CLOSED-LOOP NEUROPHARMACOLOGY FOR EPILEPSY: DISTANT DREAM OR FUTURE REALITY?

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Abstract:

Epilepsy is considered the most frequent severe neurological condition but most patients treated with medication become seizure free. The management of treatment however is highly empirical, mainly relying on observation. A closed-loop therapy for epilepsy would be very valuable for more efficient treatment regimens. Here we discuss monitoring treatment (therapeutic drug monitoring) and the potential developments in this field, as well as providing a review of potential biomarkers that could be used to monitor the disease activity. . Finally, we consider the pharmacogenetic input in epilepsy treatment.

Keywords: Biomarkers in epilepsy, therapeutic drug monitoring, pharmacogenetics, cytokines, genetic factors, refractory epilepsy, hormones.

1. INTRODUCTION

Epilepsy is one of the most prevalent neurological diseases. It is characterised by spontaneous epileptic seizures and defined either by the occurrence of two unprovoked seizures, or one seizure associated with a biological background making the risk of recurrence very likely [1]. The lifelong prevalence of the disease in the general population is one person in 26 [2] and along with seizures, this condition harbours significant morbidity and premature mortality [3]. The majority of people living with epilepsy (>70%) are treated with antiepileptic drugs (AEDs) over a long-term [4]. AEDs can control seizures in 60-80% of patients [5, 6] and in up to 50%, control of seizures is achieved with the first AED tried, after titration to reach the minimal effective dose. However, if the first AED fails, the probability of controlling seizures decreases with each additional AED tried, until after six tested AEDs, the chances of controlling seizures with medication becomes negligible [7]. Despite epilepsy treatment having evolved over a century (in terms of drugs used), the treatment basis still relies on observation to adjust or change medication [8]. If an event (seizure) occurs then treatment dosage is modified or treatment switched, but otherwise therapy continues as is. This process inevitably takes time, during which patients are exposed to seizures with all the associated risks of injury and socio-professional difficulties. The lack of reliable biomarkers that could objectively monitor the activity of the disease between or before seizures is a major limitation. Electroencephalogram (EEG), a leading tool in the diagnosis and characterisation of epilepsy provides useful prognostic values for the long-term course [9, 10], but is unable to predict the short-term evolution or response to treatment. The identification of a number of proteins (such as inflammatory cytokines) whose serum levels are associated with seizures raises hope of identifying useful biomarkers. Although these proteins were mostly shown to peak after seizures, some remained steadily increased between seizures, potentially reflecting the overall disease activity. The ability to closely follow disease activity while monitoring AED levels [11] might bring a closed-loop therapy for epilepsy for the future. The use and prescription of newer

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generation AEDs (lamotrigine and levetiracetam for instance) are continually increasing [12], and although new treatments show less pharmacokinetic variability than older generation AEDs, their bioavailability significantly changes with co-mediation or physiological modifications, such as pregnancy [13-15]. Monitoring drug levels of newer generation AEDs may therefore also be useful. In a closed-loop therapy, adjustment of the treatment would be directly correlated with disease activity allowing more efficient control (mostly in terms of speed) of the disease. Another input in the closed- loop could come from pharmacogenetics data providing the possibly to predict, in the future, response to treatment as well as risk of adverse events with view to personalized medication. Currently, the most valuable addition to treatment tailoring that pharmacogenetics provides is prediction of adverse events. [16, 17].

Overall the possibility of closed-loop therapy in epilepsy (Figure 1) allowing treatment adjustment according to biological parameters would not replace individual discussions between the patient and his/her physician, but rather contribute a helpful tool for treatment decisions. We review here the limitations of current epilepsy treatment and examine available biomarkers for disease tracking, treatment exposure, as well as genetic predictors that could be useful for future management of epilepsy patients.

