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Review

Biomarkers in the diagnosis and study of psychogenic nonepileptic seizures: A systematic review

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ABSTRACT

Objective: Video electroencephalography (vEEG) is the gold-standard method for diagnosing psychogenic nonepileptic seizures (PNES), but such assessment is expensive, unavailable in many centers, requires prolonged hospitalization, and many times is unable to capture an actual seizure episode. This paper systematically reviews other non-vEEG candidate biomarkers that may facilitate both diagnosis and study of PNES as differentiated from epileptic seizures (ES).
Methods: PubMed database was searched to identify articles between 1980 and 2015 (inclusion: adult PNES population with or without controls, English language; exclusion: review articles, meta-analyses, single case reports).
Results: A total of 49 studies were examined, including neuroimaging, autonomic nervous system, prolactin, other (non-prolactin) hormonal, enzyme, and miscellaneous marker studies. Functional MRI studies have shown PNES is hyperlinked with dissociation and emotional dysregulation centers in the brain, although conflicting findings are seen across studies and none used psychiatric comparators. Heart rate variability suggests increased vagal tone in PNES when compared to ES. Prolactin is elevated in ES but not PNES, although shows low diagnostic sensitivity. Postictal cortisol and creatine kinase are nonspecific. Other miscellaneous biomarkers (neuron specific enolase, brain derived neurotropic factor, ghrelin, leptin, leukocytosis) showed no conclusive evidence of utility. Many studies are limited by lack of psychiatric comparators, size, and other methodological issues.
Conclusion: No single biomarker successfully differentiates PNES from ES; in fact, PNES is only diagnosed via the negation of ES. Clinical assessment and rigorous investigation of psychosocial variables specific to PNES remain critical, and subtyping of PNES is warranted. Future investigational and clinical imperatives are discussed.

1. Introduction

Psychogenic nonepileptic seizures (PNES) are a form of conversion disorder defined as paroxysmal episodes resembling epileptic seizures (ES) while lacking electroencephalographic (EEG) correlation [1,2]. Changes in diagnostic methods for PNES have evolved over the years, though video electroencephalography (vEEG) is currently considered the best diagnostic option in determining ES from PNES [2]. Nonetheless, this methodology is costly, available in selected clinical environments and of value only if a typical episode occurs during monitoring. Furthermore, while vEEG assessment firmly establishes the presence or absence of epileptic discharges, PNES can only be inferred and not established. Diverse theories have been proposed to describe core psychopathological deficits, traits, or mechanisms driving PNES [3–5], but no pathognomonic biological, psychological, or social marker has been identified.

Given the aforementioned limitations in the use of vEEG for diagnosing PNES, it is prudent to continue to understand the value of other diagnostic modalities. Much emphasis has recently been applied to presumed neurobiological underpinnings of PNES [6], with various lines of investigation seeking candidate biomarkers elucidating pathophysiology, in turn informing potential therapies [7]. Such a clear marker indicating the presence (or absence) of PNES is always in demand amongst clinicians. In order to assess the relative values of potential biomarkers, we conducted a systematic

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review of the diagnostic and investigational utility of all candidate PNES biomarkers to date. We felt compelled to review this from both clinical and investigational angles as an update for the field in order to begin to prioritize diagnostic, treatment, and research imperatives relevant to PNES. Many studies are emerging focusing on the neuroimaging or other biological aspects of PNES; while we support this and feel there is a need for such investigations, we also aim to demonstrate a lack of attention to non-biological markers, such as psychosocial measures. PNES is a complex condition warranting a complex approach; we hope to illustrate with this systematic review that biological reductionism may not be useful in our investigational or (especially) clinical endeavors with PNES.

We hypothesized that this review would reveal a paucity of evidence supporting any one biomarker in the diagnosis or study of PNES and set out to test this with a systematic examination of existing literature.

2. Methods

We conducted an extensive literature search utilizing PubMed and the following terms: "psychogenic non epileptic seizures," "pseudoseizure," "non-epileptic attacks," "functional epilepsy," "hysterical seizure," "psychogenic seizure," "seizures," and "epilepsy." These terms were used in various combinations with a variety of terms used routinely in medicine as biomarkers of physiological functioning and/or disease: "hormone," "enzyme," "amino acid," "inflammatory marker," "cytokines," "cell," "neurotrophins," "neurotransmitter," "ammonia," "oxygen," "carbon dioxide," "metabolism," "heart rate," and "blood pressure." Finally, we also included terms capturing methodologies of assessment and/or sampling approaches: "galvanic skin response," "skin," "pupil," "autonomic nervous system," "serum," "cerebrospinal fluid," "computed tomography (CT)," "magnetic resonance imaging (MRI)", "functional MRI (fMRI)," and "neuroimaging." Studies published between 1980 and 2015 were screened initially, and additional articles were identified via references. Studies included had both ES and PNES patients with or without healthy controls (HC). Select case reports with pertinent findings were also included. Exclusion criteria included other forms of conversion disorder (including functional movement disorders), review articles, meta-analyses, and articles in languages other than English. We did not include electrographic studies including EEG/vEEG or single photon emission CT (SPECT) because these are neurophysiological tests currently considered to be the most robust or best available approaches to assessing ES versus PNES; vEEG specifically is considered the gold-standard of seizure diagnosis while SPECT is less commonly-utilized. Ultimately, a total of 49 articles were included for systematic review. A modified PRISMA flowchart for our systematic review methodology is given in Fig. 1. Post-review levels of evidence were applied to various

