Published in final edited form as:
Biol Psychiatry. 2008 November 15; 64(10): 907–911. doi:10.1016/j.biopsych.2008.05.035.

Elevated Neuroimmune Biomarkers in Sweat Patches and Plasma of Premenopausal Women with Major Depressive Disorder in Remission: The P.O.W.E.R. Study

Giovanni Cizza[††], **Andrea H. Marques**[*], **Farideh Eskandari**[*,&], **Israel C. Christie**[†], **Sara Torvik**[††], **Marni N. Silverman**[*,§§], **Terry M. Phillips**[§], **Esther M. Sternberg**[*], and **the P.O.W.E.R. Study Group**

††*Clinical Endocrinology Branch, NIDDK, NIH*

**Section on Neuroendocrine Immunology and Behavior, Integrative Neural Immune Program, NIMH, NIH*

&Case Western Reserve University, Department of Medicine, Division of Clinical and Molecular Endocrinology, Cleveland, OH

†*Cardiovascular Behavioral Medicine Program, Department of Psychiatry, University of Pittsburgh Medical Center*

§§*Prince of Wales, NCCAM Director's Fellow, NIH*

§*Nanoscale Immunodiagnosis, Laboratory of Bioengineering & Physical Science, NIBIB, NIH*

Abstract

Background—Major depressive disorder (MDD) is inconsistently associated with elevations in pro-inflammatory cytokines and neuropeptides. We used a skin sweat patch, recently validated in healthy controls, and recycling immunoaffinity chromatography (RIC), to measure neuroimmune biomarkers in patients with MDD mostly in remission.

Methods—We collected blood at 8 am and applied sweat patches for 24 hours in 21 to 45 year old premenopausal women (N=19) with MDD (17/19 in remission, and age-matched healthy controls (N=17) participating in the P.O.W.E.R. Study, a prospective study of bone turn-over.

Results—Pro-inflammatory cytokines IL-1α, IL-1β, IL-6, TNFα and IL-8, and neuropeptides NPY, SP and CGRP were significantly higher, and VIP, a marker of parasympathetic activity, was significantly lower in patients compared to controls, and depressive symptomatology strongly correlated with biomarker levels. All analytes were strongly correlated in the skin sweat patch and plasma in patients ($r = 0.73$ to 0.99; $p < 0.0004$).

Conclusions—The skin sweat patch allows detection of disrupted patterns of pro-inflammatory cytokines and neuropeptides in women with MDD in clinical remission, which could pre-dispose to

Corresponding author: Esther M. Sternberg, M.D., Director, Integrative Neural Immune Program, Section on Neuroendocrine Immunology & Behavior, NIMH/NIH, 5625 Fishers Lane (MSC-9401), Rockville, Maryland 20852 U.S.A., Tel. #: 301-402-2773 or 496-9255, Fax #: 301-496-6095, Email: sternbee@mail.nih.gov.

FINANCIAL DISCLOSURES
All authors reported no biomedical financial interests or potential conflicts of interest.

medical consequences, such as cardiovascular disease, osteoporosis and diabetes. This method permits measurement of cytokines in ambulatory settings where blood collection is not feasible.

Keywords

depression; interleukins; pain; autonomic nervous system; vasoactive intestinal peptide; neuropeptide Y; substance P; calcitonin-gene-related peptide

INTRODUCTION

Elevated cytokine levels have been reported in subjects with major depressive disorder (MDD) with inconsistent results (1,2). Reported alterations in the hypothalamic-pituitary-adrenal (HPA) axis, and autonomic and pain mediators, including vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), substance P (SP) and calcitonin-gene-related peptide (CGRP) could contribute to this immune dysregulation (3,4).

Elevated cytokines in MDD have been linked to osteoporosis, diabetes, cardiovascular disease, sleep disturbances, and decreased pain threshold (5). We recently reported low bone mass (5), increased levels of prothrombotic factors (6) and various pain syndromes (4) in a prospectively assembled cohort of young premenopausal women with MDD.

The aim of the current study was to evaluate neuroimmune biomarkers in sweat in women with MDD, mostly in clinical remission. We measured pro-inflammatory cytokines (IL-1α, IL-β, IL-6, TNFα, IL-8), and NPY, VIP, SP and CGRP in a non-invasive manner using a sweat patch recently validated in normal controls (7). We collected blood to verify that analyte levels in sweat correlated with plasma levels. We used RIC, a highly sensitive and specific methodology, to measure multiple analytes in minute volumes (8,9).