2. Why monitor disease activity?

The decrease in seizure frequency as a measure of treatment efficacy is based on the patient's report. Personal reporting of seizure in the doctor's surgery could be biased by particular circumstances such as needing to hold on to a driver's licence. Beyond conscious misreport, recognition of seizures can also be an issue. Indeed, Evidence from EEG monitoring shows that seizures are commonly underreported as patients fail to report 55% of all recorded seizures in EEG monitoring units. Nocturnal seizures are the most underreported (85%) [18]. The study concluded that failure to report some seizures was due to postictal seizure confusion. Further results from another study in an EEG monitoring unit showed similar findings with only 44.5% of complex partial and secondarily generalized tonic-clonic seizures recognized by epilepsy patients [19].

Assessing the outcome or the effect of a therapeutic intervention based on a relative decrease of the seizure frequency is also flawed by other features. The duration of observation to sense a significant change in seizure frequency is influenced by the stochastic fluctuations in seizure frequency and misinterpretation due to the phenomenon of *regression to the median* is common place. This phenomenon is well described by Spilker and Segreti [20] following their observation of "oscillations" of seizure frequency in a controlled clinical trial of cinromide versus placebo in patients with focal epilepsy [21]. They suggested several factors that could cause different rhythms of seizure frequency, for instance, hormonal cycles of menstrual periods, circadian rhythms, as well as phases of the moon. The authors performed three analyses of changes in seizure frequency from six previous studies (in placebo arms) and confirmed presence of regression of seizure frequency towards the median, observed in all types of epilepsy. Overall, patients who had high seizure frequencies tended to experience a reduction in frequency, while patients with low seizure frequencies rather experienced an increase. This study showed that epilepsy patients manifest changes in seizure frequency irrespective of treatment. This finding becomes more relevant in the assessment of response to a new treatment as patients are often started on increased dosage or new medication when they experience an increase in seizure frequency, making it very likely that whatever medication is used, a decrease in seizure frequency will be observed as part of the regression to the median. This regression can appear as a *honeymoon*, as the medication is considered initially efficacious but then the effect fades away [22]. Consequently, response to a medication cannot be fully assessed using common standards used in regulatory studies, such as 50% seizure frequency reduction over 3 months[23].

The International League Against Epilepsy (ILAE) proposed the "rule of three"[24] to define seizure freedom as 'without seizure for three times the pre-intervention inter-seizure duration or at least one year' [25]. The rule of three was recently reassessed [26] and in some cases of refractory epilepsy (the probability of success of an intervention being 5%), 'six times the pre-intervention inter-seizure duration' would be more adequate, supporting the ILAE's definition of the shortest period to assess the response to the treatment

as 1 year in all cases. Although it appears robust, the ILAE definition of drug response, leads to long periods of observation so that predicting treatment response in the short-term is not possible.

The poor understanding of the natural course of epilepsy also makes it difficult to assess response to treatment. Some types of epilepsy have spontaneous transitory remission periods, as in genetic generalized epilepsy after adolescence, or definitive remission in childhood epilepsies, like childhood absence epilepsy or myoclonic epilepsy in infancy that enter remission during adolescence [27, 28]. In refractory epilepsy, some patients may experience a pattern of intermittent remission alternating with relapses [29]. Some of these periods of remission are likely to be spontaneous and not treatment related [30]. Aging also seems to increase drug responsiveness in patients with drug-resistant epilepsy [3]. Altogether these data suggest that the natural course of epilepsy cannot be considered as stable and its fluctuations cannot currently be predicted.

The points discussed above highlight the difficulties of predicting and monitoring the course of epilepsy and its response to medication. These difficulties renders management of people with epilepsy mostly empirical with help of a few tools.

3. Current tools for follow-up of epilepsy

EEG is a major tool in the assessment of epilepsy, but its usefulness in epilepsy follow-up of is arguable. Several studies did not find predictive usefulness of EEG patterns in neonates, children with West syndrome and febrile seizures [31, 32] and there is only limited data on its predictive value on the course of the disease, despite its widespread use. A study carried out in 39 patients with epilepsy investigated the relationship between serial EEG recordings and clinical outcome over 15 years follow-up, concluded that patients with epileptiform discharges on repeated EEG recordings had a worse outcome compared to whose with normal initial EEG in terms of seizure control and social outcomes [10]. Absence of generalized epileptiform activity in the EEG was associated with a good prognosis in a community study [9].

