future study for the treatment of generalized convulsive SE.

Based on the pathophysiology of convulsive SE, as it progresses synaptic GABA-A receptors are internalized whereas an increase in the number and expression of NMDA receptors on the membranes of post-synaptic neurons is seen.[95] Hence, using an NMDA-receptor antagonist to block glutamatergic transmission early-on may be beneficial for the treatment of convulsive SE. NMDA receptor activity leads to increased neuronal influx of calcium, enhancing AMPA-receptor mediated excitatory transmission. Thus, blocking the NMDA receptors could block the self-reinforcing nature of seizures during SE. Further, NMDA receptors mediate neuronal loss during SE and blocking them could be neuroprotective.[96,168] As described in section 1.6.3, ketamine is an NMDA antagonist that has been extensively studied for its anesthetic properties. In addition, ketamine has been found to be safe and effective, and has been shown to have high response rates in refractory SE in observational studies. While there are two randomized controlled studies currently underway to evaluate and compare the efficacy of ketamine for the treatment of refractory SE with currently used medications including MDZ, thiopental and propofol[169,170], there is some evidence that earlier use of ketamine as second-line treatment may be beneficial. A

retrospective analysis of 16 patients who were administered parenteral ketamine for MDZ-resistant seizures by paramedics found sustained termination of seizure activity in 15 (93.7%) patients.[246] Further, advanced airway management in the form of endotracheal intubation was only required in one of the 16 cases.[246] While the sample size is small, these results are encouraging and along with other evidence of safety and efficacy of ketamine for SE, a future study of convulsive SE should test the use of ketamine in combination with a second-line treatment. Taken together, the evidence supports the use of ketamine among the currently available treatments as it offers a potential for high response rates with minimal adverse events, can be safely administered as a rapid infusion and commercial IV formulations are readily available.

Success on the ESETT primary outcome was based on clinical cessation of seizure with improved responsiveness at 60 minutes post-administration of the second-line treatment, without additional rescue therapy. Of the 460 unique enrollments, 243 failed the primary outcome. Of these 243, a total of 58 failed due the lack of improved responsiveness at 60 minutes. As rapid EEG data was not available in a majority of these patients, it was not possible to determine whether these patients were simply unconscious after the second-line treatment or if they had underlying NCSE. As discussed in section 1.2, this phenomenon of convulsive SE followed by NCSE has been reported before.[54,55] A multicenter, observational study evaluating the clinical impact of rapid EEG found that it improved the sensitivity and specificity of a physician's seizure diagnosis and enabled timely and more accurate assessment of patients in the critical care setting.[247] Hence, a future study of convulsive SE must include rapid EEG measures to comprehensively evaluate the response to treatment. The EEG system developed by Ceribell Inc. (Mountain View, CA) offers

rapid EEG acquisition and conversion of EEG signals to sound using a proprietary algorithm. The mean time to setup the rapid EEG system is reported to be 6 minutes and is significantly faster than traditional EEG[248] without impacting the quality of signals.[249] Further, rapid EEG is easy to interpret and has been found to significantly impact treatment decision.[248] The data acquired using rapid EEG can be interpreted by an available on-site specialist or by an expert at a central facility, 24-hours-a-day. Alternatively, it is possible to specially design an artificial intelligence algorithm to interpret the EEG readings.

Based on the considerations discussed above, a future study of convulsive SE treatment should assess the use of ketamine to augment the second-line treatment with LEV in patients who failed first-line treatment with benzodiazepines. Like ESETT, this study will be a multicenter, randomized, controlled, double-blind and conducted in the ED setting. This study will test at least two doses of ketamine to maximize the chance of seeing a benefit with the early use of ketamine, if one exists, and to allow better evaluation of exposure-response relationship for ketamine. Adults and children (aged 2 years and older) who continue to experience convulsive seizures despite receiving a cumulative adequate dose of benzodiazepines will be randomized to one of the following treatment groups:

Group 1: LEV + placebo

Group 2: LEV + low dose ketamine

Group 3: LEV + high dose ketamine

The **specific aim** of this study will be to determine if group 3 (LEV+ high dose ketamine) or group 2 (LEV+ low dose ketamine) is better than group 1 (LEV alone) for the treatment of benzodiazepine-refractory SE.