We found that cytokines and neuropeptides quantified in sweat patches, closely correlate with plasma levels. Women with MDD, mostly in clinical remission, exhibited substantial increases in pro-inflammatory cytokines and sympathetic and sensory neuropeptides. Biomarker levels were strongly correlated with depressive symptomatology, and could account for the increased co-morbidities associated with depression.

MATERIALS AND METHODS

Participants and Study Design

This was an ancillary study to the POWER (Premenopausal, Osteopenia/Osteoporosis, Women, Alendronate, Depression), Study, a prospective study of bone turnover in 21-to 45-year-old premenopausal women with MDD (5,6). A convenience sample of twenty consecutive women with MDD and nineteen consecutive healthy control women wore two skin patches for 24 hours. Part of the data from nine of the healthy control subjects described here, was previously reported (7). Psychiatric evaluation was conducted using the SCID (Structured Clinical Interview for DSM-IV). Current severity of depression and anxiety was evaluated with the Hamilton Depression (Ham-D) and Anxiety Scale (Ham-A). Inclusion criteria were previously described (6). This study was approved by the IRB of the National Institute of Mental Health and registered under Clinicaltrials.gov; Identifier: NCT00006180. Written consent was obtained.

Materials

Sweat patches, manufactured by Pacific Biometrics. Inc. (Irvine, CA, USA; PharmChem Inc., Fort Worth, Texas) were previously validated for measurement of pyridinoline and deoxypyridinoline in sweat and urine (10) and sweat cytokines (7).

Assays for cytokine and neuropeptide measures

IL-1α, IL-1β, IL-6, TNFα, IL-8, VIP, NPY, SP, and CGRP were measured in the sweat patch and plasma by RIC coupled with laser-induced fluorescence detection, as previously described (8,11). Analyses were performed by TMP (NIBIB, NIH) who was blinded to group allocation. Analyte identity was confirmed by mass spectrometry and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) using recombinant IL-1α, IL-β, IL-6, TNFα, IL-8, VIP, NPY, SP and CGRP as standards. Previous studies comparing RIC to commercially available ELISA assays showed $r^2 = 0.9151–0.9855$ (least-squares linear regression analysis) (8,9,11) with limit of sensitivity for RIC = 1.6 – 2.8 pg/mL for analytes reported here. Intra- and inter-assay coefficients of variation < 6.03 ± 0.33.

Assays for cortisol and catecholamines

All analyses were performed at the NIH Clinical Center Department of Laboratory Medicine and Mayo Medical Laboratory. Serum 8AM cortisol was measured with the DPC Immulite-2000 chemiluminescent immunoassay (Los Angeles, CA). Urinary epinephrine, norepinephrine, dopamine and cortisol free 24h were performed by high-performance liquid chromatography (HPLC).

Data Analysis

Differences between groups were tested using independent sample t-test or Mann-Whitney U test for continuous variables, and Fisher's exact test for categorical variables. Spearman correlations were used to describe the bivariate relationships between sweat and plasma levels of cytokines and neuropeptides, as well as between Ham-D and Ham-A scores and biomarker levels. Hierarchical multiple regression analyses was performed. Differences between groups in sweat and plasma analytes were confirmed by testing the percent variance gained by adding a dummy coded group predictor, after controlling for BMI and age (ANCOVA).

RESULTS

Women in the MDD group had similar demographic characteristics and a slightly higher BMI compared to healthy controls (Table 1). Seventeen out of 19 patients were mildly depressed at the time of sampling, as indicated by Ham-D scores below 20. One patient had a Ham-D score of 22, and one of 30. As depicted in Table 2, IL-1α, IL-1β, IL-6, TNFα, IL-8, NPY, SP and CGRP were significantly higher, and VIP was significantly lower, by several fold in the MDD group compared to healthy controls in both sweat patch eluates and plasma ($p < 0.0001$).

