# Pharmacokinetic variability, clinical use and therapeutic drug monitoring of antiepileptic drugs in special patient groups

Thesis for the degree of Philosophiae Doctor (PhD)

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# Content

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# Abbreviations

AED(s): Antiepileptic drug(s)

C/D ratio: Concentration/dose ratio

CLB: Clobazam

CNS: Central nervous system

CYP: Cytochrome P450

EMA: European Medicines Agency

FDA: U.S. Food and Drug Administration

GABA: γ-aminobutyric acid

GBP: Gabapentin

HPLC-UV: High-pressure liquid chromatography with ultraviolet detection

ILAE: International League Against Epilepsy

LCM: Lacosamide

NCLB: N-desmethylclobazam

NorPD: The Norwegian Prescription Database

PM: Poor metabolizers

RLS: Restless legs syndrome

SSE: The National Center for Epilepsy, Oslo University Hospital

TDM: Therapeutic drug monitoring

UGT: uridine glucuronyl transferases

UHPLC-MS/MS: Ultra-high pressure liquid chromatography with tandem mass spectrometry

VPA: Valproate, valproic acid

# List of papers

**Paper 1:**

Burns ML, Baftiu A, Opdal MS, Johannessen SI, Landmark CJ. Therapeutic drug monitoring of clobazam and its metabolite - impact of age and comedication on pharmacokinetic variability. Ther Drug Monit. 2016;38(3):350-357.

**Paper 2:**

Burns ML, Nikanorova M, Baftiu A, Rasmussen JB, Johannessen SI, Johannessen Landmark C. Pharmacokinetic variability and clinical use of lacosamide in children and adolescents in Denmark and Norway. Ther Drug Monit. 2019; 41(3):340-347.

**Paper 3:**

Johannessen Landmark C, Burns ML, Baftiu A, Huuse Farmen A, Lossius MI, Johannessen SI, Tomson T. Pharmacokinetic variability of valproate in women of childbearing age. Epilepsia. 2017;58(10):e142-e146.

**Paper 4.**

Burns ML, Kinge E, Stokke Opdal M, Johannessen SI, Landmark CJ. Therapeutic monitoring of gabapentin in various indications. Acta Neurol Scand. 2019;139:446–454.

## Summary

**Background**: Treatment with antiepileptic drugs (AEDs) is a cornerstone in the management of epilepsy, and many of these drugs are also used extensively in other indications. Despite the availability of more than 25 AEDs, almost 1/3 of patients with epilepsy fails to achieve seizure freedom. Pharmacokinetic variability is pronounced for most AEDs, and is a major contributor to variability in response. Therapeutic drug monitoring (TDM) by measurement of serum concentrations combined with clinical interpretation of the result, can detect unexpected pharmacokinetics and is a tool to individualize and optimize treatment. Furthermore, it can be utilized to study pharmacokinetic variability in special patient groups that are particularly vulnerable when it comes to the fine-tuned balance between efficacy and tolerability.

**Aims**: Our aims were to examine pharmacokinetic variability and clinical use of selected AEDs; clobazam in patients with difficult-to-treat epilepsies, lacosamide in children and adolescents, valproate in women of childbearing age and gabapentin which is often used in elderly and in non-epilepsy indications. Furthermore, we wanted to evaluate the use of TDM in a group of patients receiving gabapentin for restless legs syndrome (RLS).

**Methods**: Main sources of data included results from routine TDM combined with supplementary information from request forms or clinical records, as well as data from the national prescription databases in Norway and Denmark. We also conducted a small, prospective observational study on the use of TDM in treatment with gabapentin for RLS.

**Results and conclusions**: Extensive pharmacokinetic variability was observed for all the studied drugs. Serum concentrations of clobazam and/or its active metabolite N-desmethylclobazam were influenced by a number of drug-drug interactions as well as by pharmacogenetics and age. The observed variability in dose-adjusted serum concentrations of

valproate in women of childbearing age indicates that serum concentrations provide a better measure of exposure than the dose alone. Since its approval for use in children, lacosamide is increasingly used in this age group. We observed a retention rate that compares favorably to other AEDs, and it was higher in patients not concomitantly using other sodium channel blockers. Gabapentin is increasingly used in the elderly, who have reduced clearance of the drug compared to younger patients. In the studied group of patients with RLS, TDM was used to evaluate adverse effects or to support dose increases. Because of varying dosing regimens, it is essential that sufficient clinical information is available when interpreting the results of serum concentration measurements.

The observed pharmacokinetic variability makes these four drugs good candidates for TDM, particularly in special patient groups and in challenging treatment situations as described in the included studies. Including serum concentrations in future research will provide more comprehensive insights when examining efficacy and tolerability in epilepsy and in other indications, and when studying teratogenic effects of AEDs.

# 1. Introduction

## 1.1 Epilepsy

Epilepsy is one of the most common chronic neurological diseases. It is characterized by a predisposition to generate epileptic seizures, can occur at any age and has many different presentations and causes (1). The prevalence of epilepsy has been estimated to be between 0.4 and 1%, but results vary between areas and due to methodological differences between studies (2). Indeed, a recent meta-analysis found a point prevalence of active epilepsy of 6.38 per 1000 persons (95% CI 5.57-7.30), but noted significant heterogeneity between studies (3).

Accounts of what may have been epileptic seizures can be found in several ancient scriptures, dating back some 4000 years (4, 5). Around year 400 BC, Hippocrates placed the origin of epilepsy in the brain and challenged the beliefs of the time that epileptic seizures were caused by actions of demons or gods (4). In modern time the pathophysiology of seizures has been explained by a disruption of the normal balance between excitation and inhibition in the brain, but this is now considered an oversimplification (1, 6). Rather the imbalance in excitatory and inhibitory activity is conceptualized to occur within a neuronal network, which can then function in an excessive, hypersynchronous, oscillatory manner that can disrupt normal neuronal processing and other neuronal networks (7, 8). Enhanced connectivity, enhanced excitatory transmission, failure of inhibitory mechanisms and changes in intrinsic neuronal properties are all factors implicated in greater spread and neuronal recruitment (1). Furthermore, ongoing research is aiming at better understanding the involvement of other factors such as neurodegeneration, neurogenesis, gliosis, inflammation and disruption of the blood-brain barrier on the pathogenesis and perpetuation of epilepsy (9). A number of genetic mutations (modulated by epigenetic factors), insults to the brain, (possibly mediated through inflammatory cytokines), and malformations in cortical development have all been implicated

in the development of epilepsy (10), but in many patients etiology remains unknown. Despite the advancements in the understanding of the disease and the underlying mechanisms, epilepsy continues to be associated with stigma and prejudice. In 1997 the World Health Organization, International League Against Epilepsy (ILAE, a world-wide professional organization) and International Bureau for Epilepsy (the equivalent lay organization) established the project Global Campaign against Epilepsy: Out of the Shadows "to improve acceptability, treatment, services and prevention of epilepsy worldwide" (11, 12).

Correct diagnosis and classification is the key initial step in determining when and how to initiate treatment for epilepsy (13). The definition of epilepsy has evolved over time, and the most recent definition by ILAE recognizes that the diagnosis of epilepsy under some conditions can be made already after the first seizure. The current definition depends on the presence of any of the following conditions: "At least two unprovoked (or reflex) seizures occurring >24 hours apart, or one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years, or diagnosis of an epilepsy syndrome (14)." The classification of seizures was updated in 2017 and distinguishes between seizures that are generalized from the onset, originating within, and rapidly engaging, networks that are bilaterally distributed, and focal-onset seizures that originate in networks limited to one hemisphere (15, 16). The classification and terminology of epilepsies was also updated (17, 18), and includes three levels of classification; seizure type, epilepsy type and syndrome. Cause (structural, genetic, infectious, metabolic, immune or unknown) and comorbidities should be identified at each stage (7, 18).

The burden of comorbidities in people with epilepsy is high, with many somatic, psychiatric and behavioral disorders being more common than in the general population (19, 20). For example, a study from the UK General Practice Research Database showed that psychiatric

disorders occurred twice as often in adults with epilepsy (21). In fact, it has been suggested that epilepsy could be related to a systemic dysfunction also manifesting as other diseases (20, 22). The presence of comorbidities can have great impact on quality of life, and can complicate assessment and management (1). Not only will comorbidities impact on treatment choices for the epilepsy; the susceptibility to seizures also has to be considered when administering drugs to treat comorbidities (23). Furthermore, a number of challenges not directly associated with seizures such as tiredness, memory or concentration problems, headaches and feeling depressed adds to the burden of epilepsy (24). Therefore, the ILAE in 2005 proposed defining that epilepsy is characterized by "an enduring predisposition to generate epileptic seizures, and by the neurobiologic, cognitive, psychological, and social consequences of this condition" (14).

## 1.2 Pharmacological treatment of epilepsy

The overall goal of the treatment of epilepsy is to ensure the best possible quality of life according to the patient's individual circumstances; ideally by ensuring reliable freedom from seizures without adverse effects (25). Antiepileptic drugs (AEDs) are the mainstay of treatment, and about two thirds of patients become seizure free on such therapy (26-29). Even though several neurobiological processes have been proposed as potential targets for disease-modifying therapies (7), so far the only approved antiepileptogenic therapy available is the mammalian target of rapamycin (mTOR) inhibitor everolimus for the treatment of refractory epilepsy associated with tuberous sclerosis complex (30, 31). Drugs used in epilepsy are therefore really seizure suppressing drugs, but the term "antiepileptic" is conventional terminology (32). Acute treatment of prolonged seizures and treatment of status epilepticus,

as well as other treatment options in epilepsy such as surgery, neurostimulatory interventions or dietary treatments will not be covered in this thesis. An overview is provided in e.g. (6, 33).

In the centuries before Christ, treatment of epilepsy included ingestion of various substances, such as seal genitals, crocodile feces and tortoise blood (34). During the Middle Ages magical approaches prevailed, but with the Enlightenment came more rational pathophysiological hypotheses concerning seizures and epilepsy. However, it wasn't until the introduction of potassium bromide in 1857 that the first pharmacological treatment with proven benefit was introduced (34). At the National Center for Epilepsy in Norway, patients were served bread baked with bromide until 1960, but this treatment was unfortunately associated with significant adverse effects, including bromism (35). In 1912 a reduction in seizures was reported in a patient given phenobarbital for sedation, and the drug was found to be a better and safer treatment of epilepsy than bromides (36). During the 1900s more medications were gradually used to prevent seizures; phenytoin (1938), trimethadione (1946), and in the 1950s and 60s primidone, ethosuximide, sulthiame, carbamazepine and valproate (VPA) (37). In the 30 years that has passed since the introduction of vigabatrin in 1989, 17 new AEDs have been introduced (38, 39). This has provided more choice, but also made it more difficult to select the optimal drug for individual patients, as each drug has advantages and limitations (40). Nevertheless, the broad range of therapeutic options may lead to better personalized medicine in epilepsy (10).

In addition to the drugs found serendipitously to have antiseizure activity, such as e.g. phenobarbital and VPA, compounds with antiseizure activity have been identified though either random screening of chemicals, developing structural variations of known AEDs or targeting of seizure-inducing mechanisms (41, 42). The maximal electroshock seizure test and the subcutaneous pentylenetetrazole test have been widely used animal models in AED discovery, and the 6-Hertz psychomotor seizure model in mice and kindling models also have

a place in preclinical testing (43). An example of modification of an existing AED is the development of oxcarbazepine, and later eslicarbazepine acetate, based on carbamazepine, with the aim to avoid auto-induction, strong enzyme inducing properties and adverse effects. Vigabatrin on the other hand, was developed to target a specific mechanism of action, namely irreversible inhibition of γ-aminobutyric acid (GABA) transaminase, but has limited clinical use today because of retinal toxicity (35, 42).

The result of these different development strategies is a varied group of AEDs with different chemical structures and pharmacological targets (40). Their mechanisms of action are not fully understood, and most AEDs have multiple actions (1). In broad terms they involve modulation of voltage-gated ion channels, enhancement of synaptic inhibition or inhibition of synaptic excitation (44). Figure 1 provides an illustration of some targets for AEDs. More details regarding mechanisms of action of the different AEDs can be found in comprehensive textbooks such as (45, 46) and recent reviews e. g. (32, 40, 44, 47-49).

In some patients, identifying specific gene variants permits rational selection of AEDs, such as using sodium channel blockers for epilepsies due to gain-of-function SCN8A mutations, ketogenic diet for glucose transporter 1 deficiency or avoidance of sodium channel blocking drugs in SCN1A-related Dravet syndrome (50). Our current understanding of pathophysiological mechanisms and modes of action of individual drugs, is however, not sufficient to allow for a fully mechanistic approach to AED therapy for most patients (51). Drugs are rather chosen on clinical grounds, and selected according to efficacy, tolerability, drug interaction profile, and ease of use (6, 52). There are guidelines to give clinicians an overview over the current evidence for treatment of different seizure types or epilepsy syndromes (e.g. (53, 54)), but individual needs, lifestyle, comorbidities, comedication and preferences must also be taken into account when tailoring therapy.

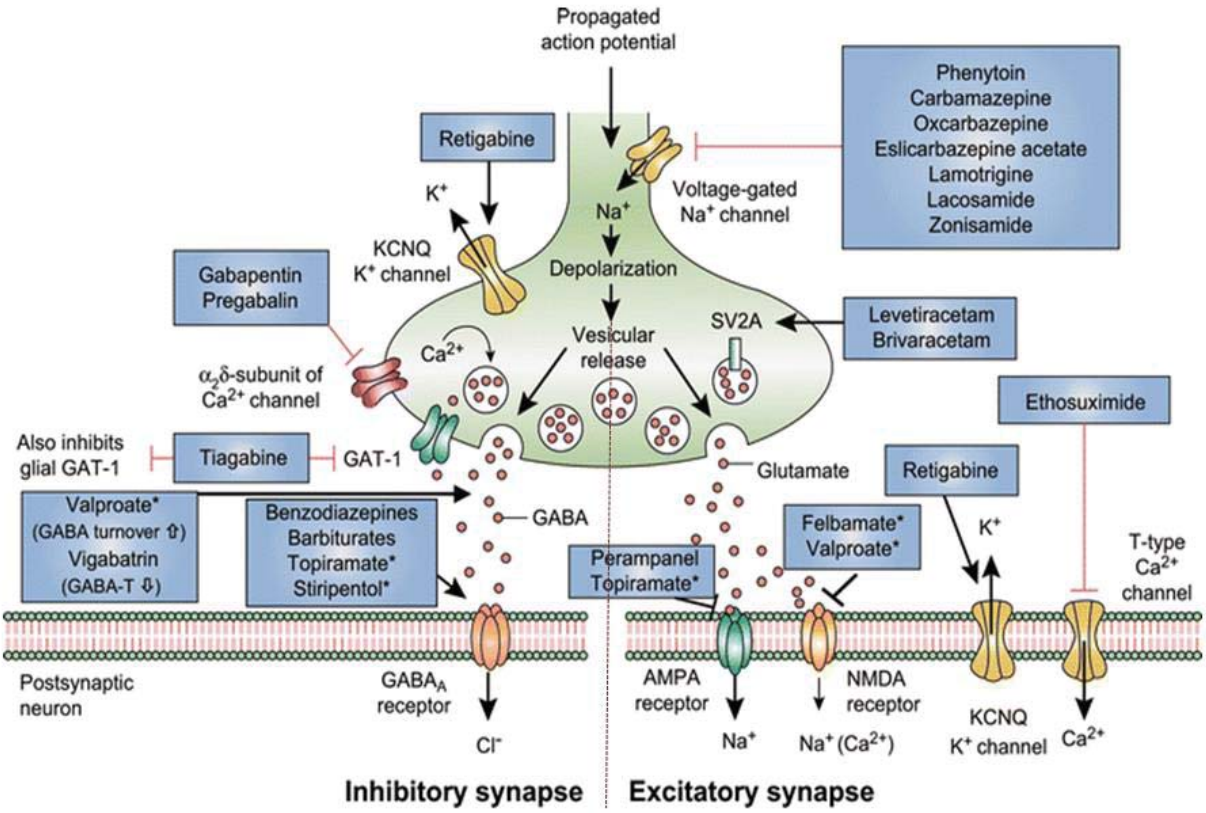


**Figure 1** – Proposed mechanisms of action of some antiepileptic drugs. *Drugs marked with asterisks act by multiple mechanisms, not all shown here. GABA: γ-aminobutyric acid, GABA-T: GABA aminotransferase, GAT: GABA transporter, SV2A: synaptic vesicle protein 2A, NMDA: N-methyl-D-aspartate, AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, KCNQ a family of voltage-gated potassium channels (also known as the Kv7 family). Figure adapted (insertion of division line between inhibitory and excitatory synapse) from Löscher et al (55) (available from https://doi.org/10.1007/s40263-016-0384-x), under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/).*

Despite the introduction of several new AEDs in the last decades, the proportion of patients becoming seizure-free on treatment does not seem to have changed substantially over the past 50 years (42, 56). Even though the new AEDs have generally not been proven to be more efficacious than older agents in newly diagnosed epilepsy, there are patients who do not respond to the old drugs that may do well with one of the new AEDs (57). Furthermore, improvements in pharmacokinetics can mean safer and easier regimens, and there is a trend for some new AEDs to exhibit better tolerability than older agents; which can be of great

importance for the quality of life of patients with epilepsy (10, 57). Another improvement is that some of the newer AEDs seem to be associated with lower risk of adverse fetal effects when used during pregnancy (58).