The **hypothesis** for this study will be that participants randomized to LEV+ketamine groups will have a significantly higher success rate on the primary outcome than those randomized to LEV alone.

The **primary outcome** will be lack of clinically evident or electrographic seizures and improved responsiveness at 60 minutes after the start of study drug infusion without additional antiseizure medication. The primary outcome is similar to ESETT except it will also incorporate results from rapid EEG to assess for electrographic seizure activity. In cases where rapid EEG data is not available, the primary outcome result will be based on clinical seizure cessation alone i.e. these patients will be assumed to have no electrographic seizure activity. The **primary safety outcome** will be the rate of endotracheal intubation. Other safety outcomes measured will include mortality, Richmond agitation and sedation scale at 60 minutes after the start of study drug infusion, recurrence of seizures within 12 hours and acute anaphylaxis.

The **consenting** process used for this study will be similar to that in ESETT and will employ the EFIC regulations for emergency research.[135]

Since the current treatments have a response rate around 50%, a 15% difference in response rates as used in ESETT should be used for this study. **Sample size** required to detect a 15% difference in response between the three groups with 90% power will be based on the expected variability in response. Further, a **Bayesian response-adaptive randomization** with early stopping criteria similar to ESETT can also be utilized in this study.

Weight-based **dosing** capped at 75 kg will be used for this study. Capping the dose for LEV is justified because ESETT found that a 60 mg/kg (maximum of 4500 mg) dose was

safe and tolerable in the large cohort of adults and children with established SE. Further, in order to be able to compare the response rates for LEV from this study to ESETT, the LEV doses will need to be similar. And lastly, given its known safety and efficacy, this dose of LEV will most likely be acceptable to the FDA/NIH study section. On the other hand, while two doses of ketamine (one high and one low) will be used for this study, the decision of capping ketamine doses will depend mainly on the safety of rapid administration of the high dose. However, several other factors need to be considered. If ketamine dose is not capped, then it will not be possible to administer ketamine and LEV from a single vial. Further, this will largely increase the logistical challenges for manufacturing and tracking the separate formulations for LEV, low dose ketamine and high dose ketamine. Additionally, if separate vials for adults and children are to be provided, to avoid potential overdosing of children using the adult dose as seen in ESETT, it will further complicate the study and increase the chance of dosing errors. Ketamine doses between 1-5 mg/kg have been used for the treatment of refractory SE.[166] Exact doses to be used for the study will be based on prior experience with ketamine, currently available safety information and the desired exposures (including peak concentrations, duration, etc.). Simulations based on published population pharmacokinetic models for ketamine can be used to estimate the expected early exposure of ketamine with different dosing regimens and guide dose selection. Based on the doses of ketamine selected, the compatibility and feasibility of manufacturing a combined ketamine and LEV formulation will need to be assessed. While a 10-minute IV infusion as used in ESETT was found to be safe for LEV, faster administration of LEV (e.g. as IV bolus or infused over five minutes) is known to be

safe. Hence, a 5-minute infusion should be used for this study if it's safe for the rapid administration of the selected high dose of ketamine.

Three blood samples (~3 mL each) will be collected from each patient within the first two hours after the completion of study drug infusion to measure concentration of LEV and ketamine. The blood samples collected must be at least five minutes apart and at least one sample blood sample will be collected ≥ 5 minutes before and after the assessment of the primary outcome at one hour. Since this is a blinded study, an LC-MS/MS method for the simultaneous analysis of LEV and ketamine, will need to be developed, validated and used for the analysis of plasma samples collected in this study. As described in chapter 3, while two blood samples were planned in ESETT, in about one-third of the patients only one sample could be collected. Collecting a third blood sample in this study should help better estimate early drug exposure.[242] Further, I hypothesize that the additional flexibility of blood sample collection and use of newer technologies like PIVO™ as described above will help increase the number of patients from whom samples can be collected and the number of samples collected per patient.