In the MDD group, all biomarkers showed strong and significant correlations between sweat patch and plasma levels (Table 3: $r = 0.73$ to 0.99; $p < 0.001$). In the control group, these correlations were more variable and less robust partly because of lower values. IL-1α, IL-1β, TNFα and IL-8 strongly correlated in plasma and sweat patch eluates but there was little or variable correlation for IL-6, VIP, NPY, SP and CGRP in controls (Table 3). These correlations remained after controlling for age and BMI (Table 4). Bivariate analysis showed a strong correlation between biomarker levels and symptom severity for both depression and anxiety, as measured by Ham-D and Ham-A scales, which were remarkably consistent between plasma and sweat, even after controlling for age and BMI (Table 5).

There were no differences in 8 am serum cortisol, UFC, and urinary NE, Epi and dopamine (data not shown) between clinical groups.

DISCUSSION

Pre-menopausal women with MDD, mostly in remission, exhibited several fold elevations of pro-inflammatory cytokines, sympathetic (NPY) and sensory (SP and CGRP) neuropeptides, and diminished parasympathetic-associated neuropeptide, VIP, in sweat. These levels strongly correlated with depressive and anxiety symptomatology, even after controlling for BMI, suggesting that symptom severity rather than disease classification per se may be related to biomarker expression. The skin patch coupled with RIC, a highly sensitive analytical method for multiple biomarker measurement, previously validated in healthy controls (7) allowed identification of a specific pattern of neuroimmune dysregulation not previously detected in mildly depressed women. Analytes in the sweat patch strongly correlated with plasma levels, supporting this approach as a valid method for biomarker measurement. This methodology avoids confounds to biomarker measurements associated with previous methods of sweat collection (exercise (12), sauna heat (13) and blood drawing (2)). Our findings of elevated pro-inflammatory cytokines are consistent with previous reports in patients with MDD (14), although conflicting results have been described (2). An elevation in pro-inflammatory cytokines of this magnitude substantially increases medical morbidity including osteoporosis, cardiovascular disease and metabolic disorders (15). Cytokines also regulate neurotransmitters, hormones, and neuropeptides (16) and modulate many behaviors, including mood, pain, which are altered in patients with depression (1).

The lower VIP levels are consistent with reduced parasympathetic tone that has been reported in depression, and with the effectiveness of parasympathetic vagal stimulation in treatment of refractory depression (17).

The elevated sympathetic and sensory-associated neuropeptides in both sweat patch eluates and plasma in subjects with mild MDD are consistent with their role in depression, although lower CSF NPY has been reported in first-episode depressed patients (18). Since most patients were pharmacologically treated, and antidepressants up-regulate central NPY synthesis, increased NPY in these subjects could be related to use of these medications.

This pattern of higher levels of pro-inflammatory cytokines, lower VIP (parasympathetic activity) and higher NPY (sympathetic activity) in patients with MDD in remission, could be associated with increased cardiovascular risk in these patients.

The elevated levels of SP and CGRP are consistent with previous reports of these peptides' role in pain perception, and of painful somatic symptoms correlating with depression severity in up to two thirds of patients with MDD (4).

The normal plasma and urinary cortisol and urinary catecholamine levels observed here have been reported elsewhere (5) in MDD and are consistent with these patients being mostly in a state of remission.

The limitations of the current study include small sample size and treatment with antidepressants, which can in some cases modulate the inflammatory response (19). Some studies indicate that antidepressants inhibit pro-inflammatory and stimulate anti-inflammatory cytokine production, although others show varying effects (19). Although we found significant biomarker alterations in this small sample, larger studies in patients on and off medication are needed to confirm and extend these results.

In summary, we found, using a skin sweat patch combined with RIC, that women with mild MDD, treated and in remission, show patterns of elevated pro-inflammatory cytokines and altered neuropeptides that could pre-dispose not only to osteoporosis as we recently reported in this cohort (5), but also to cardiovascular diseases, diabetes and other medical consequences.

Furthermore, levels of biomarkers correlate strongly with symptoms of depression and anxiety. This non-invasive method can be used to measure a variety of biomarkers simultaneously and is a valid alternative when blood collection is unfeasible.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The study was fully supported by the NIH Intramural Research Program: NIMH, Section on Neuroendocrine Immunology and Behavior, Integrative Neural Immune Program; NIDDK, Clinical Endocrinology Branch; and NIBIB, Nanoscale Immunodiagnosis, Laboratory of Bioengineering & Physical Science. The following individuals were investigators of the P.O.W.E.R. Protocol (Premenopausal Osteoporosis Women Alendronate Depression): Giovanni Cizza (Principal Investigator), Ann Berger, Marc R. Blackman, Karim A. Calis, George Csako, Bart Drinkard, Farideh Eskandari, Philip W. Gold, McDonald Horne, Christine Kotila, Pedro Martinez, Kate Musallam, Terry M. Phillips, James. C. Reynolds, Nancy G. Sebring, Esther Sternberg, Sara Torvik.

