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Post-traumatic stress disorder: A biopsychosocial case-control study investigating peripheral blood protein biomarkers

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ABSTRACT

Experiencing traumatic events is unfortunately commonplace and, in some cases, may lead to the onset of debilitating mental health disorders, such as post-traumatic stress disorder (PTSD). Current diagnostic criteria for PTSD results in high depression and anxiety comorbidity. Better understanding of biological mechanisms and pathways underlying PTSD could aid in more accurate case identification and stratification of treatments. Recent meta-analysis has identified chronic PTSD to be associated with increased expression of pro-inflammatory cytokines and alterations in neuronal structures which contribute to an overall reduction in brain volume. Despite this, there are currently no biological markers in clinical use to identify PTSD or monitor treatment. This case-control study (n = 40) aimed to identify differences in peripheral blood biomarkers, and biomarker combinations, able to distinguish PTSD participants from controls, and examine in a biopsychosocial framework. The levels of 5/37 biomarkers investigated were significantly altered in the serum of PTSD participants: HDL and LDL cholesterol, tPA, IL-8 and EGF. Biomarkers could be used in combination with psychological criteria, in a biopsychosocial model, to support clinical management decisions and ensure appropriate individual treatment pathways.

Introduction

Post-traumatic stress disorder (PTSD) is a debilitating psychiatric condition which may result following exposure to actual or threatened serious injury, death or sexual violence (American Psychiatric Association, 2013). Pre-existing factors which contribute to PTSD susceptibility include female gender, prior trauma exposure, prior psychological problems, experience of adverse life events and lower socio-economic status (Brewin et al., 2000; Xue et al., 2015). Around 4% of the general population will develop PTSD in their lifetime, but this is much greater in populations exposed to high levels of trauma such as military personnel and first responders (Bennett et al., 2004; Eiche et al., 2019; Hoge et al., 2014; Petrie et al., 2018).

Defined causative events and symptom onset one month after exposure would typify PTSD as a condition suitable for rapid diagnosis and intervention; however, to date this has proved elusive. This may be due to difficulty differentiating post-trauma symptoms of distress that will self-resolve, from those that will become chronic or those which are non-specific from other psychiatric disorders, such as anxiety and depression (Germain, 2013; Milanak et al., 2019; Shalev et al., 2019; Visser et al., 2017). For example, experiencing insomnia immediately post-trauma is predictive of PTSD at one year (Germain, 2013). However, in a recent study of 2647 US adults exposed to a potentially traumatic event, 54.2% of the non-PTSD group experienced trauma-related disturbances to sleep (Milanak et al., 2019).

One of the major limitations of DSM-5 PTSD criteria is the specificity, or lack thereof, of PTSD symptoms which often co-occur in a dysphoric type symptom factor in PTSD models and have been known to drive

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comorbidity with depression and anxiety disorders. In response to the treatment challenges comorbidity represents in PTSD, the latest (11th) edition of the International Classification of Diseases (ICD-11) criteria for PTSD, due to be adopted into clinical practice from January 2022, has introduced changes to recognized PTSD symptomatology with the specific aim of reducing depression and anxiety comorbidity (World Health Organisation, 2018). Changes include the exclusion of symptoms common to all three disorders, and instead only include symptoms considered unique to PTSD such as intrusive memories, dissociative flashbacks, nightmares, avoidance of external reminders, thoughts and feelings associated to the event, hypervigilance and exaggerated startle response (Barbano et al., 2019). In addition, ICD-11 distinguishes PTSD from a sister disorder, complex PTSD, which is differentiated by repeated trauma exposure (Hyland et al., 2016). However, recent work comparing ICD-10 and ICD-11 criteria has noted no difference in the diagnosis of comorbid anxiety or depression disorders (Barbano et al., 2019). Changes to the dimensional structure of PTSD have great clinical implications both for initial diagnosis as well as treatment planning. This is best exemplified by the introduction of DSM-5 which saw a high number of military personnel who previously met the criteria for PTSD under DSM-4, no longer meeting the DSM-5 criteria, affecting their access to treatment and support services (Hoge et al., 2014). Examining the underlying biological mechanisms that lead to the manifestation of PTSD, depression and anxiety disorders is one potential avenue with promise to aid clinicians when performing differential diagnosis.

Although there have been numerous studies into the biological processes that may underpin PTSD and indeed predispose, or contribute to its development, a defined universal alteration in biological process or systems has not materialised (Bandelow et al., 2017, 2016; Daskalakis et al., 2016; Speer et al., 2019). Due to the complex presentation of symptoms, it is likely that multiple biological systems are involved in PTSD. Previous research has posited changes in hypothalamic pituitary adrenal (HPA) axis functioning, chronic increase in sympathetic nervous system activity, increased inflammatory status and physical changes in neurological structures all to be associated with PTSD (Colvonen et al., 2017; Jakovljevic, 2019; Kim et al., 2019). However, the intricacies and directionality of these changes are not fully understood. Much of the literature is conflicting, particularly regarding the activity of the HPA axis, where attenuation, elevation and no difference has all been assigned to glucocorticoid (GC) expression in PTSD (Domingo-Fernández et al., 2019; Speer et al., 2019). What has been established through meta-analysis, is that an increase in pro-inflammatory cytokine expression and a decrease in brain volume, volumes of the hippocampus, insula and anterior cingulate is present in PTSD (Bromis et al., 2018; Passos et al., 2015).

