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The Function of IL-33 in Patients with Guillain-Barré Syndrome

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Abstract: In recent years, although the development of imaging has greatly facilitated the diagnosis of neurological diseases, it has been less helpful in the diagnosis of peripheral nervous system diseases. The gold standard for diagnosis is difficult to develop due to patient acceptance and ethical constraints. Therefore, the use of immunological methods to detect markers in human body fluids and disease-related markers has become the most important method to study diseases, diagnose and evaluate prognosis. This paper mainly focuses on the analysis and research on the role of IL-33 in patients with Guillain-Barré syndrome. This paper mainly compared the levels of IL-33 and ST2 in different parts of GBS patients with ODINs group, VE/VM group and healthy control group. In the research results, it was found that the incidence of GBS was mostly concentrated in winter and spring, accounting for 30.43% and 33.69%, respectively, followed by summer, accounting for 19.56%, and finally summer, accounting for 16.3%.

1. Introduction

GBS, also known as Guillain-Barré syndrome, is an autoimmune disease caused by viral infection or secondary infection. The clinical manifestations are loose and symmetrical paralysis of the extremities. Its specific pathogenesis has not yet been elucidated. It is generally believed that the pathological mechanism is that after macrophages invade the peripheral nervous system, under the combined action of T cells and activated cytokines, they use Schwann cells and the surface components of myelin as targets of attack, causing immune response and damage, resulting in The myelin sheath and surrounding glial structures are detached, resulting in demyelination. The second subtype of GBS is AMAN. The pathogenesis of AMAN is that the Fc receptor of the antibody mediates the immune response between ganglioside antigens on the surface of the coaxial membrane and invades the axon below the structure through the node of Ranvier, causing axonal damage [1-2].

In the study of IL-33 and Guillain-Barre syndrome, Alkan et al. reported a case of systemic lupus erythematosus associated with Guillain-Barre syndrome. MR imaging showed marked enhancement of the conus medullaris and cauda equina [3]. These MR observations may help confirm the

diagnosis of Guillain-Barré syndrome. Pietka et al. compared the responses of single- and double-deficient IL-33/ST2C57BL/6JBomTac mice in a well-established model of calcipotriol-induced atopic dermatitis [4]. The findings showed that lack of interleukin 33 and its receptor ST2 did not prevent the development of AD-like skin inflammation.

This paper firstly compared the levels of IL-33 and ST2 in different parts of GBS patients with ODINs group, VE/VM group and healthy control group [5-6]. Select ONIDs to compare the levels of IL-33 and its receptor ST2 in plasma and cerebrospinal fluid of GBS patients and patients with other autoimmune diseases to analyze whether the expression of cytokines IL-33 and ST2 is different between different neuroimmune diseases [7-8]. Comparison of plasma and cerebrospinal fluid levels of IL-33 and its receptor ST2 between GBS patients and VE/VM patients to analyze the role of cytokines IL-33 and ST2 in autoimmune disease-mediated neuroimmune diseases and infection directly mediated Is there any difference in expression between inflammatory diseases of the nervous system [9-10]. At the same time, the plasma levels of IL-33 and its receptor ST2 were compared between GBS patients and healthy controls to analyze whether the expression of cytokines IL-33 and ST2 was different between GBS and healthy people [11-12].

2. The Role of IL-33 in Patients with Guillain-Barré Syndrome

2.1 The Role of IL-33 in Guillain-Barré Syndrome

(1) Data collection

The concentrations of IL-33 and ST2 in the plasma and cerebrospinal fluid of the experimental group and the control group were detected by ELISA method (see below).

(2) Main reagents

Goat anti-human IL-33 antibody;
Mouse anti-human ST2 antibody;
Goat anti-human IL-33 biotinylated antibody;
Goat anti-human ST2 biotinylated antibody;
Recombinant human IL-33 standard;
Recombinant human ST2 standard;
Bovine serum albumin (BSA);
Horseradish peroxidase (HRP)-labeled streptavidin;
TMB;
Concentrated sulfuric acid;
Hydrochloric acid.

2.2 Main Reagent Preparation Method

(1) 0.15M PBS buffer: (PH=7.4)

NaCl	8g
KCl	0.2g
KH_2PO_4	0.24g
Na_2HPO_4-$12H_2O$	2.88g

Add double distilled water to 800ml, adjust the PH value to 7.4, and finally add double distilled water to make up to 1000ml.