It is widely agreed that the hallmark of epilepsy is an imbalance between cortical excitability and inhibition [33]. Levels of EEG activity synchrony were found to correlate with antiepileptic drug changes, suggesting that this would reflect on the cortical excitability (34). Transcranial magnetic stimulation (TMS) similarly showed motor threshold and cortical excitability on recovery curves predicting response to medication. A decrease in cortical excitability occurred in the seizure-free patients indicated by increased motor thresholds and intracortical inhibition on recovery curve analysis compared to pretreatment values [34]. TMS combined with EEG was studied as a diagnostic tool in 25 patients with genetic generalized epilepsy, comparing to 11 controls and was able to reliably differentiate patients with epilepsy from control subjects. Furthermore, patients with drug resistant epilepsy had higher amplitude late TMS-EEG responses compared to controls [35]. In another study TMS combined with EEG performed on 8 patients with periventricular nodular heterotopia showed an increased late cortical motor response compared to the matched controls, which was suggested to reflect the cortical hyperexcitability due to altered connectivity [36]. These measurements however are very liable to influence from many other factors, such as the point in the menstrual cycle in women [33]. Some authors wondered if the increased excitability found in TMS could be a biomarker of epilepsy [37], but the large inter-individual variability seems to preclude establishment of normative values [38]. Variations on an intra-individual basis could however be useful to monitor cortical excitability.

More recently, high frequency oscillations (HFO) in invasive intracranial EEG recordings were found to be tightly co-localised with the seizure onset zone and were suggested to be a biomarker of epilepsy [39]. These signals were reported to be recordable on the scalp in a non-invasive setting (although this technique may not be flawless [40, 41]), potentially opening up to widespread use [42]. There is currently limited data on the evolution of high frequency oscillations over the course of epilepsy though. A recent study showed correlation between the number of ripples and the risk of developing seizures in children with rolandic spikes. The presence of more than two ripples (in a 10-minute EEG recording) related to rolandic spikes increases the risk of having seizures. More than five ripples predicted a malignant course in rolandic epilepsy [43]. The same group published a retrospective study showing that patients with fast ripples in the

intraoperative corticogram had higher risk of seizure recurrence [44].

Overall, the management of epilepsy subjects is currently largely a process of trial and error. It is likely to remain so in the near future at least, given the great heterogeneity of epilepsies and individual patient situations. Improved monitoring of disease activity on treatment would however make the process more efficient, determining, for instance, early in the course of a treatment if it is likely to be efficacious or not.

4. Potential epilepsy biomarkers

There are numerous systemic changes reported during seizures, beyond the classical increase in serum creatinine kinase[45] and lactate[46].

Seizures have an enduring effect on multiple somatic systems, leading to the premature occurrence of somatic co-morbidities and premature mortality independent of the pre-existing health [3]. This association between widespread systemic changes and seizures suggests that seizures lead to the release of circulating mediators that could be detected in biological samples such as serum. There are suggestions that at least some of these widespread changes are associated with increased inflammatory parameters [47].

4a. Cytokine changes

Here we discuss cytokine changes induced by seizures, however the immune system and associated inflammatory reactions also play an important role in epileptogenesis [48]. We will concentrate on cytokine changes associated with established epilepsy. A comprehensive review and meta-analysis of this aspect can be found in the excellent paper of De Vries et al. [49].

Several studies assessed serum and cerebrospinal fluid (CSF) IL-1 levels in patients with focal and genetic generalized epilepsy in the first hours post seizure and in the interictal period compared to control subjects. Results were mostly negative, with no significant changes in the IL-1β serum concentrations in a 24h period after tonic-clonic seizures compared to control subjects [50-54]. Increased interictal serum IL-1 levels were at times reported in patients with temporal lobe epilepsy compared to extra-temporal lobe epilepsy and control subjects [55].