Neurobiological research into PTSD, and indeed other psychiatric disorders, has to date failed to yield substantial changes in treatment practices (Deacon, 2013). It has been suggested that a more integrated research approach which incorporates psychosocial as well as biomedical methods, may lead to advances that improve patient care (Davies and Roache, 2017; Deacon, 2013; Hašto et al., 2013). In the context of PTSD, the success of psychotherapies, as well as the core etiology of PTSD, would suggest that a successful examination of the biological aspect of the disorder should be in the context of the psychosocial characteristics (Davis et al., 2011; Hašto et al., 2013; Kar, 2011). For a number of disorders, the biopsychosocial model seems to provide promise to better define the disorder and indeed improve patient outcomes, such as depression and dementia (Becker et al., 2015; Gagliese et al., 2018; Schotte et al., 2006). However, application of a biopsychosocial approach in PTSD research has been limited (Wafa et al., 2019). Clarifying signatures representative of underlying molecular pathology of PTSD is important to implement and interpret a biopsychosocial health model that accurately reflects PTSD (Hašto et al., 2013; Schotte et al., 2006). Reaching such a milestone could not only lead to the development of new therapies, and make better use of existing ones, but also assist with stratifying patients presenting with PTSD into tailored treatment pathways, thus improving access to the most appropriate support services, in a timelier manner. To date, no clinically validated biomarker or biomarker combination has been found to assist in the diagnosis, treatment and management of PTSD (Bandelow et al., 2017, 2016; Friend et al., 2020). A biomarker tool which can accurately differentiate PTSD from non-PTSD would likely prove an essential aid to clinicians currently utilising the DSM-5, as well as aid in any crossover discrepancies if, and when, changes are made to the latent structure of PTSD moving beyond DSM-5 criteria. Symptom crossover and comorbidity with other neuropsychiatric disorders such as depression and anxiety, suggest that a single biomarker is unlikely to be diagnostic (Maslov et al., 2009).

The aim of this study was to identify differential expression of a number of biomarkers, measurable in peripheral blood, in control and PTSD participants. Differential expression was investigated to determine whether a biomarker or combination of biomarkers, in tandem with clinical observations, could be used to assist clinicians in identifying patients at risk of PTSD, and build better biopsychosocial models.

Materials and methods

Study participants

This case-control pilot study involved 40 (20 controls; 20 PTSD) age and sex-matched participants recruited in the US between January and June 2019, by Discovery Life Sciences (DLS), California USA, and PrecisionMed, California, USA. Venous blood samples and a detailed clinical history were collected from each study participant. The study conformed to all Data Use Agreements (DUA). Patient samples were de-identified and publicly available and are thus exempt from the requirement of the Institutional Review Board (IRB) approval (Exempt Category 4). A clinician and/or psychologist performed a clinician-administered PTSD scale for DSM-5 (CAPS-5) assessment on PTSD participants (Weathers et al., 2018). CAPS-5 scores were available for all 20 individuals with PTSD, and a PTSD checklist (PCL-5) score was available for the 20 control individuals (Ibrahim et al., 2018; Weathers et al., 2013).

Psychological assessment

CAPS-5 assessment tool is widely used in clinical interview assessments for PTSD, with demonstrated strong interrater and test-retest reliability (Weathers et al., 2018). Similarly, the PCL-5 self-assessment tool has also demonstrated strong psychometric properties such as strong internal consistency and test-retest reliability (Blevins et al., 2015). CAPS-5 total symptom severity score is calculated by summing severity score for the 20 DSM-5 PTSD symptoms, as is PCL-5 total severity. A CAPS-5 or PCL-5 score of < 33 was considered negative for PTSD. The CAPS-5 and PCL-5 have shown strong correlation and high diagnostic concordance rates (Weathers et al., 2018). Therefore, the data in this study is presented using the assumption that both CAPS-5 and PCL-5 methodologies are similar for identifying PTSD.

Clinical characteristics

Socio-demographic and clinical factors were collected from each participant and included age, gender, ethnicity, tobacco and alcohol use, BMI, pulse rate, systolic and diastolic blood pressure, current medications, comorbidities e.g. diabetes, hypertension, depression, anxiety, panic disorder, attention deficit disorder (ADD), bipolar disorder, substance abuse, and suicidal ideation.