REFERENCES

1. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol 2006;27(1):24–31. [PubMed: 16316783]
2. Marques-Deak AH, Neto FL, Dominguez WV, Solis AC, Kurcgant D, Sato F, et al. Cytokine profiles in women with different subtypes of major depressive disorder. J Psychiatr Res 2007;41(1–2):152–159. [PubMed: 16375926]
3. Marques-Deak A, Cizza G, Sternberg E. Brain-immune interactions and disease susceptibility. Mol Psychiatry 2005;10(3):239–250. [PubMed: 15685252]
4. Hartman JM, Berger A, Baker K, Bolle J, Handel D, Mannes A, et al. Quality of life and pain in premenopausal women with major depressive disorder: The POWER Study. Health Qual Life Outcomes 2006;4(1):2. [PubMed: 16420706]
5. Eskandari F, Martinez P, Torvik S, Phillips T, Sternberg E, Mistry S, et al. Low Bone Mass in Premenopausal Women with Depression. The Archives of Internal Medicine 2007;167(21):2329–2336.
6. Eskandari F, Mistry S, Martinez PE, Torvik S, Kotila C, Sebring N, et al. Younger, premenopausal women with major depressive disorder have more abdominal fat and increased serum levels of prothrombotic factors: implications for greater cardiovascular risk, The Power Study. Metabolism 2005;54(7):918–924. [PubMed: 15988701]
7. Marques-Deak A, Cizza G, Eskandari F, Torvik S, Christie IC, Sternberg EM, et al. Measurement of cytokines in sweat patches and plasma in healthy women: validation in a controlled study. J Immunol Methods 2006;315(1–2):99–109. [PubMed: 16942779]
8. Phillips TM. Multi-analyte analysis of biological fluids with a recycling immunoaffinity column array. J Biochem Biophys Methods 2001;49(1–3):253–262. [PubMed: 11694283]
9. Phillips TM, Krum JM. Recycling immunoaffinity chromatography for multiple analyte analysis in biological samples. J Chromatogr B Biomed Sci Appl 1998;715(1):55–63. [PubMed: 9792497]
10. Sarno M, Sarno L, Baylink D, Drinkwater B, Farley S, Kleerekoper M, et al. Excretion of sweat and urine pyridinoline crosslinks in healthy controls and subjects with established metabolic bone disease. Clin Chem Lab Med 2001;39(3):223–228. [PubMed: 11350019]
11. Castle PE, Phillips TM, Hildesheim A, Herrero R, Bratti MC, Rodriguez AC. Immune profiling of plasma and cervical secretions using recycling immunoaffinity chromatography. Cancer Epidemiol Biomarkers Prev 2003;12(12):1449–1456. [PubMed: 14693736]
12. Jones AP, Webb LM, Anderson AO, Leonard EJ, Rot A. Normal human sweat contains interleukin-8. J Leukoc Biol 1995;57(3):434–437. [PubMed: 7884315]
13. Sato K, Sato F. Interleukin-1 alpha in human sweat is functionally active and derived from the eccrine sweat gland. Am J Physiol 1994;266(3 Pt 2):R950–R959. [PubMed: 8160891]

14. Thomas AJ, Davis S, Morris C, Jackson E, Harrison R, O'Brien JT. Increase in interleukin-1 beta in late-life depression. Am J Psychiatry 2005;162(1):175–177. [PubMed: 15625217]

15. Carney RM, Freedland KE. Depression, mortality, and medical morbidity in patients with coronary heart disease. Biol Psychiatry 2003;54(3):241–247. [PubMed: 12893100]

16. Silverman MN, Pearce BD, Biron CA, Miller AH. Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. Viral Immunol 2005;18(1):41–78. [PubMed: 15802953]

17. Gjerris A, Rafaelsen OJ, Vendsborg P, Fahrenkrug J, Rehfeld JF. Vasoactive intestinal polypeptide decreased in cerebrospinal fluid (CSF) in atypical depression. Vasoactive intestinal polypeptide, cholecystokinin and gastrin in CSF in sychiatric disorders. J Affect Disord 1984;7(3–4):325–337. [PubMed: 6241214]

