The Roles of S100B , IL-1β and IL-2 as Neuroinflammation Biomarkers in Generalized Anxiety Disorder

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Short report

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Abstract

Introduction: S100B is a neurotrophic factor regulates neuronal growth and plasticity via activating astrocytes and microglia through production of neuro-inflammatory molecules like interleukin (IL)-1β involved in many mental disorders, few studies have combined S100B and cytokines to explore their roles as neuro-inflammatory biomarkers in Generalized Anxiety Disorder (GAD).

Methods: Serum S100B and cytokines (IL-1β , IL-2, IL-4 and IL-10) of 108 untreated GAD cases and 123 healthy controls were determined by enzyme linked-immuno-sorbent assay (ELISA) and then compared, while Hamilton Anxiety Rating Scale (HAMA) scores were measured to evaluate anxiety severity.

Results: The serum S100B and IL-1β, IL-2 levels of GAD cases were lower than HC significantly (P<0.001), the IL-4 level of GAD were higher than HC (P<0.001), while IL-10 had no significant difference between two groups (P=0.215). The ROC area of S100B, IL-1β, IL-2 and IL-4 in diagnosis of GAD was (0.740 ± 0.032) , (0.900 ± 0.021) , (0.920 ± 0.018) and (0.696 ± 0.037) , all of them suggested a good predicting value (P < 0.001) , while the ROC area of IL-10 was (0.544 ± 0.038) (P = 0.251). The sensitivity of S-100B, IL-1β, IL-2 in diagnosis of GAD was 73.1%, 80.6%, 85.2%, while the specificity was 61.0%, 86.2%, 80.5%. The combination ROC area of S100B, IL-1β , IL-2 and IL-4 was (0.985 ± 0.006)(P < 0.001). Serum S100B was positively correlated with IL-2 and IL-4 (P <0.05)., while was negatively with HAMA scores (P <0.001).

Conclusion: The serum S-100B, IL-1β, IL-2 levels of GAD were down-regulated while IL-4 was up-regulated, both IL-2 and IL-4 had a good diagnosis value in GAD separately while the combination of S100B and cytokines had a better diagnosis value which means the neuro-inflammation in GAD is a network regulated by many factors.

1. Introduction

Generalized anxiety disorder (GAD) is a chronic psychiatric disease characterized by persistent, excessive worry which impair patients' social and cognitive function seriously with a lifetime prevalence up to 6.2% in the United States of America[1] and 5.3% in urban China[2]. Lifetime prevalence of GAD varies from 0.1% in Nigeria to 6.2% in New Zealand [3], and varies from 0.3%[4] to 4.1-4.6% in China[5]. Excessive, uncontrollable worry in the absence of direct stimuli or a disproportionate sense to potentially risk is the key diagnostic criterion of GAD[6]. So far no distinct laboratory test or brain scan or any other bio-marker is available to distinguish GAD from other mental disorders.

Neither genetics[7] nor protein bio-markers (such as BDNF and GDNF)[8] can not applied to trait bio-markers because of anxiety disorders may have several subtypes with different etiopathogenesis [9]. Early stressful life events and dysfunction of hypothalamic-pituitary-adrenal (HPA) axis have a significant role in the onset of anxiety[10], and chronic inflammation has been proved to be involved in the pathogenesis of anxiety disorders which reflected by the abnormalities of cytokines (e.g., IL-1β, IL-2, IL-6) both in peripheral and CNS[11]. The neuro-inflammatory responses initiated by inflammatory cytokines

and the subsequent neuronal dysfunction can lead to further glial activation and continued over expression of pro-inflammatory cytokines, which is metabolized by microglia and astrocyte[12][13].

S100 calcium-binding protein B (S100B) is one member of the S100 family proteins, secreted by astrocytes in gray matters while expressed by oligodendrocytes in white matter , which modulates the proliferation and differentiation of neurons and glia[14]. In the developing CNS, S100B acts as a neurotrophic factor and neuronal survival protein. In the adult brain, it is usually upregulated in response to stress[15]. A meta-analysis by Schroeter et al[16] revealed that S100B serum levels were consistently increased in acute major depressive or manic episodes and decreased during antidepressant treatment if clinical improvement was sufficient. As is known to all, GAD and depression often co-occur, Brown et al [17] estimated the lifetime comorbidity of MDD in GAD and GAD in MDD at 64% and 9% respectively which suggests GAD and depression may share certain etiology and pathogenesis mechanisms. Compared to the studies of S100B in depression, the research of S100B on anxiety especially in GAD is rather limited. Meryem[18] reported decreased S100B levels in the hippocampus and prefrontal cortex in diabetic rats with anxiety like-behaviours which can revised by melatonin. As glia originated protein, S100B activates microglial IL-1β production through mitogen-activated protein kinase (MAPK) pathways[19]. Studies had evaluated the ability of IL-8, IL-10, and S100B to differentiate between levels of alcohol consumption and cutoff values to identify low risk and unhealthy alcohol use groups[20]. But whether S100B in different brain regions interacts with different cytokines induces different affective diseases even distinct GAD subtype is still a question[21].

