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WONOEP appraisal: Biomarkers of epilepsy-associated comorbidities

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SUMMARY

Neurologic and psychiatric comorbidities are common in patients with epilepsy. Diagnostic, predictive, and pharmacodynamic biomarkers of such comorbidities do not exist. They may share pathogenetic mechanisms with epileptogenesis/ictogenesis, and as such are an unmet clinical need. The objectives of the subgroup on biomarkers of comorbidities at the XIII Workshop on the

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Neurobiology of Epilepsy (WONOEP) were to present the state-of-the-art recent research findings in the field that highlighting potential biomarkers for comorbidities in epilepsy. We review recent progress in the field, including molecular, imaging, and genetic biomarkers of comorbidities as discussed during the WONOEP meeting on August 31–September 4, 2015, in Heybeliada Island (Istanbul, Turkey). We further highlight new directions and concepts from studies on comorbidities and potential new biomarkers for the prediction, diagnosis, and treatment of epilepsy-associated comorbidities. The activation of various molecular signaling pathways such as the "Janus Kinase/ Signal Transducer and Activator of Transcription," "mammalian Target of Rapamycin," and oxidative stress have been shown to correlate with the presence and severity of subsequent cognitive abnormalities. Furthermore, dysfunction in serotonergic transmission, hyperactivity of the hypothalamic-pituitary-adrenocortical axis, the role of the inflammatory cytokines, and the contributions of genetic factors have all recently been regarded as relevant for understanding epilepsy-associated depression and cognitive deficits. Recent evidence supports the utility of imaging studies as potential biomarkers. The role of such biomarker may be far beyond the diagnosis of comorbidities, as accumulating clinical data indicate that comorbidities can predict epilepsy outcomes. Future research is required to reveal whether molecular changes in specific signaling pathways or advanced imaging techniques could be detected in the clinical settings and correlate with epilepsy-associated comorbidities. A reliable biomarker will allow a more accurate diagnosis and improved treatment of epilepsy-associated comorbidities.

Keywords

Neurobehavioral comorbidities; Cognition; Depression; Imaging; Polymorphisms; Epilepsy

Epilepsy is a chronic neurologic disorder with a spectrum of conditions comprising many types of seizures, syndromes, and comorbidities. Comorbidity is defined as "any distinct clinical entity occurring during the clinical course of an index disease" (i.e., epilepsy).[1] Comorbid condition(s) can arise before, concomitantly, or after epilepsy diagnosis; they may be cause or consequence of epilepsy, or they can be associated with epilepsy because they share common pathologic risk factors (genetic, environmental, molecular, or morphologic), or a pathogenic process, or they can be discordant conditions.[1] Neurobehavioral comorbidities are frequent and shown to be higher in epilepsy patients compared to the general population.[2,3] The prevalence of such comorbidities varies significantly across the types and severity of epilepsy and include psychiatric (mood disorders, anxiety, depression, attention deficit hyperactivity disorder, and psychosis) and cognitive (deficits in learning and memory, academic underachievement, and impairments in executive functions) dysfunctions.[2,3] Other comorbidities are migraine, sleep disorders, dementia, vascular pathology and stroke, brain tumors, and even extracranial pathologies including metabolic disorders and cardiovascular and respiratory diseases.[4] Comorbidities in patients with epilepsy constitute a significant burden for patients and their families, and are associated with a poor prognosis, poor responses to antiepileptic drugs (AEDs), poor health outcomes, decreased quality of life, and higher mortality.[4]

Due to the significant consequences of comorbidities and their relation to the pathogenesis of epilepsy, complications, and response to treatment, there is an unmet need to identify

reliable biomarkers that will allow the early and accurate diagnosis, facilitate prevention, enable reliable follow-up, and offer novel and effective treatments of comorbidities.

A biomarker in epilepsy has been defined as an objectively measured characteristic that reflects the activity of epileptogenesis or ictogenesis.[5] Ideal biomarker(s) of epilepsy-associated comorbidities are expected to predict the development, severity, and progression of a comorbid condition, reflect the underlying mechanism, provide guidance for a specific treatment, and allow follow-up on treatment efficacy (Table 1).

To identify a biomarker, experimental approaches and animal models are required for a better understanding of the neural mechanisms underlying comorbidities, and their association with epileptogenesis and ictogenesis in different disease models, and sex and age groups. Although no reliable biomarker has been identified so far, recent advances in the study of genetic alterations and molecular signaling, as well as technological improvements in brain imaging modalities, and increased sensitivity of assays for the detection of molecular changes in blood and brain tissue augment our understanding of epilepsy and expect to facilitate the identification of novel biomarkers. Several biomarker categories in epileptogenesis and ictogenesis have been proposed, including electrophysiologic, imaging, behavioral, molecular, and cellular measurements. Because comorbidities may share pathogenic mechanisms with epileptogenesis and ictogenesis, one may consider testing the capacity of epileptogenic/ictogenic biomarkers to predict, diagnose, and follow-up comorbidities as well. For epilepsy, pathologic high-frequency oscillations (pHFOs), functional magnetic resonance imaging (fMRI), transcranial magnetic stimulation (TMS), alpha-methyl-tryptophan (AMT) positron emission tomography (PET) imaging, gene expression, and microRNA (miRNA) profiles are being investigated as potential biomarkers.[5] However, none has yet been adequately validated. A better understanding of the bidirectional, causal, and resultant relationships between epilepsy and comorbidities will no doubt facilitate the identification of reliable biomarkers. Research on biomarkers of comorbidities may also facilitate a better understanding of shared mechanisms of epileptogenesis, ictogenesis, and comorbidities, and offer yet unforeseen new treatments.