Drug resistant epilepsy was defined by an ILAE task force in 2010 as "failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" (59). In a recent meta-analysis, the pooled prevalence proportion of drug resistant epilepsy among epilepsy patients was 0.3 (95% CI 0.19-0.42), but there were wide variations in the case definition of the condition in the included studies (60). Even when the established definition is applied, making the diagnosis of drug resistant epilepsy is not easy: In a recent study, 19% of patients classified by the enrolling neurologist to have drug resistant epilepsy according to the ILAE definition were defined by the expert panel as rather having "undefined responsiveness" (61). To meet the need of patients with pharmacoresistant epilepsy, and because currently available AEDs are not without adverse effects, the search for new and better drugs is clearly warranted (10). Bialer et al. have reviewed the AEDs currently in development (62, 63).

## 1.3 Other uses of antiepileptic drugs

AEDs are also used in a number of non-epileptic conditions, primarily in pain disorders and psychiatry (64, 65). Some drugs have approved indications in other illnesses, such as VPA, carbamazepine and lamotrigine in bipolar disorder, pregabalin for generalized anxiety disorder, gabapentin (GBP), pregabalin and carbamazepine for neuropathic pain, and topiramate as migraine prophylaxis (66, 67). AEDs are also used off label in a number of different indications both in adults and in children. Examples include, but are not limited to, restless legs syndrome (RLS), dystonia, insomnia, schizophrenia, fibromyalgia, multiple

sclerosis, headache, essential tremor, nausea, pruritus, chronic cough and alcohol use disorders (38, 64, 66, 68, 69). Use in non-epilepsy disorders accounts for more than half of the utilization of AEDs in Norway, and has increased over the last few years (65, 67, 70). It is particularly high in the elderly, where neuropathic pain is the main indication for use of AEDs (65). A recent Swedish study found that in children and adolescents on the other hand, AEDs are still used mainly in epilepsy, accounting for approximately 70% of prescriptions (66).

Also in many non-epilepsy conditions, AEDs are thought to act through effects on ion channels, by promoting inhibition mediated by $GABA_A$ receptors or decreasing glutamatergic excitatory neurotransmission (64, 71). In the treatment of neuropathic pain, the effect on ion-channels in the brain and spinal cord are thought to be of particular importance (71). For the therapeutic activity in psychiatry, effects on neurotransmitters such as serotonin and dopamine may also be important (72), but long-term benefit in these indications are proposed to be because of effects on the structural integrity of neurons and the enhancement of synaptic plasticity mediated through second messenger systems (64).

In one of the studies included in this thesis, we examined the use of TDM in treatment with GBP for RLS. RLS is a relatively common disease, with prevalence estimated to be approximately 5-15% in Caucasian populations (73). Patients experience an urge to move the legs, usually accompanied or caused by unpleasant sensations or pain in the legs. This occurs at rest, is worse in evening or night and is relieved by movement (74). It can cause great distress and disturbance of sleep (75). GBP has been found to be beneficial in RLS (76), but this is currently not an approved indication for the drug. The use of off-label treatment puts an additional responsibility on the prescriber, but other therapies for RLS have significant limitations, such as augmentation with dopaminergic drugs and dependence and tolerance with opioids (76). Optimizing safety and efficacy of treatment with GBP in these patients is therefore of great value.

## 1.4 Pharmacokinetic variability of antiepileptic drugs

There is pharmacokinetic variability of AEDs both between and within patients, and for most of the drugs it is extensive (38, 77-79). This pharmacokinetic variability is an important determinant of differences in response to AEDs (57). It can be a result of differences in absorption, distribution, metabolism and excretion, and is determined by genetic factors, age, physiological states, pathological conditions, environmental factors and interactions with other drugs (77). An illustration of pharmacokinetic processes relevant to AEDs is provided in Figure 2.

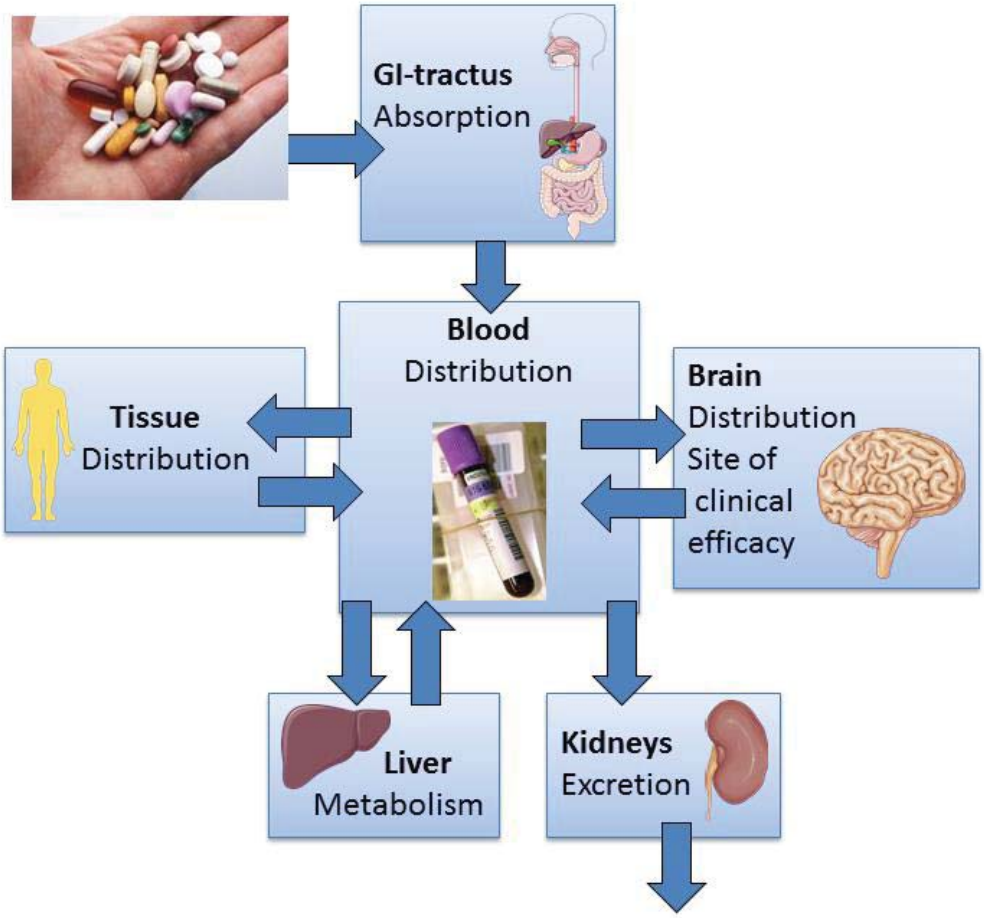


**Figure 2** – An overview of pharmacokinetic processes relevant to antiepileptic drugs. *Figure adapted with permission from Elsevier and Cecilie Johannessen Landmark (77). Medical illustrations added from https://smart.servier.com, under the terms of the Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).*

### 1.4.1 Variability in pharmacokinetic processes

In general, absorption is extensive and bioavailability high for most AEDs (77). GBP, however, displays dose-related absorption and resulting variability in bioavailability (80).

Most AEDs are lipid soluble so that they can cross the blood-brain barrier, and are generally widely distributed in the body (77). The degree of protein binding varies between drugs, and has recently been determined for most AEDs (81). AEDs that were shown to be >90% protein bound, include phenytoin, VPA, stiripentol, perampanel, clonazepam, clobazam (CLB) and tiagabine (81). For highly bound AEDs alterations in protein binding, and proportion of free, non-protein-bound pharmacologically active drug, can occur in a number of situations. Examples include hypoalbuminemia, chronic liver or renal disease, pregnancy and displacement from binding sites by other highly protein-bound drugs or endogenous substances (e.g. in uremia) (82, 83).

Most AEDs undergo extensive metabolism, mainly through oxidative reactions catalyzed by the cytochrome P450 (CYP) enzymes (phase I reactions) or by conjugations like glucuronidation by uridine glucuronyl transferases (UGTs) (phase II reactions) (77, 84). Exceptions include levetiracetam and rufinamide which undergo hydrolysis, and GBP, pregabalin and vigabatrin which are renally excreted unchanged (77). There is established evidence that polymorphisms in CYP2C9 and CYP2C19 genes can have significant effects on serum concentrations of phenytoin and N-desmethylclobazam (NCLB, the active metabolite of CLB) (85). CYP and/or UGT polymorphism has also been proposed to affect the clearance of VPA, lamotrigine, phenobarbital and zonisamide, but data are limited and in part conflicting (85-88). For drugs that are predominantly eliminated through renal excretion, such as for example GBP and pregabalin, changes in renal function is an important determinant of pharmacokinetics (77).

### 1.4.2 Gender-related pharmacokinetic variability

A number of factors could lead to differences between males and females in serum concentrations obtained after a given dose. For example, women have on average lower body weight and different body composition than men, and differences in the activity of some metabolizing enzymes and renal clearance have been demonstrated (89, 90). Yet, gender-specific or weight-adjusted dosing in adults are rarely seen (89). In addition to physiological factors, studies suggest that adherence is affected by gender (91). Even though gender differences in serum levels per dose have been reported for some AEDs (92), a review of the available evidence suggested that gender does not influence the absorption, distribution and elimination of AEDs in a consistent and clinically relevant manner (77).

Data on the impact of fluctuations in endogenous hormones and of the effect of menopause on serum concentrations of AEDs are limited (92, 93). While most studied AEDs have not been shown to vary significantly throughout the menstrual cycle (94-99), concentrations of phenytoin have been found to be lower during the menstrual phase in patients with catamenial epilepsy (100-102). Results regarding effect of perimenopausal age on lamotrigine clearance have been conflicting (95, 103, 104), whereas carbamazepine and licarbazepine (the active metabolite of oxcarbazepine) have not been found to be affected (103, 104).

Important gender-related differences in pharmacokinetics are changes occurring during pregnancy and interactions between AEDs and drugs only used in males or females (92). Examples of the latter include decreased serum levels of lamotrigine and VPA with oral contraceptive pills (92, 105-107), and reduced concentrations of lamotrigine with hormone replacement therapy (108, 109).

Pregnancy is a time of particularly rapid and pronounced pharmacokinetic changes. Absorption can be affected by physiological changes or vomiting, volume of distribution can

change as a result of increases in body water and fat stores, and free fractions of highly protein bound drugs may increase (110-112). Furthermore, the activity of drug-metabolizing enzymes is altered during pregnancy; while the activity of UGT1A4 and 2B7 and CYP2B6, 2C8, 2C9, 2D6, 2E1 and 3A4 has been shown to increase, decreased activity has been demonstrated for CYP1A2 and 2C19 (111, 112). In addition, renal blood flow and glomerular filtration rate increase, affecting clearance of renally excreted drugs (110-112).

Documented gestation-induced changes in kinetics of AEDs have recently been reviewed by Tomson et al., who describe a decline in concentration of lamotrigine, levetiracetam, phenytoin, phenobarbital, licarbazepine, topiramate and total carbamazepine and VPA, but that data are limited or lacking for a number of other AEDs (110). These changes are believed to primarily be caused by increased metabolism and to some extent renal clearance (92, 110). However, the effects of pregnancy on serum concentrations vary considerably between individuals, as they are also influenced by a number of patient and environmental factors (110, 111). Because of the change in free fraction of highly protein bound AEDs, free concentrations of these drugs should be monitored during pregnancy where possible, as changes in total concentrations can be misleading in regards to the effective concentration and fetal exposure (110, 113, 114).

In addition to the physiological changes occurring during pregnancy, adherence has been shown to be low in pregnant women with epilepsy (115). This could partially be related to the risk of adverse fetal effects of AEDs, such as major congenital malformations, growth restriction, cognitive impairment and behavioral abnormalities (58). The teratogenic potential varies substantially among AEDs, and for some drugs it is related to dose. Whereas prenatal exposure to VPA carries the greatest risk, lamotrigine, levetiracetam and oxcarbazepine are probably associated with the lowest risk of adverse fetal effects (58).

### 1.4.3 Age-related pharmacokinetic variability

With the exception of the first few weeks of life, differences in absorption of AEDs are generally not considered a major factor in age-related pharmacokinetic variability (116). Body composition changes significantly throughout life, leading to changes in volume of distribution, and an increased free fraction of highly protein-bound drugs can be seen in neonates or elderly (83, 116). Clearance is often reduced in neonates, especially for drugs that are eliminated through renal excretion or glucuronide conjugation, whereas older infants and children usually eliminate drugs at a faster rate than adults (78). Hepatic metabolism and renal excretion can be increased because of larger relative size of these organs in children compared to adults, and the ontogeny of metabolizing enzymes is relevant for some drugs (117-119). However, all these developmental changes are influenced by genetic, environmental and pathophysiological factors (118). In the elderly clearance is generally lower than in younger adults, either as a result of decreased renal function and/or less efficient drug-metabolizing activity, but factors such as frailty, nutritional status and comorbidities common to old age also play important roles (78). In addition to pharmacokinetic considerations, etiologies and certain epilepsy syndromes may be age-specific, and pharmacodynamic responses differ with age (116). An important result of this plethora of potential age related changes in pharmacokinetics and pharmacodynamics is increased variance between individuals at the extremes of life (83).

### 1.4.4 Drug interactions affecting pharmacokinetic variability

There is potential for numerous drug-drug interactions both within the AED group and with other drugs (51, 120-123). Approximately 20% of all patients, and more than half of patients

with refractory epilepsy, use AEDs in combinations (124). In addition to patients requiring multiple AEDs to optimize seizure control, many patients receive pharmacological treatment for associated or intercurrent conditions. Furthermore, a substantial proportion of patients with epilepsy take over-the-counter medications, herbs and dietary supplements, which pose further risks of interactions (125). Pharmacokinetic interactions can be identified by a change in the serum concentration of the relevant drug (51), whereas pharmacodynamic interactions occur at the site of action of the drugs. The latter can be additive, synergistic or antagonistic, and can lead to both beneficial or adverse effects (51).