Interleukin 6 (IL-6) is a multifunctional cytokine regulating inflammatory responses. It was shown to be increased in several neurological conditions such as Alzheimer's disease, trauma and meningitis [56]. Serum and CSF IL-6 levels have been studied in epilepsy patients with recent seizures (<72h), where levels were found to be higher than in seizure-free patients and controls [55]. Serum IL-6 levels were increased interictally within 24h of a seizure, particularly in patients with temporal lobe epilepsy [54, 57, 58]. The levels were shown to decrease after resection of the epileptogenic lesion in these patients [59]. CSF and serum IL-6 were also reported to be correlated with epilepsy severity, assessed in terms of seizure frequency (measured by seizure diaries), as well as seizure intensity, scored using the National Hospital Seizure Severity Scale (NHS3) and the Veterans Administration Seizure Frequency and Severity Rating Scale score (VA score) in 1, 218 patients with symptomatic epilepsy compared to 200 control subjects [50, 60].

The IL-17A receptor (IL-17RA) is highly expressed in focal cortical dysplasia, a major cause of epilepsy [61]. In one prospective study, interictal serum and CSF IL-17A levels were increased in 70 patients with focal non-lesional epilepsy compared to 68 healthy controls. Patients with somatic co-morbidities were excluded. Serum IL-17A levels were independently associated with seizure severity, evaluated by the NHS3 and the VA score)[60] and seizure frequency (measured by seizure diaries) [61]. IL-17A serum levels were also reported to correlate with interval to the next seizure in focal epilepsy [50]

Other inflammatory markers have been studied, for example Interferon λ 3 (IFN λ) CSF and serum levels which were found to be correlated with seizure severity and interval to next seizure in temporal lobe epilepsy [50]. Serum levels of interferon (IFN) γ and IL-8 were also suggested to correlate with seizure severity (NHS3 scale and VA score) [50]; Increased levels of C reactive protein (CRP) were also found in serum of patients with refractory temporal lobe epilepsy compared to

controls [62]; this increase was more marked 3-6 hours after a generalized tonic-clonic seizure.

Inflammatory mediators appear to have important potential as biomarkers for epilepsy. However, although there are consistent results showing changes in inflammatory mediators in epilepsy, none of the changes are specific to the disease and some of them may be the consequence of the underlying condition. Increased inflammatory mediators do not appear to be related to traumatic consequences of seizures as changes were demonstrated in EEG monitoring units, where trauma due to seizures is much less likely than in everyday life. Another potential pitfall in use of inflammatory mediators as part of a biomarker panel is their relatively low concentrations which may lead to difficulties in detecting them by standard proteomic techniques.

3. 4b Hormonal changes

Hormonal changes (mostly sexual hormones in females) are well known to interact with epilepsy.

Mesio-temporal epilepsy has been shown to deregulate luteinising hormone secretion in one study [63] where secretion of luteinizing hormone was detected interictally and postictally in women with mesio-temporal epilepsy during two 24-hour epochs (an interictal baseline and in the postictal period) compared to males. The authors found that seizures provoked timing irregularity in luteinizing hormone secretion, whereas chronic epilepsy was related to modifications in luteinizing hormone pulse frequency, amplitude and mass. Even though the exact mechanism of this dysregulation is not clear, it is probable that mesial temporal epilepsy alters hypothalamic functions. Epileptic discharges in the left temporal region have been associated with increased LH/FSH ratios and testosterone levels [64]. This altered LH/FSH ratio and testosterone levels might conceivably be a biomarker of mesio-temporal lobe epilepsy. Fluctuations of sexual hormones in women are known to modulate cortical excitability and therefore the occurrence of seizures. Studies using TMS showed cortical excitability varies according to the phase of the menstrual period in healthy women [65, 66]. Cortical hyperexcitability is increased in the luteal phase in female patients with catamenial seizures compared to control women[33].