Herein we review recent advances on molecular, genetic, and imaging biomarkers of comorbidities discussed during the Workshop on the Neurobiology of Epilepsy (WONOEP) on August 31–September 4, 2015, in Heybeliada Island (Istanbul, Turkey). This review also points to new directions in research and emerging concepts arising from recent research on molecular and imaging studies and comorbidities as biomarkers for epilepsy. The potential of electrophysiologic indicators as biomarkers of comorbidities will not be discussed here, since this topic has been recently covered by comprehensive reviews.[6,7]

Potential Molecular Biomarkers of Comorbidities

Cognitive and memory deficits

Cognitive dysfunction is a frequent and debilitating comorbidity of many acquired epilepsies, particularly temporal lobe epilepsy (TLE). Cerebral insults such as complex febrile seizures in early ages, traumatic brain injury (TBI), stroke, hypoxia–ischemia (HI), and status epilepticus (SE) can lead to epilepsy. The extent of cerebral injury has been

shown to correlate positively with the extent of neurologic morbidity including the degree of cognitive dysfunction and the occurrence of acquired epilepsy.[8,9] It is notable that the epileptogenesis process itself (i.e., the modifications occurring within the local cortical network after the insult and before spontaneous seizures occur) and/or focal nonpropagating, nonconvulsive seizures may actually be the mechanism underlying cortical dysfunction conveyed as a cognitive difficulty. Future research, however, should confirm (or reject) the hypothesis that shared molecular mechanisms and/or brain networks underlie both neurocognitive deficits and seizures in individual patients/disorders. Similar to epileptogenesis, at present there are no biomarkers to predict the risk or severity of cognitive deficits in at-risk patients, and there is no targeted therapy available.

Potential new biomarkers may be considered due to brain injury research discoveries of signaling pathways that are activated following an insult, and are considered part of brain repair mechanisms but may also contribute to epileptogenesis and cognitive comorbidities. Two of such pathways are the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) and the mammalian target of rapamycin (mTOR) pathways. Of interest, although both pathways are activated after an insult, they are also involved in mediating cellular physiologic mechanisms critical for memory formation, long-term depression (LTD), and long-term potentiation (LTP). Similarly, recent research has implicated oxidative stress and mitochondrial dysfunction as potential contributing factors to both epileptogenesis and associated cognitive dysfunction. The proinflammatory transforming growth factor beta (TGFβ) signaling was also shown to be activated after injury, and to not only underlie increased excitability and epileptogenesis,[10] but also to be involved in pathologic synaptic plasticity.[11] However, whether the detection of activation of either, few, or all of these pathways may serve as potential biomarkers of cognitive comorbidities in acquired epilepsies remains to be investigated.

JAK/STAT pathway

The JAK/STAT pathway has been shown to be a critical mediator of LTD,[12] and is known to be activated after many types of epileptogenic injuries including TBI, SE, HI, and stroke.[13–15] The JAK/STAT pathway regulates expression of genes critical for essential physiologic functions, including cell proliferation, differentiation, neurogenesis, learning and memory, and regulation of the γ-aminobutyric acid (GABA) type A receptor ($GABA_AR$) subunit expression. JAK/STAT-mediated decreases in α1 subunit–containing $GABA_ARs$ in hippocampus have been demonstrated after experimental SE and TBI,[14–16] and are thought to contribute to hippocampal hyperexcitability after injury and subsequent epileptogenesis.

Using the controlled cortical impact (CCI) model of posttraumatic epilepsy in mice, the JAK/STAT pathway has been shown to be differentially activated depending on the severity of brain injury, and to correlate with the presence and severity of subsequent cognitive comorbidities. The degree of impact generated during CCI correlates with postinjury lesion size, levels of several $GABA_AR$ subunits, activation of the JAK/STAT pathway, and functional neurologic outcomes.[15] Specifically, the levels of phosphorylated STAT3 (pSTAT3) were differentially increased in the injured hippocampus 6 h after CCI depending of the severity of injury (Fig. 1A). Furthermore, there was a positive correlation between the

severity of injury, the extent of cerebral damage, and memory and vestibular motor dysfunction as assessed by novel object recognition and rotarod testing (Fig. 1B,C).[15] These findings suggest that the extent of JAK/STAT activation may provide a molecular biomarker of the severity of the injury and of subsequent cognitive and motor impairment. It is notable that transient inhibition of the JAK/STAT pathway immediately after CCI improved long-term vestibular motor recovery, but did not significantly affect memory performance (although there was a trend toward improvement).[15] This study was limited, however, as the modulation of JAK/STAT pathway activation was performed with only a single pharmacologic agent (WP1066), and a single dosing paradigm, and did not obtain complete inhibition of pSTAT3. In addition, only a single test of memory was utilized (novel object recognition), so many aspects of memory, particularly hippocampal spatial memory, were not specifically assessed. Therefore, the degree and specificity of pharmacologic inhibition of JAK/STAT could not be correlated with the molecular and functional changes.

mTOR pathway

In rodent models, epileptogenic brain injuries including SE and TBI also trigger an immediate and long-lasting increase in the phosphorylation of the S6 ribosomal protein (pS6) in the hippocampus, which is a downstream marker of excessive activation of the mTOR complex 1 (mTORC1) pathway.[17–19] In addition, structural or dysplastic lesions associated with infantile spasms in rodents or humans also demonstrate overactivation of the mTORC1 pathway in the epileptogenic cerebral cortex.[20] Research in cognitive neuroscience has shown that excessive activation of the mTORC1 pathway in transgenic rodents or in acquired models of epilepsy is associated with cognitive and behavioral deficits, which are reversed following treatment with the mTOR inhibitor rapamycin.[21,22] This suggests that aberrant mTORC1 activation may be a candidate mechanism associated with the memory deficits seen in acquired or certain genetic forms of epilepsies, and that phosphorylation of the S6 ribosomal protein in the hippocampus or other epileptogenic regions, may serve as a molecular biomarker of cognitive dysfunction following epileptogenic brain injuries. Brewster et al.[18] examined the effects of rapamycin on the hippocampal-dependent spatial learning and memory deficits found following pilocarpine-induced SE. Rapamycin-treated SE rats performed significantly better than the vehicle-treated controls in two spatial memory tasks, the Morris water maze and the novel object recognition test. Taken together, these findings suggest that mTORC1 signaling may contribute to hippocampal-dependent spatial learning and memory deficits associated with SE-induced epileptogenic injury, and that phosphorylation of the S6 ribosomal protein in the hippocampus may serve as a molecular biomarker of cognitive dysfunction following epileptogenic brain injuries.