Early drug exposure of LEV and ketamine will be estimated as described in chapter five using concentration data from plasma samples collected in this study and prior information on LEV and ketamine in the form of published population pharmacokinetic models. The exposure metric used will be pAUC (0-2 hours). These early exposures will be correlated with treatment response to develop exposure-response relationships. Treatment response can be either the results of the primary outcome (efficacy analysis) or the primary safety outcome (safety analysis). LEV early exposure measures and treatment response for patients randomized to Group 1 will be used to develop **exposure-response relationship**

for LEV and potentially guide future dosing of LEV. Similarly, early exposures for both LEV and ketamine and response data collected from patients randomized to Groups 2 and 3 will be used to assess if higher early exposure of ketamine increases the likelihood of success on the primary outcome, after adjusting for early exposure of LEV or does higher early exposure of ketamine significantly increase the likelihood of endotracheal intubation at 60 minutes. Several patient-specific covariates including age, weight, sex and underlying etiology of SE will be tested to assess if they have a significant effect on the exposure-response relationship for both LEV (group 1) and ketamine after adjusting for LEV (groups 2 and 3).

The results obtained from this study will be pivotal in guiding future treatment of SE. First, consider the possibility that addition of ketamine even at a high dose (Group 3) to second-line treatment with LEV does not significantly increase the likelihood of primary outcome success. Even so, if the exposure-response analysis data from Group 1 (LEV only) shows a relationship between LEV early exposure and response, it will provide useful information to optimize LEV dose used in clinical practice and for future studies. Further, this analysis may also help identify subgroups of patients in whom treatment with LEV is more effective (e.g. children vs. adults) and guide clinical practice. The results from Groups 2 and 3, while not significantly different from Group 1, will still provide useful insights on the use of ketamine for the management of early SE. The exposure-response analysis will provide valuable information on the early exposure of ketamine, its variability and its potential relationship with response (both with primary outcome and safety outcome) in this patient population. The subgroup analyses may reveal that higher ketamine concentrations are needed for seizure cessation overall or in certain subgroups. On the other hand, these results

may indicate that ketamine is not useful as a second-line treatment of SE. These findings can be used to guide future studies of convulsive SE with ketamine.

Alternatively, if addition of ketamine to augment second-line treatment with LEV significantly improves the response rate as compared to LEV alone, these results will provide conclusive evidence to warrant early use of ketamine for the management of convulsive SE. The exposure-response analysis using data from Groups 2 and 3 can be used to determine if there is a relationship between the ketamine exposure and response. Further, this exposure-response analysis will potentially help identify subgroups of patients in whom treatment with ketamine is more effective. Conversely, it is possible that the analysis identifies a subgroup of patients susceptible to higher rates of endotracheal intubation with higher early exposure of ketamine. All these potential findings will provide the clinicians with an effective treatment alternative and also guide the choice of a safe and effective dose of ketamine. The study results will inform future studies focusing on evaluating the use of ketamine in the identified subgroup and further advance the knowledge in this field. Additionally, as rapid EEG data will be collected as a part of the study, it will be possible to compare the onset of effect of LEV vs. LEV+ketamine. Using these data, it will be possible to get insights into the time needed to elicit a response for ketamine. If the LEV+ketamine group has a much faster response time, it may provide the basis for a follow-up study to compare LEV vs. ketamine for second-line treatment of SE.

6.3 Conclusions

Using data from ESETT, which was comparative-effectiveness study of FOS, LEV and VPA for second-line treatment of patients with benzodiazepine-refractory convulsive SE, my research shows that despite the availability of evidence-based guidelines, benzodiazepines used as first-line treatment of convulsive SE are underdosed in the EMS and ED settings. Increasing the awareness of appropriate benzodiazepine dosing and implementing the suggested measures will potentially improve the performance of first-line treatment with benzodiazepines and reduce the number of SE episodes progressing to treatment-refractory stages and provide significant benefit to patients. Capping the weight-based dose of FOS, LEV and VPA at 75 kg did not have a significant effect on ESETT primary outcome. Further, the concentrations of PHT, LEV and VPA that I reported from ESETT samples will provide clinicians with some perspective on expected early concentrations after a high IV dose of these drugs in young children with benzodiazepine-refractory SE. While the number of samples collected in ESETT were inadequate to relate drug exposure to treatment response, the novel method for estimating early drug exposure using two plasma concentrations and a population pharmacokinetic modeling approach can be applied in other emergent conditions or situations where early drug exposure may be of interest. My research work provides valuable information to facilitate the design and conduct of a future study of convulsive SE treatment in the ED setting.

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