Taken together, the existing evidence suggests that GAD patients may have abnormal serum S100B and cytokines levels, but the association between S100B and cytokines in GAD is still a question, and S100B may exerts its role with some specific cytokines in the neuro-inflammation mechanisms of GAD in Chinese population. So we conducted this study.

2. Materials And Methods

2.1. Subjects

One hundred and seventy-seven patients who met the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria for GAD were recruited from Huzhou Third Municipal Hospital between June 2018 and June 2019. Inclusion criteria: (1) Han nationality, (2) age range 18-65 years, (3) Hamilton Rating Scale for Anxiety (HAMA) scores ≥ 17 and 17-item Hamilton Rating Scale for Depression (HAM-D_{17}) ≤ 14, (4) free of major psychotropic drugs or psychotherapy for at least 4 weeks before inclusion. Exclusion criteria: (1) any mental illness requiring medical intervention such as dementia, schizophrenia, delusional disorder, bipolar disorder, major depression disorder and so on, (2) substance abuse disorders, (3) obvious abnormality in laboratory examination, (4) failure to complete examination and questionnaires , (5) with severe physical diseases (including epilepsy, severe cardiopulmonary, hepatorenal insufficiency, malignant tumor or hematopathy, autoimmune diseases), (6) taking immunomodulatory drugs, such as glucocorticoids, immunomodulators, antipyretic and analgesic drugs,

etc., within half a year, (7) pregnant or lactating women. Healthy controls recruited from the local community were assessed by a psychiatrist using the Structural Clinical Interview for DSM-IV Disorders (SCID). The HAMA scores of all control subjects were ≤ 7. Those with a history of any psychiatric disorder were excluded. The protocol was permitted by the Ethics Committee of Huzhou Third Municipal Hospital. Written informed consent was obtained from all participants before enrolled.

2.3. Rating symptoms

The severity of the GAD symptoms was rated using HAMA, which was administered by a single trained rater. The rater and laboratory workers were blind to the purpose of the study. In addition, the rater was blind to the laboratory data, and the HAMA scores were not disclosed to the laboratory workers. To maintain blindness, a trained research coordinator managed all data and schedules.

2.4. Determination of serum S100B and cytokines

In GAD patients, 10ml blood samples were collected into two sterile tubes between 7 and 8 AM using a standard sterile preparation before treatment and the blood samples abstained from HC on the same time and centrifuged for 15 min at 2500rpm. The cell free-serum was pipetted and aliquoted in 2 ml standard freezer vials which were then stored within 2 hours at -80° C to determine the serum S100B and cytokines levels. The inflammatory cytokines were measured by ELISA including IL-1β, IL-2, IL-4, IL-10. The ELISA kits were manufactured by Shanghai Yaoyun Biotechnology Limited Company. And S100B ELISA kit were manufactured by Wuhan USCN Business Co., Ltd. The sensitivity by this assay for detecting S100B, IL-1β, IL-2, IL-4 and IL-10 is 1.0 pg/L. To minimize inter-assay variations, S100B and cytokines was determined after all samples were collected.

For S100B, the inter- and intra-assay coefficients of variation were 5% and 6.5%, respectively. For cytokines, the inter- and intra-assay coefficients of variation were <10%.

2.5. Statistical analysis

SPSS 19.0 for Windows was used to analyze the collected data. Data were generally reported as mean ± SD. The distributions of all variables were checked by the Kolmogorov–Smirnov test, and all showed normal and equal or nearly equal distribution. Student's t-tests were performed for the comparisons of some demographic data, including the serum S100B/cytokines levels between GAD patients and HC. ROC (receiver operating characteristic curve) was applied to compare the predicting value of baseline S100B and cytokines levels in GAD, and the sensitivity, specificity, Yorden index and Kappa value of S100B/cytokines in diagnosis were calculated. Relationships between S100B/cytokines and clinical variables (age, HAMA scores and illness duration) were evaluated using Pearson correlations. A difference was considered significant at two tailed $P < 0.05$.