18. Hou C, Jia F, Liu Y, Li L. CSF serotonin, 5-hydroxyindolacetic acid and neuropeptide Y levels in severe major depressive disorder. Brain Res 2006;1095(1):154–158. [PubMed: 16713589]

19. Kubera M, Kenis G, Bosmans E, Kajta M, Basta-Kaim A, Scharpe S, et al. Stimulatory effect of antidepressants on the production of IL-6. Int Immunopharmacol 2004;4(2):185–192. [PubMed: 14996410]

20. Appenzeller BM, Schummer C, Rodrigues SB, Wennig R. Determination of the volume of sweat accumulated in a sweat-patch using sodium and potassium as internal reference. J Chromatogr B Analyt Technol Biomed Life Sci 2007;852(1–2):333–337.

Table 1

Table 1 shows demographic and clinical characteristics of study participants. Inclusion criteria required at least one episode of MDD within the last three years (DSM-IV). All medications were recorded. The control group was matched by age (three years) and BMI (two units). All patients and controls were in good general health. Data from three subjects were excluded due to (a) a cutaneous reaction in the area where the sweat patches were applied; (b) a subsequent diagnosis of a chronic pain disorder; and (c) the research chart could not be located.

Variable	MDD (n=19)	Healthy Controls (n=17)	p
Age –(years) (range)	33.26±6.5 (23–44)	33.2±6.9 (23–44)	0.99 [a]
BMI (Kg/m^2) (range)	27.7±6.2 (17–39)	24.0±3.4 (20–33)	0.03[a]
Caucasian	17/19 (89%)	15/17 (88%)	1.0[b]
Years of Education (range)	17±1.8 (12–20)	16.5±1.8 (12–20)	0.38[a]
Married	7/19 (37%)	10/17 (59%)	0.3[b]
Current Smokers	2/19 (10%)	2/17 (12%)	1.0[b]
Use of psychotropic	18/19 (95%)	0/17	n/a
Birth control	2/19(10%)	5/17 (29%)	0.2[b]
Current MDD	4/19 (21%)	0/17	n/a
Comorbidity of psychiatric diagnosis	11/19 (58%)	3/17 (18%)	0.02[b]
Hamilton depression scale	8.9±7.4	1.6±2.2	<0.0001[a]
Clinical Remission*	17/19	n/a	
Hamilton anxiety scale (range)	6.2±4.5	1.4±1.8	0.0003[a]
GAF	59.4±8.9	79.9±3.2	<0.0001[a]

Data reported as mean ± SD and Ratio / Percent ratio/

[a] t-test

[b] Fisher's test

* Clinical remission is defined here as a Hamilton Depression score below 20, which usually defines mild depression.

Table 2

Table 2 shows concentrations of plasma and sweat patch cytokines (pg/ml) and neuropeptides in patients with MDD and in healthy controls. Data are reported as median and range, MDD vs. Healthy Control differences in plasma (Mann-Whitney U tests, p 's<0.0001) and in sweat (p's <0.0001) for all analytes. As vacuum extraction was used to recover analytes from the patch, standardization of sweat volume was performed by measuring total protein rather than using sodium and potassium as internal references as previously described (20). For 11 subjects, levels of IL-1 alpha were below the assay cut-off limit of 0.5 pg/ml. As a result data from these subjects were omitted from the analysis and degrees of freedom were corrected to account for the lower sample size.

Analytes	Plasma		Sweat	
	MDD	Healthy Control	MDD	Healthy Control
IL-1α	52.6 30.5–85.9	5.9 2.5–9.8	57.5 39.8–85.2	7.6 3.7–13.6
IL-1β	139.9 33.2–305.9	10.5 4.9–16.4	160.5 50.5–292.7	10.9 6.9–18.4
IL-6	101.8 66.7–223.7	7.8 5.1–13.5	133.8 66.3–246.5	10.4 6.9–15.5
TNFα	158.8 55.5–320.8	11.1 5.9–16.5	177.9 66.5–361.3	12.8 9.3–21.1
IL-8	50.7 10.5–160.4	2.7 0.6–5.5	63.2 16.5–153.8	2.9 1.5–6.1
VIP	5.3 1.7–22.7	17.2 9.5–32.6	6.2 2.9–28.4	22.5 20.5–36.1
NPY	46.7 5.4–69.8	1.1 0.6–2.6	50.8 14.2–73.2	1.9 0.8–2.9
SP	77.2 24.1–163.5	3.3 1.3–6.8	88.6 66.2–180.7	3.8 1.6–7.2
CGRP	55.6 13.5–101.8	1.65 0.7–4.6	60.5 18.9–125.2	2.1 1.1–3.8

Table 3

Table 3 shows plasma - sweat patch correlations of cytokines and neuropeptides in patients with MDD and in healthy controls.