The most common clinically relevant pharmacokinetic interactions affecting AEDs are at the level of metabolism and occur through hepatic enzyme induction or inhibition (77). Carbamazepine, phenytoin and phenobarbital are known to be potent inductors of a variety of CYP enzymes (e.g. CYP2C9 and 3A4), UGT and epoxide hydrolase (120, 126). However, phenobarbital has been suggested to induce CYP3A4 less than the other two drugs (127). Other AEDs can have selective or mixed effects, for example the induction of CYP3A4 and inhibition of 2C19 by felbamate and oxcarbazepine. Still others have primarily been shown to inhibit metabolic enzymes, such as stiripentol and VPA (even if induction of certain enzymes also has been suggested for VPA) (120, 126). To complicate the picture further, for some drugs the effects appear to be dose dependent, for example topiramate, where enzyme-inducing properties are seen in daily doses above 200 mg (120, 126). These effects will of course not only affect metabolism of other AEDs, but also other drugs undergoing hepatic metabolism. One example of the latter is the accelerated metabolism of oral contraceptive pills by AEDs such as carbamazepine, phenobarbital, phenytoin and oxcarbazepine (128). As a group, the newer AEDs are generally considered to have less interaction potential than the old AEDs due to their pharmacokinetic properties (122). However, interactions involving the newer drugs are becoming increasingly apparent as further research is conducted (128).

Interactions at the level of absorption are less common, but examples are reduced absorption of GBP when co-administered with magnesium oxide (129), and an observed 44% increase in the area under the curve for GBP when co-administered with morphine, proposed to be a result of reduced intestinal motility and increased absorption (130). Highly bound AEDs may be involved in interactions involving displacement from protein binding sites; one example being the displacement of phenytoin by VPA (126). Interactions at the level of renal excretion are not commonly described, but the clearance of felbamate is reduced with co-administration of GBP (131).

## 1.5 Therapeutic drug monitoring

As described in the previous chapter, numerous factors contribute to pharmacokinetic variability of AEDs, and the net effect in the individual patient can be hard to predict. However, it can be determined by measuring serum concentrations of the drug(s) in question. Furthermore, based on the assumption that clinical effect correlates better with drug concentrations than dose, therapeutic drug monitoring (TDM) can be used to tailor treatment to the patient (25, 132-138). In TDM, quantification of drug levels in blood or serum is combined with information on pharmaceutical properties, patient characteristics and a clinical evaluation to individualize treatment (137). This is particularly useful for medications with a narrow therapeutic index and extensive intra- or inter-individual pharmacokinetic variability (139). When TDM is used to guide medication doses the drug must have a reversible action, the drug's concentration at the site of sampling should correlate with the concentration at receptor sites and if the drug has active metabolites these should be measured as well (25). TDM can also be used to examine pharmacokinetics, discover drug interactions and determine

adherence, as well as to investigate adverse effects or toxicity, and in the treatment of overdoses and intoxications (139).

In 1960, Buchthal and Svensmark measured phenytoin and phenobarbital in blood samples, and related concentrations to dose, effect and adverse effects (140). From the 1970s, routine measurements of AEDs have been available in many laboratories, and have been used as part of the comprehensive care approach in epilepsy (79). There are several reasons why TDM has become a commonly used tool to optimize treatment in this disease: Most AEDs are subject to pronounced pharmacokinetic variability, treatment of epileptic seizures are prophylactic, with seizures occurring at unpredictable intervals, there are no reliable surrogate markers of effect, and therapeutic failure can have drastic consequences (79, 141). Furthermore, signs and symptoms of toxicity can be subtle and difficult to distinguish from the illness itself (132). Although the solid evidence for the usefulness of TDM in improving clinical outcome in epilepsy treatment is lacking (142-144), long experience indicates that it is useful both in treatment with new and old AEDs, if used appropriately (145).

ILAE issued guidelines for TDM in 1993 (146), these were updated in 2008 (139) and the first author of the reports published a further updated overview in 2018 (38). In the guidelines, the "reference range" was defined as "a range of drug concentrations, which is quoted by a laboratory and specifies a lower limit below which a therapeutic response is relatively unlikely to occur, and an upper limit above which toxicity is relatively likely to occur" (139). However, it was recognized that many patients can achieve therapeutic benefit at concentrations outside these ranges, and hence that the "therapeutic range" of AEDs could only be determined on an individual basis, as "the range of drug concentrations which is associated with the best achievable response in a given person" (134, 139). When the patient serves as his/her own control over time, TDM can be utilized even if a population based reference range is not established (134, 135, 147). At the initiation of treatment, and in certain

situations, such as in patients with very infrequent seizures or inability to communicate the occurrence of adverse effects, an individual therapeutic range can be difficult to establish. If the reference range has been determined based on appropriate and extensive research, the therapeutic range will lie within, or close to, this range for many patients and can be a useful aid in such situations (139). Because of limitations with population based reference ranges, and because individual therapeutic ranges may change over time, e.g. due to disease progression or development of tolerance, it is recommended that serum concentrations are not used as a sole reason for dosage adjustments, but rather in conjunction with a careful assessment of the patient's clinical state (139). Furthermore, it is important to remember that population based reference ranges cannot encompass the large number of different indications for AED therapy and the numerous different combinations of drugs that are in use. Reference ranges have recently been harmonized in Norway, to ensure that they are based on up-to-date research and to prevent differences between laboratories (148).

For TDM to be a useful tool, it is essential that it is applied in an appropriate manner, both when it comes to "how" and "when". For samples to be comparable with measurements from other patients or the same patient over time, a standardized sampling time is important. Samples should generally be collected immediately before the next dose (trough) in the morning, at steady-state (about five half-lives after starting or changing dose) (139). For some indications for TDM, however, it can be appropriate to collect samples at other times; for example at the time of expected peak concentrations with transient symptoms of dose-related toxicity or as soon as the patient presents in the case of overdose or status epilepticus (139). Either way, knowledge of sampling time and dosage history is required in order to interpret the result.

TDM should only be used on clear indications (79), and Table 1 provides some examples of situations where TDM is considered useful. When alterations in protein-binding of highly

bound AEDs are suspected, measurements of free, unbound concentrations should be performed where available, or if not available, carefully considered when interpreting the results in the clinical context (82, 139).

**Table 1** – Examples of situations where therapeutic drug monitoring is considered useful (79, 139).

| Situation | Rationale |
|---|---|
| **After initiation of therapy** | Detect unexpected pharmacokinetics |
| **When treatment outcome is satisfactory** | To establish individual therapeutic range |
| **When seizure control is not achieved despite apparently adequate dosage** | Determine actual exposure to drug |
| **Unexpected change in seizure control** | Aid in diagnosis and management |
| **Change in dose** | Especially if AED displays non-linear pharmacokinetics |
| **Change in other treatment (AEDs or other drugs)** | Potential introduction or removal of pharmacokinetic interactions. Establish new therapeutic range in case of pharmacodynamic interactions. (Measurement of free concentration when combining highly bound AEDs.) |
| **Suspected adverse events, toxicity or overdose** | Aid in diagnosis and management |
| **In infants, children or elderly** | Change in pharmacokinetic parameters over time (117). Large pharmacokinetic variability at extremes of age (78). Difficulties communicating adverse effects in small children. Presence of comorbidities in many elderly. (Measurement of free concentration for highly bound AEDs in infants/elderly). |
| **During and after pregnancy** | Ensuring adequate therapy in mother, while minimizing exposure to the fetus. Changes in pharmacokinetics expected for many AEDs, but large individual differences (110). (Measurement of free concentrations for highly bound AEDs (113).) |
| **Comorbidities** | Altered organ function (liver/kidney) that can affect pharmacokinetics of drugs. (Measurement of free concentrations for highly bound AEDs.) Use of concomitant drugs that may cause drug interactions. Difficulty/inability to communicate adverse effects. |
| **Change in drug formulations** | Potential change in serum concentrations. |
| **Examine adherence** | Patients often take medications differently to how they are prescribed (91, 149). Non-adherence has been shown to be an important cause of hospitalizations in patients with epilepsy (150). |
| **Emergency situations, status epilepticus** | Aid in diagnosis and management |

For AEDs used in non-epilepsy conditions, TDM has yet to play a major part (151). Reference ranges have been suggested for some AEDs in mood disorders (137, 152) and for carbamazepine in neuralgias (153, 154). However, establishing a therapeutic range can be difficult when the end point of treatment is subjective, as is the case for many non-epilepsy indications for AEDs (151). The concept of individual therapeutic concentrations may be applied in many situations, and serum concentration measurements to investigate variable adherence, misuse/diversion, unexpected pharmacokinetics, drug interactions, suspected toxicity or safety of a dose increase are independent of therapeutic indication. However, factors potentially affecting pharmacokinetics and pharmacodynamics of the drugs and dosing regimens need to be carefully considered when interpreting the results (137).

## 1.6 Use of therapeutic drug monitoring data to investigate pharmaco-kinetic variability

As clinical trials are performed on carefully selected populations and over limited periods of time, documenting real life use of drugs after marketing is important (155, 156). TDM data from routine practice have been used as a tool to study pharmacokinetic variability, and the effect of gender, age and/or comedication on serum concentrations for a number of AEDs. Some examples include, but are certainly not limited to the following references: (157-173). Even so, the current knowledge regarding pharmacokinetic variability in clinical practice is still incomplete for a number of both older and newer AEDs, especially in certain patient groups such as children or the elderly. Furthermore, data from routine TDM can provide signals regarding potential interactions that have not yet been investigated in clinical trials (124). Studies examining and documenting these conditions are therefore useful, but lack of standardization is a major limitation in much pharmacokinetic literature. Trough serum

concentrations at steady state should be used in such studies (174), as is recommended in clinical practice (139).

## 1.7 The drugs studied in the thesis

The four drugs studied in this thesis first received marketing approval in the EU/US at different times; VPA in 1967, CLB in 1975, GBP in 1993 and lacosamide (LCM) in 2008 (40). Yet, for a number of reasons they have all recently become particularly relevant; use of CLB has increased with the 2011 FDA approval (175), VPA has come into focus with recent limitations to its use in women of childbearing potential (176), LCM has received extension of marketing approval to include young children (177), and GBP is increasingly used in the elderly and in non-epilepsy indications, and at the same time concerns have been raised regarding potential for misuse (178). Furthermore, all the included studies focus on treatment in challenging clinical settings and vulnerable patients groups – "difficult-to-treat" epilepsies, polytherapy, use outside approved indication and in children, elderly or women of childbearing age. For all the four drugs, there have been gaps in the knowledge regarding the pharmacokinetic variability in clinical practice in these patient groups. Table 2 provides an overview of some important characteristics of the studied drugs.

**Table 2** – Characteristics of the studied drugs

| Drug | Clobazam | Lacosamide | Valproate | Gabapentin |
|---|---|---|---|---|
| **Presumed main mechanism of action (10, 40)** | GABA potentiation | Enhanced slow inactivation of voltage-gated $Na^+$ channels | Not fully elucidated. GABA potentiation, glutamate inhibition, $Na^+$ and $Ca^{2+}$ channel blockade has been proposed | $Ca^{2+}$ blockade (α2δ subunit) |
| **Approved use in epilepsy (FDA, EMA) (40)** | Lennox-Gastaut syndrome | Focal onset seizures with or without secondary generalization (monotherapy or adjunctive therapy) from 4 years of age | Focal, generalized and absence seizures | Focal seizures with or without secondary generalization. |
| **Other uses approved (FDA, EMA) (40)** | Anxiety disorders | | Bipolar disorder, migraine prophylaxis | Neuropathic pain e.g. diabetic neuropathy, post-herpetic neuralgia |
| **Elimination (77, 179)** | CLB and the active metabolite NCLB are metabolized via CYP3A4 and CYP2C19 | CYP2C19, 2C9, 3A4 | CYP2A6, 2C9, 2C19, 2B6, UGT 1A2, 2B7 | Renal excretion |
| **Limitations** | Drug-drug interactions and pharmacogenetic variability, sedative, tolerance | | Teratogenic, hepatotoxicity, drug-drug interactions, weight gain | Variable absorption, weight gain |
| **Reference range in Norway (148) µmol/L (mg/L)** | 0.1-1.0 (0.03-0.3) CLB<br>1-10 (0.3-3.0) NCLB | 10-40 (3 -10) | 300-700 (43-101) | 20-120 (3-21) |
| **Conversion factor (F): µmol/L = F x mg/L (139)** | 3.33 CLB<br>3.49 NCLB | 3.99 | 6.93 | 5.84 |

*FDA: U.S. Food and Drug Administration, EMA: European Medicines Agency, GABA: γ-aminobutyric acid, CLB: clobazam, NCLB: N-desmethylclobazam*

### 1.7.1 Clobazam

CLB is a 1,5-benzodiazepine acting as a $GABA_A$-agonist, potentiating inhibitory neurotransmission (180). The active metabolite NCLB contributes to therapeutic effect and can cause adverse events, but the relative potency of the metabolite has not been well documented (174). CLB is metabolized primarily by CYP3A4 to NCLB, which is metabolized by CYP2C19 to inactive metabolites (181). Because of its use in many "difficult-to-treat" epilepsies, including Lennox-Gastaut and Dravet syndrome (174, 182, 183), there is a substantial risk for drug-drug interactions with AEDs and other psychotropic drugs commonly used in this group (128, 184, 185). However, the resulting effects on serum levels of CLB and NCLB are not fully understood. Furthermore, some patients may be poor metabolizers in the metabolic step through CYP2C19, which can result in an accumulation of the metabolite (186, 187).

### 1.7.2 Lacosamide

LCM is a serine-analogue that was initially approved by the European Medicines Agency as add-on therapy for focal onset seizures with or without secondary generalization in patients 16 years or older, but the indication was later extended to include monotherapy and children >4 years (177, 188). In contrast to other sodium channel-blocking AEDs, it has been proposed that LCM exerts its effect by selectively enhancing slow inactivation of voltage-gated sodium channels (189, 190). Because of its recent approval in children (2017), there is limited evidence regarding use and pharmacokinetic variability in this patient group.

### 1.7.3 Valproate (valproic acid)

VPA is an effective AED with a broad spectrum of clinical activity, probably resulting from its combined actions on several pharmacological targets (49, 191, 192). It has been widely used to treat almost all types of seizures and epilepsy syndromes, but is particularly useful for the management of generalized epilepsies (192, 193). In addition to its use in epilepsy, approved indications are bipolar disorder and migraine prophylaxis (40). Furthermore, as VPA modulates DNA transcription through inhibition of histone deacetylases, it is under investigation as a positive modulator of chemotherapy in cancer treatment, and the possible therapeutic role of VPA-induced neuroprotection is being explored (194).

Studies have consistently found that compared to other AEDs, exposure to VPA during pregnancy carries the greatest risk both for major congenital malformations and cognitive and behavioral abnormalities (58). Restrictions have therefore been put in place for use in women of childbearing age (176). For some women with generalized epilepsies, however, VPA may be the only effective medication (195, 196). In these women, it is advocated to use the lowest effective dose, as risks of negative fetal affects have been shown to be dependent on maternal dose (195).

### 1.7.4 Gabapentin

The calcium channel blocker GBP is approved for use in epilepsy and peripheral neuropathic pain in Europe (197). It is also used in numerous other conditions, such as restless legs syndrome (RLS), fibromyalgia, trigeminal neuralgia, multiple sclerosis, headache, anxiety, post-operative pain, nausea, pruritus, chronic cough and alcohol use disorders (38, 68, 69). In Norway, GBP is used more in neuropathic pain than in epilepsy and its use has been increasing over the last few years, especially in the elderly (65, 67). Inter- and intraindividual

differences in dose-to-plasma concentrations have been observed for GBP (161, 198, 199). Sources of pharmacokinetic variability include variable, dose-dependent absorption due to saturability of active absorption by the L-amino acid transporter (80, 200), interactions with some antacids and analgesics (122, 123) and differences in renal function (201). Because of concerns related to misuse, GBP (and pregabalin) was reclassified as class C controlled substances in the UK from April 2019 (178).