Oxidative stress

Oxidative stress is known to occur after brain injury and during epileptogenesis in the pathogenesis of acquired epilepsies, but its functional role and contributions to cognitive comorbidities remain to be fully elucidated. Recent work by Pearson et al.[23] demonstrate that markers of oxidative stress correlate with memory dysfunction following pilocarpine-induced SE, and appear to contribute to cognitive decline during epileptogenesis in a rodent experimental model of TLE. The authors demonstrated that animals that were exposed to

pilocarpine and developed SE (but not those that were treated with pilocarpine and did not develop SE), had elevated markers of oxidative stress, and developed TLE as well as experienced learning and memory deficits. Pharmacologic removal of reactive oxygen species (ROS) using a synthetic catalytic antioxidant (Mn^{III}TDE-2-ImP^{5+}), prevents oxidative stress, deficits in mitochondrial oxygen consumption rates, hippocampal neuronal loss, and cognitive dysfunction.[23] Of interest, antioxidant treatment had no effect on the intensity of the initial SE or on frequency or severity of subsequent spontaneous seizures, suggesting that learning and memory improvement was not due to a reduction in the severity of the initial precipitating injury or overall seizure burden, and that biomarkers predicting and mechanisms underlying cognitive dysfunction may be distinct from those for epileptogenesis. Taken together, these data suggest that injury-induced ROS production may provide a biomarker for cognitive dysfunction following epileptogenic brain injuries and implicate oxidative stress as a novel mechanism by which cognitive dysfunction can arise during epileptogenesis and a potential disease-modifying therapeutic approach.

Epilepsy-associated depression

Depressive disorder represents by far the most common comorbidity of epilepsy, whereby depression is two to five times more frequent among patients with epilepsy than in the nonepilepsy populations.[24] Similar to the stand-alone major depressive disorder (MDD), epilepsy-associated depression presumably reflects perturbations in various signaling pathways. Such perturbations may be used as biomarkers of the comorbidity. Several examples are discussed below and summarized in Table 2.

Serotonin 1A (5-HT1A) receptors

Dysfunctional serotonergic transmission has been identified as a key mechanism of MDD. Among the 14 known 5-HT receptors, 5-HT1A receptors are the most abundant in the brain and are either presynaptic (i.e., autoreceptors located in raphe nucleus, and responsible for the negative feedback-based autoinhibition of 5-HT release) or postsynaptic (e.g., those in the forebrain structures such as the hippocampus and frontal lobes). In patients with TLE, depressive symptoms were suggested to be due to a decreased number of postsynaptic 5-HT1A receptors, as supported by brain imaging using 5-HT1A radioligands (e.g., [^{18}F] (N-{2-[4-(2-methoxyphenyl)piperazino]}-N-2-pyridinyl)trans-4-fluorocyclohexanecarboxamide), [^{18}F] FCWAY, and [^{18}F] 2′-methoxyphenyl-(N-2′-pyridinyl)-p-fluoro-benzamidoethyipiperazine, [^{18}F]MPPF).[25,26]

In an animal model of TLE and comorbid depression, however, diminished function (i.e., the ability to activate G protein) of the postsynaptic 5-HT1A receptors was found using autoradiography employing agonist-stimulated [^{35}S] guanosine-5′-triphosphate (GTP)γS binding.[27] In contrast to postsynaptic receptors, the function of presynaptic 5-HT1A receptors is increased in animals with epilepsy-associated depression, a consequence being strengthened by autoinhibition of 5-HT release and ultimate insufficient neurotransmitter supply into target forebrain areas (e.g., prefrontal cortex and hippocampus).[27]

Stress hormone axis

MDD is closely associated with the hyperactivity of the hypothalamic-pituitary-adrenocortical axis (HPA-A), which is a failure of the negative feedback stress-related hormone loop to adequately regulate the release of cortisol (or corticosterone in rodents) by the adrenal gland. HPA-A dysfunction is detectable using simple laboratory tests (e.g., radioimmunoassay of plasma or serum cortisol/corticosterone). However, free levels of circulating cortisol/corticosterone are not necessarily elevated in MDD patients. Instead, to reveal the HPA-A dysfunction, either the dexamethasone (DEX) suppression test or the combined DEX-corticotropin-releasing hormone (CRH) test is administered.[28] A positive DEX suppression test consists of the inability of DEX to suppress plasma glucocorticoid level, whereas a positive combined DEX/CRH test is characterized in addition by the exacerbated rise in glucocorticoid in response to exogenously administered CRH. Using the latter assay, the hyperactivity of the HPA-A was shown both in TLE patients with concurrent depression and in an animal TLE/depression model.[29,30]

Inflammation

The connection between depression and chronic inflammation has been repeatedly documented. Inflammatory cytokines, such as interleukin (IL)-1β, IL-2, IL-6, interferon-γ, and tumor necrosis factor alpha (TNF-α) are present in the blood of patients with MDD.[31] An astroglia-specific S100 calcium-binding protein β (S100β) has been shown to be consistently elevated in the serum of patients with MDD. Several prospective studies have suggested that plasma levels of C-reactive protein (CRP) may serve as a potential biomarker to predict the onset of MDD.[31] At the same time, brain inflammation has been well established in patients with TLE and corroborated in various animal models of chronic epilepsy.[32] Elevated blood levels of CRP, S100β, and IL-6 have been observed in patients with epilepsy,[33–35] whereas increased plasma levels of IL-1β have been specifically connected with epilepsy-associated depression.[36] Similarly, increased levels of plasma IL-1β, IL-6, TNF-α, CRP, and S100β protein have been detected in an animal TLE model.[37,38] Although the latter studies did not directly connect inflammatory biomarkers with depressive impairments, the employed epilepsy model is consistently characterized by depressive behavior.[27,30]

In conclusion, several lines of evidence suggest that in experimental models of acquired epilepsy, activation of specific cell signaling pathways is associated with cognitive decline or comorbid depression. Because the same signaling pathways are often critical regulators of cellular mechanisms underlying synaptic plasticity, their detection may become a promising approach for identifying the risk of cognitive comorbidities following epileptogenic brain injuries. Their brain detection, however, may also be challenging. Future studies are awaited to determine if levels of pSTAT3, pS6, and/or ROS in cerebrospinal fluid (CSF) similarly rise following brain injury, and if these levels correlate with cognitive outcome with sufficient specificity to provide a more clinically accessible biomarker of future risk of cognitive dysfunction. Moreover, it remains to be seen to what extent these biomarkers are specific for epilepsy-associated comorbidities, or are present in patients with epilepsy notwithstanding comorbid conditions.