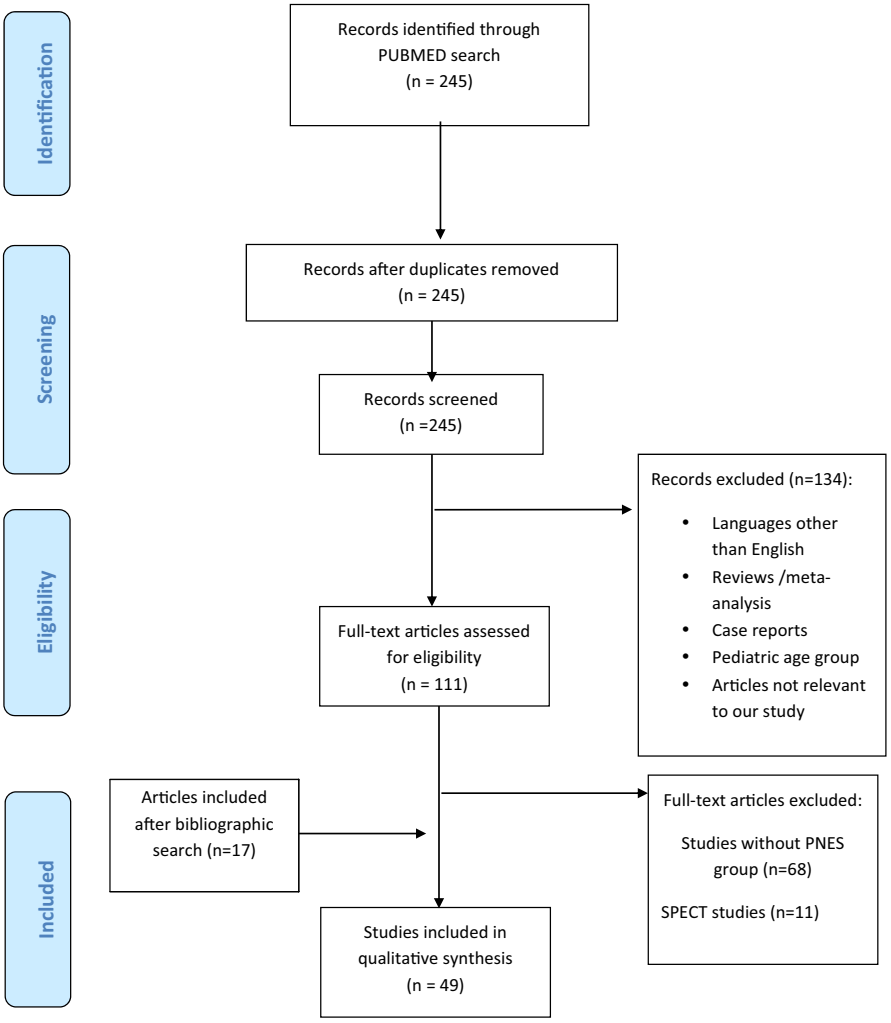

Fig. 1. Modified PRISMA flow diagram for the methodology.

biomarkers based on a scoring system provided by consensus experts (explained and cited in discussion section).

3. Results

The findings of our review are organized under various headings as follows:

3.1. Neuroimaging markers

A number of structural neuroimaging approaches have been used to study abnormalities associated with PNES (Table 1).

Structural central nervous system (CNS) abnormalities on CT and MRI have been demonstrated to varying degrees in patients with PNES when compared to ES, although studies have been

Table 1
Neuroimaging studies in PNES.

Ref.	Study	Imaging	*n*	PNES	ES	HC	Study type	Results/findings	Strengths/limitations/ comments
[8]	Benbadis et al. (2000)	MRI	4	4	0	0	Case series	All abnormal MRI (mesial temporal sclerosis), while PNES on EEG; MTS not specific to ES	Very small sample size, no HC group
[9]	Devinsky et al. (2001)	CT, MRI	201	79	122	0	Retrospective	85% PNES ≥1 finding, unclear significance (+ RT-sided laterality)	No HC group
[10]	Reuber et al. (2002)	MRI (plus EEG and neuropsychiatric evaluation–NPS)	329	329	123	0	Retrospective	PNES 22% ≥1 finding (EEG 9%, MRI 10%, NPS 10%) PNES + ES: 92% ≥1 findings (EEG 71%, MRI 60%, NPS 53%) MRI abnormalities: PNES + ES > PNES ($p < 0.0001$)	Retrospective, missing MRI/ NPS testing in 55% of PNES group, no HC group
[11]	Labate et al. (2012)	MRI (voxel based morphometry, cortical thickness)	60	20	0	40	Case control	PNES gray matter thinning in RT premotor region, bilateral cerebellum > HC ($p < 0.05$);	No ES group; no psychiatric comparator
[12]	Wieshmann et al. (2002)	CT, MRI	495	18	477	0	Cross sectional	0% PNES with findings; 51% ES ≥1 finding	No HC group
[13]	Ristic et al. (2015)	MRI	74	37	0	37	Case control	PNES cortical thickness LT insula, b/l OFC > ES ($p < 0.05$); PNES b/l insular sulcal depth > HC ($p < 0.001$); PNES cortical surface area = HC	No ES group; no psychiatric comparator
[14]	Lee t al (2015)	Diffusion tensor imaging	32	16	0	16	Case control	PNES white matter connectivity corona radiata, LT temporal gyrus, LT internal/external capsules, LT UF > HC ($p < 0.05$)	Sample size, no ES group, no psychiatric comparator
[15]	Ding et al. (2013)	fMRI (structural, functional & DT)	37	17	0	20	Case control	PNES decreased FC/structural connectivity (decreased coupling); "lattice-like" organization > HC ($p < 0.001$)	Sample size, no ES group, no psychiatric comparator
[16]	Ding et al. (2014)	fMRI	37	17	0	20	Case control	PNES increased FC between lateral middle frontal gyrus, ACC, SMA, b/l median cingulate, insula, occipital cortex > HC ($p < 0.05$)	Small sample size, possibility of head motion artifact, no psychiatric comparator
[17]	Li et al. (2014)	fMRI (insular subregions)	37	17	0	20	Case control	PNES increased FC between insular sub regions and sensorimotor network, ingual gyrus, superior parietal gyrus, putamen > HC ($p < 0.05$).	Same patients as in Ding et al., 2013/2014 studies; small sample size; no psychiatric comparator
[18]	Van der Kruijs et al. (2011)	fMRI	23	11	0	12	Case control	PNES increased FC in insula, inferior frontal gyrus, parietal sulcus > HC ($p < 0.05$); higher dissociation > HC ($p < 0.05$)	Findings confounded by dissociation; no psychiatric comparator
[19]	Van der Kruijs et al. (2014)	fMRI	48	21	0	27	Case control	PNES increased coactivation of cingulate/insular network; cingulate superior parietal lobe, pre/post central gyri, SMA network; and precuneus network > HC; higher dissociation > HC ($p < 0.05$)	Findings confounded by dissociation; no psychiatric comparator
[20]	Arthuis et al. (2014)	FDG-PET	32	16	0	16	Case control	PNES hypometabolism RT inferior parietal/central and b/l ACC > HC ($p < 0.001$).	Retrospective, small sample, PTSD/anxiety not controlled for; no psychiatric comparator
[21]	Hernando et al. (2015)	Diffusion tensor imaging	16	8	0	8	Case control	PNES greater UF streamlines in right hemisphere > HC ($p < 0.03$).	Sample size, due to technical limitations were not able to include all connecting streamlines belonging to UF, minor pathways not included, female preponderance