A case study assessed plasma levels of prolactin, noradrenaline, vasopressin and oxytocin during and after focal secondarily generalized seizures [67]. Prolactin levels were found to increase at the beginning of aura until the end of the generalized tonic- clonic seizure, this increase was correlated with the intensity and the duration of the seizure [68]. Levels of noradrenaline, vasopressin and oxytocin remained stable during the aura, but increased in the generalized phase [67].

Recently, hair cortisol levels were postulated as a biomarker of chronic stress in the months preceding a first seizure. Twenty two subjects with a first epileptic seizure were compared to twenty nine control subjects. Increased hair cortisol-levels were found within twenty four hours following seizure compared to the control group, possibly reflecting hyperactivity of the hypothalamic-pituitary- adrenal axis [69]. A positive relationship between cortisol levels and epileptiform discharges on long-term (> 24h) EEG recordings was also reported in patients with focal epilepsy, who reported seizures related to stress [70]. A recent review assessing the occurrence of seizures according to the circadian cycle, found that seizures were more frequent in the morning, possibly linked to the peak of cortisol[71].

Although hormonal changes are well demonstrated *consequences* of seizures, there is less evidence on interictal changes. Interictal hormonal changes may indeed be the consequences of AED medication-induced liver induction[72]. Natural short term fluctuations (circadian and menstrual cycles) may render hormonal levels potentially less useful for long-term course biomarkers of disease.

4. 4c. Other biological and physical changes

People with epilepsy (mainly those with TLE) have been shown to have interictally decreased heart rate variability [73-75] independent of antiepileptic medication that could be related to seizure frequency [76, 77]. One study assessed autonomic functions (heart rate variability) in 21 patients with juvenile myoclonic epilepsy and 21 with temporal lobe epilepsy, treated with carbamazepine, valproate or phenytoin compared to control subjects. Patients with temporal lobe epilepsy treated with carbamazepine had lower heart rate variability than controls, suggesting decreased

sympathetic tone [75]. There was no significant heart rate variability difference in patients with juvenile myoclonic epilepsy compared to controls. A recent study demonstrated that patients suffering from temporal lobe epilepsy had more marked reduced heart rate variability at night than in daytime, and nocturnal heart rate variability increase did not appear. This suggested a suppression of circadian heart rate dynamics in patients with temporal lobe epilepsy [78].

There is some evidence that seizures induce an elevation of troponin I, a marker of cardiac injury [79]. In a small series of 11 people assessed for epilepsy surgery, no troponin I elevation was observed after mostly complex partial seizures [80]. A study of 30 complicated (followed by significant systemic repercussions such as desaturation or hypotension) seizures compared to 30 uncomplicated generalized tonic-clonic seizures [81] found significantly higher troponin I values after complicated rather than uncomplicated seizures; all values were, however, within the normal range. Finally a recent large study of 741 consecutive people admitted to hospital with generalized tonic-clonic seizures, [82] found an elevation of troponin I in 6.7% after seizures. None of the 6.7% had known ischemic heart disease, and troponin I elevation was asymptomatic in all cases. There was no obvious explanation for these elevations; Takotsubo cardiomyopathy was excluded by echocardiography, and serial ECGs and monitoring were unremarkable in these people.
Most cardiovascular changes seem to be short-term consequences of seizures and are therefore unlikely to represent useful biomarkers for long-term disease activity. Cardiac autonomic dysfunction (heart rate variability) may be a chronic feature associated with epilepsy, but it may also be the consequence of repeated seizures rather than the underlying disease activity.