Imaging Biomarkers of Comorbidities

Neuroimaging is commonly used in epilepsy since it informes about the diagnosis, localization, and etiology of the disease. Here, we review the evidence supporting the utility of imaging studies as biomarkers for the frequently encountered neuropsychiatric comorbidities of epilepsy.

Intellectual disability (ID)

ID is a common comorbidity in patients with seizures, particularly those with intractable epilepsy. An intelligence quotient <79 is found in nearly 60% of patients with medically refractory epilepsy.[39] Notably, epilepsy severity cannot fully predict the degree of ID, even in those with the same genetic disorders.[40,41] Standard neuroimaging with computed tomography (CT) and MRI has not yielded insight into this association of epilepsy and ID. However, imaging modalities evaluating brain microstructural changes may be used in the future to predict cognitive impairments in patients with epilepsy. Volumetric MRI quantifies the size of the whole brain and regional structures using anatomic guides. Voxel-based morphometry uses automated comparisons of the distribution of gray and white matter among groups. Investigations using these techniques in TLE patients with concomitant deficits in verbal learning and memory demonstrated decreased hippocampal volumes and to a lesser extent decreased volumes of the thalamus, amygdala, and the mammillary bodies.[42] Changes in global white matter volume may also predict cognitive deficits in epilepsy, although results are variable.[43,44] Investigations of specific white matter tracts using diffusion tensor imaging (DTI) and tractography may provide a better marker of this association. DTI allows the evaluation of water diffusivity and therefore a quantitative assessment and visualization of predetermined white matter tracts. For example, in a cohort of patients with frontal or temporal lobe epilepsy and ID, white matter connectivity between brain regions is less robust and less well organized compared to those with epilepsy and normal IQ.[45] Investigations among patients with epilepsy and ID using MR spectroscopy (MRS) evaluating cerebral metabolic parameters, or fMRI evaluating resting state connectivity are limited, but have the potential to become novel biomarkers for specific cognitive impairments in epilepsy.[42]

Anxiety and attention-deficit/hyperactivity disorder (ADHD)

Akin to neuroimaging findings in patients with epilepsy and ID, patients with epilepsy and anxiety demonstrate alterations in volumes of gray matter structures. In children with epilepsy and an anxiety disorder, the volume of the amygdala was increased and the thickness of orbitofrontal cortices was reduced.[46] Widespread changes in gray matter are also observed in children with epilepsy and ADHD.[47] Although studies of connectivity and white matter integrity are lacking in these groups, fMRI may afford an additional marker of comorbid disease. In children with benign childhood epilepsy and ADHD, resting-state fMRI demonstrates a decrease in the blood oxygen level–dependent (BOLD) signal in the dorsal attention network (DAN) compared to children with epilepsy without ADHD, thereby implicating changes in the interplay between sensory stimuli and control of spatial attention.[48] As with the findings described in patients with epilepsy and ID, no prospective studies are

yet available to delineate the timeline of these anatomic and functional changes with regard to the development of the comorbidity.

Depression

Perhaps the most robust body of evidence supporting the role of neuroimaging as a biomarker of comorbidities in epilepsy is that for depression in patients with TLE. Using MRI-based volumetric and voxel-based morphometry techniques, investigators have described that among TLE patients, orbitofrontal cortical thinning is associated with increasing depressive symptoms.[49] Further insight has been established with functional neuroimaging including PET and fMRI. PET evaluates functional processes using an injected biologically active molecule, or radiotracer. The most commonly used ligand in neurologic imaging is 2-deoxy-2-[^{18}F]-fluoro-D-glucose (FDG), measuring regional cerebral glucose metabolism, although additional ligands can evaluate numerous neuronal functions. In cohorts of patients with TLE and depression, PET with radiotracers directed to the 5-HT1A receptors demonstrate widespread alterations within the limbic system, with decreased receptor binding in the anterior cingulate gyrus and hippocampus ipsilateral to the epileptogenic focus, as well as within the raphe nuclei.[26,49] Similarly, investigations into network connectivity using fMRI demonstrate reductions in the BOLD signal within the hippocampal-prefrontal cortex networks in patients with TLE and depression, implicating dysregulation of frontolimbic networks.[50] Taken together, these imaging findings provide a detailed constellation that may differentiate patients with epilepsy with and without depressive symptoms. However, as with other comorbidities in epilepsy, prospective studies are required to validate these findings and to determine their association with disease onset and symptom progression.

Autism spectrum disorders

Autism spectrum disorder (ASD) is characterized by the constellation of impairments in communication, socialization, and stereotyped or repetitive behaviors. Ten percent to 30% of children with ASD develop epilepsy at some point in their lives, and up to 80% show epileptiform activity on EEG recordings.[51] Conversely, ASD is diagnosed in up to 8% of the patients with epilepsy, with its prevalence elevated in selected populations such as tuberous sclerosis complex (TSC).[52] TSC is a neurocutaneous disorder characterized by hamartomatous growths in multiple body regions, particularly the central nervous system. Nearly 95% of patients with TSC have hamartomas in the cerebral cortex with concomitant epilepsy, among whom 40% are diagnosed with ASD.[53] Standard MRI sequences establish the diagnosis of TSC and delineate the burden and location of cortical tubers. Although early investigations in small cohorts associated tubers in the temporal lobe with an increased risk of ASD, results have not been replicated in larger cohorts.[53,54] The presence and burden of a particular type of tuber, termed cyst-like tubers, may, however, predict the risk of ASD.[53] Functional imaging findings can also be associated with ASD in TSC. FDG-PET demonstrates decreased glucose metabolism in the bilateral temporal gyri and/or increased metabolism in the bilateral deep cerebellar nuclei in patients with ASD and epilepsy, although findings are modest (Fig. 2; see also Asano et al.[54]). The extrapolation of neuroimaging biomarkers of ASD to the broader population with epilepsy has been difficult,

likely attributed to the heterogeneity of both disorders. Novel investigations exploring the role of quantitative MRI and tractography may better establish this relationship.