3. Results

3.1. Demographic and serum S100B/cytokines levels of GAD cases and HC

We analyzed the data of 108 GAD cases and 123 HC. There was no significant difference in the male/female ratio and age between GAD subjects and HC.

The baseline serum S100B and IL-1β, IL-2 levels of GAD cases were lower than HC significantly (P<0.001), the serum IL-4 levels of GAD cases were higher than HC (P<0.001), while IL-10 had no significant difference between two groups (P=0.215)(see Table 1).

The correlation of serum S100B and IL-1β, IL-2, IL-4, IL-10 levels in GAD cases with age was (r = -0.065, P = 0.505), (r = -0.147, P = 0.128), (r = -0.056, P = 0.568),(r = 0.013, P = 0.893), (r = -0.113, P = 0.244) respectively.

Table 1. Demographic and clinical characteristics of GAD and HC

Characteristics	GAD (n = 108)	HC (n = 123)	Statistics	
			χ^2/t	P value
Sex (male/female)	30/78	40/83	0.612	0.434
Age (years)	49.6 ± 11.3	47.5 ± 8.4	1.567	0.119
HAMA scores	22.5±3.1	NA		
HAMD scores	9.6 ± 2.9	NA		
Illness duration(month)	24.4±37.5	NA		
S100B（pg/ml）	330.3 ± 79.9	421.8 ± 123.0	6.785	<0.001
IL-1β（pg/ml）	35.5 ± 11.3	56.1 ± 15.0	11.883	<0.001
IL-2（pg/ml）	34.7 ± 8.7	57.0 ± 16.3	13.214	<0.001
IL-4（pg/ml）	63.7 ± 21.4	46.7 ± 10.6	7.474	<0.001
IL-10（pg/ml）	42.6 ± 9.4	41.0 ± 10.6	1.242	0.215

GAD:generalized anxiety disorder, HC:healthy controls, HAMA: Hamilton Anxiety Rating Scale, HAMD: Hamilton Depression Rating Scale

3.2. The single diagnosis value of S100B, IL-1β, IL-2, IL-4, IL-10 in GAD

3.2.1 The ROC value of S100B, IL-1β, IL-2, IL-4, IL-10 in GAD

The ROC area of S100B, IL-1β, IL-2 and IL-4 in diagnosis of GAD was (0.740 ± 0.032) , (0.900 ± 0.021) , (0.920 ± 0.018) and (0.696 ± 0.037) , all of them suggested a good predicting value ($P < 0.001$) , while the ROC area of IL-10 was (0.544 ± 0.038) ($P = 0.251$). (see Figure 1,2)

3.2.2 The sensitivity, specificity, Yorden index and Kappa value of S100B , IL-1β and IL-2 in the diagnosis of GAD

Based on the ROC curve, the sensitivity, specificity, Yorden index and Kappa value of S100B and IL-1β, IL-2 were calculated, define 375pg/ml, 41pg/ml and 43pg/ml as cut point of S100B, IL-1β and IL-2 respectively, the sensitivity and specificity of IL-1β and IL-2 were all above 80%, the details of sensitivity, specificity, Yorden index and Kappa value of S100B , IL-1β and IL-2 see Table 2, all of these indexes suggested a good diagnosis value ($P < 0.001$).

Table 2 The sensitivity, specificity, Yorden index and Kappa value of S100B , IL-1β and IL-2

	Sensitivity(%)	Specificity(%)	Yorden index	Kappa value
S100B	73.1	61.0	0.341	0.338
IL-1β	80.6	86.2	0.668	0.669
IL-2	85.2	80.5	0.657	0.653

3.3 The combination diagnosis value of S100B and IL-1β, IL-2, IL-4 in GAD

Based on the single diagnosis value of S100B, IL-1β, IL-2, IL-4, IL-10, S100B, IL-1β, IL-2 and IL-4 were included in the combination diagnosis of GAD. The combination ROC area of S100B, IL-1β , IL-2 and IL-4 was (0.985 ± 0.006), the combination ROC area of S100B, IL-2 and IL-4 was (0.983 ± 0.006), the combination ROC area of S100B, IL-1β and IL-2 was (0.945 ± 0.014), the combination ROC area of S100B, IL-1β and IL-4 was (0.940 ± 0.015). The combination ROC of IL-2 and IL-4 was (0.974 ± 0.009), the combination ROC of IL-1β and IL-2 was (0.940 ± 0.015), the combination ROC of S100B and IL-2 was (0.932 ± 0.017), the combination ROC of IL-1β and IL-4 was (0.926 ± 0.018), the combination ROC of S100B and IL-1β was (0.913 ± 0.020), all of them suggested a good predicting value ($P < 0.001$) .(see Figure 3)