# 2. Aims

The overall aim of the thesis was to contribute to improved characterization of pharmacokinetic variability, clinical use and TDM of AEDs in patient groups or situations where pharmacological treatment is challenging.

The objectives for each paper were as follows:

**Paper 1:** To investigate pharmacokinetic variability of CLB in clinical practice with emphasis on the impact of comedication and age in patients with epilepsy.

**Paper 2:** To investigate the use of LCM in children and adolescents in relation to age, comedication, dose, serum concentrations, and duration of treatment, and to examine real-life pharmacokinetic variability in this population.

**Paper 3:** To investigate pharmacokinetic variability of VPA in women of childbearing age by use of TDM data to elucidate the relationship between dose and serum concentrations.

**Paper 4:** To investigate the use and pharmacokinetic variability of GBP in epilepsy and non-epilepsy indications, and to evaluate the use of TDM in a group of patients with RLS.

# 3. Materials and methods

## 3.1 Sources of data

In the included studies we examine real life use of AEDs by combining data from serum concentration measurements with clinical information and prescription trends. In all four included studies data from our TDM-database was used to examine pharmacokinetic variability. Clinical information was sourced from laboratory request forms, medical records or from a prospective study on patients in a neurological practice just outside Oslo. Paper II also included data from the Danish Epilepsy Center, Filadelfia, Dianalund. Information on national prescription trends were sourced from prescription databases. Figure 3 depicts the data sources that were used in the different studies/papers.

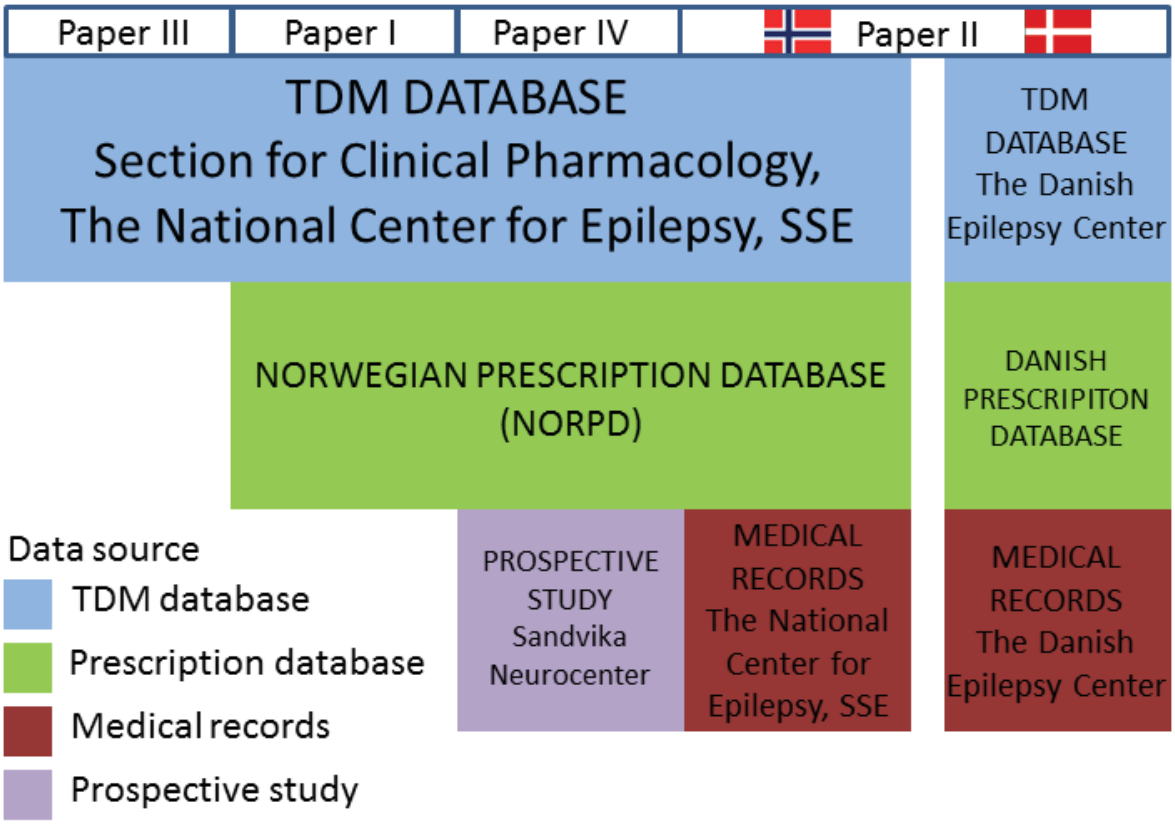


**Figure 3** – Sources of data in the included studies. *TDM: Therapeutic drug monitoring, SSE: The National Center for Epilepsy, Oslo University Hospital.*

## 3.2 The therapeutic drug monitoring database

Our laboratory was established 50 years ago, and performs approximately 20 000 analyses of AEDs yearly. We are currently organized as Section for Clinical Pharmacology, The National Center for Epilepsy (SSE), under the Department of Pharmacology, Oslo University Hospital. Our department offers routine serum concentration measurement of all relevant AEDs in use in Norway, as well as determination of free, unbound concentrations of phenytoin and VPA and drug metabolites NCLB and carbamazepine epoxide. (Vigabatrin is generally not considered a candidate for TDM because of its mechanism of action (38, 148), and is not part of the repertoire). Although other laboratories in the country measure serum concentrations of AEDs, routine measurement of some compounds, such as CLB, NCLB, sulthiame, perampanel, stiripentol and brivaracetam are only offered at our laboratory (202, 203). Utilizing results from our TDM database (Unilab) enables us to study a large number of patients and to compare gender and age groups; it reflects the complexity of clinical practice and enables us and to assess the influence of complex AED polytherapy. There is a strong tradition in Norway to use TDM as part of management of patients with epilepsy. The population of patients in our database is therefore expected to be representative of the therapeutic setting, which allows for an estimation of the degree of interindividual variability in clinical practice, due to both known and unknown factors (83).

Results from routine serum concentration measurements of CLB, NCLB, LCM, VPA and GBP were retrieved retrospectively from the TDM database. Paper II also contains data from the TDM database at the Danish Epilepsy Center, Filadelfia. Standard procedure is to draw blood for such measurements drug fasting in the morning at assumed steady-state, and samples noted not to be taken under such conditions were excluded from our studies. To avoid introducing bias from multiple samples from individual patients, the most recent

measurement with complete data was used when more than one result was available. Clinical information was retrieved from the accompanying TDM request form. For the Danish patients in Paper II such information was retrieved from medical records, as was additional data for some of the Norwegian patients in this study. All data were de-identified before further processing.

## 3.3 Bioanalytical methods

The analyses were routine measurements at the Section for Clinical Pharmacology, The National Center for Epilepsy, Oslo University Hospital. CLB and NCLB were measured by high-pressure liquid chromatography with ultraviolet detection (HPLC-UV) on an Ultimate 3000, with a 4.6 x 30 mm 3,5 µm ZORBAX Eclipse Plus C18 column. LCM analyses were performed using HPLC-UV, on an Ultimate 3000, with a 125 x 3 mm 3µm Hypersil BDS C-18 column, based on (204) until 2018. Thereafter we used ultra-high pressure liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) on a Prelude MD HPLC/Endura MD mass spectrometer, using the AEDs ClinMass TDM Platform Kit System (MS9000, MS9200) from Recipe (Munich, Germany) (205). At The Danish Epilepsy Center, Filadelfia, LCM was analyzed using an in-house UPLC-MS/MS method applying a Waters Acquity UPLC with a 2.1 x 100 mm 1.7 µm Ethylene Bridge Hybrid C18 column, in connection with a triple quadrupole mass detector. VPA was measured by immuno-assay on a COBAS C111, Roche Diagnostics, Switzerland using the Roche Valproic Acid Online TDM kit (04642473/03375790). GBP was analyzed using HPLC-UV, on a Dionex-HPLC system, with a 250 x 4 mm 5µm Mos HYPERSIL column based on (206).

The value of TDM depends on reliable measurements of serum concentrations. The first international quality control scheme for AEDs was established in London in 1972 (139), and our laboratory was one of the first to participate in such a program. The analytical methods used in the included studies are all subject to monthly, international proficiency testing, in addition to the internal controls that are part of the analysis set up.

## 3.4 The prescription databases (Paper I, II and IV)

Data on prescriptions of CLB, LCM and GBP were retrieved from the Norwegian Prescription Database (NorPD) (207). NorPD is a registry that contains information on prescriptions dispensed to patients outside hospitals and nursing homes. All pharmacies in Norway are required to report data to the registry, which is administrated by The Norwegian Institute of Public Health. Since it was established in 2004, it has been found to be a reliable source of data for pharmacological research and has been used in numerous studies (208, 209). Basic searches are publically available, but researchers can also apply for more comprehensive data files. In the included studies, the first option was used, with number of patients (overall or in selected age groups) being prescribed the drug in question as the variable requested. Further information on all variables is available from the database website (207). In Paper II, data were also sourced from the Danish prescription database (210) which contains comparable information (209). National agencies for statistics were used as sources for population sizes (211, 212).

## 3.5 Retention rates (Paper II)

In post marketing studies, measurement of retention rates or time to withdrawal is considered to provide relevant clinical information, as it is a measure of effectiveness, a composite of both efficacy and tolerability (213). In addition, it is influenced by patient willingness or ability to continue the medication. Retention rates do not distinguish between those who have complete seizure freedom and those who have just enough perceived effect to choose to continue therapy. Nor does it distinguish between effects on the epilepsy, and other potential beneficial effects, for example on mood or behavior. We believe that calculating retention rates after one year allowed sufficient time for most patients without initial effect to discontinue therapy. However, some patients with slow titration, infrequent follow up and/or questionable effect may try the drug longer. Adverse effects can occur shortly after initiation of therapy, but also later in the time course. With a time-frame of 1 year, no discontinuation due to remission was expected.

## 3.6 Prospective study (Paper III)

In the prospective, observational study carried out as part of Paper III, the treating physician collected clinical information regarding the patient, illness, treatment, serum concentrations and perceived usefulness of TDM in a predefined questionnaire during two separate consultations. The illness severity was defined according to the International Restless Legs Scale (IRLS) (0-40 points) (214). Evaluation of treatment effect and usefulness of TDM were subjective opinions. Because of the limited sample size and considerable heterogenicity between included patients, we did not consider it feasible or useful to standardize these evaluations or perform statistical tests on the material.

## 3.7 Calculations/presentation of results

Serum concentrations, daily doses, concentration/dose (C/D) ratios and C/(D/kg) ratios were presented as means with standard deviation (SD) or medians with minimum–maximum values. C/D ratios were calculated by dividing measured serum concentrations in μmol/L by daily dose in mg (or mg per kg for C/(D/kg) ratios). This corrects for the variability in serum concentrations caused by different dosing when comparing results.

Variations in C/D ratios were used as expressions for pharmacokinetic variability. The maximum/minimum ratio was used as a measurement of this variability, which means that it was affected by extreme outliers. In some instances calculations such as truncated range, coefficient of variation (CV) or interquartile range can be more appropriate, but in our studies we considered it to be important to include these outliers, as they represent patients that could potentially have great benefit from TDM and individualized dosing. Concentrations and doses were also presented graphically, to provide a visual impression of variability.

Because CLB has an active metabolite NCLB, three C/D ratios (total (CLB + NCLB) C/D ratio, CLB C/D-ratio and NCLB C/D-ratio) as well as the ratio between metabolite and parent drug (NCLB/CLB) were calculated for this drug.

The C/D ratio is affected by the weight of the patient. In adults where doses are generally not adjusted according to weight we did not include weight in the calculation of the ratio, to reflect this clinical practice. For children dose was adjusted for a 70 kg individual (for CLB in Paper I) or C/D ratio was calculated based on dose/kg (for LCM in Paper II).

## 3.8 Statistical analyses

For statistical analyses IBM SPSS Statistics version 22 or 25 (SPSS Inc, Chicago, IL, USA) was used. Several statistical methods/tests were applied, as detailed in the individual papers. Tests were chosen based on the nature of the variable and the type of comparison performed, as well as sample size and distribution of data. P-values of <0.05 were considered statistically significant for all analyses.

Unlike parametric tests, such as the Student's t-test, non-parametric tests do not require assumptions about the distribution of the population. If the sample size is >30 the violation of the assumption of normally distributed data is of less importance, according to the central limit theorem (215). Although many text books state that assumptions should be checked and tests chosen depending on whether they are met, others argue that normality has to be established for the populations under consideration or come from extra data sources (216). In our studies we examined the distributions by visually assessing histograms and quantile-quantile plots and in some instances by performing Shapiro-Wiik tests for normality.

In Paper I Student's two-sided t-test with unequal variance was used to calculate significant differences between ratios with concomitant drugs compared to the neutral group. As some of the groups were small and not normally distributed, a non-parametric test would have been more appropriate. Furthermore, p-values were not adjusted for multiple testing. A number of procedures have been developed to deal with the increasing probability of finding statistically significant results by chance when performing multiple testing, but there is continuing controversy regarding if and when these procedures should be used (217). The Bonferroni method, although conservative, has the appeal of being cautious and easy to apply (218). To explore the impact of applying these statistical methods to the same data set, we performed pairwise comparisons with Mann-Whitney rank sum test and Bonferroni correction (with a

corrected significance level of <0.006). We found results comparable to those reported, except that the difference in NCLB/CLB ratio reached statistical significance also for felbamate, VPA and sulthiame. Considering the sample size (felbamate n=6, sulthiame n=8) and/or other reported results (reduced CLB C/D ratio with VPA), this would not alter the conclusions reached.

When analyzing the effects of comedication, age and gender separately on the whole data set, there is a risk of differences in one of the other variables affecting the results. Regression models or analyses of covariance would have allowed the impact of multiple factors, such as age and comedication to be studied simultaneously. The method we chose has the appeal of being easy to apply and interpret, but the experience from this thesis has inspired us to explore utilizing more advanced statistical models in future research.

## 3.9 Ethical considerations

All studies were approved by the Regional Committees for Medical and Health Research Ethics, Norway before initiation. Number: 2009/1380 (Paper I-III) and 2015/1295 (Paper IV).

We did not obtain consent from the patients whose TDM results were included in our studies, as it was not considered practically possible to contact several hundred patients regarding the use of results that in some instances dated back several years. Since the Nurnberg codex, via the Helsinki declaration and until today, the principle of voluntary, informed consent has been an essential principle in medical research (219). However, this ethical framework was primarily developed for clinical research on humans, and it has been questioned whether it is necessary to apply equally strict rules regarding consent for research on data that have already been collected (220). Furthermore, it has been argued that only

including patients that are willing to participate in research, may introduce bias in the material (221), even if the evidence for this is limited (219). Whether or not research without informed consent is justifiable, depends on how it is performed, and potential harms and benefits to involved subjects.

Performing research on data that are already collected for clinical purposes, such as our TDM database, does not involve any direct effort, inconvenience or discomfort for the patient. The research can be performed and completed relatively quickly, so that the knowledge generated can be of direct benefit for the patients that have been included. One potential negative effect of not obtaining consent is that some patients may see the use of their data without their explicit consent as a breach of trust (219). We concluded that it was acceptable to perform the retrospective studies in this thesis without obtaining consent, which was approved by the Regional Committees for Medical and Health Research Ethics.

In the prospective, observational study, informed consent was obtained from each patient. This was collected by the treating physician, which is not optimal. To avoid patients feeling obliged to agree to participate because they are asked by their doctor, the patient should be approached by an independent person regarding consent. The risk would however, not be entirely eliminated, and such an approach would have large practical consequences. As the negative impact of participating in the study was considered minimal (mainly that the consultation would take a little longer), the suggested approach was found acceptable.