Sampling and laboratory methods

Biomarker analyses was conducted at Randox Clinical Laboratory Services (RCLS) (Antrim, UK), using cytokine arrays (Randox

Laboratories Ltd, Crumlin, UK) for the following markers: Cytokine I Array: Interleukin-1α, −1β, −2, −4, −6, −8, −10, VEGF, EGF, TNFα, IFNγ, and MCP-1; Metabolic Array I: ferritin, insulin, leptin, plasminogen activator inhibitor-1 (PAI-1), and resistin; Metabolic Array II: C-reactive protein (CRP), adiponectin and cystatin C; Cerebral Array I: Brain-derived neurotropic factor (BDNF), glial fibrillary acidic protein (GFAP), and heart-type fatty acid-binding protein (H-FABP); Cerebral Array II: D-dimer, neuron specific enolase (NSE), neutrophil gelatinase-associated lipocalin (NGAL), and soluble tumour necrosis factor receptor I (sTNFR1). Arrays were run on an Evidence Investigator© analyser according to manufacturer's instructions (Randox Laboratories Ltd, Crumlin, UK). Cholesterol (total), HDL and LDL cholesterol, homocysteine and iron were analysed on an Imola analyser (Randox Laboratories Ltd, Antrim, UK). Folic acid and vitamin B12 were analysed on a Cobas Analyser (Roche, Basel, Switzerland). Human tissue-type plasminogen activator (tPA) and human type-1 plasminogen activator inhibitor (PAI-1/tPA) complex ELISAs were obtained from AssayPro (St. Charles, Missouri, USA). Midkine ELISAs were obtained from CellMid, (Sydney, Australia). Assays were completed according to manufacturer's instructions. The limits of detection (LOD) for the biomarkers under investigation were as follows: Cytokine I - IL-2 2.97 pg/ml; IL-4 2.12 pg/ml; IL-6 0.12 pg/ml, IL-8 0.36 pg/ml; VEGF 3.24 pg/ml; IFNγ 0.44 pg/ml; TNFα 0.59 pg/ml; IL1α 0.19 pg/ml; MCP1 3.53 pg/ml; EGF 1.04 pg/ml; IL-10 0.37 pg/ml; IL-1β 0.26 pg/ml; Metabolic Array I – Ferritin 3.27 ng/ml, insulin 2.32 μIU/ml, leptin 1.10 ng/ml, PAI-1 2.34 ng/ml, and resistin 1.06 ng/ml; Metabolic Array II – CRP 0.69 mg/l, adiponectin 164 ng/ml, and cystatin C 60 ng/ml; Cerebral Array I – BDNF 0.59 pg/ml, and GFAP 0.18 ng/ml; Cerebral Array II – D-dimer 2.1 ng/ml, NSE 0.26 ng/ml, NGAL 17.8 ng/ml, and STNFRI 0.24 ng/ml. Direct HDL - cholesterol (HDL) 0.189 mmol/l (7.30 mg/dl), direct LDL – cholesterol (LDL) 0.189 mmol/l (7.30 mg/dl), cholesterol 0.865 mmol/l (33.4 mg/dl), iron 2 μmol/L, folic acid 1.2 ng/ml, homocysteine 1.74 μmol/L and vitamin B12 100 pg/ml. AssayPro ELISA – Human tPA ELISA – 0.013 ng/ml and human PAI-1tPA complex ELISA – 0.05 ng/ml. Thirty-seven biomarkers in serum were investigated. Biomarker values that reported below the assay LOD were recorded as 90% of LOD.

Statistical analyses

Statistical analyses were performed using R Version 3.5.1, and IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp, Armonk, New York) (Field et al., 2013; R Core Team, 2018). All tests were performed in duplicate. Continuous variables are presented as mean ± standard deviation (mean ± SD). Comparisons were made using the Wilcoxon rank sum test and Bonferroni correction for multiple biomarker testing ($\alpha = 0.05/37 = 0.0014$). Categorical variables are presented as percentage (%) and were compared using a chi square (χ^2) test. The Area Under Receiver Operator Curve (AUROC), sensitivity and specificity, positive and negative predictive values were calculated for each biomarker and biomarker combination. Correlations were performed using Spearman's Rho test.

Results

Participant demographics

There was no significant difference in age between the control and PTSD participants (39.0 ± 2.6 vs. 41.5 ± 11.0, $p = 0.386$, respectively) or male gender (50% vs. 45%, $p = 0.752$). However, a significant difference in ethnicity was observed; a greater proportion of study participants in the PTSD group were non-Caucasian vs. the control group (60% vs. 21.2%, $p = 0.013$). Comparison of PTSD and control group demographics are described in Table 1.

Table 1
Biological, psychological, social demographics and clinical factors of the study participants.

	Factor	Control (n = 20)	PTSD (n = 20)	p value
Biological	**Age (years)**	39.0 ± 2.6	41.5 ± 11.0	0.386
	Gender (male)	10/20 (50%)	9/20 (45%)	0.752
	Ethnicity (Caucasian)	15/19 (78.9%)	8/20 (40%)	0.013
	BMI	29.7 ± 7.9	27.9 ± 6.3	0.496
	Pulse	73.2 ± 10.4	78.3 ± 19.7	0.725
	Systolic BP	122.2 ± 14.7	133.8 ± 19.7	0.055
	Diastolic BP	79.9 ± 7.3	93.6 ± 13.7	0.010
	Hypertension	4/20 (20%)	4/20 (20%)	1.000
	Diabetes	0/20 (0%)	3/20 (15%)	0.072
	Medications	1.1 ± 1.4	3.2 ± 2.8	0.008
Psychological	**Depression**	4/20 (20%)	13/20 (65%)	0.004
	Anxiety	2/20 (10%)	16/20 (80%)	< 0.001
	ADD	0/20 (0%)	1/20 (5%)	0.311
	Bipolar Disorder	0/20 (0%)	2/20 (10%)	0.147
	Panic Disorder	0/20 (0%)	5/20 (25%)	0.017
Social	**Smoker**	13/20 (65%)	6/20 (30%)	0.027
	Cigarettes/day	6.2 ± 7.6	2.3 ± 4.7	0.048
	Smoking (years)	8.0 ± 7.9	3.2 ± 6.3	0.025
	Alcohol	15/20 (75%)	5/20 (25%)	0.002
	Substance Abuse	0/20 (0%)	4/20 (20%)	0.035
	Suicide ideation	0/20 (0%)	2/20 (10%)	0.147