3.4 Relationship between serum S100B and cytokines (IL-1β, IL-2, IL-2, IL-10) and clinical features (HAMA scores, illness duration)

The serum S100B and IL-1β, IL-2, IL-4, IL-10 was (330.3±79.9), (35.5±11.3), (34.7±8.7), (63.7±21.4), (42.6±9.4) respectively, the correlation between serum S100B and IL-1β, IL-2, IL-4, IL-10 was (r = 0.175, *P* = 0.070), (r = 0.237, *P* = 0.014), (r = 0.261, *P* = 0.006),(r = 0.055, *P* = 0.589) respectively.

The serum S100B and HAMA scores, illness duration was (330.3 ±79.9), (22.5±3.1), (24.4±37.5), respectively, the correlation between serum S100B and HAMA scores, illness duration was (r = -.386, *P* <0.001),(r = 0.080, *P* = 0.410) respectively.

4. Discussion

In this study, we explored the serum S100B and cytokines levels (IL-1β, IL-2, IL-4, IL-10) in Chinese GAD population. We compared the serum S100B and cytokines levels between GAD and HC, we also explored the diagnosis value of S100B and cytokines in GAD as well as the correlation between S100B and cytokines. The main finding in this study including as follows: (1) The serum S100B and IL-1β, IL-2 levels were lower in GAD cases compared to HC, the IL-4 level of GAD were higher compared to HC, while IL-10 had no significant difference. (2) The ROC area of S100B, IL-1β, IL-2 and IL-4 all suggested a good predicting value in diagnosis of GAD, while the ROC area of IL-10 had a poor diagnosis value. The sensitivity of S-100B, IL-1β, IL-2 was 73.1%, 80.6%, 85.2% and the specificity was 61.0%, 86.2%, 80.5% in diagnosis of GAD. (3) The combination of S100B and cytokines had a better ROC area than single index in the dianosis of GAD, in which IL-2 comined with IL-4 had a promising diagnosis value with a ROC as high as (0.983 ± 0.006). (4) Serum S100B had a positive correlation with IL-2 and IL-4 while had a negative correlation with HAMA scores.

The exact pathologies of GAD in present time has not been well established and is still a subject of debate. Many studies have concentrated on the neuro-inflammation pathogenesis of GAD based on the changes of cytokines (cytokines plays a key role in the neuro-inflammation pathway)[22, 23]. Cytokines include pro-inflammatory cytokines (such as IL-1β, IL-2, IL-6.) and anti-inflammatory cytokines (such as IL-4, IL-10) which mediated by many factors such as S100B [24]. To our knowledge, this study was one of the first to explore the relationship between S100B and cytokines (IL-1β, IL-2, IL-10) in GAD so as to investigate the characteristics of serum S100B and cytokines in GAD patients and their prognosis roles in GAD.

S100B is mainly expressed in glial cells and plays its biological roles depending on concentrations and physiological states [15, 25-27]. When it is present in nanomolar concentrations, S100B acts as a growth and differentiation factor for neurons and astrocytes. When it is present in micromolar concentrations, S100B exerts neurotoxic activity and induces the apoptosis of neurons and astrocytes[28] .The results of this study indicate that serum S100B levels are down regulated in GAD patients compared to healthy controls. Limited researches have reported controversial results of S100B on anxiety, Bergh C D[29] reported patients with elevated S100B suffered more anxiety 3-6 years after cardiac surgery. Tomas[30] et al found no correlation between cerebrospinal fluid S100B levels and anxiety symptoms. Another study found S-100B-positive cells and anxiety level were markedly increased after treatment[31].