The interplay of epilepsy and psychological and psychiatric comorbidities is undoubtedly complex. Whether these processes develop in tandem, or are a consequence of the same underlying disease, is difficult to delineate. Certainly, evidence of premorbid cognitive changes in children with new diagnosed epilepsy lends support for the utility of diagnostic and predictive biomarkers for comorbidities in epilepsy.[55] Because neuroimaging is used widely in the diagnosis of new-onset seizures, prospective and longitudinal investigations should be readily employed to establish those neuroimaging modalities that best predict severity and progression of comorbidities in epilepsy.

Genetic Biomarkers of Comorbidities

Several studies carried out in patients with epilepsy have addressed the influence of genetic background on cognitive functions, by analyzing polymorphisms related to genes previously associated with cognitive impairment in healthy aging population or in neurologic disorders and their association with the cognitive performance (Table 3).

For example, it has been shown that TLE patients with apolipoprotein E (ApoE) ε4 allele perform worse on verbal and nonverbal memory tasks.[56,57] Moreover, the combined effect of long disease duration (≥22 years) and ApoE ε4 allele greatly increased the risk of cognitive dysfunction.[56,57] The analysis of hippocampal tissue of TLE subjects stratified according to memory tests from “average” to “very severe,” showed that a genetic variant of bridging integrator 1 (BIN1)/amphiphysin 2 is associated with poor memory performance.[58] Such polymorphism is associated with high messenger RNA (mRNA) levels of BIN1.[58] Warburton et al.[59] enrolled a subgroup of subjects from the Standard and New Antiepileptic Drugs (SANAD) trial and they evaluated the presence of polymorphisms in the neuron-restrictive silencer factor (NRSF) and brain-derived neurotrophic factor (BDNF) genes and their association with cognitive functions. Cognitive performance was evaluated in newly diagnosed epilepsy patients (baseline) and again 12 months later. They found that genetic variants of *NRSF* and *BDNF* were associated with greater impairment in memory functions and in psychomotor speed either at epilepsy diagnosis and in the subsequent follow up (e.g., 12 months later).[59]

MicroRNA (miRNA) are noncoding small RNA emerging as the master regulator of gene expression. A relatively novel area of research concerns the regulation of miRNA by learning and memory, as well as in patients with psychiatric disorders. Accordingly, miRNAs involved either in synaptic plasticity[60] or in psychiatric disorders[61] have been identified. Histone and nucleotide modifications (acetylation and methylation, among others) have also been associated with psychiatric syndromes.[62] Such epigenetic modifications might be investigated in epilepsy-associated comorbidities both in experimental models and in human specimens. Genetic and epigenetic markers may have great potential as biomarkers of comorbidities, since DNA is readily available.

Comorbidities as Biomarkers

Accumulating clinical evidence suggests that comorbidities can predict epilepsy outcome. In accordance, presurgical memory deficits and psychiatric disorders are associated with an unfavorable postsurgical seizure freedom (Fig. 3A),[63–65] although other studies failed to confirm such association.[66] Differences in the behavioral phenotype and/or in the sample size analyzed might explain these variable results.

Hitiris et al.[67] analyzed the data of patients with newly diagnosed epilepsy during a 20-year follow-up period in search of clinical factor(s) that predict pharmacoresistance. They found that psychiatric comorbidities preexisting to, or concomitant with, epilepsy, particularly depression, are associated with lack of response to AEDs.[67] Moreover, pharmacoresistant patients exhibited more severe comorbidities than patients who respond to an AED.[68] This evidence was confirmed in experimental models[69]: epileptic rats not responsive to phenobarbital (PB) displayed more marked hyperexcitability, anxiety-like behavior, and cognitive deficits in spatial learning tasks (Fig. 3B) compared to rats responsive to PB, as assessed in the open field, elevated-plus maze, and Morris water maze, respectively.[69] Although the mechanism(s) underlying these differences are presently unknown, one hypothesis is that the presence of comorbidities reflects a more severe disease state characterized by a more extensive epileptogenic area.[63–65] Because comorbidities are associated with poor functional outcomes (unfavorable postsurgical seizure freedom, lack of response to AEDs), the availability of biomarkers that reliably predicts their occurrence in patients at risk for epilepsy (i.e., after brain insults and during suspected epileptogenesis), may predict patient response to treatment and pharmacoresistance.

Preclinical[70–72] and clinical[73,74] data indicate that “premorbid” behavioral deficits occur before the onset of spontaneous seizures or at the onset of epilepsy, thus representing potential early marker of the disease. In this context, it has been shown that psychotic-like behaviors in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a validated model of absence epilepsy, were already present in 6-week-old animals, and therefore prior to the emergence of spike-and-wave discharges.[72] However, differences in epilepsy severity between GAERS colonies were reported recently, highlighting the potential impact of environmental conditions on the severity of epileptic and behavioral phenotypes in this rodent model of epilepsy. In adult rats exposed to pilocarpine-induced SE, spatial but not nonspatial memory was altered before the occurrence of spontaneous convulsive seizures.[70,71] Of interest, psychotic-like behaviors in GAERS were associated with an increase in gamma oscillation in the cortex,[72] whereas in pilocarpine-treated rats, a positive correlation was found between the decrease in theta power in the hippocampus and the cognitive deficits.[71] These EEG rhythms are relevant for cognitive processes.[6]

The preclinical data are consistent with clinical studies where the behavioral profile of patients (mainly with unknown etiologies) was assessed before or at epilepsy diagnosis. Cognitive and psychiatric dysfunctions as well as academic underachievement have been described in children with new-onset epilepsy as compared to classmate healthy individuals or sibling.[55,73,75,76] The presence of such impairments has been documented either at pre-treatment baseline[73] or before the diagnosis of epilepsy.[55,75,76] Similarly, deficits in

attention, memory, executive functions, language,[74] and psychiatric disorders[77] were found in newly diagnosed untreated adult epileptic patients.