Continuous variables were expressed as mean ± SD or as % for categorical variables. The difference in continuous variables were analysed using Wilcoxon rank sum test, while chi square ($\chi 2$) test was used for categorical variables. A $p < 0.05$ was considered significant. BMI – body mass index; BP – blood pressure; ADD – attention deficit disorder.

BMI, pulse, hypertension (HTN), systolic and diastolic blood pressure

There was no statistically significant difference in BMI, pulse rate, or HTN diagnosis between the control and PTSD participants. However, a significant increase in diastolic blood pressure was observed for PTSD participants (93.6 ± 13.7 vs. 79.9 ± 7.3; $p = 0.010$) and a trend level increase in systolic blood pressure (133.8 ± 19.7 vs. 122.2 ± 14.7; $p = 0.055$) (Table 1).

Psychological comorbidities and medications

Comorbid psychiatric conditions were more prevalent among the PTSD participants than control participants. Notably; depression (control 4/20 (20%) vs. PTSD 13/20 (65%) $p = 0.004$), anxiety (control 2/20 (10%) vs. PTSD 16/20 (80%), $p < 0.001$) and panic disorder (control 0/20 (0%) vs. PTSD 5/20 (25%), $p = 0.017$). PTSD participants unsurprisingly were prescribed more medications when compared to control group (3.2 ± 2.8 vs. 1.1 ± 1.4; $p = 0.008$, respectively). Three main categories of medications prescribed included anti-depressants, anti-anxiety, and pain management drugs.

Social demographics

In this study, the control participants smoked more (6.2 ± 7.6 vs. 2.3 ± 4.7 cigarettes/day, $p = 0.048$) and for longer periods of time (8.0 ± 7.9 vs. 3.2 ± 6.3 years, $p = 0.025$). Furthermore, there were more participants that smoked in the control vs. PTSD group (13/20 (65%) vs. 6/20 (30%); $p = 0.027$, respectively). Alcohol consumption was also significantly greater in the control vs. PTSD group (15/20 (75%) vs. 5/20 (25%); $p = 0.002$, respectively). However, the incidence of substance abuse was greater amongst PTSD participants (4/20 (25%) vs. 0/20 (0%); $p = 0.035$) (Table 1).

A greater proportion of the PTSD group were single (15/20 (75%) vs. 3/19 (15.8%); $p < 0.001$, respectively), and fewer were married

compared to the control group (4/20 (20.0%) vs. 12/19 (63.2%), p = 0.006, respectively). The divorce rate was not significantly different between the control and PTSD group (4/19 (21.1%) vs. 1/20 (5.0%); p = 0.134, respectively). In addition, 2/20 (10%) PTSD participants reported previous suicide attempts. This was not reported by any of the control participants (Table 1).

PTSD assessment and symptom severity

Total severity of PTSD symptoms was determined by summing the severity of each DSM-5 PTSD symptom clusters. Mean total severity of PTSD symptoms for the PTSD group was 47.7 ± 7.6. In the control group, mean total severity of PTSD symptoms, as assessed by PCL-5 was 10.1 ± 7.8. Symptom severity of each individual PTSD symptom cluster is detailed in Table 2. The number of endorsed PTSD symptoms (symptoms whose severity is equal to or greater than a score of 2/4) was 16.6 ± 1.6 and 1.8 ± 2.2 in the PTSD and control participant groups, respectively.

Biomarker analysis

In total, 37 serum biomarkers were investigated (Table 3). Five biomarkers (5/37 (13.5%)) were identified as significantly altered in PTSD participants when compared to the control group; HDL cholesterol, LDL cholesterol, EGF, tPA and IL-8 (Table 3). LDL and HDL cholesterol had the highest Area Under the Receiver Operator Curve (AUROC) for differentiating between the control vs. PTSD group. However, as the study participants had not been asked to fast prior to their venous blood sampling, HDL and LDL cholesterol were not included in any further analyses. Of the remaining 3 significant biomarkers, EGF was significantly lower in the PTSD group (13.3 ± 14.3 pg/ml vs. 73.2 ± 35.7 pg/ml, p < 0.001) (Fig. 1A) whereas, tPA and IL-8 were significantly elevated in PTSD; tPA (5.1 ± 2.9 ng/ml vs. 1.7 ± 0.7 ng/ml, p < 0.001) (Fig. 1B) and IL-8 (13.0 ± 5.6 pg/ml vs. 8.1 ± 2.8 pg/ml, p = 0.037) (Fig. 1C). The AUROCs of the three individual biomarkers and in combination are shown in Fig. 1D.