PNES, psychogenic nonepileptic seizure; ES, epileptic seizure; HC, healthy control; CT, computational tomography; MRI, magnetic resonance imaging; MTS, medial temporal sclerosis; NPS, neuropsychiatric scale; FC, functional connectivity; fMRI, functional magnetic resonance imaging; DT, diffusion tractography; FDG-PET, fluorodeoxyglucose-positron emission tomography; UF, uncinate fasciculus; ACC, anterior cingulate cortex; OFC, orbitofrontal cortex; SMA, supplementary motor area; RT, right; LT, left; b/l, bilateral.

retrospective in design or rather small in size [8–10]. Devinsky et al. [9] found a preponderance of right hemisphere structural abnormalities in a cohort of 79 PNES patients when compared to the ES group, although this is of unclear significance. In a group of 20 PNES patients, brain MRI revealed abnormal gray matter thinning in the right premotor region which positively correlated with incidence of depression [11]. Other studies, however, have found no increased incidence of structural brain abnormalities in PNES as compared to ES [12]. Ristic et al. [13] showed that patients with PNES possessed increased cortical thickness in the left insula and bilateral medial orbitofrontal cortex, as well as increased sulcal depth in bilateral insula and right cingulate when compared to HC, but no differences in terms of cortical surface area or cortical curvature. Similarly, Lee et al. [14] revealed increased white matter connectivity (as measured by fractional anisotropy) in the left corona radiata, internal/external capsules, temporal gyrus, and uncinate fasciculus amongst PNES patients when compared to HC. Although these indicate some potential differences between patients with PNES and HC in terms of brain morphology, various limitations are to be noted (see next paragraph and Section 4).

At least seven studies have used fMRI techniques to investigate PNES patients. In two studies sampling the same subjects ($n = 17$), Ding et al. have shown increased functional connectivity between various cortical areas as well as a "lattice-like" organization in functional and structural connectivity networks in PNES subjects when compared to HC [15,16]. Li et al., utilizing once again the same cohort as Ding et al., have demonstrated hyperlinked functional connectivity in insular subregions in PNES [17]. van der Kruijs et al. have shown increased coactivation of cingulate and insular cortices in PNES when compared to HC, and identified a correlation between such coactivation and both dissociation and emotional dysregulation [18,19]. Positron emission tomography (PET) has revealed a pattern more akin to anxiety or posttraumatic stress disorders (PTSD), characterized by hypo-metabolism in the right inferior parietal and bilateral anterior cingulate cortices [20]. Hernando et al. [21] utilized diffusion tensor tractography to observe dense right hemisphere uncinate fasciculus involvement in PNES as compared to HC. Though these imaging studies are not meant for differentiating PNES from ES, they may contribute insights into the neural basis of PNES. However, they are limited by heterogeneous methodologies, small sample sizes, a lack of ES comparison groups, and a lack of psychiatric comorbidity control. These limitations will be discussed further in a later section.

3.2. Autonomic nervous system

Table 2 summarizes studies examining autonomic nervous system (ANS) changes in PNES as compared to ES. Autonomic dysfunction has long been studied in epilepsy, as passage of epileptic discharges through subcortical limbic structures such as the amygdala, hippocampus, hypothalamus, and insula results in centrally-mediated sympathetic and parasympathetic nervous system changes [22]. Cortical areas such as the insular, anterior cingulate, posterior-orbitofrontal and pre-frontal cortices are similarly implicated in centrally-mediated changes of the ANS [23].

Studies have compared heart rate (HR) and heart rate variability (HRV) responses to seizure events in ES and PNES groups. One study showed that maximal ictal HR above 130 beats per minute (BPM) differentiated ES from PNES with a reported sensitivity of 83%, specificity of 96%, and positive predictive value of 97% [24]. Oliveira et al. also reported ictal tachycardia in 100% of ES (complex partial) patients as compared to zero PNES subjects [25]; the authors replicated this in another cohort, finding ictal and postictal HR elevations in ES (complex partial seizures; CPS) but not PNES groups [26]. Reinsberger et al. found both pre ictal and postictal HR (but not ictal HR) was significantly higher in ES (complex partial) than PNES [27].

Table 2
Autonomic nervous system studies in PNES.