(2) Washing buffer (PH=7.40.15M PBST)

0.05%Tween-20 0.5ml

Add PBS buffer to 1000ml

(3) Blocking buffer

Bovine Serum Albumin (BSA) 1.0g
Add 0.15M PH=7.4 PBS to 20ml
(4) TBS buffer: (PH=7.3)
Tris-base 2.42g (20mM Tris base)
NaCl8.78g (150mMNaCl)
Double distilled water to 1000ml, pH adjusted to 7.3
(5) Stop solution: (0.5mmol/l H_2SO4)
In 20ml of double-distilled water, add 2.7ml of concentrated sulfuric acid dropwise, stir while adding, dilute to 100ml after dissipating heat, and store at room temperature.
(6) IL-33 standard dilution

Bovine Serum Albumin (BSA)	0.1g
PH=7.4	0.15M PBS 100ml

(7) Goat Anti-Human IL-33 Biotinylated Antibody Diluent

TBS	50ml
BSA	0.05g

(8) ST2 standard dilution: PBS
Mouse anti-human ST2 antibody (coating antibody) diluent: PBS
Goat Anti-Human ST2 Biotinylated Antibody Diluent:

TBS	30ml
BSA	0.03g
2% heat-inactivated goat serum	0.6g

(9) IL-33 coated antibody blocking solution

PBS	100ml
BSA	1g

(10) ST2 coated antibody blocking solution

PBS	100ml
BSA	1g
5% sucrose	5g
0.05%NaN3	0.5g

(11) Sub-packaging of antibodies
1) Goat anti-human IL-33 antibody 100ug:
Add 1ml of goat anti-human IL-33 biotinylated antibody diluent to obtain a concentration of 100ug/ml, and divide into ten tubes, each with 100μl.
2) Mouse anti-human ST2 antibody 500ug:
Add 1ml of sterile PBS to obtain a concentration of 500ug/ml, and divide into ten tubes, each with 100μl.
3) IL-33 biotin antibody 50ug:
Add 1ml of sterile PBS to obtain a concentration of 50ug/ml, and divide into ten tubes, each with 100μl.
4) ST2 biotin antibody 50ug:
Add 1 ml of goat anti-human ST2 biotinylated antibody diluent to obtain a concentration of 50 ug/ml, and divide into ten tubes, each with 100 μl.
5) Recombinant human IL-33 standard 10ug:
Add 1ml of IL-33 standard dilution solution to obtain a concentration of 10ug/ml, which is divided into ten tubes, each tube of 100μl.
6) Recombinant human ST2 standard 100ug:
Add 1ml of sterile PBS to obtain a concentration of 100ug/ml, and divide into ten tubes, each with 100μl.

Main instruments and equipment
Quartz Automatic Double Water Distiller
Cryogenic high-speed centrifuge
pH meter
Electronic Analytical Balance
Electric heating constant temperature drying oven
Microplate reader
Kang's oscillator
-80℃ low temperature refrigerator

2.3 Logistic Regression Analysis Technology

Statistical regression analysis is mainly used to analyze dependent variables, such as variable classification (such as disease remission, rating, etc.), and independent variables can be categorical variables or continuous variables. This method can select from multiple independent variables that affect the transition dependence and can give the same prediction. The binary logistic regression analysis method can screen out the influencing factors related to the dependent variable (the response results of the research object only take two values of 0 and 1), and it has been widely used in many fields such as medical analysis. This article uses it to explore risk factors for having a certain chronic disease. The dependent variable Y is a binary variable with a value of 1 or 0, where 1 means suffering from a certain chronic disease, and 0 means not suffering from the chronic disease. Suppose there are k independent variables, denoted as x1,...,xk, then the probability of suffering from the chronic disease is denoted as p, p=p(y=l|xi.X2,...,xk), then the general model is as follows:

$$p = p(y = 1 \mid x_1, x_2, \cdots, x_k) = \frac{\exp(\alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k)}{1 + \exp(\alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k)} \tag{1}$$

After logit transformation, it becomes linear form:

$$\log it(p) = \ln(\frac{p}{1-p}) = \alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k \tag{2}$$

Among them, α is a constant term, and βk is a regression coefficient, indicating that each unit of change in the value of xi can make logit (p) change by βi, and determine the direction of the correlation. The odds ratio is written as OR:

$$OR = \exp(\beta_i) \tag{3}$$

The OR represents a relative risk, and the magnitude of the OR value can determine the strength of the correlation, which can be calculated by statistical software.

3. Study on the Role of IL-33 in Patients with Guillain-Barré Syndrome

3.1 Collection of Samples

(1) Collection of venous blood samples
All the enrolled cases collected 10ml venous blood samples with heparin as anticoagulant at 7:00 (if the samples were chyle blood or hemolysis and re-collected the next day), no patients had fever or other infectious diseases when the blood samples were collected, and the blood samples were collected within 1 hour. The whole blood samples were centrifuged at 1000g/min at 4°C for 10 min

to separate the plasma. The plasma was subpackaged and stored in a -80°C refrigerator until ELISA detection.