Correlation matrix chart

A correlation matrix chart was completed to test for correlations between all significant biomarkers, and between significant biomarkers and CAPS-5 assessed total PTSD symptom severity. EGF had the strongest correlation with CAPS-5 assessed total PTSD severity (r = −0.79).

Table 2
Summary of the PTSD scale for DSM-5 (CAPS-5) and PCL-5 score.

∑	Control (PCL-5) (n = 20)	PTSD CAPS-5 (n = 20)	p value	Control (median)	PTSD (median)
PTSD Total Symptom Severity	10.1 ± 7.8	47.7 ± 7.6	< 0.001	7.0	45.0
Endorsed PTSD Symptoms	1.8 ± 2.2	16.6 ± 1.6	< 0.001	1.0	17.0
I total	2.1 ± 2.2	13.3 ± 2.4	< 0.001	1.5	13.0
Av total	1.0 ± 1.2	5.8 ± 1.0	< 0.001	0.5	6.0
C total	2.8 ± 3.0	16.6 ± 3.1	< 0.001	2.0	16.0
Ar total	4.2 ± 3.1	11.9 ± 3.2	< 0.001	4.0	11.0

Threshold for CAPS-5/PCL-5 indication of PTSD total score ∑IAvCAr ≥ 33. The difference in continuous variables were analysed using Wilcoxon rank sum test, data is presented as mean ± SD; a p < 0.05 was considered significant.
I total – intrusion symptoms; Av total – avoidance symptoms; C total – cognitions and mood symptoms; Ar total – arousal and reactivity symptoms.

Table 3
Comparison of mean serum biomarker levels in control vs. PTSD participants.

Biomarker	Control (n = 20)	PTSD (n = 20)	p value
LDL Cholesterol (mmol/l)	3.4 ± 0.9	1.3 ± 0.3	< 0.001
HDL Cholesterol (mmol/l)	1.3 ± 0.4	3.2 ± 1.0	< 0.001
EGF (pg/ml)	73.2 ± 35.7	13.5 ± 14.3	< 0.001
tPA (ng/ml)	1.7 ± 0.7	5.1 ± 2.9	< 0.001
IL-8 (pg/ml)	8.1 ± 2.8	13.0 ± 5.6	0.037
PAI-1 (ng/ml)	35.0 ± 12.0	24.0 ± 8.1	0.111
D-dimer (ng/ml)	50.1 ± 38.6	28.1 ± 27.9	0.222
Cystatin C (μg/ml)	1.2 ± 0.2	1.0 ± 0.2	0.259
Homocysteine (μmol/l)	12.5 ± 7.4	8.9 ± 2.8	1.000
Midkine (pg/ml)	107.0 ± 42.2	482.4 ± 979.9	0.851
Folic Acid (pg/ml)	12.7 ± 8.5	15.1 ± 5.9	1.000
PAI-1/tPA (ng/ml)	12.4 ± 5.9	9.2 ± 5.6	1.000
Resistin (ng/ml)	5.6 ± 2.4	4.4 ± 1.4	1.000
IFNγ*	2/20 (10%)	10/20 (50%)	0.222
Vitamin B12 (ng/ml)	460.5 ± 256.2	572.4 ± 292.8	1.000
Ferritin (mg/l)	43.7 ± 36.3	79.6 ± 85.5	1.000
GFAP (ng/ml)	0.6 ± 0.4	1.0 ± 0.7	1.000
CRP (mg/l)	3.6 ± 4.8	8.0 ± 12.5	1.000
Insulin (pmol/l)	34.6 ± 36.9	20.3 ± 16.3	1.000
NGAL (ng/ml)	826.9 ± 196.6	758.5 ± 197.7	1.000
Adiponectin (μg/ml)	4.1 ± 4.0	3.9 ± 2.3	1.000
IL-1β (pg/ml)	1.8 ± 0.3	2.2 ± 1.0	1.000
Leptin (mg/l)	12.9 ± 13.9	12.3 ± 14.1	1.000
IL-4 (pg/ml)	2.6 ± 0.4	2.4 ± 0.4	1.000
IL-1α (pg/ml)	0.5 ± 0.1	0.4 ± 0.1	1.000
MCP-1 (pg/ml)	188.8 ± 117.3	186.2 ± 71.7	1.000
sTNFR1 (ng/ml)	0.8 ± 0.2	0.8 ± 0.2	1.000
IL-2 (pg/ml)	3.3 ± 0.8	3.5 ± 1.0	1.000
Iron (μmol/l)	12.2 ± 5.2	13.2 ± 9.7	1.000
IL-6 (pg/ml)	2.4 ± 3.0	2.7 ± 2.8	1.000
IL-10 (pg/ml)	1.3 ± 0.2	1.7 ± 1.3	1.000
BDNF (pg/ml)	7099.5 ± 743.9	6407.7 ± 1704.1	1.000
VEGF (pg/ml)	80.5 ± 52.6	95.1 ± 94.8	1.000
H-FABP (ng/ml)	1.4 ± 0.5	1.4 ± 0.7	1.000
NSE (ng/ml)	4.0 ± 1.6	4.8 ± 3.3	1.000
Total Cholesterol (mmol/l)	5.1 ± 1.0	5.1 ± 1.1	1.000
TNFα (pg/ml)	2.2 ± 0.4	2.4 ± 0.8	1.000

*IFNγ results were converted to nominal data with values below the limit of detection (LOD) assigned 0 and any value above the LOD assigned 1. Chi square ($\chi 2$) test was used for this categorical variable. Continuous variables were expressed as mean ± SD. Bonferroni correction for multiple testing was applied. A p < 0.05 was considered significant. LDL and HDL markers are reported but excluded from analysis as participants had not been asked to fast prior to venous blood sample collection. Values below the LOD were assigned a value of 90% of the LOD.