The identification of cognitive and emotional deficits prior to or at the time of epilepsy onset suggests that their presence in patients cannot be merely attributed to seizures or to treatment with AEDs. In summary, comorbidities are increasingly recognized as early symptoms of epilepsy possibly sharing with the disease common pathophysiologic mechanisms and/or be a symptom of the disease as well as seizures.[24] It is presently unknown whether these early behavioral deficits correlate with epileptogenesis, and to what extent the same pathogenic mechanisms(s) and/or brain networks are involved in the generation of seizures and in neurocognitive deficits. The experimental evidence showing that cognitive deficits and the propensity to develop epilepsy after SE both increase with age,[78] rein-forces the hypothesis that they may share common molecular changes.

To better address the question to what extent comorbidities may become biomarkers of epileptogenesis, experimental models are required in which epilepsy develops only in a cohort of animals. In this respect, the induction of SE in 21-day-old rats is a highly valuable model for determining factors associated with epileptogenesis, since only 60–70% of animals develop epilepsy in adulthood, although SE is similar in all rats. Using this model, it has been recently shown that reduced rate of learning and accelerated forgetting are two specific features of rats prone to develop epilepsy, and these deficits anticipate the onset of spontaneous seizures.[79] These results provide a proof-of-principle demonstration that assessment of cognitive abilities following potential epileptogenic injuries may represent clinically meaningful biomarkers for early identification of individuals at high-risk for developing epilepsy.

Conclusions and Future Directions

Comorbidities associated with epilepsy may share pathologic mechanism(s) in common with epileptogenesis, may affect ictogenesis, and thus have a profound effect on seizure frequency, response to therapy, and patients' quality of life. The identification of specific biomarkers is thus required for the accurate and early diagnosis of epilepsy-related comorbidities, their prevention, and follow-up treatment efficacy. In attempting to summarize the discussion at the workshop, it is useful to recall the definition and utility of biomarkers in epilepsy (and associated comorbidities).[5] Some of the data presented at the workshop represented mechanistic studies, and it is reasonable to assume that ideal biomarkers will reflect underlying processes. However, there are also utilitarian requirements for biomarkers, when directed to monitor the progression of disease, effect of therapy, and so on. In this regard, translation and implementation of biomarker research for the diagnosis and therapy of specific comorbidities require that the approach be (1) assessable by minimally invasive measures such as structural or functional neuroimaging, biochemical measures in body fluids, or physiologic measures such as scalp EEG; and (2) sensitive and *specific* to the comorbidity under study, and not a general measure of processes involved in neuronal and network adaptation. The challenge in biomarker research on epilepsy comorbidities is to dissect and differentiate (if possible) those that reflect the presence or progression of network changes that underlie recurrent seizures (i.e., epilepsy)

from those that reflect a specific comorbidity. For example, signaling pathways such as the JAK/STAT or the mTOR may be extremely important in mediating epileptogenesis and the evolution of comorbidities, without being specific for a particular comorbidity. On the other hand, a derangement of serotonergic traffic from the raphe nucleus to the prefrontal cortex or the noradrenergic traffic from the locus ceruleus to the prefrontal cortex may be specific to depression or ADHD/impulsivity, respectively, in parallel to (or independent of) the epileptogenic changes. Future research is required to confirm the notion that mechanism-based biomarkers are specific to patients with epilepsy and have added value compared to the existing clinical assessment (e.g., for symptoms of depression). Preclinical and clinical data suggest that such a biomarker-driven approach hints at specific mechanisms that are shared between epileptogenesis and comorbidities, thus highlighting specific novel therapeutics for the prevention of epileptogenesis, reducing the burden of comorbidities, and improving patients' response to AEDs. Identification of some biomarkers (e.g., neuroinflammation) may have broader implications. Cytokine signaling may interact with the JAK/STAT and mTOR pathways, and can potentially affect the course of epilepsy and its cognitive/psychiatric comorbidities. In specific context, inflammatory biomarkers may have more specific prognostic value. For example, IL-6 in pregnant mice may be predictive of autism in the off-spring by itself, whereas the combined effect of IL-6 and IL-1β may be required for epileptogenesis.[80]

In addition to these selected signaling pathways, advances in genetics or epigenetics epilepsy research have identified numerous molecular targets that could be implicated in the pathogenesis of epilepsies and their comorbidities. The development of biomarker assays to measure these targets in accessible tissue (e.g., blood, CSF), the availability of imaging modalities to monitor their expression and/or function centrally in the brain in vivo is essential for bringing these advances to the clinic. Some of these newer bio-marker assays are discussed in this review. The evidence that comorbidities per se can predict epilepsy outcomes suggests that a systematic neuropsychological analysis may be beneficial early after epilepsy diagnosis, before medical treatment is initiated. However, prospective studies are required to validate these findings.

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Biography

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Key Points

- Comorbidities in patients with epilepsy constitute a significant burden for patients and their families
- The identification of biomarkers for the diagnosis, prevention, and treatment of comorbidities in epilepsy is an unmet clinical need
- Perturbations in various signaling pathways can be used to develop new therapeutic approaches
- Potential genetic and imaging biomarkers of epilepsyassociated comorbidities have been identified
- Comorbidities can predict epilepsy outcomes