But our study found serum S100B was negatively correlated with anxiety syndromes. Buschert J et al[32] reported elevated S100B levels increase behavioral and neural plasticity in response to acute environmental stimuli, but chronic mild stress induces decreased S100B in hippocampal and cerebrospinal fluid and can be revised by fluoxetine[14]. S100B may be a protective factor in the acute stage of anxiety when individuals facing stress, but with the development of disease, the down-regulation of S100B may be a result of decompensation which closely related to the chronic pathologicalgenesis of GAD, and the illness duration of GAD always lasts more than 6 months (chronic anxiety). S100B is mainly expressed in astrocytes and oligodendrocytes in the white matter[15], at a nanomolar physiological concentration, S100B stimulates the neurite growth, increases neuronal maturation and glial cell proliferation. Reduced white matter volumes in dorsolateral PFC, anterior limb of the internal capsule, and midbrain was observed in GAD patients[33]. The reduced white matter volumes may help to explain the down regulation of S100B levels in GAD patients but the interaction between the two factors needs further studies.

Both central and peripheral immune dysregulation has been researched as a etiology of anxiety disorders. The results of our study reveals GAD patients have lower serum pro-inflammatory cytokines of IL-1β and IL-2, higher anti-inflammatory cytokines of IL-4 while IL-10 had no dysregulation, compared the consistent hyper activation of inflammatory cytokines in depression, the results in anxiety are always controversial. Zhen Tang[34] found Serum levels of CRP, IL-1α, IL-2, IL-6, IL-8, IL-12, IFN-γ, and GM-CSF were significantly higher in the GAD group in comparison to the control group, while Vogelzangs found [35] no associations were found with IL-6 or TNF-a in anxiety disorders including GAD. Among people with alcohol use disorder, IL-10 was negatively associated with anxiety score[36], Labaka Ainitze et, al[37] reported chronic social instability stress induced anxiety-like behavior and decreased IL-10 expression in the hippocampus of the female mice, while no differences in pro-inflammatory cytokine expression such as IL-1β and IL-6. Our study found decreased pro-inflammatory cytokines of IL-1β and IL-2 as well as increased anti-inflammatory cytokines IL-4 while IL-10 showed no obvious difference between GAD and controls. Both IL-2 and IL-4 showed a promising diagnosis value in GAD based on ROC, but the sensitivity and specifity were all below 90%, while IL-2 combined with IL-4 showed a ROC as high as 0.983, and added with S100B and IL-1β, the ROC area was more high. Considering the complex interaction of inflammatory mediators, cytokines in depression and other mental disorders are usually defined as a network, a single specific cytokine leads to very limited influence on the whole net, but certain key cytokine may play huge impact in a specific disorder[38]. Undoubtedly, IL-2 and IL-4 were key plots in the neuro-inflammation pathogensis of GAD compared to IL-1β and IL-10 which deserving more attention in the future study.

In addition, inflammatory cytokines are influenced by BMI index, anxiety severity, duration, age of onset, subtype, depression comorbidity, alcohol use and many other factors in anxiety disorder[23]. We only analysed the relation between age and cytokines but found no positive conections. So the results of this work need more larger sample studies to verify in order to explore the trait characteristic of inflammatory cytokines in GAD. This study also found serum S100B was positively correlated with IL-2 and IL-4, while had no correlation with IL-1β and IL-10. Evidence suggests that S100B stimulates mitogen-activated protein kinase (MAPK) pathways and then induces increase of microglial IL-1β production, and each

MAPK to IL-1β production depended on the activating stimulus[19]. which suggests that different glial activators use distinct sets of signaling pathways to induce different inflammatory cytokines changes and finally develop into different CNS diseases in microglia. Preliminary studies also show that S100B upregulates IL-1β and TNF-αexpression in microglia via receptor for advanced glycation end products and later induces upregulation of COX-2 and eventually causes brain damage[24]. Compared to IL-1β, S100B had a closer association with IL-2 in GAD in our study, although no direct evidence had been found between S100B and IL-2, based on the results of our study, it can be inferred that the roles of S100B in the neuro-inflammation pathway of GAD may mainly via the activating of IL-2 upregulation, so the downregulation of S100B coordinated with IL-2 in our work maybe interpreted from this point. But the interconnection of inflammatory cytokins and mediators is a network which needs to be confirmed by basic research.

In conclusion, our study adds to the literature by showing that serum S100B can serve as a protective role in the neuro-inflammation pathophysiology of GAD. The serum S-100B, IL-1β, IL-2 levels of GAD were down-regulated while IL-4 was up-regulated, both IL-2 and IL-4 had a good prognosis value in GAD separately while the combination of S100B and cytokines had a better diagnosis value which means the neuro-inflammation in GAD is a network regulated by many factors. As concluded by Chong Z Z[15] “It seems that S100B is not a specific marker for any pathological process due to its similar pattern of changes in different diseases and therefore, it is currently far from conclusion that S100B is regarded as a standard biomarker to monitor the pathological process and evaluate the prognosis of diseases practically”. More studies are required to demonstrate the relationship between S100B and GAD via neuro-inflammation pathway.