Changes in microRNA (miRNA) expression were described to be associated with epilepsy. The analysis of miRNA profiles in autopsy hippocampal tissue found 165 miRNAs up-or-down-regulated in patients with temporal lobe epilepsy compared to healthy control tissue [83]. Recent studies showed that miRNAs may play an important wide role in epilepsy and its treatment. Animal studies demonstrated that silencing a specific miRNA (miR-134) with antisense oligonucleotides had an anti-seizure effect, while deletion of miR-128 caused refractory epilepsy. Studies on DNA-methylation revealed epigenetic regulation of miRNA levels and synthesis [84]. This was seen in patients with mesio-temporal epilepsy who had altered DNA methylation of several miRNAs (miRNA 27A, miR-193a-5p and miR-876-3p), thought to contribute to neuronal development and remodeling [85]. MiRNAs could be potential epilepsy biomarkers due to their tissue-specific expression [86], as well as their stability and ease of detection in most biological materials, such as serum. At this stage further work is needed to understand the relationship between potential alterations in miRNA expression and disease activity in terms of seizure frequency.

4d. Future of epilepsy biomarkers

The systemic changes induced by seizures make it likely at some point to monitor disease activity. Table 1 summarizes the potential epilepsy biomarkers and their relationship with seizures. Changes demonstrated as present interictally are obviously more relevant than modifications only found post-ictally. None of the changes listed above are specific to the disease; the future lies probably in a combination of markers that together reach sufficient specificity and sensitivity. The usefulness of such combined biomarkers will need to correlate with statistically robust clinical outcome, such as seizure freedom defined by the rule of 3 [26]. One question remaining is will these markers reflect on past occurrence of seizures or monitor the propensity of new seizures to come, which would be more useful. Some of the changes discussed above (maily elevated cytokines) are present even between seizures, which raises the hope of monitoring the underlying disease activity that triggers seizures. Current evidence is however insufficient to ascertain that interictal markers predict the occurrence of future seizures. Studies addressing the effects of medication on these markers are scarce (33-34-35).

5. Why monitor drug exposition?

Therapeutic drug monitoring (TDM) comes from the observation that the effects of some drugs correlate better to the circulating concentration than to the administered dose. TDM encompasses both drug quantification in a sample and pharmacological

interpretation with dosage adjustment if needed. Drugs in which monitoring is particularly valuable are those that display large inter-individual and low intra-individual pharmacokinetic (PK) variability, as well as good correlation between blood concentrations and the clinical response/side effects [87]. Some AEDs (both older and newer generation) show significant inter-individual PK variability, due to interactions with or polymorphisms in hepatic cytochrome or glucuronidase substrates (phenytoin, valproate, lamotrigine, lacosamide, zonisamide) [87]. Phenytoin may present nonlinear pharmacokinetics (unpredictable loss of correlation between dose and attained blood levels) at usual doses due to saturation of its elimination pathways [88]. TDM can provide a useful tool to adjust the dose of medications in relation to clinical response. However, some newer generation AEDs have lower inter-individual variability because of unaltered renal excretion (gabapentin, pregabalin and levetiracetam), for which the TDM usefulness is less likely.

5a. Therapeutic drug monitoring of older generation AEDs

TDM of older generation AEDs is more studied than that of newer generation AEDs and is widely considered validated.

In 1960, Buchthal et al. established a positive correlation between the clinical effect of phenytoin (PHT) and its serum levels in a small group of inpatients with epilepsy (12), suggesting phenytoin serum level ranges between 10-20 µg/ml [89]. For these inpatients, plasma PHT level higher or equal to 10 µg/mL, was associated with clinical improvement over the course of 3 to 4 weeks. Later, a three-year prospective study in outpatients (n=32) with generalized epilepsy treated with PHT showed that a phenytoin range of 10 and 20 µg/ml was the most appropriate for seizure control and avoiding adverse events [90]. The influence of plasma levels of phenytoin, carbamazepine and phenobarbital were evaluated in 78 patients according to their seizure type. Mean plasma levels of AEDS were higher for controlling focal than generalized tonico-clonic seizures [91].