Analytes (pg/ml)	MDD		Healthy Control	
	r	p	r	p
IL-1α	0.94	0.0001	0.78	0.02
IL-1β	0.97	0.0001	0.65	0.005
IL-6	0.92	0.0001	0.36	0.15
TNFα	0.95	0.0001	0.67	0.003
IL-8	0.99	0.0001	0.63	0.006
VIP	0.76	0.0002	−0.006	0.98
NPY	0.92	0.0001	0.22	0.4
SP	0.83	0.0001	0.43	0.08
CGRP	0.73	0.0004	0.36	0.2

Table 4

Table 4 shows hierarchical regression analysis relating age and BMI to plasma and sweat levels of cytokines and neuropeptides. Both plasma and sweat values were natural log transformed prior to estimation in order to normalize the distribution and to correct heteroscedasticity. Analyses were performed using Prism version 3:0 for Windows (GraphPad Software, San Diego, CA) and the R statistical computing environment (version 2.3.1; R Development Core Team, 2006). An α of 0.05 was used in all significance tests.

	Plasma			Sweat		
Analytes	**B**	**ΔR^2**	**p**	**B**	**ΔR^2**	**p**
IL-1α	2.26	0.54	<0.0001	2.00	0.50	<0.0001
IL-1β	2.46	0.66	<0.0001	2.51	0.70	<0.0001
IL-6	2.70	0.80	<0.0001	2.48	0.77	<0.0001
TNFα	2.56	0.74	<0.0001	2.47	0.73	<0.0001
IL-8	3.02	0.67	<0.0001	2.87	0.72	<0.0001
VIP	−0.96	0.35	<0.0001	−1.33	0.54	<0.0001
NPY	3.07	0.67	<0.0001	3.00	0.74	<0.0001
SP	3.10	0.73	<0.0001	3.20	0.78	<0.0001
CGRP	3.14	0.77	<0.0001	3.33	0.79	<0.0001

Table 5

Table 5 - shows Bivariate Correlation between Scores in Hamilton Depression Scale (HAM-D) and Hamilton Anxiety Scale (HAM-A) and levels of sweat and plasma cytokines and neuropetides in patients with MDD and healthy controls

Analytes (pg/ml)	HAM-D		HAM-A	
	Sweat	Plasma	Sweat	Plasma
IL-1α	r = 0.61 p= 0.0001	r = 0.61 p= 0.0001	r = 0.57 p= 0.0003	r = 0.56 p= 0.0004
IL-1β	r = 0.69 p <0.0001	r = 0.65 p<0.0001	r = 0.55 p= 0.0004	r = 0.52 p= 0.0012
IL6	r = 0.67 p<0.0001	r = 0.63 p<0.0001	r = 0.52 p= 0.001	r = 0.49 p= 0.002
TNF-α	r = 0.72 p<0.0001	r = 0.74 p<0.0001	r = 0.56 p= 0.0004	r = 0.6 p= 0.0001
IL-8	r = 0.61 p= 0.0001	r = 0.48 p= 0.003	r = 0.50 p= 0.002	r = 0.41 p= 0.014
VIP	r = − 0.51 p= 0.0014	r = − 0.6 p= 0.0001	r = − 0.55 p= 0.0005	r = − 0.58 p= 0.0002
NPY	r = 0.58 p= 0.0002	r = 0.59 p= 0.0002	r = 0.59 p= 0.0001	r = 0.67 p<0.0001
SP	r = 0.66 p< 0.0001	r = 0.73 p< 0.0001	r = 0.64 p< 0.0001	r = 0.76 p<0.0001
CGRP	r = 0.68 p< 0.0001	r = 0.73 p< 0.0001	r = 0.60 p= 0.0001	r = 0.69 p<0.0001

Sperman correlation