There are a few limitations to our study. (1) The sample size is too small to prove that S100B is a biomarker of GAD and its value in neuro-inflammation pathway of GAD.(2) It would have been better to analyze the results in subgroups (sex and age), but the power would have decreased. Samples well-matched in age and sex would be ideal for subsequent studies.(3) Although we tried to eliminate the effects of depression syndromeS, GAD has a high comorbidity with MDD, and stricter inclusion criteria could be helpful in future studies.(4) If we can acquire more dynamic changes of S100B and cytokines levels in the treatment (we did not collect blood sample post-intervention), the dynamic role of S100B and cytokines in neuro-inflammation pathway of GAD will be more clear.(5) There are so many cytokines, and cytokines(inflammatory status) affected by mass factors, so weather the results in this study can be duplicated in subsequent trials with other cytokines is still a question.

Abbreviations

BMI: Body mass index

CNS: Central nervous system

COX-2: Cyclooxygenase-2

DSM-IV: Statistical Manual of Mental Disorders-IV

GAD: Generalized Anxiety Disorder

IL-1β: Interleukin-1β

IL-2: Interleukin-2

IL-4: Interleukin-4

IL-10: Interleukin-10

ELISA: enzyme linked-immuno-sorbent assay

HAMA: Hamilton Anxiety Rating Scale

HAMD: Hamilton Rating Scale for Depression

HC: Healthy controls

HPA: hypothalamic-pituitary-adrenal

MAPK: mitogen-activated protein kinase

MDD: Major depression disorder

PFC: Prefrontal cortex

ROC: Receiver operating characteristic curve

S100B: S100 calcium-binding protein B

TNF-α: Tumor necrosis factor-α

Declarations

Ethics approval and consent to participate

The protocol was permitted by the Ethics Committee of Huzhou Third Municipal Hospital. Written informed consent was obtained from all participants before enrolled.

Consent for publication

Not applicable

Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

No Competing interests are declared.

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Authors' contributions

Contributions: (I) Conception and design: Yonggui Yuan, Xinhua Shen, (II) Administrative support: Zhongxi Shen, (III) Provision of study materials or patients: Zhongxi Shen, Lie Ren, (IV) Collection and assembly of data: Zhongxi Shen, Mincai Qian, (V) Data analysis and interpretation: Lijun Cui, (VI) Manuscript writing: All authors, (VII) Final approval of manuscript: All authors.The first two authors contributed equally to this article.