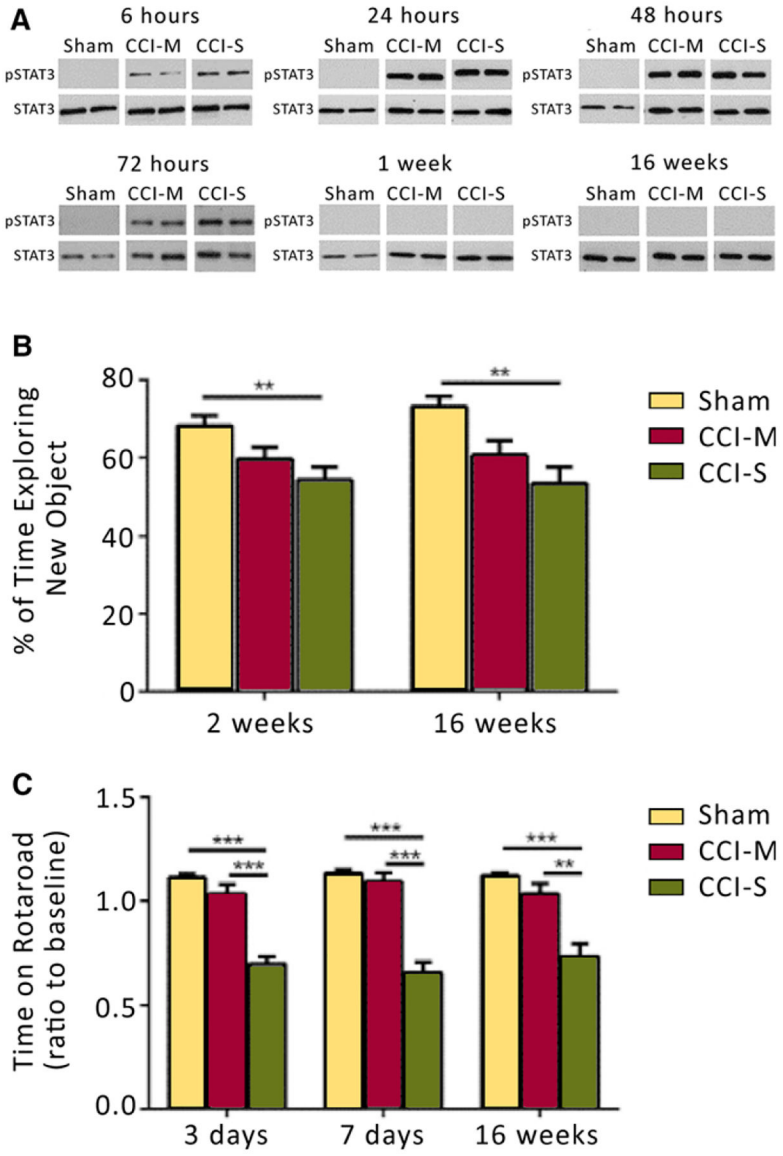

Figure 1.

Phosphorylated STAT3 levels in injured hippocampus correlate with injury severity and cognitive and motor performance after controlled cortical impact (CCI) in mice. (**A**): Representative Western blots of protein homogenates from whole ipsilateral hippocampus (relative to side of CCI) of mice at 6–72 h, 1 week, and 16 weeks after CCI probed with pSTAT3 and STAT3 antibodies. The pSTAT3 levels were higher in both the severe CCI (CCI-S) and moderate CCI (CCI-M) injured groups from 6 to 72 h postinjury when compared to sham-injured controls. Notably at 6 h post-injury, the CCI-S group had statistically higher levels of pSTAT3 than those detected in the CCI-M group. (**B**): Quantification of average time spent exploring the new object during Novel Object Recognition testing shows that the CCI-S injured mice performed statistically worse than the sham-injured controls or CCI-M injured mice. (**C**): Quantification of average time spent on

the rotarod apparatus showed that the CCI-S injured mice performed significantly worse than the CCI-M injured mice or sham-injured controls. *$p < 0.05$, **$p < 0.01$.

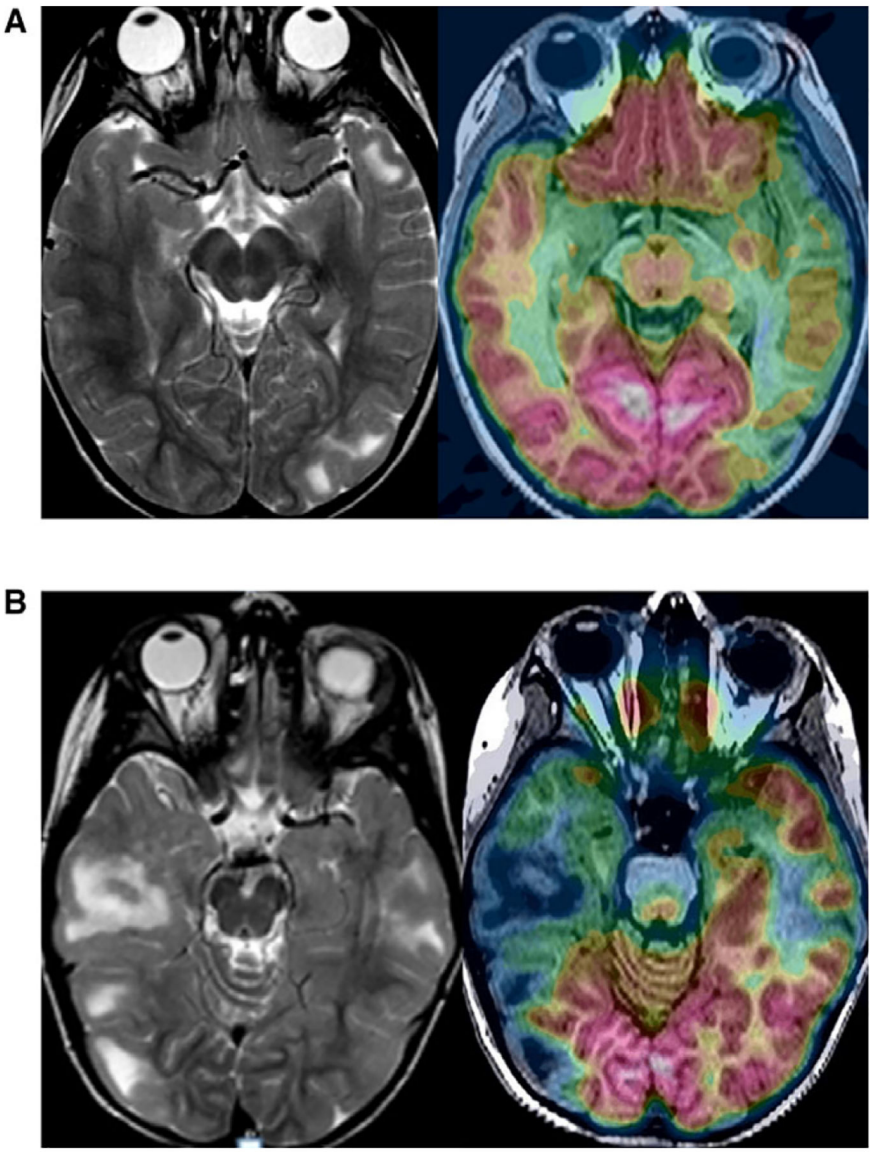

Figure 2.