Data collected in our studies were de-identified before processing, but were still considered sensitive because of the possibility of re-identification. As such it was treated according to the hospital policy for sensitive information. As the prospective study included very few patients, we took measures to avoid identification of individuals, such as using approximate age and disease duration when publishing the results.

# 4. Results

**Paper I:** The study included 550 patients, aged 1 to 86 years. We found extensive interindividual pharmacokinetic variability of CLB, illustrated by a 100-fold variability in total (CLB+NCLB) C/D-ratio (0.03-3.29 (μmol/L)/mg). The CLB C/D-ratio was lower in young children (2-9 years) than in adults, reflecting higher clearance in this group. Several co-administered medications appeared to affect the metabolism of CLB. The NCLB/CLB ratio was seven in patients receiving monotherapy or comedication considered interaction neutral, but 200%-950% of this value in patients receiving stiripentol, phenytoin, carbamazepine, oxcarbazepine or eslicarbazepine acetate, phenobarbital or zonisamide. In patients receiving stiripentol, felbamate, phenytoin or zonisamide there was a high mean NCLB C/D ratio (130%-230% of mean in neutral group, 0.24 (μmol/L)/mg) and/or mean total C/D-ratio (160%-200% of neutral group, 0.29 (μmol/L)/mg). Patients receiving phenytoin, VPA, carbamazepine or phenobarbital had lower CLB C/D-ratios than those in the neutral group (20%-70% of mean in neutral group, 0.047 (μmol/L)/mg). NCLB/CLB, total and NCLB C/D ratios were high in 3 patients known to be CYP2C19 poor metabolizers (PMs).

The pharmacokinetic variability of CLB and NCLB in clinical practice was extensive and influenced by drug-drug interactions, age and pharmacogenetics, indicating that therapeutic drug monitoring can be valuable in patient management.

**Paper II:** In this study we describe the use of LCM in 124 children and adolescents in two Scandinavian countries. Median age was 15 years (range 2-17 years) and data on weight was available for 76 patients. Pharmacokinetic variability was demonstrated as the C/(D/kg) ratios ranged from 1.3 to 9.4 (μmol/L)/(mg/kg). Furthermore, this ratio was lower in young children compared to adolescents. The doses (median 300 mg/day) and resulting serum concentrations (median 18 μmol/L) were low or moderate for many of the patients. Polytherapy was

common, with 86% of patients concomitantly using 1-3 other AEDs. Use of LCM in children and adolescents has increased over the last few years in both Norway and Denmark. More than two thirds of patients (71%) continued treatment beyond one year, and all of those patients had serum concentrations within the defined reference range. One-year retention rates were higher in those not using other sodium channel blocking AEDs compared to those who did (82% vs 56%).

The observed retention rates suggest reasonable effectiveness of LCM in this patient group, and the demonstrated pharmacokinetic variability indicates the potential usefulness of TDM.

**Paper III:** Data from 643 non-pregnant women of childbearing age using VPA demonstrated significant pharmacokinetic variability, as measured by the 14-fold range in C/D ratios (0.11-1.52 (μmol/L)/mg). The variability was more pronounced at low doses (<700 mg/day), compared to higher doses. Mean dose and serum concentration of VPA in the included patients were 968 mg/day and 411 μmol/L, respectively. VPA was combined with other AEDs in 59% of patients, and C/D ratios were lower with concomitant use of carbamazepine. The demonstrated pharmacokinetic variability means that dose is a poor approximation of drug exposure, and therefore measurement of serum concentrations can be useful in developing more refined strategies for safer treatment for those women who need VPA to control their epilepsy.

**Paper IV:** The study demonstrated that the number of patients using GBP in Norway increased by 30% from 2014 to 2017 (32181 to 42675 users), with extensive use in the elderly. TDM-data from 120 patients showed a 22-fold pharmacokinetic variability in C/D ratios (0.0036-0.0800 (μmol/L)/mg). This ratio was elevated in elderly patients (≥65 years) compared to younger adults, and the majority of elderly used GBP for non-epilepsy

indications. A prospective study on TDM in patients using GBP for RLS found that in these patients, intake in the evening/night only was common due to nocturnal symptoms, and as such, drug fasting concentrations the following morning did not reflect concentrations at the time of required therapeutic effect. Despite this limitation, TDM was found useful in most patients; to support dosage increase or to evaluate adverse effects.

# 5. Discussion

## 5.1 Pharmacokinetic variability

We considered that the pharmacokinetic variability observed in the included studies was extensive. However, there is no clear definition of what should be considered extensive in this context, and the term is therefore open for interpretation. In the included studies the variability in C/D ratios ranged from approximately 7-fold for LCM in children and adolescents, to 100-fold for overall use of CLB. The dosing recommendations for LCM states that 200 mg/day is considered "initial therapeutic dose", which can be increased to a maximum dose of 600 mg/day in monotherapy or 400 mg/day as adjunctive therapy in patients weighing more than 50 kg (188). This indicates that the "therapeutic dose" is considered to vary 2-3-fold, and a similar variation is stated in the pr/kg dose for smaller patients. In light of this we consider a 7-fold variability in C/D ratio among children and adolescents to be extensive. In women of childbearing age using VPA, the observed variability means that some outliers using low doses still have high exposure to the drug.

When a drug is subject to extensive pharmacokinetic variability, it is difficult to anticipate exposure in the individual patient based on dose alone. Unless the drug in question has a very wide therapeutic index and both therapeutic benefit and adverse effects can be easily determined clinically, this indicates the potential usefulness of TDM. In clinical practice this means that in patients who do not attain and maintain seizure freedom (or other desired outcome) without adverse effects at "standard" doses, measurement of serum concentrations and clinical interpretation of the result can provide a tool to adjust and optimize therapy. Furthermore, in studies on efficacy and tolerability (and possibly also teratogenic effects), such measurements can help determine thresholds for effect and safety that can translate to more rational use. We did not examine whether TDM improves clinical outcome in any of the

included papers, which would need to be evaluated in properly designed and conducted studies. It has been suggested that documentation of the potential value of TDM of new AEDs is affected by this being considered unfavorable for the marketing of a new drug (57), indicating the importance of independent, post marketing studies.

### 5.1.1 Influence of gender (Paper I, II, IV)

None of the three studies that included both males and females (I, II and IV) found differences in C/D ratios between genders. Very few pregnant women were included in the studies, and the use of drugs only prescribed to males or females were rarely noted in the included patients. Considering that 30-40% of women of childbearing age in the Nordic countries use hormonal contraceptives (222), it is likely that there was underreporting of such use in our material. (The completeness of clinical data is further discussed in the section on methodological considerations).

### 5.1.2 Influence of age (Paper I, II, IV)

We observed an effect of age on the C/D ratios of CLB, LCM and GBP. While apparent oral clearance was increased in children taking CLB and LCM, it was reduced in elderly patients taking GBP, in line with pharmacokinetic changes occurring throughout life (77, 78, 117). The increase in clearance of LCM in young children is reflected by the recommended dosing per kilo being higher in the lower weight ranges (188). Whether the observed increase in C/D ratios of GBP in elderly patients was solely due to reduction in renal function in this group could not be determined from the available data. In all the included studies there was also pharmacokinetic variability within the different age groups, pointing to the influence of other factors as well.

### 5.1.3 Influence of comedication (Paper I-IV)

**Clobazam**

Concomitant use of a number of AEDs led to increases in NCLB/CLB ratios, as well as increases in total and NCLB C/D-ratios or decreases in CLB C/D-ratios. These demonstrated interactions were in line with the known induction of CYP3A4 and/or inhibition of CYP2C19 by carbamazepine, phenobarbital, phenytoin, stiripentol, felbamate, oxcarbazepine and eslicarbazepine acetate (120, 128, 174, 223-226). The main pathways for metabolism of CLB and NCLB are depicted in Figure 4, with the demonstrated effects of inducers/inhibitors indicated in the relevant metabolic step. The interactions with VPA and zonisamide are possibly also related to effects on CYP2C19 and/or 3A4. Although VPA has generally been considered a broad enzyme inhibitor (120), it has also been demonstrated *in vitro* to up-regulate CYP3A4 (227). Our data did not include measurement of free, unbound concentrations of CLB or NCLB, but in future research, it would be interesting to examine the potential of an interaction at the site of protein binding, as VPA, phenytoin, CLB and NCLB are all highly protein bound (81). According to the prescribing information CLB and NCLB are p-glycoprotein substrates, and interactions at this level are also possible (228).

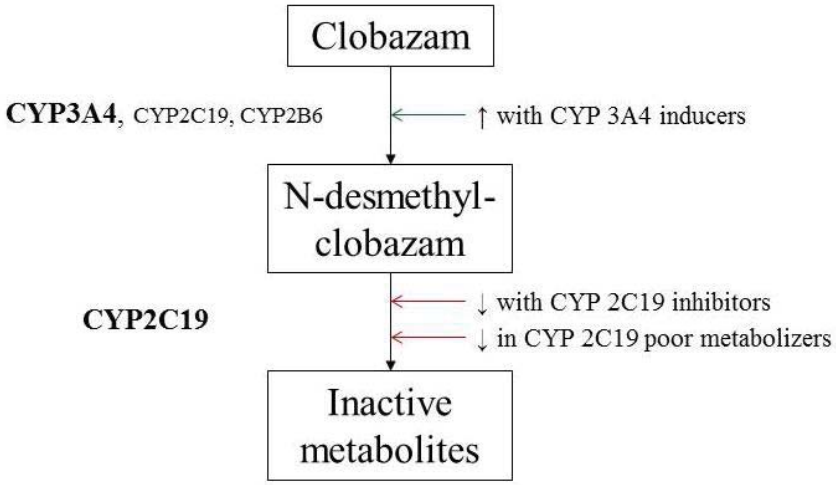


**Figure 4** – The main pathways for metabolism of clobazam and N-desmethylclobazam with demonstrated effects of inducers/inhibitors and pharmacogenetics. *CYP: Cytochrome P450.*

**Lacosamide**

We did not observe any changes in LCM C/D ratio with concomitant use of VPA, which is in line with previous studies (163-165, 170). Complete data were only available for three patients concomitantly using enzyme inducing AEDs (carbamazepine, phenytoin and/or phenobarbital), which did not allow for any conclusions to be drawn regarding interactions. It has been suggested that CYP2C19, CYP3A4 and CYP2C9 are involved in metabolism of LCM to its O-desmethyl metabolite (179). Furthermore, carbamazepine, phenobarbital and phenytoin are known inductors of CYP2C9 and CYP3A4 enzymes (120), and others have observed increased clearance and reduction in serum LCM concentrations by about 20-40% when combined with these drugs (163-165, 170). An even more pronounced effect (46%) was seen in children in another study (164).

In Paper II we also examined the potential pharmacodynamic interaction between LCM and other sodium channel blockers, by comparing one-year retention rates in patients using and not using such drugs in combination. In adults, studies have suggested decreased tolerability of LCM in combination with other sodium channel blocking drugs (229-232), but results regarding children have been conflicting (233, 234). We found a significantly higher retention rates in patients not concomitantly using other sodium channel blockers (82% vs 56%). This would suggest decreased tolerability with such combinations, alternatively a lack of efficacy in patients also using other drugs acting on the sodium channel.

**Valproate (valproic acid)**

A lower VPA C/D ratio was observed in patients concomitantly using carbamazepine, in line with previous findings (120, 171). As phenobarbital and phenytoin were used in a limited number of patients (10 and 3 patients respectively), interactions with these drugs could not be

reliably assessed. No significant effect of concomitantly used oxcarbazepine/eslicarbazepine acetate was observed.

**Gabapentin**

In line with the low interaction potential of GBP due to lack of hepatic metabolism (122), no changes in C/D ratios were detected with lamotrigine, levetiracetam or carbamazepine; the three most commonly used concomitant drugs. However, interactions can occur in pharmacokinetic processes other than metabolism, and in the summary of product characteristics of lamotrigine, a potential interaction through effect on the organic cation transporter 2 (OCT2) has been suggested (235). As stated, we did not observe any pharmacokinetic interaction between the two drugs in our material. It is worth noting that interactions between GBP and some antacids and analgesics have been reported previously (123). Even though many patients being prescribed GBP are elderly patients with pain syndromes, concomitant use of these groups of drugs was rarely reported in our study.

### 5.1.4 Influence of pharmacogenetics (Study I)

In Paper I we included data on 3 patients known to be CYP2C19 poor metabolizers (PMs), demonstrating high NCLB/CLB, total and NCLB C/D ratios. It is known that CYP2C19 PMs can experience an accumulation of NCLB and potential for adverse effects (187, 236). The effect of genotypes coding for functional CYP3A5 enzymes or increased function of CYP2C19 (ultrarapid metabolizers) on metabolism of clobazam is not established (174, 236). A recent review estimated that approximately 2% of Caucasians and 15% Asians are CYP2C19 PMs (237). With relatively low toxicity of NCLB, the availability of TDM services and a low proportion of patients expected to have PM genotypes, it is unlikely that routine *a priori* testing for such mutations in patients starting CLB will be justified in Norway. We do,

however, offer and recommend testing *a posteriori* when mutations are suspected from serum concentration measurements. In addition to the importance for dosing of CLB, the results will have implications for any other treatment with drugs metabolized by this enzyme.

VPA and LCM are also metabolized by enzymes that are subject to genetic variability (Table 2), however, genotypes were not known for patients included in Paper II and IV on these AEDs.

## 5.2 Use of the studied drugs

To place the findings from our studies in a wider context and to provide some perspective on the sample sizes, nation-wide data on prescription of the studied AEDs are presented. The total number of patients prescribed CLB, LCM, VPA and GBP in Norway in 2014 and 2018 is shown in Table 3. In the years immediately prior to the publication of Paper I, 2009-2014, on average 664 patients were prescribed CLB yearly in Norway. Since then the use has gradually increased to 934 users in 2018. In Paper II we demonstrated increased use of LCM from 2014 to 2017 in patients <20 years, and that more patients aged 5–14 years were prescribed LCM in 2017 compared with previous years. As the drug received EU approval for pediatric use in September 2017, we expected this number to increase further in 2018. While the number of users <10 years and between 15 and 19 years of age remained relatively stable from 2017 to 2018, the number of patients in the 10-14 year age group increased from 25 to 34. The overall use of LCM also increased from 2014 to 2018 (Table 3). The increase in number of users of GBP observed in Paper IV continued into 2018, when 46001 patients, including 18096 elderly, were prescribed the drug, compared to 42675 patients of whom 16562 were elderly in 2017. The continuing increase in the use of these drugs underpins the relevance of improving knowledge to help facilitate safe and efficacious use.

The number of users of VPA was not described in Paper III, but has decreased somewhat from 2014 (when EMA strengthened restrictions on the use of VPA (238)) to 2018 (when further measures to avoid valproate exposure in pregnancy were endorsed (176)). It is worth noting that there were still 1956 women aged 15-44 using the drug in 2018, even if this is somewhat fewer than the 2328 reported in 2014.

**Table 3** – Use of clobazam, lacosamide, valproate and gabapentin in Norway based on prescription data (239).

| | Clobazam | Lacosamide | Valproate | Gabapentin |
|---|---|---|---|---|
| **Users in Norway in 2014** | 751 | 500 | 15184 | 32181 |
| **Users in Norway in 2018** | 934 | 770 | 14988 | 46001 |

## 5.3 Retention rates (Paper II)

Retention rate is used as an outcome measure both in prospective and retrospective studies, and can be used to compare different treatments or to relate effectiveness to other factors. We found that 71% of patients continued LCM for more than one year, comparable to 65% and 73 % observed in other studies on LCM in children (233, 240). Neither of these studies included serum concentration measurements of LCM. In our study patients who discontinued LCM before one year had lower serum concentrations than those who continued treatment for more than one year. This is an interesting finding that should be further explored and related to clinical effect in future studies. Although not directly comparable due to differences in methodology and clinical use of the drugs, results from studies of other AEDs may provide some perspective on the magnitude of the observed retention rates. In adults with different types of epilepsy, retention rates at one year was found to be 45%-61% for topiramate, levetiracetam,

oxcarbazepine and zonisamide, 75% for lamotrigine and 83% for eslicarbazepine acetate (167, 241). In children with refractory epilepsy, retention rates of 37% for topiramate, 49% for levetiracetam, 51% for CLB, and 69% for lamotrigine have been observed (242, 243). This indicates reasonable effectiveness of LCM, as measured by one-year retention rates.