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Figures

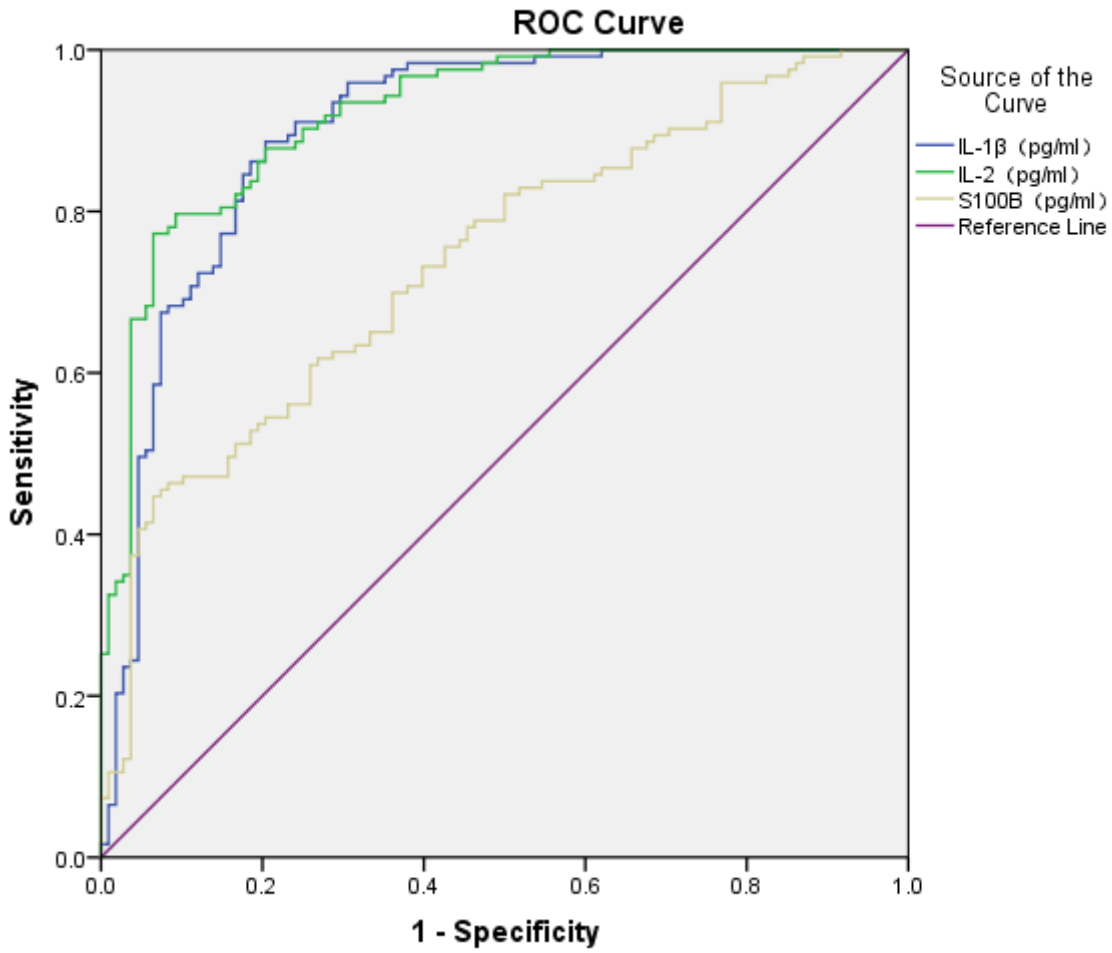

Figure 1

ROC of S100B、IL-1β, IL-2 in diagnosis of GAD