Ref.	Study	Bio-marker	*N* (events)	PNES (events)	ES (events)	HC	Study type	Results/findings	Strengths/limitations/ comments
[24]	Opherk et al. (2002)	HR	105	38	67	0	Cross sectional	ES ictal onset HR > PNES ($p < 0.05$) ES ictal HR > PNES ($p < 0.001$) ES PI HR > PNES ($p < 0.001$)	No age/gender demographics noted
[25]	Oliveira et al. (2007)	HR	59	20	39	0	Retro-spective	CPS ictal/PI HR rise > SPS, PNES (no rise; $p > 0.05$)	Retrospective
[26]	Oliveira et al. (2009)	HR	143	50	93	0	Retro-spective	ES mild/mod/severe ictal HR > PNES mild/mod ictal HR ($p < 0.05$) (only PNES severe ictal HR increased, but return to baseline while no return in ES)	Retrospective
[27]	Reinsberger et al. (2012)	HR	88	42	46	0	Retro-spective	ES preictal and PI HR > PNES ($p < 0.006$ and $p < 0.015$) ES ictal HR = PNES ($p < 0.493$)	Retrospective; age distribution not mentioned
[28]	Mungen et al. (2010)	SSR	75	25	30	20	Cross- sectional	ES UE SSR > PNES ($p < 0.05$)	Not all patients had ictal VEEG
[29]	Ponnusamy et al. (2011)	HRV	129	52	42	35	Cross-sectional	ES SDNN < PNES ($p < 0.01$) PNES/ ES HRV < HC ($p < 0.03$) PNES CSI > HC ($p < 0.003$)	Various potential confounders (comorbid conditions, AED effects, pre-/ post-ictal effects)
[30]	Ponnusamy et al. (2012)	HRV	50	24	26	0	Retro-spective	ES CSI > PNES ($p < 0.001$). ES: High ictal HRV changes = high sympathetic/reduced vagal tone	Retrospective study design
[31]	Reinsberger et al. (2015)	EDA	20	9	11	0	Case control (pilot study)	ES EDR > PNES ($p = 0.0074$)	Pilot study; comorbid psychiatric conditions in various subjects (PTSD, GAD, etc.)

PNES, psychogenic non epileptic seizure; ES, epileptic seizure: HC, healthy control; GTCS, generalized tonic clonic seizure; CPS, complex partial seizure; SPS, simple partial seizure; IC, interictal; PI, postictal; HR, heart rate; BPM, beats per minute; HRV, heart rate variability; VEEG, video EEG; AED, anti-epileptic drug; UE, upper extremity; SSR, sympathetic skin response; SDNN, standard deviation of NN interval; CSI, cardio sympathetic index; FFT technique, fast Fourier transformation technique; EDA, electrodermal activity; EDR, EDA responses.

Other measures of ANS function have been conducted in ES and PNES cohorts. Mungen et al. assessed sympathetic (galvanic) skin response, finding significantly-prolonged response latency in upper extremities of ES both postictally and interictally when compared to PNES [28]. Ponnusamy et al. used time and frequency domains of HRV as measures of vagal tone, correctly identifying 86% of HC subjects from PNES patients using various HRV parameters, though only one parameter (the standard deviation of NN intervals) showed statistically-significant difference between ES and only a subgroup of PNES patients [29]. A second investigation by Ponnusamy et al. examined resting and ictal ANS changes and found that ES (CPS) groups had higher sympathetic tone than PNES cohorts and showed that 88% of ES and 73% of PNES diagnoses were identified correctly using their HRV parameters [30]. These studies in combination suggest that HRV is a superior ANS measure than HR alone in differentiating ES from PNES.

As an additional measure of ANS function, one recent pilot case control comparison of 9 ES and 11 PNES subjects suggested increased electrodermal responses (EDR) in ES when compared to PNES, although the study was limited by sample size and various uncontrolled psychiatric comorbidities [31].

3.3. Hormones

Various hormones have been suggested as biomarker candidates in the differentiation between ES and PNES. Tables 3 and 4 summarize studies involving prolactin and other hormones, respectively.

3.4. Prolactin

Prolactin (PRL), a polypeptide secreted by lactotrophic cells of the anterior pituitary gland, was first isolated in 1970s [32]. Ohman et al. [33] first reported serum PRL elevations following ES in the context of electroconvulsive therapy (ECT), and elevations following spontaneous ES were later described [34]. In ES, propagation of epileptic discharges from mesial temporal structures to the hypothalamus likely results in postictal PRL releases [35].

Multiple studies have revealed elevated PRL levels in ES groups (but not PNES groups) as early as 10–20 min postictally, remaining elevated for up to 2 h thereafter [36–46]. ES patient groups had significantly elevated PRL levels than PNES. With capillary PRL assays, one study found 69% sensitivity (SS) and 93% specificity (SP) in differentiating ES from PNES [47] while another group reported 100% positive predictive value (PPV) in the diagnosis ES [48].

Two of the selected studies, however, failed to support use of PRL as a biomarker. One study found elevated postictal PRL in up to 20% of the PNES cohort [49], while a second group found no statistically-significant difference in postictal PRL between ES and PNES subjects [50].

3.5. Other hormones

Cortisol, a polypeptide hormone integral to stress processing, is released from the adrenal cortex in response to adrenocorticotrophic hormone (ACTH), itself released in response to hypothalamic secretion of corticotrophin releasing hormone (CRH) [51]. Cortisol is known to act on glucocorticoid receptors of the hypothalamus, pituitary, hippocampus, and amygdala to maintain complex homeostatic needs [52].

Studies on postictal cortisol levels have shown mixed results [37,39,42,53]. Tunca et al. reported a rise in cortisol levels during PNES events when compared with basal cortisol levels in HC [54]. Zhang and Liu found a typical pattern of pre-ictal fall and postictal rise of cortisol in ES but no such changes in PNES [55]. Other studies by Bakvis et al. have demonstrated PNES patients possess basal hypercortisolism positively correlated with both traumatic histories and threat vigilance [56,57].

Regarding other hormone candidates, Rao et al. [39] found serum elevations in a number of pituitary hormones such as growth hormone and thyrotropin releasing hormone along with PRL and cortisol following ES but not PNES. Adrenocorticotropic hormone (ACTH) is yet another stress-processing hormone whose level has been observed to be higher in ES but not in PNES postictally in older studies with small sample sizes [53,58]; despite this, the previously-mentioned Zhang and Liu study did not demonstrate this difference [55], calling into question the utility of ACTH in the differentiation of ES from PNES.

3.6. Enzymes

Table 5 lists studies evaluating enzymes as potential biomarkers differentiating ES from PNES.

3.7. Creatine kinase

Creatine kinase (CK) is a muscle-specific enzyme that catalyzes the phosphorylation of creatine to form creatine phosphate, a major energy reserve. Four studies have studied the utility of serum CK in differentiating ES from PNES, and they suggest some utility in postictal CK levels, with a sensitivity of 75%, specificity of 85.5%, positive predictive value of 62.5%, and negative predictive value of 91.4% [43,59,60,61]. These results should be interpreted with caution as CK may be elevated in a number of other clinical situations [62,63].