Other biomarker correlations identified between CAPS-5 assessed total PTSD severity included tPA (r = 0.59) and IL-8 (r = 0.33). Biomarker correlations are described in Fig. 2.

Discussion

Exposure to traumatic events is common in the general population with a lifetime rate for PTSD of approximately 4% (Frissa et al., 2013; Kilpatrick et al., 2013; Shalev et al., 2019). In occupations such as Emergency Medical Services (EMS), police, fire services and the military, where exposure to traumatic events is more common, the lifetime rate for PTSD is higher than that of the general population (Eiche et al., 2019; Hoge et al., 2014; Petrie et al., 2018). In this study, we used a participant cohort with a heterogeneous trauma type. Differences in trauma type may relate to differences in symptom severity. Therefore, using a heterogeneous participant cohort may prove more beneficial in identifying potential biological signature(s) that are common across PTSD.

Comparison of baseline social and biological demographics of the PTSD and control participant groups identified several significant differences. Namely, the control participants consumed more alcohol, smoked more, and for longer. However, alcohol abuse was more prevalent in the PTSD group. There is extensive literature documenting the

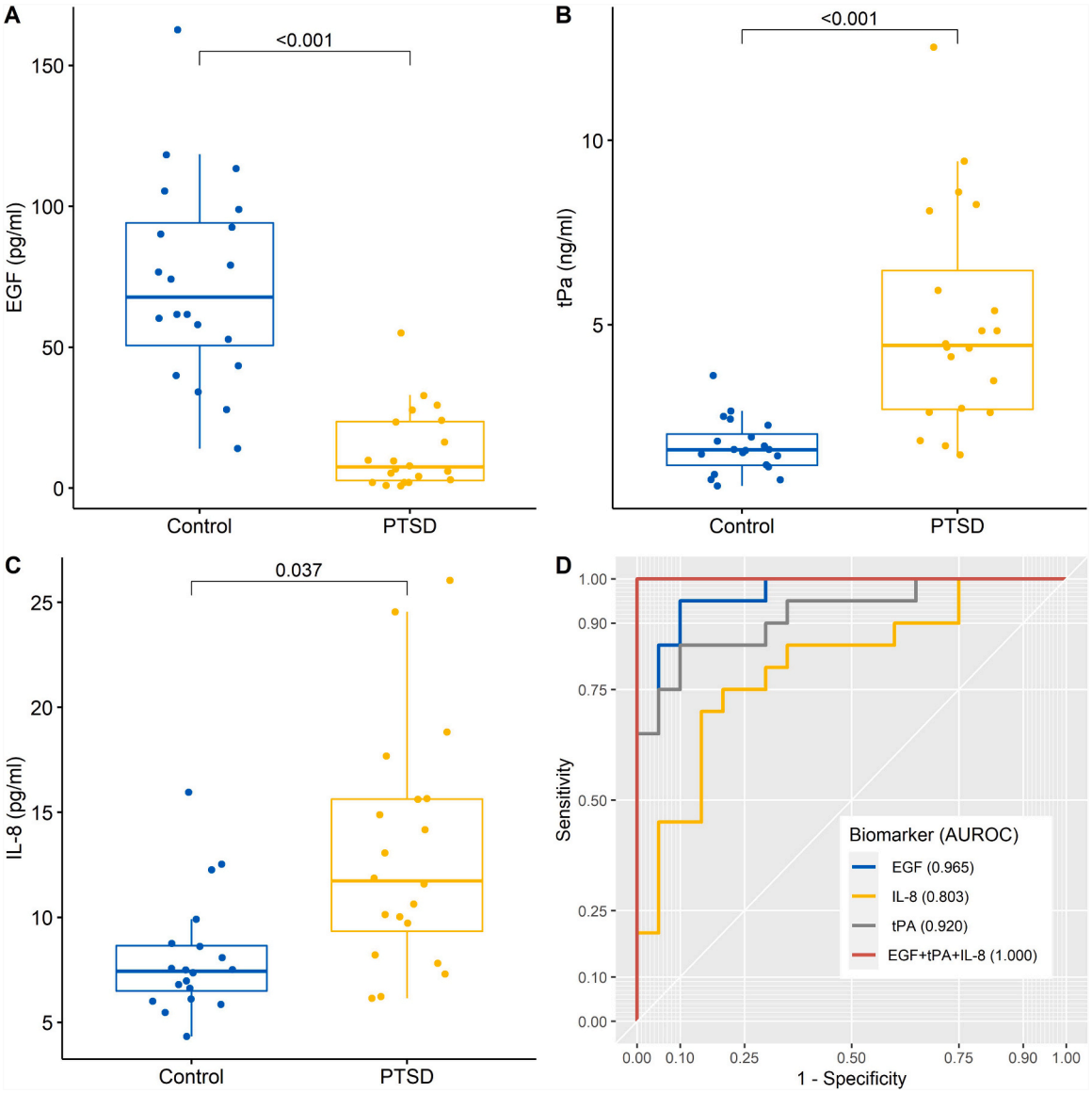

Fig. 1. Boxplots describing serum markers significantly differentiated in control vs. PTSD participants. Panel A: EGF, Panel B: tPA, Panel C: IL-8. Panel D describes the AUROC curves for individual significant biomarkers and in combination. *Bonferroni corrected $p < 0.05$ considered significant.*

relationship between PTSD and excessive alcohol use (Angkaw et al., 2015; Miller et al., 2017). Moreover, the hypothesis of alcohol misuse as 'self-medication' to attenuate psychological suffering has gained wide popularity (Khantzian, 1997). However, studies investigating alcohol and cigarette use in chronic PTSD have reported that weekly alcohol consumption was substantially below that of the general population, in agreement with our findings (den Velde et al., 2002).

Recent meta-analysis has identified that PTSD is associated with poorer lifestyle factors such as increased obesity and smoking, and these factors likely contribute to an increased cardiovascular disease (CVD) risk in this population (van den Berk-Clark et al., 2018). PTSD is also associated with hypertension, which further contributes to CVD risk (Abouzeid et al., 2012; Balint et al., 2016; Gradus et al., 2015; Scherrer et al., 2019; Sumner et al., 2016). Therefore, it was unsurprising that PTSD participants in this study presented with an elevated blood pressure (BP), when compared to controls; albeit, a 24-hour ambulatory BP measurement would be considered a more reliable indicator of true BP.