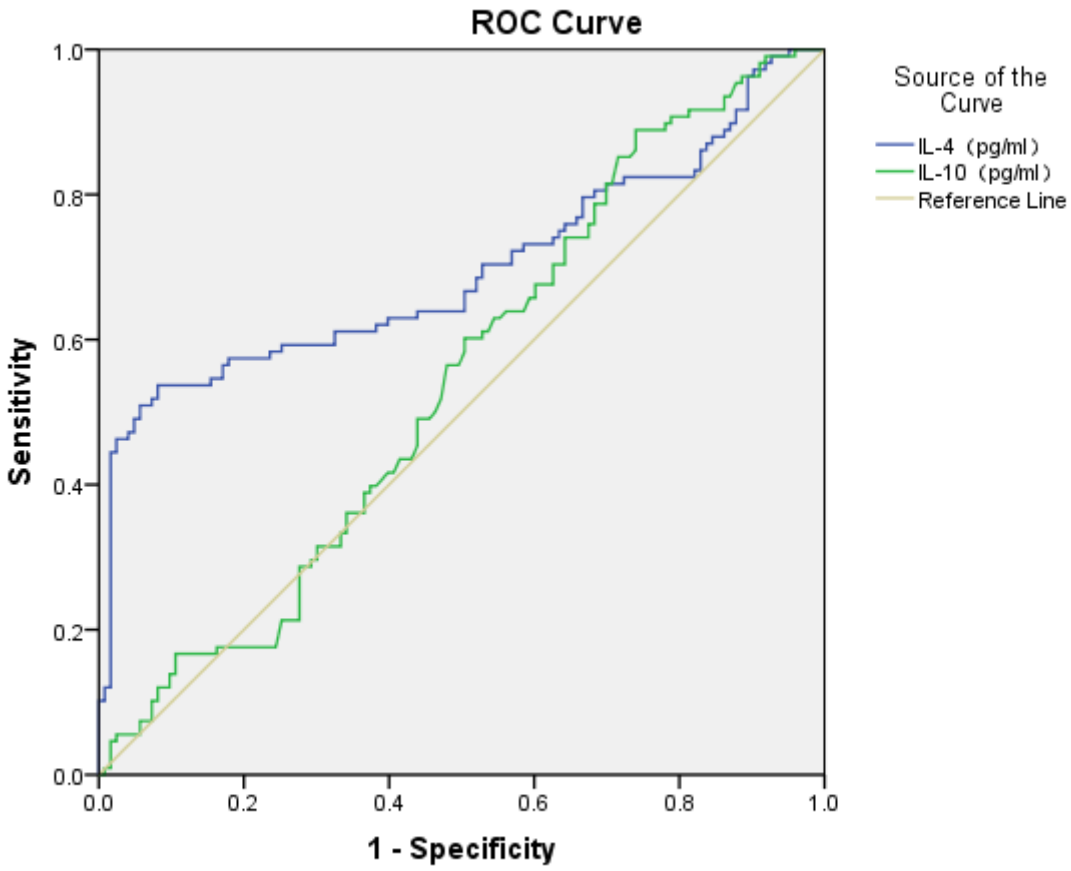

Figure 2

ROC of IL-4, IL-10 in diagnosis of GAD

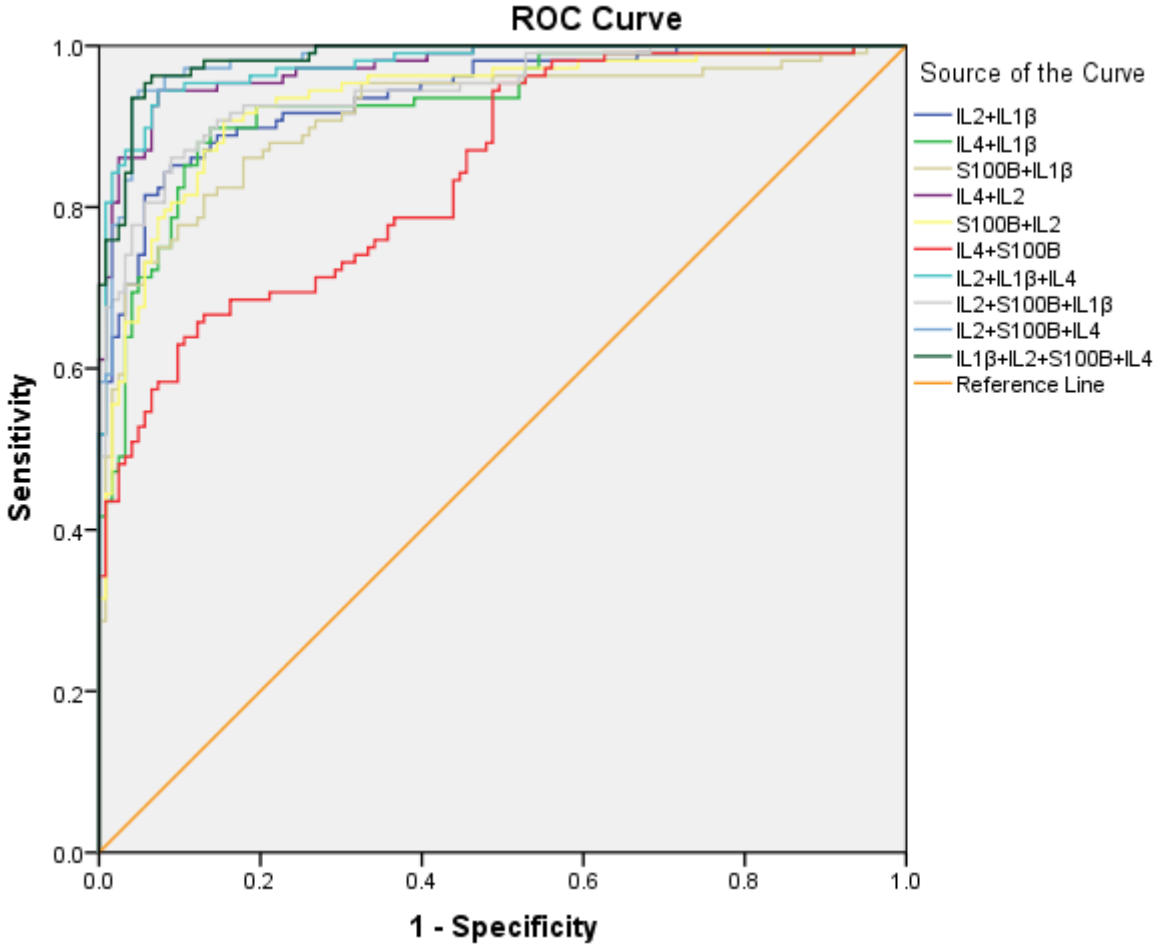

Figure 3

Combination ROC of S100B and IL-1β, IL-2, IL-4 in GAD