However, a randomized clinical trial including 127 patients with epilepsy concerning carbamazepine, ethosuximide, phenobarbital, primidone, or valproate failed to demonstrate that serum concentration within a therapeutic range contributed to decreased frequency of seizures. Patients were randomized into a group where drug blood levels were communicated to the treating neurologist and the dose was adjusted to reach the reference range, or into a group where blood levels were not reported to the neurologist and the dose was adjusted based on the clinical response. Results were negative, showing that routine AED TDM and adjustment of dosage to achieve therapeutic levels did not improve the treatment of patients with epilepsy [92]. Another multicenter randomized controlled trial also failed to demonstrate the benefit of routine TDM of older generation AEDs [93]. Despite the conclusions of these two randomized clinical trials, routine TDM continues to be used following other nonrandomized studies that showed that TDM is useful in AED treatment in some situations [94].

5b. Therapeutic monitoring of newer generation AEDs

The use of a newer generation AEDs is increasingly replacing the older generation AEDs [95, 96]. Although newer generation AEDs have a better therapeutic index due to their better tolerance, some (lamotrigine, topiramate, tiagabine, zonisamide, and felbamate) may be good candidates for TDM [11]. These particular drugs have large inter- and intra-individual pharmacokinetic variability and their metabolism is influenced by age, pregnancy, associated disease and some concomitant medication [97]. There are suggestions that the effects of newer generation AEDs are correlated to their serum levels [94, 98]. TDM of lamotrigine during pregnancy is widely recommended because of the significant drop in concentrations over the course of the pregnancy [99]. Current practice guidelines for TDM [100] now recommend its use in : dose optimization of the first prescribed AED to reach a target concentration, increased seizure frequency, suspected toxicity due to accumulation, pregnancy, suspected poor compliance, risk of interactions, cases of disturbed bioavailability of the AED and whenever there is an unexpected change in clinical response.

5c. Future of TDM in epilepsy

Despite current limited evidence of its usefulness (randomized trials are ongoing for newer generation AEDs), TDM will remain an important tool in the treatment of subjects with epilepsy. Several new developments however should improve its value.

We need to define a better correlation between drug levels and clinical effects using more robust outcomes, such as remission. Stable patients in remission (as defined by the ILAE) not uncommonly have drug levels below the reference range, suggesting a ceiling effect, above which no remission patient is found [101, 102]. Such observations could prove to be useful for medication titration.

The widespread use of ultra-performance liquid chromatography coupled with tandem mass spectrometry will improve reliability [103] of the measures and the use of saliva samples will make samples more readily available[104].

Computer applications should soon be ready for use to facilitate AED prescription. They would use population pharmacokinetic models [105, 106], describing the time course of drug exposure for a patient according to factors considered to contribute to variability (weight or other medication for example). Models are then combined with drug levels to calculate the most probable pharmacokinetic parameters for the patient (Bayesian approach) and thereby propose, if necessary, more suitable dosages.

Although there is currently little evidence on the tangible benefit of TDM in epilepsy, it remains an invaluable tool to assess and make normalized drug exposures of individual patients and as such is likely to be an important part of closed-loop therapy.

6. Another input to the loop: pharmacogenetics

Pharmacogenetics could potentially provide precious data on the dosing, response and tolerance of AEDs. However, for now this field has lots of non-replicated studies. A recent paper reviewed the evidence level of findings [107]. Genetic variation can influence the effects of AEDs through pharmacokinetic and pharmacodynamic changes, as well as through other mechanisms involved in overall drug resistance [107]

6a. Genetic biomarkers for pharmacological effects: pharmacodynamic aspects

There are relatively few demonstrated effects of genetic variations on drug targets. Functional *polymorphisms (IVS5-91 G>A)* in the *SCN1A* (sodium voltage-gated channel) gene were shown to be associated with varying prescribed doses of phenytoin and carbamazepine spontaneously used in clinical practice [108]. Other polymorphisms of *SCN1A (rs3812718)* were also shown to modify the effect of carbamazepine on the cortical silent period (probably by GABA modulation) in TMS studies [109].