## 5.4 Therapeutic drug monitoring in restless legs syndrome (Paper IV)

In the prospective study of patients using GBP for RLS, most patients were noted to take the medication in the evening or at night only. Considering the short half-life of GBP (5-7 hours (200)), the serum concentration the next morning is therefore not representative of the concentration at the time of need for symptomatic relief. Sampling at night to measure concentrations that can be related to therapeutic effect is practically challenging in the outpatient setting, but may be feasible if self-sampling (e.g. of dried bloods spots) is available (244). The applications of TDM that were perceived useful by the treating physician were to evaluate dose-related adverse effects during the day or the likelihood of such effects occurring with a dose increase. Assessment of adherence or overuse can also be useful applications of TDM in this setting, especially when considering the concerns regarding abuse potential of GBP (178) or alternative analgesics. Interpretation of the measured serum concentration needs to take into accounts the dosing strategies, and can be complicated when the daily dose is distributed in an unequal fashion (245). It is therefore essential that sufficient clinical information is available when interpreting the results – both regarding therapeutic indication, dosing schemes, renal function and indication for TDM. Furthermore, it has been suggested that repeating serum concentration measurements of GBP to assess intrapatient variability can be useful when assessing side effects and symptom relief also in indications such as RLS or neuropathic pain (198).

## 5.5 Methodological considerations

All the included studies have strengths and limitations, some of which have already been discussed in the chapter on materials and methods. In the studies, data from serum concentration measurements were combined with clinical information (even if limited in some cases) and prescription trends, enabling a more complete picture to be drawn than if considering each type of data separately. A major strength of paper I was our ability to examine a complete national data set of serum concentration measurements of CLB and NCLB, and the 550 patients included reflect the complexity of clinical practice. In paper II data was sourced from two national epilepsy centers in Norway and Denmark, which enabled the inclusion of 124 children and adolescents.

Major limitations of the studies are related to the retrospective nature and completeness of clinical data, as clinical information primarily was retrieved from the accompanying TDM request form. In a recent study, we demonstrated good correspondence between information retrieved in such a manner regarding dose, steady-state conditions and comedication, and information from medical records at the National Center for Epilepsy in Norway (165). However, both inpatients and outpatients form the entire country were included in our studies, and doctors at other practices, clinics and hospitals may be less stringent in providing such information. In Study I, we observed that some patients in the neutral medication group had very high NCLB/CLB ratios (>25), which can indicate interactions with comedication not listed, or unknown CYP2C19 PM status (174). In paper II, data on 1-year retention rates of LCM were unfortunately only available for 63 patients, with unknown duration of treatment in the other patients. Furthermore, information on reason for discontinuation was not available. As patients were identified from the TDM database, early discontinuation, before serum concentration measurement, may result in overestimation of retention rates. In paper

III, it is likely that information on use of oral contraceptives was missing in a number of patients. In paper IV, many patients used GBP for other indications than epilepsy, and the requesting physicians may not be as familiar with the importance of a standardized sampling time and inclusion of relevant information.

Poor adherence cannot be controlled for in a retrospective and naturalistic setting, especially among outpatients. It is known that many patents with epilepsy take their medications differently to how it is prescribed (91, 149), and non-adherence is an important cause of hospitalizations (150). Furthermore, since the laboratory database and not medical records was used, the serum concentration measurements could not be matched with seizure control.

In the prospective study on patients using GBP for RLS in paper IV the sample size was small, but to the best of my knowledge, the use of TDM in this patient group has not previously been described. The evaluation of effect of GBP and usefulness of TDM were subjective opinions of the treating physician and should be interpreted accordingly.

Reflections regarding the statistical methods are presented in the Material and Methods section

## 5.6 Impact of the presented results

### 5.6.1 Paper I

CLB is an old AED, but few thorough pharmacokinetic studies in real-life have been performed by measuring both CLB and NCLB. The numerous drug-drug interactions demonstrated in Paper I are of great clinical importance, especially when considering that many patients using CLB have refractory epilepsies requiring treatment with multiple AEDs.

By awareness of such interactions, changes in serum concentration when altering relevant comedication can be monitored (by TDM) and compensated for through dose adjustments. Demonstrating the real life interaction potential of CLB in independent, post marketing studies is important in view of the claims by some that the drug has low potential for drug-drug interactions (246).

Most of the demonstrated drug-drug interactions resulted in changes involving NCLB, highlighting the importance of measuring this active metabolite as part of TDM of CLB. Indeed, we have been made aware that our study prompted another specialty laboratory in Europe to add measurement of NCLB to their TDM service of CLB. Furthermore, the study served to validate the usefulness of NCLB/CLB and the three C/D ratios in examining pharmacokinetic variability of CLB, as suggested by deLeon et al. (174). These ratios have since been used by other researchers to further study CLB in children (247). In our laboratory we have observed a continuing increase in the number of serum concentration measurements of CLB and NCLB performed over the last few years. The experience and reference material generated is also relevant to emerging treatments; for example the drug cannabidiol, which has recently been approved in the USA (248), can impair metabolism of CLB and in particular NCLB (249-251). This interaction is noteworthy because CLB is frequently used in epileptic encephalopathies for which CBD appears to be a promising new treatment (252).

### 5.6.2 Paper II

The study describes the initial experience with LCM in children and adolescents, and documents increasing use over the studied period. It is complementary to another study on LCM in primarily adult patients with refractory epilepsy performed at our hospital (165). The demonstrated pharmacokinetic variability indicates that serum concentration measurements

can aid in establishing the right dose for these patients. Furthermore, reasonable effectiveness in clinical practice is observed, especially when the drug is not combined with other sodium channel blockers. This knowledge can aid clinicians and decisions makers regarding the use and follow-up of a new drug in a vulnerable patient population. Additionally, in conducting this study we further consolidated an international collaboration between specialized hospitals and laboratories, and demonstrated how this can help generate knowledge regarding treatment in small patient groups.

### 5.6.3 Paper III

In 2018, new restrictions on the use of VPA in women were issued by the European Medicines Agency (EMA). Use of VPA to treat migraine or bipolar disorder was banned during pregnancy, and the treatment of epilepsy only allowed if there is no other effective treatment available. In addition, all women of childbearing potential have to meet the condition of a new pregnancy prevention program in order to use VPA (176). However, in a select group of women, VPA may still have a place in treatment of their epilepsy (196).

In population-based studies the risks of adverse fetal effects have been related to maternal dose, but a safe upper dose has not been established (196). We highlighted that serum concentrations among women of childbearing age are unpredictable, and may still be high in some women using low doses of VPA. Furthermore, some patients using high doses of VPA have low serum concentrations, and a reduction in dose may not be possible without loss of seizure control. Hence, measurement of serum concentrations of VPA would provide a better estimate of exposure before and at conception, both in studies and in clinical practice. After conducting this study, we went on to examine measurements of VPA during pregnancy, finding that because of pregnancy induced pharmacokinetic changes, free, unbound

concentrations should be measured for better safety evaluation in both the mother and fetus (113). The results of both these papers call for further studies to elucidate relationships between actual exposure and outcomes.

### 5.6.4 Paper IV

The paper highlights the widespread use of GBP in the elderly and in non-epilepsy conditions, and adds to the limited number of real-life pharmacokinetic studies in these important patient groups. It was inspired by a previous project, where we documented our experience with TDM and gender aspects of GBP and pregabalin from 2009 to 2013 (161). The demonstrated pharmacokinetic variability, combined with the extensive use in the elderly who often have reduced renal function, points to potential usefulness of TDM.

In the study on patients with RLS we were only able to include 10 patients, reflecting the difficulties with performing prospective studies in such a setting. Nevertheless, the paper describes potential uses and challenges in the use of TDM when GBP is prescribed for non-epilepsy conditions such as RLS, which has not previously been studied. It can also be useful as a pilot relevant to larger studies on patients using AEDs for pain conditions. Furthermore, the paper emphasizes the importance of access to sufficient clinical information regarding dosing and indication when interpreting serum concentration measurements.

# 6. Conclusions

In the present thesis, the following main findings have been demonstrated:

- Extensive pharmacokinetic variability in clinical practice for CLB, LCM, VPA and GBP (Paper I-IV)
- No effect of gender on C/D ratios of CLB, LCM or GBP (Paper I, II, IV)
- Increased clearance of CLB and LCM in young children and decreased clearance of GBP in the elderly (Paper I, II, IV)
- Pharmacokinetic interactions between CLB and numerous AEDs used concomitantly in clinical practice, and between VPA and carbamazepine (Paper I and III)
- No pharmacokinetic interactions between LCM and GBP and the most used concomitant AEDs, although too few patents used LCM and enzyme-inducers to assess this potential interaction (Paper II and IV)
- Reasonable effectiveness, as measured by 1-year retention rates of LCM in children and adolescents, but this decreased with concomitant use of other sodium channel blockers (Paper II)
- Increased use of CLB, LCM and GBP in Norway over the last few years (Paper I, II, IV)
- Perceived usefulness of TDM in a group of patients using GBP for RLS, in spite of practical challenges related to such practice (Paper IV)

The knowledge generated in the included studies can be useful in individualizing and optimizing treatment, and indicates usefulness of TDM in special patient groups and challenging treatment situations. Furthermore, including serum concentrations in future research will provide more comprehensive insights when examining efficacy and tolerability in epilepsy and in other indications, and when studying teratogenic effects of AEDs.

# 7. Future perspectives

This thesis is part of an ongoing effort to study AED use in special patient groups and refractory epilepsies, as well as to document the clinical use and pharmacokinetic variability of the newest AEDs. As a specialized laboratory we have an extensive repertoire of AED analyzes, and a close collaboration with clinicians at the National Center for Epilepsy. To provide the best possible service to patients with epilepsy, it is essential that research and routine goes hand-in-hand. Through our research we aim to improve our own TDM service, as well as to contribute knowledge to others in our field, with the ultimate goal to facilitate safer and more efficacious treatment with AEDs in vulnerable patient groups. Although this thesis has contributed some pieces in the puzzle, many questions remain to be elucidated.

The impact of TDM on clinical outcomes was not examined in the current projects. There have been calls for randomized controlled trials determining such effects; however this is associated with a number of challenges. In addition to practicalities regarding design and conduction, it must be considered whether true equipoise exists for the indication for TDM that is to be studied. Furthermore, randomized controlled trials are not without flaws and bias (253), and alternative designs should also be considered.

Real-life studies examining the clinical efficacy and tolerability of AEDs in relation to serum concentrations are of great value in improving TDM services. To address the limitation of incomplete clinical data in the included studies, we are establishing more projects in close collaboration with clinicians treating patients with epilepsy. The relevance of biochemical markers to monitor adverse effects, and pharmacokinetic interactions at the level of protein binding for highly protein-bound AEDs are other planned research topics that will further build on the knowledge generated in this thesis.

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# 9. Errata

**Paper I**: In the methods section on page 351 the measuring range for NLCB should have read 0.3-30 µmol/L, not 3.0-30 µmol/L.

P. 353 «The «neutral» group contained 171 patients.» - should have read «The «neutral» group contained 170 patients.»

Legend to figure 3 on page 355 "Mean NCLB/CLB ratio in the neutral group = 7.23 (n = 171)" – should have read "Mean NCLB/CLB ratio in the neutral group = 6.96 (n = 170)".

The number of patients in the group (170) is correctly identified in Table 1, p 352, and the correct NCLB/CLB ratio was used in the calculations and when generating Figure 3. The errors therefore did not impact on the discussion or the conclusions.

**Paper I and III:** Data from the TDM database are described as anonymized, but this should have read de-identified or coded.

**Paper III:** In the discussion it is stated that "Other AEDs were used in a limited number of patients, and other drug-drug interactions could not be assessed." This sentence is imprecise, and should have read: "As phenobarbital and phenytoin were used in a limited number of patients (10 and 3 patients respectively), interactions with these drugs could not be reliably assessed."

In Table 1 C/D ratio for patients using <700 mg/day is stated to range from 0.16-1.60, this should have read 0.16-1.52.

**Paper I-IV:** Where it is stated that results are presented as (range) it should have read (minimum – maximum).

# 10. Papers I-IV

IV

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ORIGINAL ARTICLE

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# Therapeutic drug monitoring of gabapentin in various indications

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**Objectives:** Gabapentin has been increasingly used in various indications in recent years. Despite variable pharmacokinetics, therapeutic drug monitoring (TDM) is scarcely described in other indications than epilepsy. The aim of the study was to investigate the use and pharmacokinetic variability of gabapentin in epilepsy and non-epilepsy indications and to further evaluate the use of TDM in patients with restless legs syndrome (RLS).

**Materials & Methods:** Population-based data from the Norwegian Prescription Database, retrospective TDM data from the section for Clinical Pharmacology, the National Center for Epilepsy, Norway, and prospective observational data on patients with RLS were used.

**Results:** The use of gabapentin increased by 30% from 2014 to 2017 (32 181 to 42 675 users). TDM data from 120 patients showed a 22-fold pharmacokinetic variability in concentration/dose ratios, and this ratio was elevated in elderly patients (≥65 years). The majority of elderly used gabapentin for non-epilepsy indications. In patients with RLS, intake in the evening/night only was common due to nocturnal symptoms, in contrast to regular dosing regimens in epilepsy. Thus, drug fasting concentrations do not reflect concentrations at the time of required therapeutic effect. TDM was still found useful in most patients to support dosage increase or evaluate adverse effects.

**Conclusion:** Due to extensive pharmacokinetic variability, TDM can benefit patients using gabapentin. Challenges with applying TDM in new indications such as RLS include different dosage regimens and consequently different interpretation of serum concentrations. Thus, TDM should be requested on clear clinical grounds and the service tailored according to the therapeutic indication.

KEYWORDS
elderly, epilepsy, gabapentin, pain, restless legs syndrome, therapeutic drug monitoring (TDM)

## 1 | INTRODUCTION

The approved indications for gabapentin (GBP) in Europe are epilepsy and peripheral neuropathic pain,[1] and this use is well established in international guidelines.[2,3] In Norway, the use in neuropathic pain greatly surpasses that in epilepsy and has been increasing over the last few years, especially in the elderly.[4,5] GBP has also been used off-label in a number of other conditions, such as restless legs syndrome (RLS), fibromyalgia, trigeminal neuralgia, multiple sclerosis, headache, anxiety, post-operative pain, nausea, pruritus, chronic

cough, and alcohol use disorders.[6-8] The drug will be reclassified as class C controlled substances in the UK as of April 2019 due to concerns related to misuse.[9]

Gabapentin has variable absorption and nonlinear pharmacokinetics, which leads to large interindividual differences in dose-to-plasma concentrations and makes it an excellent candidate for therapeutic drug monitoring (TDM).[7,10,11] It is not protein bound or metabolized, but rather cleared entirely by renal elimination, with an elimination half-life of 5-7 hours in healthy subjects.[10,12] The interaction potential is therefore seen as low, even though interactions with some antacids and analgesics have been reported.[13,14]

Therapeutic drug monitoring is a commonly used tool to optimize treatment of epilepsy[7] and has been used as a part of the comprehensive care approach in epilepsy for 50 years.[15] The reference range for GBP in epilepsy in Norway is 20-120 µmol/L under drug fasting conditions at steady state,[16] similar to international recommendations.[7] The potential benefits of TDM in chronic pain have not been fully realized,[17] despite the widespread use of drugs such as GBP for this indication. Even though treatment with GBP has been found to have clinical effect in neuropathic pain and RLS,[18,19] real-life data on serum concentrations and effect are limited. As there is no established therapeutic or reference range for GBP in non-epilepsy indications, the concept of individual therapeutic concentrations, where the patient serves as his/her own control over time, is particularly useful.[20] Furthermore, serum concentration measurements to investigate variable adherence, misuse/diversion, unexpected pharmacokinetics, suspected toxicity, or safety of a dose increase are independent of therapeutic indication.

