



The Molecular Dichotomy Between Epileptic and Functional Seizures

Association of Epileptic and Nonepileptic Seizures and Changes in Circulating Plasma Proteins Linked to Neuroinflammation

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Objective: To develop a diagnostic test that stratifies epileptic seizure (ES) from psychogenic nonepileptic seizure (PNES) by developing a multimodal algorithm that integrates plasma concentrations of selected immune response associated proteins and patient clinical risk factors for seizure. **Methods:** Daily blood samples were collected from patients evaluated in the epilepsy monitoring unit (EMU) within 24 hours after EEG confirmed ES or PNES and plasma was isolated. Levels of 51 candidate plasma proteins were quantified using an automated, multiplexed, sandwich ELISA and then integrated and analyzed using our diagnostic algorithm. **Results:** A 51 protein multiplexed ELISA panel was used to determine the plasma concentrations of ES patients, PNES patients, and healthy controls. A combination of protein concentrations, TRAIL, ICAM-1, MCP-2, and TNF-R1 provided a probability that a patient recently experienced a seizure with TRAIL and ICAM-1 higher in PNES than ES, and MCP-2 and TNF-R1 higher in ES than PNES. The diagnostic algorithm yielded an AUC of 0.94 ± 0.07 , sensitivity of 82.6% (95% CI: 62.9-93.0), and specificity of 91.6% (95% CI: 74.2-97.7). Further, expanding the diagnostic algorithm to include previously identified PNES risk factors enhanced diagnostic performance with AUC of 0.97 ± 0.05 , sensitivity of 91.3% (95% CI: 73.2-97.6), and specificity of 95.8% (95% CI: 79.8-99.3). **Conclusions:** These 4 plasma proteins could provide a rapid, cost-effective, and accurate blood-based diagnostic test to confirm recent ES or PNES. **Classification of Evidence:** This study provides Class III evidence that variable levels of 4 plasma proteins, when analyzed by a diagnostic algorithm, can distinguish PNES from ES with sensitivity of 82.6% and specificity of 91.6%.

Commentary

I believe the results of the study¹ require a brief recap: 51 serum proteins were investigated but only 4 (TRAIL, ICAM-1, MCP-2, and TNF-R1) were found to be significantly different between groups and fulfilled the aim to aid in the differentiation between recent epileptic and functional seizures. Both diagnoses were confirmed by the gold standard diagnostic method—video/electroencephalogram (EEG) monitoring. The other proteins were either not different between the diagnostic groups or their levels were very low and barely detectable. Of the proteins that were different, TRAIL and ICAM-1 were higher in functional and MCP-2 and TNF-R1 in epileptic seizure patients. When these proteins were used in the diagnostic algorithm for correct detection/identification of epileptic seizures, there were 87.2% overall accuracy, 82.6% sensitivity, and 91.7% specificity. However, when clinical characteristics of functional seizures were added to the algorithm (ie, practitioner was still needed to collect the relevant clinical information), the algorithm performed even better with 94% overall

accuracy, 91.3% sensitivity, and 95.8% specificity. Thus, overall, the performance of the algorithm that included specific protein biomarkers TRAIL, ICAM-1, MCP-2, and TNF-R1 and the clinical features of functional seizures correlated with very high sensitivity and specificity with the diagnostic group.

However, let us first discuss the 2 molecules, MCP-2 and TNF-R1 that were significantly higher in patients with epileptic rather than in patients with functional seizures. First, MCP-2 has been shown to have ~60% structural overlap with MCP-1 that has been documented to be induced by, for example, excitotoxicity or hypoxic-ischemic injury.^{2,3} Similar to MCP-1, MCP-2 has been well recognized to participate in immune-regulation via binding to chemokine receptors and activation chemotaxis in lymphocytes T, natural killer (NK) cells, and monocytes therefore contributing to the pathogenesis of monocyte-dependent tissue injury.⁴ Hence, MCP-2 overexpression could result in an increased immune response. Further, since increased levels of MCP-2 have been observed in patients with Alzheimer's disease, this may further support the