3.8. Neuron-specific enolase

Neuron-specific enolase (NSE) is a dimeric intracytoplasmic glycolytic enzyme present in neurons and cells of neuroectodermal origin [64]; it is not physiologically secreted, yet released under pathological conditions such as neuronal injury with detection in peripheral circulation as a result of blood–brain barrier disruption [65,66]. NSE has been evaluated as a biomarker in various neurological conditions [67–69] as well as major depressive disorder [70]; it has been found to be elevated in both febrile seizures [71] and other ES [72–74].

Two studies have compared postictal NSE levels amongst ES and PNES patients [43,75], each failing to show any postictal elevation from baseline in PNES cohorts. In addition, this biomarker is limited by falsely elevated levels in any hemolytic conditions [76,77].

3.9. Miscellaneous

Additional studies involving other biomarkers including neuropeptides and cell counts are summarized in Table 5.

3.10. Neuropeptides

Ghrelin and nesfatin-1 have gained importance due to their role in appetite regulation. Ghrelin exerts anticonvulsant action by acting on the receptor-growth hormone secretagogue receptor 1a, in turn increasing gamma-aminobutyric acid (GABA) concentration in the hypothalamus [78]; nesfatin depolarizes paraventricular and arcuate nuclei neurons [79].

Aydin et al. have reported elevated postictal serum and salivary nesfatin in ES but not in PNES, whereas they detected lower postictal serum ghrelin levels in ES when compared to PNES [44]. Unfortunately, no difference was found in the levels of these biomarkers between PNES and HC groups, calling into question

Table 3
Prolactin studies in PNES.

Ref.	Study	Bio-marker	*N* (events)	PNES (events)	ES (events)	HC	Sample timing (min/h)	Results/findings	Strengths/limitations/ comments
[36]	Collins et al. (1983)	PRL	36	8	28	0	PI: 5, 15, 30, 60, 90, 120, 180 min	AUC/CMAX ES > PNES ($p < 0.001$)	No BL measure
[37]	Pritchard et al. (1985)	PRL	12	6	6	0	BL; PI: 15, 30, 45, 60 min	PI PRL ES > PNES [15 min ($p < 0.02$); 30 min ($p < 0.005$), 45 min ($p < 0.05$), 60 min ($p < 0.02$)]	Small sample; all PNES patients were male; no control for AED effects
[38]	Laxer et al. (1985)	PRL	85	21	64	0	PI: 20 min, 24 h	PI PRL rise ES > PNES ($p < 0.001$); PI PRL rise SPS > PNES ($p < 0.1$)	Significant findings
[39]	Rao et al. (1989)	PRL	40	6	6	28	Ictal, PI: 0–2 h, q15 min	PI PRL decrease ES > PNES	Small sample size
[40]	Wroe et al. (1989)	PRL	33	10	23	0	q2 h during admission; PI: 0, 2, 5, 10, 20, 30, 60, 120 min	PI PRL ES > PNES/absence SZ	Most PRL assessments of all
[41]	Bauer et al. (1992)	PRL	78	11	47	20	PI: q 20 min × 24 h	PI PRL ES > PNES	Small sample size
[42]	Mehta et al. (1994)	PRL	97	9	78	10	IC; PI: 10, 20, 30, 60, 120 min	PI PRL ES > PNES (GTCS: 68.33%, PS: 11.11%, PNES: 0%)	Small sample size
[43]	Willert et al. (2004)	PRL	60	12	32	16	Daily sampling, unclear timing	PI PRL ES (10 min – 24 h) > PNES (92% ES vs 41% PNES)	Unclear sampling methods, small size
[44]	Aydin et al. (2011)	PRL	70	16	34	20	PI within 5 min; 1, 24, 48 h	PI PRL ES > PNES/HC ($p < 0.05$)	Significant findings; only male study group
[45]	Shah et al. (2001)	PRL	340	102	238	0	BL, PI within 40 min	PI PRL ES > PNES ($p < 0.01$)	No mention of age distribution
[46]	Anzola (1993)	PRL	59	19	40	0	Admission/BL, 1 hr following; AM day two, AM day three	PI PRL ES > PNES ($p < 0.005$); PI > 60 min: no stat. sig. difference	PNES = "syncopal attacks" (despite excluding cardiac causes)
[47]	Ehsan et al. (1996)	PRL (+ cap)	50	14	36	0	PI: 15, 75 min	PI PRL ES > PNES (SS: 69%, SP: 93%)	No baseline; assessed venous + capillary PRL
[48]	Fisher et al. (1991)	PRL (+ cap)	20	4	16	0	PI: within 20 min	PI Venous PRL ES/ PNES > capillary PRL ES/PNES ($p = 0.5$) capillary PRL PPV 100% GS, 71% CPS, 100% PNES (NPV)	Small PNES size; no baseline; assessed venous + capillary PRL
[49]	Alving (1998)	PRL	58	20	38	0	PI 15 min; 2 h	PI PRL rise ES > PNES ($p < 0.001$); 20.4% PNES relative increase >2x	Low SS/SP/PPV in determining ES vs PNES
[50]	Shukla et al. (2004)	PRL	36	19	17	0	PI: 15–20 min	PI PRL ES = PNES ($p = 0.24$)	Single PI PRL level; no BL measure; no stat. sig. difference

SZ, seizure(s); PNES, psychogenic non epileptic seizure; ES, epileptic seizure; HC, healthy control; PRL, prolactin; GTCS, generalized tonic clonic seizures; GS, generalized seizure; CPS, complex partial seizure; PS, partial seizure; PI, postictal; IC, interictal; AUC, area under curve; CMAX, maximum concentration; TMAX, time taken to attain maximum level; BL, baseline; VEEG, video EEG; PPV, positive predictive value; min, minute(s); h, hour(s); cap, capillary; SS, sensitivity; SP, specificity.

Table 4
Hormonal studies in PNES (prolactin excluded).