FDG-PET coregistration with MRI in a patient with ASD, intellectual disability, and epilepsy. Representative images showing the color spectrum correspondent to FDG uptake, with red indicative of high uptake and blue indicative of low uptake, in a patient with ASD, intellectual disability, and TSC. Note several areas of decreased FDG uptake in the bilateral temporal lobes (**A** and **B**).

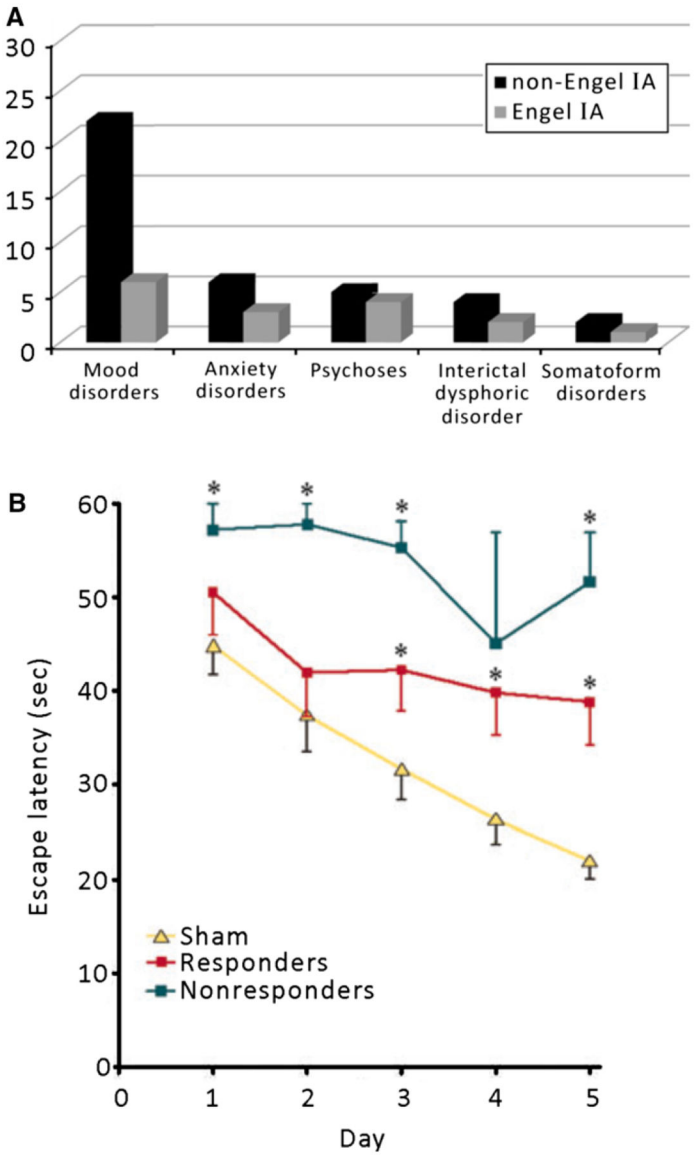

Figure 3.

Clinical and experimental evidence showing the utility of comorbidity assessment to predict epilepsy outcomes. (**A**): Bar gram depicting the evidence that the presence of psychiatric problems before surgery in patients with temporal lobe epilepsy is associated with an unfavorable postsurgical seizure freedom (non-Engel class IA). (**B**): Cognitive performance in epileptic rats as assessed by Morris water maze. Epileptic rats not responsive to phenobarbital (PB) (*Nonresponder*) displayed more marked learning deficits than epileptic rats responsive to PB (*Responder*), as assessed by the longer escape latency measured (i.e., the time needed to reach the hidden platform). Sham-implanted rats are used as controls.

Table 1.

Utility of biomarkers of comorbidity in epilepsy

• To predictthe development, severity, and progression of comorbid condition(s)
• To predictthe progression ofagiven comorbidityafter its development
• Topredicttreatmentefficacy
• To optimize/guide therapeutic strategies
• To identify permanent (i.e., related to epileptogenesis) vs. transient (i.e., related to seizure occurrence and/or medications) impairments

Table 2.

Biomarkers of epilepsy-associated depression

Biomarker	Assay	Change in epilepsy-associated depression
5-HT1Areceptor: postsynaptic	PET (forebrain)	Decreased number
	Autoradiography (forebrain)	Decreased function
5-HT1Areceptor: presynaptic	Autoradiography (Raphe nuclei)	Increased function
HPA-A	Radioimmunoassay (plasma/serum)	Positive DEX suppression test; Positive combined DEX/CRH test
Inflammation: IL-1 β; IL-6; CRP;S100B	ELISA (plasma, serum)	Elevated

5-HT1A, serotonin 1A; HPA-A, hypothalamic-pituitary-adrenocortical axis; PET, positron emission tomography; DEX, dexamethasone; CRH, corticotropin-releasing hormone; ELISA, enzyme-linked immunosorbent assay.

Table 3.

Polymorphisms associated with cognitive dysfunction in epileptic subjects

Gene	SNP or polymorphism	Consequences on comorbidities
ApoE	ε4 allele	Poor verbal and nonverbal memory performance
NRSF	rs1105434 (b), rs2227902 (b), rs2227902 (l)	Greater impairment in memory functions (Ray AVLT delayed score)
NRSF	rs3796529 (l)	Greater deficit in psychomotor speed (VRT)
BDNF	rs1491850 (b), rs2030324 (b) rs11030094 (b), rs12273363 (l)	Greater impairment in memory functions (Ray AVLT delayed score)
NRSF/BDNF	rs2227902G-allele (*NRSF*) associated with rs6265 A-allele (*BDNF*)	Greater impairment in memory functions (Ray AVLT delayed score)
BINI	rs744373 C-allele	Poor verbal and nonverbal memory performance

ApoE, apolipoprotein E; NRSF, neuron-restrictive silencer factor; BDNF, brain-derived neurotrophic factor; BIN1, bridging integrator 1; Ray AVLT, Ray auditory verbal learning task; VRT, visual reaction time; b, baseline; l, longitudinal analysis.