Comorbid psychiatric disorders such as depression and anxiety in patients with PTSD is often the rule rather than the exception (Price et al., 2019). As discussed previously, the overlap in disorder symptomology is likely to contribute to PTSD (Barbano et al., 2019; Price et al., 2019). Considering PTSD participants in this study were identified as such using the DSM-5 based CAPS-5 assessment, it was unsurprising that a high proportion had comorbid depression and anxiety.

Identification of biological signatures that reflect or identify PTSD are dependent on understanding the pathophysiological systems which underly the development and progression of its pathology. The current thinking in the literature for PTSD has focused on dysregulation of the HPA-axis, its stress response and the subsequent effect this has on the immune system (Daskalakis et al., 2016; Kim et al., 2019; Michopoulos et al., 2015; Yehuda et al., 2014). The HPA-axis is the main coordinator of the neuroendocrine response to stress and altered levels of glucocorticoids (GCs) have been consistently identified in PTSD (Daskalakis et al., 2016; De Kloet et al., 2005; Schmidt et al., 2013; Van Zuiden et al., 2012, 2011; Yehuda et al., 2014). Primarily a reduction in circulating GCs is observed in chronic PTSD, although elevations have also been reported (Pervanidou and Chrousos, 2012; Sarapas et al., 2011; Schmidt et al., 2013; Tegeler et al., 2017). Elevated GCs are likely attributable to differences in time since trauma, gender and sampling conditions (De

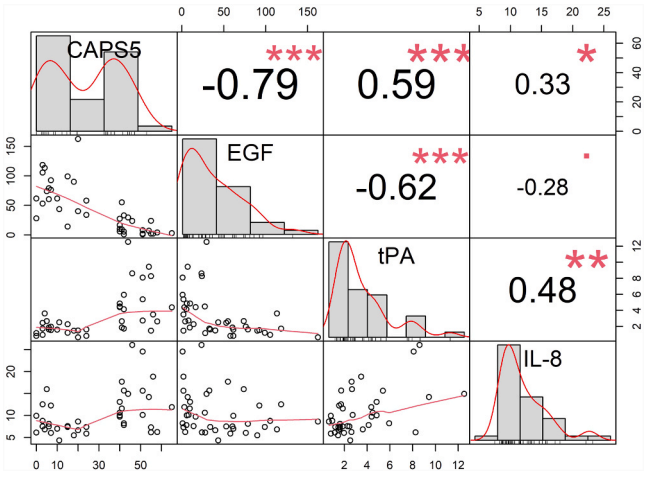

Fig. 2. Correlation Matrix Chart for significant biomarkers and CAPS-5. Top - the (absolute) value of the correlation (R) plus the result based on Spearman's rho as stars. Bottom - the bivariate scatterplots, with line fitted. Stars of significance shown as follows; $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = *** *$.

Quervain et al., 2016; Kim et al., 2019; Shirazi et al., 2015; Szeszko et al., 2018). Moreover, genetic studies have shown polymorphisms in FKBP5 gene, a functional inhibitor of the glucocorticoid receptor (GR), predispose children who experience trauma in early childhood to an increased risk of PTSD as an adult, with more severe symptoms (Binder et al., 2008). Low GCs may contribute to PTSD susceptibility, related to stressful life events.