Ref.	Study	Biomarker	*n*	PNES	ES	HC	Sample timing	Results/findings	Limitations/comments
[37]	Pritchard (1985)	Cortisol	12	6	6	0	Baseline; PI: 15, 30, 45, 60 min	Baseline ES > PNES; PI cortisol ES > PNES ($p < 0.001$)	No HC group; small sample size
[39]	Rao et al. (1989)	Cortisol, TRH, GH, catecholamine (NE), melatonin	40	6	6	28	IC/PI: 0–2 hr, every 15 min; 24 hr: every 3–4 hrs 3 additional samples hourly	PI PRL & TRH ES > PNES ($p > 0.05$); PI GH ES (and decrease) > PNES ($p < 0.02$); Cortisol ES > PNES; Mean NE ES/PNES < HCl	Small sample size
[42]	Mehta et al. (1994)	Cortisol	97	9	78	10	PI: 10, 20, 30, 60, 120 min; IC	PI cortisol PNES > ES (no stat significance)	Small sample (PNES) size
[53]	Gallagher et al. (1984)	ACTH, cortisol	37	5	23	9	1st: 8–9.30 AM, every 5 min 2nd: 2–4PM, every 5 min	ACTH ES > PNES ($p < 0.05$); cortisol mean and rate no statistical significance	No demographics; only AM level compared between ES/PNES; 11 ES patients post-lobectomy
[54]	Tunca et al. (2000)	Cortisol	26	18	0	8	IC, circadian (11–12AM, 1–2PM, 4–5PM); day two 8AM	IC/AM/PM cortisol PNES > HC ($p = 0.22$)	No ES control group
[55]	Zhang et al. (2008)	ACTH, cortisol	47	11	36	0	8AM (wake), 12AM (sleep)	ACTH changes ES = PNES; PM cortisol PNES slightly > wake level ($p > 0.05$)	No HC group
[56]	Bakvis et al. (2009)	Cortisol (saliva, as correlated to AB	56	19	17	20	40 min pre-AB task (angry faces)	Correlation PNES vs ES: $Z = 1.64$ ($p = 0.05$); PNES vs HC: $Z = 1.52$ ($p = 0.064$)	No difference in correlations
[57]	Bakvis et al. (2010)	Cortisol (saliva)	37	18	0	19	Awakening cortisol: 0, 15, 30, 45, 60 min; post DST	CAR PNES = HC ($p < 0.279$); post DST PNES = HC after controlling for confounders (smoking, psychotropic medications)	No ES control group
[58]	Gallagher (1987)	ACTH	10	5	5	0	8–9.30AM, every 5 min	Mean ACTH ES > PNES ($p < 0.05$)	No HC group; small sample size

PNES, psychogenic nonepileptic seizure; ES, epileptic seizure; HC, healthy control; PI, post ictal; IC, interictal; GTCS, generalized tonic clonic seizure; PS, partial seizure; ACTH, adrenocorticostimulating hormone; CAR, cortisol awakening response; DST, dexamethasone suppression test; SS, statistical significance; NE, nor epinephrine; AB, attentional bias.

Table 5
Miscellaneous (enzyme, cellular) biomarker studies in PNES.

Ref.	Study	Biomarker	*n*	PNES	ES	HC	Sampling time	Results/findings	Limitations/comments
[43]	Willert et al. (2004)	NSE, CK	60	12	32	16	Baseline; PI 10, 20, 30 min & 1, 6, 12, 24 h	PI NSE/CK ES > PNES; ($p = 0.017$);	Early termination of study missing delayed CK release
[44]	Aydin et al. (2011)	Nesfatin-1, ghrelin	70	16	34	20	PI within 5 min, 1, 24, 48 hr	PI nesfatin ES > PNES/HC ($p < 0.05$); PI ghrelin ES < PNES/HC ($p < 0.05$); +nesfatin/PNES	Only male study group; unclear significance
[45]	Shah et al. (2001)	WBC count	340[a]	102[a]	238[a]	0	Baseline, PI	PI WBC ES > PNES ($p < 0.01$)	Unclear demographics; no HC group
[59]	Wyllie et al. (1985)	CK	22	6	16	0	Daily random samples, quantity	PI CK ES > PNES ($p = 0.05$)	Unclear sampling time/ quantity; no HC group; small sample size
[60]	Holtkamp et al. (2006)	CK	18	8	10	0	PI (within 24 hr)	Low CK level in PNES than ES; PNES 38 U/l vs ES 699 u/ l($p = 0.001$) ($n = 8$ each)	Retrospective study design; small sample size; no HC group
[61]	Petramfar et al. (2009)	CPK	82	20	20	20 (+ 22 VVS)	PI (within 12–15 hrs)	PI CPK ES > PNES/HC ($p < 0.0001$).	No baseline level; diagnostic heterogeneity (vasovagal HC group)
[75]	Rabinowicz et al. (1996)	NSE	43	10	15	18	Baseline; PI	Baseline NSE ES = PNES; PI CNSE ES = PNES	No significant findings
[84]	LaFrance et al. (2010)	BDNF	44	12	15	17	Unknown sampling methods	BDNF PNES < HC ($p < 0.001$); BDNF ES < HC ($p < 0.001$); BDNF PNES = ES ($p < 0.868$)	Unclear sampling time/ quantity; HC average 20 years younger than ES/PNES
[87]	Cupello et al. (2008)	Platelet membrane serotonin transport (SERT)	66	16	27	23	PI, within 4 days	Paroxetine binding ES within 4 days < HC/ES past 4 days ($p < 0.01$); paroxetine BMAX ES < HC ($p < 0.001$) & PNES ($p < 0.01$)	Single sample; no baseline level for controls; ES group with seizure past 4 days data taken from previous Cupello et al. study (2005)

PNES, psychogenic non epileptic seizure; ES, epileptic seizure; HC, healthy control; PI, postictal; NSE, neuron specific enolase; CK, creatine kinase; CPK, creatine phosphokinase; VVS, vasovagal syncope; BDNF, brain derived neurotropic factor; WBC, white blood cell; BMAX, density of serotonin uptake sites.
[a] Events.

their utility; their use in differentiating ES from PNES remains to be determined via replication studies.