existence of the bidirectional relationship between neurodegeneration and seizures/epilepsy.⁵ The other molecule—TNF-R1—is a membrane receptor that binds TNF- α which plays a major part in mediating neuroinflammatory response. Human brain specimens obtained during, for example, temporal lobe epilepsy resections have also shown inflammatory responses in the perivascular spaces of the ictal onset zone and in the adjacent parenchyma.^{6,7} Taking all of this into account, the notion that neuroinflammation is the key pathology behind focal epileptic seizure initiation and maintenance (and seizure refractoriness) and that the dynamic and adaptive process of neuroinflammation is associated with blood–brain–barrier disruption and glial activation is no longer a surprise.⁶ Thus, the increased levels of MCP-2 and TNF-R1 in patients with epilepsy when compared to functional seizures should not surprise anyone as the first is frequently associated with neuroinflammation, while the second one is not thought of as a neuroinflammatory condition. However, is this dichotomy correct and justified?

Interestingly, the 2 proteins with lower levels in patients with epilepsy (or higher in patients with functional seizures) are proteins that are also involved in the neuroinflammatory cascade. As the authors of the current report indicate, TNF-related apoptosis-inducing ligand (TRAIL) is known in some situations to induce tissue injury in humans and to activate other molecules, for example, ICAM-1 and interleukin 8 (IL-8).⁸ Further, TRAIL expression is repressed in the context of limiting neuroinflammation by molecules produced by T cells and microglia.⁹ TRAIL can activate ICAM-1 that is an adhesion molecule the expression of which is regulated by the TNF- α signaling pathway; this molecule aids in the attachment of leucocytes to the endothelium and in immune response. Further, as the authors of this report indicate, degraded ICAM-1 molecule potentiates pro-inflammatory pathways. Could the increases of TRAIL and ICAM-1 signify the presence of neuroinflammation in patients with functional seizures? Further, it begs a question whether the structural abnormalities observed in some studies such as cortical thinning¹⁰ or white matter abnormalities¹¹ in patients with functional seizures could be at least, in part, the result of TRAIL/ICAM-1 protein overexpression in these patients?

Another 28 proteins were detected in the study, but the differences between the groups were not significant so these proteins were not included in the algorithm. So what is the significance of these not-different proteins, for example, IL-6? Interleukin 6 is known to be elevated in patients with epileptic seizures and has been shown to decrease with cessation of seizures after temporal lobectomy.¹² What are we to think when the neuroinflammatory proteins that are clearly linked to epilepsy and seizure intractability are not different in patients with functional seizures? Is there a link between neuroinflammation and functional seizures and could the dichotomy eluded to above be incorrect? Can studies like the one discussed here help us to decide this? Are there different inflammatory pathways involved in epileptic versus functional seizures? Are


there modalities other than molecular analyses, for example, neuroimaging that could help us with these questions?

Well, let us get off the neuroinflammatory soapbox and back to the study. There have been many studies published, some of them referenced by the authors of this report, that focused on predicting the diagnostic category based on clinical presentation of patients and their comorbidities. Features such as pan-positive review of systems, presence of multiple somatic complaints, psychiatric comorbidities, sex, history of abuse/trauma and so on have been used for this purpose. While none of them came as close to the sensitivity and specificity of the molecular biomarkers in the present study, all of them constitute a set of clinical features we as clinicians rely on in establishing the final diagnosis. As epilepsy specialists, in patients with functional seizures, we have EEG to aid us in the diagnosis. But, others who diagnose and take care of patients with other functional neurological disorders don't have that comfort—could a set of molecular biomarkers in addition to clinical and historical features help us quickly determine the diagnosis so that we don't have to spend years treating patients for what they don't have—functional seizures and/or other functional neurological disorders?

The authors are absolutely correct that the algorithm that includes the quantification of these protein molecules and clinical features could aid in rapid and accurate test to confirm, especially in the emergency room setting, whether the observed or reported event was an epileptic or a functional seizure. However, these remain very early findings and need to be validated and reproduced. As all biomarker research, a multitude of potential confounding variables could have accounted for the observed differences. Before we implement this algorithm, we need to complete the prospective and blinded studies testing this algorithm. If eventually robust and validated, we will need to set up the necessary assays so that they can be run rapidly in every hospital laboratory and clinic, and publish the calculator that gives us the probability of which type of event was experienced by the patient. Until we meet these needs, my job as an epileptologist monitoring patients with video and EEG is safe.

By Jerzy P. Szaflarski, MD, PhD 

ORCID iD

Jerzy P. Szaflarski, MD, PhD  <https://orcid.org/0000-0002-5936-6627>

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