RLS is a disorder characterized by an urge to move the legs, usually accompanied or caused by uncomfortable and unpleasant sensations in the legs, which occurs at rest, is worse in evening or night and is relieved by movement.[21] It is relatively common and can cause great distress and disturbance of sleep.[22]

The aim of this study was to investigate the use and pharmacokinetic variability of GBP in epilepsy and non-epilepsy indications and to further evaluate the use of TDM in a group of patients with RLS.

## 2 | MATERIAL AND METHODS

### 2.1 | Population-based data on the use of gabapentin

Data on nationwide use of GBP were collected from the Norwegian prescription database and population data from Statistics Norway.[23,24]

### 2.2 | Retrospective TDM data

Patients who had serum concentrations of GBP measured from 2014-2017 were retrospectively identified from the TDM database at the National Center for Epilepsy (SSE), Oslo University Hospital, Norway. Patients with at least one serum concentration measurement were included. Our database contained a total of 462 samples from 285 patients. Where more than one serum concentration measurement was available for a patient, the latest measurement with complete data was selected. Additional clinical data were listed on the request form for some of the patients. Samples were excluded if under the lower limit of quantification and either requested by mistake (n = 3), intake without prescription was suspected (n = 1), there was information on acute intoxication and no information on therapeutic use (n = 3), or where no further information was available (n = 15) (Figure 1). Standard procedure in Norway is to perform

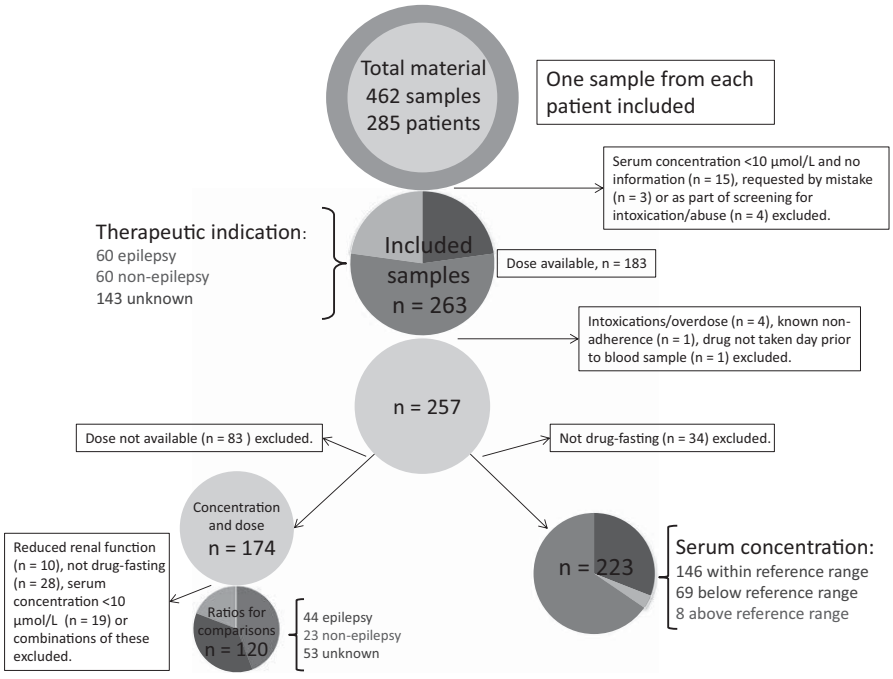


**FIGURE 1** Overview of included patients/samples

TDM on samples drawn drug fasting (directly before the intake of the next dose) in the morning at assumed steady state. Samples were considered not to be drug fasting if it was indicated on the request form that the sample was not collected under such conditions, or if there was information on intake of GBP less than 8 hours before the sample time. When a therapeutic indication for GBP was not listed on the request form, we extrapolated it to be epilepsy when other antiepileptic drugs (AEDs) were used concomitantly, except for carbamazepine or lamotrigine, which are commonly used in non-epilepsy indications. Non-epilepsy indication was considered most likely if concomitant medication or clinical information indicated the presence of pain. When examining the effect of age on pharmacokinetics, the patients were divided into three groups: <18, 18-64, and ≥65 years of age. To explore whether the most commonly used AEDs in the included patients affected the pharmacokinetics of GBP, we compared concentration/dose (C/D) ratios in patients using the drug in question (lamotrigine, levetiracetam, or carbamazepine) to all other included patients.

## 2.3 | Drug analysis

The analyses were routine measurements at the Section for Clinical Pharmacology, The National Center for Epilepsy, Oslo University Hospital as measured by HPLC-UV (Dionex, HPLC-system). The method is a modified method for quantification of pregabalin[25] and is subject to monthly, international proficiency testing.

## 2.4 | Prospective observational study on patients with RLS

To examine the use of TDM in patients with RLS, we conducted an open, prospective, observational study including patients attending

**TABLE 1** Patient characteristics and results from therapeutic drug monitoring of gabapentin

| | |
|---|---|
| All included patients (n = 263) | Gender: 163 female, 100 male<br>Age: Median 55 y, range 5-90 y.<br>Serum concentration: Median 30 μmol/L, range <10-279 μmol/L.<br>Prescribed daily dose, available in 183 patients:<br>All 183 patients: Median 1500 mg, range 43-6000 mg<br>Patients with epilepsy, n = 51: Median 1800 mg, range 200-4200 mg<br>Patients with other indication, n = 45: Median 1500 mg, range 43-6000 mg |
| Clinical indications for the use of gabapentin | Epilepsy (n = 60), non-epilepsy (n = 60)<br>Non-epilepsy indications include: Peripheral neuropathic pain, headache, multiple sclerosis, other pain syndromes, restless legs syndrome, irretraceable cough, pruritus, and rare diseases/syndromes |
| Reason for serum concentration measurements on the request forms, available in 75 of 263 patients. | Routine monitoring (n = 55)<br>Adverse effects (n = 2)<br>Acute intoxications/suspected overdose or overuse (n = 6)<br>Dose change (n = 8)<br>Suspected drug-drug interactions (n = 1)<br>Therapy failure (n = 9)<br>(Or combinations of these) |
| C/D ratio general considerations (n = 120) | 78 female, 42 male<br>Age: Median 53 y, range 18-88 y.<br>Serum concentration: Median 28 μmol/L, range 10-103 μmol/L<br>Prescribed dose: Median 1800 mg, range 200-5400 mg<br>C/D ratios:<br>Mean 0.021 (SD 0.015) μmol/L/mg<br>Median 0.0167 (range 0.0036-0.0800) μmol/L/mg |
| C/D ratio and age | Elderly (n = 35): Mean 0.028 (SD 0.017) μmol/L/mg<br>Adults <65 y (n = 85): Mean 0.018 (SD 0.012) μmol/L/mg<br>($P < 0.002$) |
| C/D ratio and gender | Females (n = 78): Mean 0.021 (SD 0.013) μmol/L/mg<br>Males (n = 42): Mean 0.021 (SD 0.018) μmol/L/mg<br>No difference in mean C/D ratio |
| C/D ratio and comedication | Mean C/D ratio and (SD) in patients using vs not concomitantly using:<br>Lamotrigine (n = 20/100): 0.024/0.020 (0.015/0.015) μmol/L/mg<br>Levetiracetam (n = 14/106): 0.022/0.021 (0.015/0.015) μmol/L/mg<br>Carbamazepine (n = 9/111): 0.021/0.021 (0.012/0.015) μmol/L/mg<br>No statistically significant differences |
| C/D ratio and clinical indication | Epilepsy (n = 44): Mean 0.021 (SD 0.015) μmol/L/mg<br>Other indications (n = 23): Mean 0.024 (SD 0.017) μmol/L/mg<br>No statistically significant differences |

C/D ratio, concentration/dose ratio; SD, standard deviation

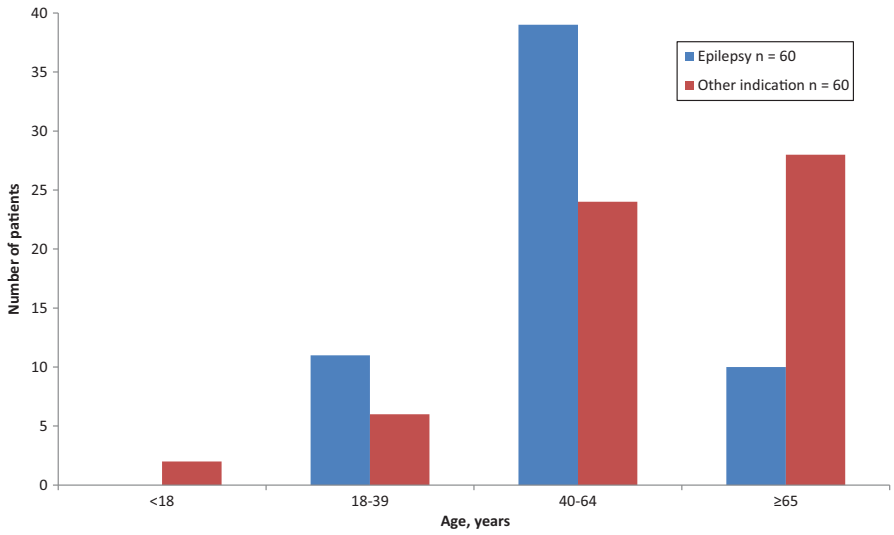


**FIGURE 2** Age distribution of patients using gabapentin for epilepsy and non-epilepsy indications, based on therapeutic drug monitoring data in Norway

Sandvika Neurocenter. Patients were invited to participate if they were treated with GPB for RLS. Informed consent was obtained, and the treating physician collected information in a predefined questionnaire on duration of illness, effect and adverse effects of GBP on a Likert scale, score on the International Restless Legs Scale (IRLS) (0-40 points),[26] concomitant and past treatment, comorbidities, and serum concentrations of GBP, during two separate consultations. Furthermore, the physician provided comments on how serum concentration measurements were used in the management of the patient and made an evaluation of the usefulness of TDM in each case. The study was approved by the Regional Ethics Committee.

### 2.5 | Calculations

Serum concentrations, doses, and C/D ratios were calculated as means with standard deviations (SD) or medians with minimum-maximum range to express variability. For statistical analyses, IBM SPSS Statistics version 25 (SPSS Inc, Chicago, IL, USA) was used. Comparisons were performed by the non-parametric Mann-Whitney rank sum test for data that was not normally distributed. For normally distributed data, the Students' two-sided *t* test with unequal variance was used to compare means. *P*-values of <0.05 were considered statistically significant for all analyses.

## 3 | RESULTS

### 3.1 | Use of GBP in Norway

According to the Norwegian prescription database (NorPD), 42 675 patients (25 670 women and 17 005 men) in Norway were prescribed GBP in 2017, representing 8.1 users per 1000 inhabitants. Of these, 39% were elderly patients over the age of 65 (n = 16 562); 18.5/1000 inhabitants in this age group. As there were 32 181 users in 2014, including 12 256 elderly, there had been an increase of 33% in number of patients and 35% in number of elderly patients being prescribed GBP over the period.

### 3.2 | Patients and clinical characteristics for retrospective TDM data

Samples from 263 patients were included. Patient characteristics, prescribed doses, clinical indication, measured serum concentrations, and reason for such measurements are presented in Table 1. Epilepsy was the listed (n = 30) or extrapolated (n = 30) indication in 60 patients, and non-epilepsy indications in 60 patients (47 listed, 13 extrapolated). The age distributions in these two groups are shown in Figure 2. Patients were prescribed a wide range of daily doses of GBP (Table 1), with no statistically significant difference between patients using the drug in epilepsy (n = 51 patients with known dose) or other indications (n = 45).

Excluding patients with known non-adherence, suspected acute intoxications and samples not taken drug fasting, 146 of the remaining 223 patients (65%) had serum concentrations between 20-120 µmol/L, that is, within the recommended reference range, while 69 patients (31%) had concentrations below the lower limit of this range (Figure 1). GBP was used in combination with 14 different AEDs, various analgesics and other medicines. The most common AEDs were lamotrigine (n = 28), levetiracetam (n = 17), and carbamazepine (n = 15).

### 3.3 | Pharmacokinetic variability from retrospective TDM data

The prescribed dose of GBP was available for 183 patients. In nine patients, there was information on intoxication, overdose or suspected overuse, known non-adherence or presence of a disease/syndrome that was considered to possibly affect the pharmacokinetics of GBP. The daily dose and measured serum concentrations of GBP in the remaining 174 patients are shown in Figure 3A. Reduced renal function or renal failure was known to be present in 10 patients according to the request form. In 28 patients, there was information that the sample had been collected less than 8 hours after intake of GBP or it had been indicated on the request form that the sample was not drug fasting. One of these patients also had reduced renal function. Figure 3B depicts the dose and serum concentrations in

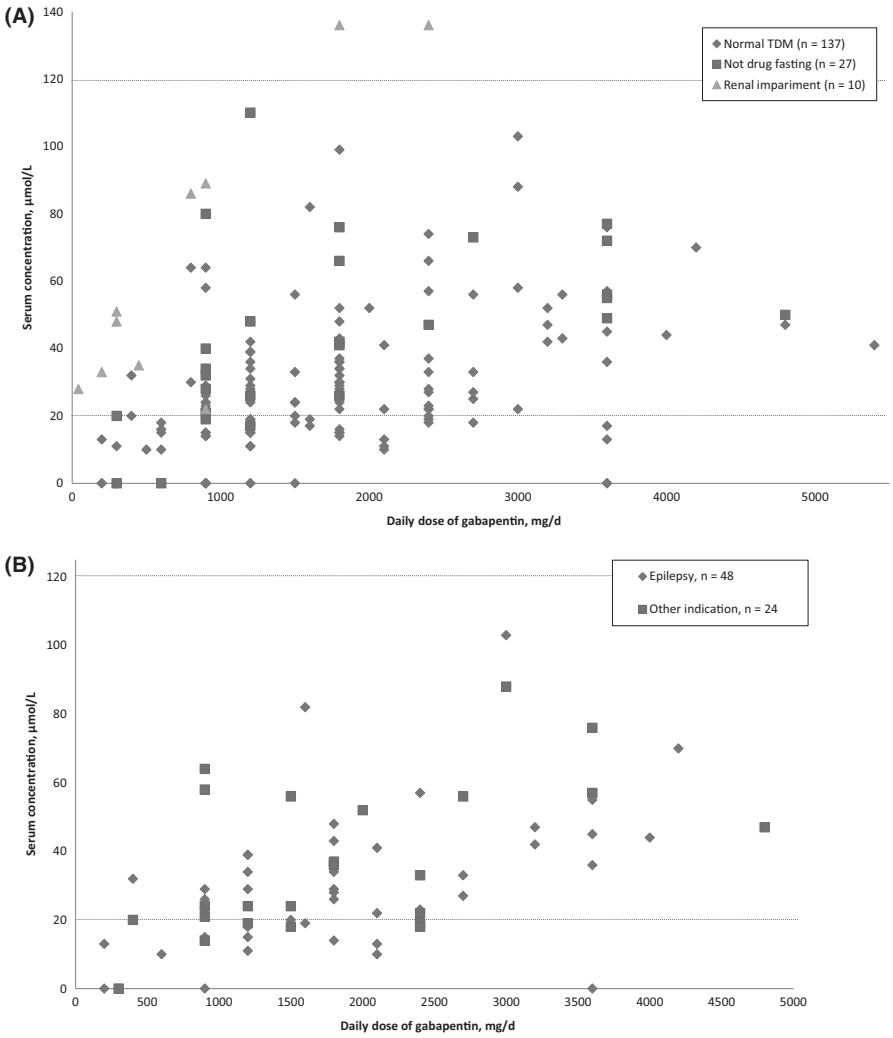


**FIGURE 3** A and B, Doses and serum concentrations of gabapentin in patients based on therapeutic drug monitoring data in Norway Patients with known non-compliance, suspected overuse or acute intoxications are excluded. Serum concentrations below the limit of quantification (10 µmol/L) were set to 0 for illustration purposes. The reference range, 20-120 µmol/L, is indicated in dotted lines. A, Doses and serum concentrations (n = 174) in "normal" samples (diamonds), samples known not to be drug fasting (squares) and from patients with known renal impairment (triangles). B, Doses and serum concentrations (n = 72) in patients with epilepsy (diamonds) or other indications (squares) for gabapentin

patients with known indication (epilepsy, n = 48 and other indications, n = 24) after excluding these patients.