3.11. Brain derived neurotrophic factor

Brain derived neutrotrophic factor (BDNF) is a neurotrophin with important effects on neurogenesis and neuronal plasticity [80], abundantly present in the hippocampus and the entorhinal cortex [81]. Increased serum BDNF has been demonstrated in children with epilepsy [82], while lowered amounts were noted in conversion disorder and major depressive disorder [83]. Only one study by Lafrance et al. demonstrated lower postictal BDNF in patients with ES and PNES when compared to HC [84], thereby lacking specificity in differentiating seizure types.

3.12. Leukocytosis

Shah et al. reported transient rise in peripheral white blood cell (WBC) count following 36% of generalized seizures, 7% of complex partial seizures, and zero cases of PNES [45]. It is believed that excessive muscular activity secondary to catecholamine surges in ES result in WBC margination [85,86]. This biomarker is limited by cortisol effects and numerous other clinical situations resulting in transient WBC elevations.

3.13. Platelet membrane serotonin transporter

Serotonin function has been implicated in numerous neuropsychiatric disorders, and platelets have long been investigated as peripheral markers of CNS serotonergic activity. Cupello et al. used a paroxetine binding system to study the density of platelet membrane serotonin transporter (SERT) in both ES and PNES groups [87]. The study showed a significant decrease in platelet SERT density in ES patients and no changes in the PNES group. The exact mechanism for this is unclear.

4. Discussion

Many biomarkers have been assessed as candidates in the differentiation of ES from PNES. Our review indicates varying degrees of utility in these measures, each with inherent strengths and limitations.

Structural neuroimaging (CT, MRI) studies comparing ES and PNES populations are few; different analyses suggest contradictory findings and deficits (some with right-sided laterality and others with left-sided predominance); very little can be confidently extrapolated from these studies due to methodological limitations and small sizes. Functional neuroimaging of PNES groups repeatedly reveal impaired cortical and subcortical connectivity at rest when compared to HC; nonetheless, these studies are unfortunately limited by heterogeneous findings (different CNS regions of interest), small sample sizes, no ES comparison group, and the lack of psychiatric controls. Perez et al. [7] have recently summarized neuroimaging studies in both PNES and functional movement disorders, concluding that psychiatric controls with commonly-comorbid conditions (anxiety/mood disorders) will be needed to confirm whether such functional neuroimaging findings are specific to PNES or secondary to commonly occurring psychiatric co-morbidities.

Histories of childhood trauma, abuse, and/or neglect are commonly found in patients with PNES as well as in populations with mood and anxiety disorders, undoubtedly contributing to frequent association of PNES with these disorders. While this demands careful psychiatric co-morbidity studies of PNES candidate biomarkers [88–93], fear-based anxiety disorders such as posttraumatic stress disorder (PTSD) offer relevant psychobiological models of PNES. A recent meta-analysis [94] of neuroimaging studies comparing PTSD subjects with HC reveals statistically-significant increased activation of certain CNS structures (right anterior insula, bilateral precuneus, left supplementary motor area, left superior temporal gyrus, left premotor anterior cingulate

cortex, anterior orbitofrontal cortex) in PTSD when compared to trauma-naïve HC. As many of these structures are also found to be activated in PNES, future neuroimaging studies would require comparison arms with ES as well as PTSD/other psychiatric populations. Beyond these confounders, fMRI remains an impractical diagnostic tool in the differentiation of ES from PNES due to its high cost and limited availability.

ANS studies measuring HR, HRV, and SSR collectively suggest marked sympathetic and cardiovascular postictal changes in ES groups as compared to PNES cohorts. These studies are hampered by design (half are retrospective [25,26,27,29]) and methodology (various comorbid and other confounders [29,30]); furthermore, distal markers of increased postictal sympathetic activity are crude measures with multiple other potential causes, restricting their specificity. Despite these limitations, certain indicators of ES may prove useful (e.g. prolonged return of HR to baseline postictally [26]) as adjunct diagnostic tools alongside other findings.

Of all the biomarkers examined in this review, PRL appears to possess the highest and most consistent sensitivity in diagnosing ES from PNES. This is long-known and postictal PRL levels remain a useful and often-utilized diagnostic aid. All but two [49,50] of the 15 studies examined here confirm such utility, reporting at times sensitivity as high as 93% [47] and negative predictive value as high as 100% [48]. According to the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology [95] PRL offers an approximate diagnostic sensitivity of 96% for all types of ES. The Subcommittee recommends administering the PRL test postictally within 10–20 min and promote it as a useful adjunct in the differentiation of ES from PNES in both adults and children.

Eight studies assessed cortisol as a biomarker in the differentiation of ES from PNES; although some studies showed increased postictal cortisol in ES groups when compared to PNES [37,39], others showed the reverse [42] or no statistically-significant difference at all [53]. Of all the study groups, these investigations showed the highest methodological heterogeneity, particularly varying collection times and approaches. Given cortisol's reactivity in a number of physiological/diurnal and pathological states, its specificity is rather low and thus should not be used as a diagnostic aid in the diagnosis of PNES. The same can be said about related hormones, such as ACTH and TRH.

Other protein and cellular biomarkers were examined. CK/CPK showed the highest promise as an adjunct diagnostic tool, as several studies [43,59–61] indicated elevated postictal CK/CPK levels in ES groups as compared to PNES cohorts. In addition, a delayed CK/CPK release was noted, indicating some value in later, postictal phases of assessment. As mentioned earlier, these results should be interpreted with caution due to multiple confounding conditions and states that may elevate CK/CPK. However alongside other clinical features, this marker may assist in differentiating seizure types, particularly in the absence of other diagnostic aids or in cases of unwitnessed events. Other protein and cellular biomarkers either failed to demonstrate any difference or are isolated studies requiring replication prior to confirmation of their diagnostic utility.