Decreased GC activity in chronic PTSD, in tandem with a decrease in parasympathetic nervous system stimulation and increased sympathetic nervous system activity, results in a chronic elevation of proinflammatory cytokines (Daskalakis et al., 2016; Kim et al., 2019). GCs suppress lymphocyte production and their subsequent secretion of proinflammatory cytokines (Boumpas et al., 1993). Therefore, decreased GC has been suggested to contribute to the increase in inflammatory biomarkers observed in PTSD (Kim et al., 2019). The results presented here, where PTSD patients exhibited a significant elevation in the proinflammatory cytokine IL-8, support these findings. Table 4.

Cytokines can pass across the blood brain barrier by passive diffusion, or by binding to and activating receptors on endothelial cells, which then induce local neuronal production of proinflammatory cytokines (Kebir et al., 2007). This inflammation has been hypothesised to contribute to structural and functional impairment of areas in the brain of PTSD patients (Kim et al., 2019).

Stress-related fear memories induced by GCs, are facilitated by BDNF, which is activated from its precursor and is dependent on the activity of tPA (Revest et al., 2014). The conversion of proBDNF to BDNF by tPA is inhibited by PAI-1 (Revest et al., 2014). In the current study, we observed increased levels of tPA in the PTSD group. Previous work has reported similar tPA findings, in an exclusively female adolescent PTSD population (Aksu et al., 2018). However, we did not observe any significant differences in the concentration of BDNF. This may be due to differing precursor availability as we did not measure proBDNF.

The expression profile of tPA has been characterised in depression and have been observed to change following antidepressant therapies (Chen et al., 2017; Jiang et al., 2017). It is noteworthy that tPA and PAI-1 have opposite expression profiles in PTSD compared to depression, where a decrease and increase in expression has typically been reported, respectively (Chen et al., 2017; Jiang et al., 2017). We hypothesise that tPA may be providing a measure of the ability of GCs to enhance fear memories.

Table 4
Area Under the Receiver Operator Curve (AUROC), sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV) for biomarkers identified that differentiated control from PTSD participants.

Biomarker	AUC	Sensitivity	Specificity	PPV (%)	NPV (%)
EGF (pg/ml)	0.965	0.950	0.900	90.5	94.7
tPA (ng/ml)	0.920	0.850	0.900	89.5	85.7
IL-8 (pg/ml)	0.803	0.750	0.800	78.9	76.2

The clinical utility of the three biomarkers (EGF, tPA and IL-8) that were differentially expressed between the control and PTSD group, was assessed individually and in combination. Combining the three biomarkers into a single model differentiated control from PTSD and correctly identified all control and all PTSD patients.

Adopting a biopsychosocial model for PTSD into clinical use would need to provide benefit to clinicians and also improve patient treatment and outcomes. One of the challenges currently faced in the PTSD diagnostic process is the high comorbid rate of depression and anxiety disorder. The new ICD-11 PTSD diagnostic criteria aims to address these comorbidities however, recent research has failed to provide supportive evidence (Barbano et al., 2019). The use of the biomarker combination in tandem with psychological symptomology assessment could potentially improve the accuracy of PTSD diagnoses providing for more evidence-based treatment pathways.

In summary, we have identified biomarkers that could be used to identify individuals with PTSD and potentially monitor treatment regimens (Colvonen et al., 2017; Kim et al., 2019). Deployment of the biomarkers to screen individuals with suspected PTSD could accelerate the diagnostic and treatment process by stratifying individuals most at risk, ensuring that triage is effective and that individuals receive the appropriate psychiatric care. The biomarker combination could aid in clarifying the ambiguity which often surrounds borderline cases, potentially reducing misdiagnoses, where psychological symptomology may present non-specifically.

Study limitations

The main limitations of the study include (1) the small sample number of participants, (2) sampling bias (the cases and controls recruited for the study may not be truly representative) and (3) the use of the PCL-5 to assess PTSD symptoms in the control group is not a diagnostic tool. However PCL-5 has been shown to have an acceptable diagnostic concordance rate with the CAPS-5 and is regularly used in similar research (Ibrahim et al., 2018). Despite the limitations, the results suggest that biomarker-based models may offer clinical utility when diagnosing patients at potential risk of PTSD. Combining biomarker-based models with psychological criteria could potentially reduce bias, provide greater objectivity, potentially reduce under and over-diagnosis and stratify patients into the most appropriate treatment pathways.

Conclusion

Thirty-seven serum biomarkers were investigated to examine their expression profiles in control and PTSD participants. Three biomarkers were identified as differentially expressed in PTSD vs. control participants namely, EGF, tPA and IL-8. Current psychological criteria for PTSD diagnosis is hampered with high rates of comorbidities e.g. anxiety and depression. A biopsychosocial approach to PTSD which incorporates these biomarkers, psychological criteria and social factors may improve PTSD identification, diagnosis and treatment management.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests, Joanne Watt, John Lamont, Mary Jo Kurth and Mark Ruddock are employees of Randox Laboratories Ltd but hold no shares in the Company. Peter Fitzgerald is the Managing Director of Randox Laboratories Ltd. The project was funded by the Randox – Ulster University Industrial Ph. D. Academy.

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