In addition to the patients with known impaired renal function and samples known not to be drug fasting, results below the limit of quantification were excluded from considerations of C/D ratios, leaving 120 patients for such calculations (Figure 1). Patient characteristics, prescribed doses, measured serum concentrations, and C/D ratios for these patients are presented in Table 1. The C/D ratio ranged from 0.0036 to 0.0800 µmol/L/mg, that is, a 22-fold variability. The C/D ratio was significantly higher in elderly patients, compared to adults <65 years. No patients <18 years were available for comparisons of C/D ratios. There were no statistically significant differences in C/D ratio between patients using and not using the three most commonly used AEDs, between males and females or between patients using GBP for epilepsy and other indications (Table 1).

### 3.4 | Results from prospective observational study on patients with RLS

Clinical data and serum concentrations of GBP for 10 patients with RLS are presented in Table 2. A comment/evaluation from the treating physician regarding how serum concentration measurements were used in the management of the patient is included in the table. Most patients had suffered from RLS for several years, and the median IRLS score was 23 at the first (n = 10) and 19 at the second consultation (n = 8). Patients used variable doses of GBP, ranging from 100 to 2700 mg per day, but most patients took the medication in the afternoon and evening only. Six of the 10 patients were perceived to have good or excellent effect of gabapentin, while three had some effect and one none. Six patients had no experience of adverse effects, but among those who did sedation, tiredness, and unsteadiness were most commonly reported. Most of the measured serum concentrations were low compared to the reference range for treatment of epilepsy. The treating physician found that serum concentration measurement of GBP was useful in the management in most patients either to support a dose increase or to evaluate adverse effects.

## 4 | DISCUSSION

The present results demonstrate an increased use of GBP in Norway, with extensive use in the elderly. It demonstrates wide

**TABLE 2** Clinical details and use of therapeutic drug monitoring of gabapentin in patients with restless legs syndrome

| Patient number, gender, approximate age and duration of RLS | Consultation number, elapsed time | Dose gabapentin, mg/day | Serum concentration, µmol/L | IRLS score (0-40) | Effect | Adverse effects | Other treatment for RLS | Comment from treating physician |
|---|---|---|---|---|---|---|---|---|
| 1, male, 80 y, RLS for 1-5 y | 1 | 200 evening | <10 | 14 | Excellent | None | Ropinirole 0.5 mg | Patient had himself reduced the gabapentin dose between consultations, but due to decrease in effect, it was increased back to 200 mg at 2nd consultation. |
| | 2:23 wk | 100 evening | <10 | 9 | Good | Sometimes: tiredness | Ropinirole 0.5 mg | |
| 2, male, 70 y, RLS for >10 y | 1 | 300 afternoon 300 evening | 12 | 5 | Excellent | Sometimes: tiredness | Pramipexole 0.18 mg | Good effect of gabapentin, low serum concentrations gives room to increase dose with the view to wean pramipexole. Gabapentin dose is increased to 900 mg. |
| | 2:23 wk | 300 afternoon 300 evening | 21 | 6 | Excellent | None | Pramipexole 0.54 mg, to be weaned | |
| 3, male, 70 y, RLS for 1-5 y, mild renal failure | 1 | 800 evening | 62 | 23 | Some | None | Ropinirole 2 mg | Gabapentin dose was increased to 1200 mg (400 + 800), but because of increased tiredness, it was reduced back to 800 mg (400 + 400). |
| | 2:14 wk | 400 afternoon 400 evening | 86 | 17 | Some | Sometimes: tiredness | Ropinirole 2 mg, reduced to 1 mg. | |
| 4, female, 70 y, RLS for >10 y | 1 | 2700 evening | 73[a] | 24 | Some | Often: sedation | Ropinirole 3 mg, tramadol 400 mg | Because of worsening symptoms from RLS the dose of gabapentin was increased to 3000 mg at the 2nd consultation, with support in the results from the serum concentration measurement. |
| | 2:50 wk | 2700 evening | 42 | 30 | Some | Often: sedation | Ropinirole 2.5 mg, tramadol 300 mg | |
| 5, female, 40 y, RLS for >10 y | 1 | 1200 evening | 35 | 35 | Some | None | | Gabapentin was stopped between consultations because of adverse effects (dizziness, nausea, and sedation). |
| | 2:9 wk | 0 | | 25 | | | Oxycodone/naloxone 5/2.5 mg | |
| 6, female, 50 y, RLS for 1-5 y | 1 | 600 evening | <5 | 23 | Good | None | Pramipexole 0.18 mg, magnesium | Good effect with low doses of gabapentin. No particular benefit from the measurement of serum concentrations. |
| | 2:15 wk | 600 evening | <5 | | Good | None | Pramipexole 0.18-withdrawn | |
| 7, male, 60 y, RLS for >10 y | 1 | 600 afternoon 600 evening | 14 | 23 | Some | None | Pramipexole 0.63 mg | Gabapentin dose was further increased to 1800 mg, with support in low serum concentrations. |
| | 2:25 wk | 600 afternoon 900 evening | 24 | 21 | Good | None | Pramipexole 0.72 mg | |
| 8, female, 50 y, RLS for 6-10 y | 1 | 600 morning 600 afternoon 1200 evening | 22 | 16 | Good | None | | Serum concentration measurement supported dose increase, which resulted in good effect. |
| | 2:14 wk | 600 morning 600 afternoon 1500 evening | | | Excellent | None | | |

*(Continues)*

**TABLE 2** (Continued)

| Patient number, gender, approximate age and duration of RLS | Consultation number, elapsed time | Dose gabapentin, mg/day | Serum concentration, µmol/L | IRLS score (0-40) | Effect | Adverse effects | Other treatment for RLS | Comment from treating physician |
|---|---|---|---|---|---|---|---|---|
| 9, female, 80 y, RLS for >10 y | 1 | 600 morning<br>900 afternoon<br>600 evening | 41 | 24 | None | Sometimes: tiredness, unsteadiness | Pramipexole 0.18 mg, magnesium | Gabapentin dose further increased to 2700 mg. Serum concentration measurement useful to support increase in doses. |
| | 2:12 wk | 600 morning<br>900 afternoon<br>900 evening | 33 | 24 | Some | Sometimes: memory impairment, unsteadiness | Pramipexole 0.36 mg, magnesium | |
| 10, male, 70 y, RLS for 6-10 y | 1 | 600 evening | 12 | 14 | Excellent | None | Pramipexole 0.54 mg | Serum concentration measurement useful to support dose increase. Gabapentin dose increased further to 1200 mg. |
| | 2:14 wk | 900 evening | | 14 | Excellent | None | Pramipexole 0.18 mg, fentanyl patch | |

Age was rounded to closet decade. Duration of RLS was grouped as <1, 1-5, 6-10 or >10 y. Serum concentrations under the limit of quantification are reported as <5 or <10 µmol/L depending on performing laboratory.
RLS, restless legs syndrome; IRLS, International Restless Legs Scale.
[a]Sample, not drug fasting.

pharmacokinetic variability and increased C/D ratio in patients ≥65 years of age. Furthermore, the prospective study of patients with RLS highlights opportunities and challenges related to the use of TDM in non-epilepsy indications.

### 4.1 | Use of GBP in Norway

Data from the Norwegian prescription database show that the use of GBP increased by more than 30% from 2014 to 2017. The use of GBP in Norway was shown to gradually increase from 2007 to 2015,[4,5] due to an increase in use in neuropathic pain.[4] Increased use has also been demonstrated internationally, with a recent study from United Kingdom finding a large increase in the rate of patients newly treated with GBP from 2007 to 2017.[27]

### 4.2 | Pharmacokinetic variability from retrospective TDM data

We found extensive pharmacokinetic variability among the included patients as demonstrated by the 22-fold range in C/D ratios, in line with the known variability in bioavailability,[11] the effect of renal function on clearance [28] and previous findings from clinical practice.[29]

The observation that the C/D ratio was higher in elderly patients (≥65 years) also confirms previous findings.[29,30] It has been suggested that changes in renal function are responsible for age-related changes in GBP pharmacokinetics,[28] but as we did not have information on renal function at the time of serum concentration measurement, we could not examine this.

The interaction potential of GBP is generally considered low.[14] The lamotrigine Summary of Product Characteristics contains advice that co-administering lamotrigine and organic cation transporter 2 (OCT2) substrates such as GBP, can cause increased plasma levels because lamotrigine has been found to inhibit OCT2 in vitro.[31] However, a possible role of OCT2 in GBP excretion is uncertain.[32,33] We found no statistical difference in the C/D ratio of GBP in patient using and not using lamotrigine.

### 4.3 | Use of TDM for GBP in various indications

Some studies have examined the concentration-response relationships of antidepressant analgesia in chronic pain,[17,34] and reference ranges for the use of carbamazepine in neuralgias have been suggested,[35] but no data are available for GBP used in the treatment of neuropathic, or other pain syndromes. Information on the request forms in our study described the use of GBP in a wide range of clinical indications, with significant off-label use. Unfortunately, the indication was unknown in most patients. Even though the large majority of patients are being prescribed GBP for non-epilepsy indications,[4] we received the same number of requests for TDM in patients with epilepsy vs other indications. This might reflect that since we are serving the National Center for Epilepsy, we receive the majority of samples from patients with epilepsy, but it might also reflect that the use of TDM is limited in other indications. The finding that in the elderly

most samples received was for non-epilepsy indications might reflect the increased use of GBP in the elderly for pain indications.[4]

### 4.4 | Clinical experience from TDM in patients with RLS using GBP

Therapies for RLS have significant limitations, such as augmentation with dopaminergic drugs and dependence and tolerance with opioids.[19] Even though GBP has been found in reviews to be beneficial in RLS,[19] the use of off-label treatment puts an additional responsibility on the prescriber. TDM can potentially be a tool to optimize safe treatment.

A recent review concluded with doses of 800 mg being efficacious for the treatment of RLS,[19] which is lower than what is considered the effective dosing range in epilepsy (900 to 3600 mg/day).[1] The doses of GBP used by the included patients were highly variable, and both patients using the medication for RLS and epilepsy used doses outside these recommendations (Table 1 and 2, Figure 3). Most of the patients with RLS in our study took the medication in the afternoon and evening only, which reflects the need for symptomatic relief mainly at night. Because of the short half-life of GBP (5-7 hours[10]), there will be large fluctuations in the serum concentrations over the dosing interval when the drug is used only once or twice per day. Therefore, the serum concentration measured the next morning will not reflect the concentration at the time of symptoms. Furthermore, concentrations that may have resulted in adverse effects such as tiredness or dizziness during the day may be tolerable at night. The dosing strategies are thus different from that used in prophylactic treatment of epilepsy, where GBP is taken three times a day to ensure a steady state with smaller fluctuations in serum concentrations. This will then minimize the risk of adverse effects due to high concentrations, while still enabling adequate serum concentration at all times to avoid breakthrough seizures, as measured by the through concentration, drug fasting in the morning.[7,15] For patients with RLS, measuring serum concentrations at the time of maximal symptoms to establish and evaluate therapeutic levels is an option, but this is practically challenging as self-sampling is not available. The serum concentrations drawn in the morning can still be useful as an aid in evaluating tolerability or when considering whether a dose increase would put the patient at risk of adverse effects during the day, as well as to assess adherence and/or overuse.

In this study, serum concentrations were used to evaluate adverse effects in patients 3, 5, and 9 and to support the appropriateness of a dose increase in patients 2-4 and 7-10. For the latter, serum concentration measurement appears to be particularly relevant in patient 3, who had a mild degree of renal impairment, and in patients 4, 8, and 9, where doses were already high. A practical implication of this study is that the lower limit of quantification for GBP in serum samples will be lowered in our laboratory, as it will be of benefit to quantify even lower concentrations in these patients. Furthermore, it is important that the requesting physician indicate therapeutic indication for GBP on the request form, as well as clinical information (prescribed dose, comedication and indication for TDM) in order to receive optimal TDM service.

### 4.5 | Methodological considerations

Some limitations of the study need to be considered. The established practice for TDM in epilepsy in Norway is a standardized sampling time, drug fasting before the morning dose at steady state, but it cannot be assured that this is complied with at all times. This may be particularly important when considering that samples in patients using GBP for other indications may have been requested by physicians not as used to this practice. Poor adherence cannot be controlled for in a naturalistic setting, and exact time of intake of the last dose is often unavailable. Furthermore, lack of essential information on many request forms reduced the number of patients available for consideration of many important aspects.

In the prospective study on patients with RLS, the sample size was small, but to the best of our knowledge, the use of TDM in this patient group has not been described. The evaluation of effect of GBP and usefulness of TDM were subjective opinions and could have been influenced by change in other treatment as well. The results are, however, not used to determine whether or not TDM should be used, but to provide experience in order to better tailor a service that potentially can be of benefit to these patients.

## 5 | CONCLUSIONS

Due to extensive pharmacokinetic variability, TDM has the potential to benefit patients using gabapentin. Challenges with applying TDM in new indications such as RLS include different dosage regimens and consequently different interpretation of serum concentrations. Thus, TDM should be requested on clear clinical grounds and sufficient clinical information provided, so the service can be tailored according to the therapeutic indication.

### ACKNOWLEDGEMENTS

We would like to thank Marte Swakhoven at Sandvika Neurocenter for administrative assistance with the prospective study on patients with RLS and Dr. Oliver Henning at The National Center for Epilepsy, Oslo University Hospital for useful discussions of the results and manuscript.

### CONFLICT OF INTEREST

M L Burns, E Kinge, M S Opdal: none. S I Johannessen has received consultant honoraria from GW Pharma. C Johannessen Landmark has received speaker's honoraria from Eisai, GW Pharma, and Labor Krone.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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# Appendix 1

## Corrections

The following corrections have been made to the manuscript after the thesis was approved for defense and before printing:

| Page | Line | Original text | Corrected text |
|---|---|---|---|
| 9 | 14 | adolescence | adolescents |
| 21, 21, 57 | 14, 17, 21 | has | have |
| 17, 55 \| 54 | 12, 12 \| 1 | was \| were | were \| was |
| 18 | 24 | os | is |
| 20 | 4 | AEDs are… | Most AEDs are… |
| 23, 25 \| 24 | 2, 1 \| 25 | is \| are | are \| is |
| 23 | 8 | dugs | drugs |
| 26 | 19 | relative | relatively |
| 29 | 10 | needs | need |
| 29 | 19 | AED | AEDs |
| 33 | 22 | that | than |
| 34 | 2 | includes | include |
| 44 | 14 | in | is |
| 45 | 4 | CLB | The CLB |
| 50 | 7 | aetate | acetate |
| 52 | 20 | to be | are |
| 61 | 14 | adolescent | adolescents |

The following was added to Errata in the published articles, page 90:

In the methods section on page 351 the measuring range for NLCB should have read 0.3-30 µmol/L, not 3.0-30 µmol/L.