6b. "Pharmacological correction" of genetic defects

The knowledge of a specific genetic etiology might help determine the most adequate treatment. This remains an exceptional situation at present in everyday clinical practice. A well-known example is the glucose type I transporter (GLUT-1) deficiency, a genetic metabolic encephalopathy caused by a mutation in the *SLC2A1* gene, encoding GLUT-1 that provokes altered transport of glucose across the blood-brain barrier. The most effective treatment is a ketogenic diet in this case. Another example is mutations in the voltage-gated sodium channel alpha 1 subunit gene *(SCN1A)* are associated with Dravet syndrome and epilepsy with febrile seizures plus (GEFS+). The treatment of this syndrome is less evident; the combination of stiripentol, valproate and clobazam and avoidance of sodium channel blocking AEDs are deemed to be the most efficient, although formal evidence is lacking [107]. Recently, cannabinoids were also suggested to be efficacious in Dravet Syndrome [110]. There are also suggestions that antiNMDA drugs like memantine could be used in children with an early onset epileptic encephalopathy due to *GRIN2A* mutations [111].

6c. Genetic biomarkers for pharmacological effect: pharmacokinetic aspects

There is much more data on the genetic variations of AED metabolism (such as in P450 cytochromes genes), affecting their pharmacokinetic properties. For instance, carrying CYP2C9 alleles with reduced activity of *rs1057910 (CYP2C9*3)* compared to individuals homozygous for the wild-type alleles causes a high risk of toxicity upon exposure to phenytoin [112]. Although detection of these variants can help to anticipate the bioavailability of medication, they are rarely used in practice as the more readily

available serum levels provide data on the overall metabolism of the medication including drug interactions.

6d. Genetic biomarkers predicting the efficacy of AEDs

Genetic variations predicting pharmacoresistance have been investigated in several studies that mainly focused on multidrug transporters that expel AEDs from the CNS, but they did not reach consistent results. The drug transporters *ABCC1*, *ABCC2* and *ABCC5* have been shown to be overexpressed in brain capillary endothelial cells and astroglia of patients with refractory epilepsy [113, 114]. Some studies suggested an increased risk of drug resistance in patients with the *c.-24C>T ABCC2* polymorphisms compared to controls, which other studies failed to confirm [115]. miRNAs were also recently studied for drug resistance and several miRNAs *(miR-194-5p,-301a-3p, -30b-5p, -342-5p and -4446-3p)* were found to be increased in patients with refractory epilepsy compared to drug responsive patients. *MiR-3012-3p* was the most closely associated with drug-resistance, with 80.5% sensitivity and 81.2% specificity [116].

6e. Genetic prediction of adverse events

Prediction of adverse events is the area where pharmacogenetics contributes most to treatment of epilepsy. There is indeed strong evidence in Asian populations of an association between the HLA-B*1502 allele and severe rashes, such as Stevens-Johnson syndrome, provoked by treatment with CBZ [17, 117]. The HLA-A*3101 allele has also been shown to be associated with CBZ induced hypersensitivity reactions, from maculopapular exanthema to severe blistering reactions amongst patients of European ancestry and in the Japanese population [118, 119]. While these tests are clearly useful, the lengthy delay in obtaining results renders it easier for the time being to use medications that do not require this testing as shown in Asian populations[120].

7. Conclusion

Although hypothetical at this point, a closed-loop therapy for epilepsy has potential to improve the state of treatment which currently mostly relies upon trial and error. Two major points are hampering its development: lack of reliable experience with epilepsy biomarkers and limited correlation with medication levels and clinical effects. A set of biomarkers would probably need to be identified using modern systematic methods ("omics") such as proteomics and metabolomics, in cross-sectional studies comparing people with controlled vs. uncontrolled epilepsy with subsequent validation in prospective studies. Similarly, AED serum levels would need to be related to more robust clinical outcomes such as remission, before being tested prospectively.

The road for closed-loop therapy is therefore still long and probably paved with methodological issues, such as difficulties of validating the correlation of potential biomarkers with a robust clinical outcome in the first place.

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