Our review is far from comprehensive yet offers insights into the relative utility of various biomarkers in the clinical evaluation or research investigation of PNES. Strengths of this analysis include capture of an extensive array of biomarker candidate types, a large time span, and a systematic review methodology. Limitations include lack of review of neurophysiological assessments (EEG, vEEG, SPECT), clinical approaches (e.g. saline seizure induction, physical examination techniques, clinical characteristics of seizures), or psychosocial/psychological evaluations [Minnesota Multiphasic Personality Inventory (MMPI), Personality Assessment Inventory, neuropsychological testing]. Our review was intentionally limited to biomarker candidates with presumed diagnostic and investigational import so as to answer questions regarding their surrogate significance. In addition, our analysis of studies as reported in this review is meant to be cursory and limited in depth given journal limitations.

It should be noted that others have reviewed diagnostic methods utilized in differentiating ES from PNES. Cuthill and Espie [96] examined 13 studies between the years 1984 and 2000 with a range of evaluation procedures (MMPI, saline seizure induction, PRL, SPECT, ictal/postictal clinical characteristics, and pre-ictal pseudosleep), but no other neuroimaging or hormonal/cellular/protein biomarkers. The authors found varying degrees of sensitivities and specificities for these modalities, noting that clinical ictal characteristics showed the highest combined sensitivity/specificity while postictal SPECT had the lowest combined sensitivity/specificity. Other modalities such as pre-ictal pseudosleep showed high specificity but low sensitivity while other approaches (MMPI, patient-reported post-ictal symptoms) showed conflicting evidence. The reader is referred to Cuthill and Espie's work for further examination.

5. Biomarkers and significance

Our review's focus is somewhat narrower than that taken by Cuthill et al. in its emphasis on measurable biomarkers, yet our scrutiny of many more studies provides a broader assessment of candidate gauges of disease. A biomarker has been defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention," while a surrogate is understood as "a biomarker intended to substitute for a clinical endpoint [97]." Our study focused on biomarkers serving as surrogate indicators of presence or absence of epilepsy in the face of seizures; the ideal biomarker would reliably designate either a seizure episode or a seizure patient as suffering from ES versus PNES. Based on our review, postictal PRL levels may be the most reliably elevated biomarker in ES but not in PNES, suggesting its high utility in differentiating seizure types with or without EEG/vEEG confirmation.

Consensus groups have offered mechanisms to assess the levels of evidence for biomarkers. Lassere et al. [98] provide a scale of increasing validation for clinical decision-making with biomarkers on the lower end to actual patient outcomes on the other end. In this model, levels of evidence (Level 1 being the highest level of evidence, Level 5 the lowest) are determined via a scoring system assessing the target in question, studies of this target and their designs, statistical strengths, and any penalties due to lack of evidence or contrary evidence. Although findings from our analysis do not translate directly to this approach, biomarker candidates assessed in this review only approximate mid-range levels of evidence at best (e.g. PRL alone achieves a Level 3 given reliable findings across numerous studies, whereas other biomarkers achieve Level 4 or 5 level of evidence). On a clinical dimension, these biomarkers may have varying degrees of diagnostic utility, yet none have been correlated with actual patient outcomes. Similarly, from an investigational standpoint the biomarkers assessed in this review remain inconclusive in elaborating the actual pathomechanism of PNES, as none of the markers definitively indicate presence of PNES disease and/or such markers have not been compared with ES cohorts (as in the case with functional neuroimaging studies).

Ultimately, biomarker candidates in PNES have failed to offer much more than diagnostic negation (PNES is diagnosed by the absence rather than the presence of any biological measure). Less

attention has been directed at clinical interventions with measurable outcomes, although these have been summarized and certain psychopharmacological (sertraline) and psychotherapeutic (cognitive behavioral therapy, psychodynamic therapy, group therapy) options have shown promise [2]. PNES has been conceptualized as a truly biopsychosocial condition [4] and as such may require complex diagnostic [99], investigational [100], and therapeutic [101] approaches that only minimally rely on biomarkers. Our study and its lack of a reliable, PNES-specific biomarkers thus supports the use of clinical and psychosocial (not neurobiological) determinants in the diagnosis and treatment of this condition, even when vEEG-confirmation is available.

6. Conclusions and future directions

This review has examined biomarker candidates that may assist clinically in the differentiation between ES and PNES. In addition, this review examined which indicators may benefit from future investigational analysis. As a whole, there are few if any biomarkers with reliable utility in differentiating ES from PNES; we thus feel there is indeed a paucity of evidence-based support for the use of any biomarkers in the diagnosis and/or study of PNES. PRL does offer consistent negative predictive value; its relatively low cost and risk burden continues to solidify its position as an attractive adjunct to clinical diagnosis. Other markers are understudied or fail to show utility. Finally, PNES neuroimaging studies require significant replication in larger samples and require comparison groups with both ES and psychiatric comparisons. Such direct comparison with psychiatric populations should extend beyond neuroimaging studies to include ANS and hormonal domains. As mentioned, a promising psychiatric model for PNES pathophysiology is PTSD; beyond the aforementioned neuroimaging deficits shared by both PNES and PTSD, certain other psychobiological derangements have been demonstrated in these conditions, including sympathetic nervous system dysregulation [102] and cortisol elevations [103]. Dissociation has also been identified in both PNES [18,19] and PTSD [104], suggesting one of many potential psychobiological targets linking each condition. A variety of other psychological processes (fear sensitivity, alexithymia, emotional dysregulation, etc.) may also be examined in the context of these comorbidities, as suggested by certain proposed psychobiological conceptualizations [3]. Ultimately, there may not be a unifying biomarker for PNES given its phenotypic heterogeneity, yet the validation of certain psychobiological subtypes (dissociative subtype, autonomic subtype, fear-sensitive subtype, etc.) may prove a worthy investigational exercise for future researchers.

In conclusion, biomarkers have offered limited benefit in the diagnostic and investigational needs surrounding PNES; specifically, PRL levels offer only negative predictive value. Complex psychosocial dimensions intrinsic to PNES will continually prevent sole or meaningful use of cellular, hormonal, autonomic, or neuroimaging findings in clinical care until further research is performed. Clinically, vEEG remains the preferred modality in differentiating ES from PNES, but in the absence of this diagnostic modality, multidimensional biopsychosocial assessments remain most valuable to the practicing clinician.

Conflict of interest statement

On behalf of all authors, the corresponding author states that there is no conflict of interest. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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