(2) Collection of cerebrospinal fluid samples

The cerebrospinal fluid samples were collected from GBS patients within 7 days of admission, 1-14 days after the initial symptoms, and the patients were reexamined in the 2-4 weeks after admission, 15-32 days after the initial symptoms. After centrifugation for 1 min, the samples were stored in a -80°C refrigerator for testing.

Cerebrospinal fluid samples were collected from other patients within 7 days of admission. The collected cerebrospinal fluid was quickly centrifuged at 4°C, 1000g/min for 1min, and then stored in a -80°C refrigerator for testing.

3.2 ELISA Detection of Cytokine Levels

(1) Preparation of reagents

1) Return all reagents to room temperature before use;

2) Preparation of standards: prepare immediately before use. Gently shake the standard for 5min and add an appropriate amount of diluent. The concentrations of the reconstituted standards are: IL-3310000pg/ml, ST28000pg/ml.

3) Double dilution of the standard: prepare seven test tubes, add 900μl of standard diluent to S1-S7, S1, 500μl of standard diluent to S2-S7, add 500μl of standard prepared in 2) to S1, After mixing, take 500 μl from S1 and add to S2, after mixing, take 500 μl from S2 and add to S3, and so on, and finally take 500 μl from S7 and discard. The concentration of IL-33 standard after doubling dilution from S7-S1 is: (78, 156, 312, 625, 1250, 2500, 5000pg/ml) and the concentration of ST2 standard from S7-S1 is: (62, 125, 250, 500, 1000, 2000, 4000pg/ml).

4) Preparation of biotin antibody: prepare immediately before use. In clean, dry glass tubes, IL-33 and ST2 biotin antibodies were diluted in PBS.

5) Preparation of HRP-labeled streptavidin: use dilution buffer to prepare the required amount at a ratio of 1:100, and prepare within 1 hour before use.

3.3 Operation Steps

(1) IL-33, ST2 coating

IL-33

1) Dilute the goat anti-human IL-33 antibody (0.8μg/ml) with PBS, add 0.1 ml to the reaction well of a polystyrene plate, overnight at 4°C.

2) The next day, pour off the solution and rinse with washing solution 3 times, every 3 minutes. One last time, pat dry with paper.

3) Blocking: Add 300 μl/well of IL-33-coated antibody blocking solution, and incubate at room temperature for 1 hour.

4) Wash the board 3 times and let it dry. Can be stored at 4-8°C for two months.

(2) Steps of ELISA to detect IL-33

1) Add sample: adjust the hollow hole, standard hole and sample hole for testing. Add 100 μl of diluent sample to blind wells, and add 100 μl of standard sample or sample sample to other wells, respectively. Be careful not to create air bubbles. When adding the sample, place the sample under the well of the ELISA plate, try not to touch the wall of the well, and shake gently to mix. To ensure the validity of the experimental results, a new standard solution was prepared for each experiment.

2) Remove the water from the wells, dry and wash the plate 3 times with 200μl/well of washing solution.

3) To each reaction well, add 100μl (0.2μg/ml) of biotin-containing antibody activity solution

(prepared within one hour before use), and react at room temperature for 60 minutes.

4) Remove the water from the wells, dry and wash the plate 3 times with 200μl/well of washing solution, soak for 1-2 minutes each time and air dry.

5) To each reaction well, add 200 μl of avidin activity solution with horseradish peroxidase label (shake gently, prepare within one hour before use), and react at room temperature for 20 minutes.

6) Remove the water from the wells, wash the plate 3 times with 200μl/well of washing solution, soak for 1-2 minutes each time and air dry.

7) In each reaction well, add 200 μl of substrate solution (TMB) at the beginning, and let the color develop for 30 minutes at room temperature in the dark (in 30 minutes, the first 3-4 standard wells may appear pure gradient blue, The mood can stop when the color does not appear after 3-4 pores).

8) End of inversion: To each reaction completely, add 0.5MH2SO4 100μl solution solution in the order of adding the substrate solution (the blue immediately turns yellow at this time). In order to ensure the accuracy of the experimental results, the relapse should be terminated as soon as possible after the substrate suspicious period.

9) Result judgment: Within 15 minutes after installing the stent solution, use an enzyme detector with a wavelength of 450nm to configure the system empty control, and measure the optical density (OD value) of each well.

4. Analysis of the Role of IL-33 in Patients with Guillain-Barré Syndrome

4.1 Analysis of the General Data of GBS Patients

(1) Gender

A total of 92 people, 52 males and 40 females, accounted for 56.52% and 43.48% respectively.

(2) Age

Among the 92 patients, the youngest was 1 year and 9 months old, and the oldest was 81 years old, with an average age of 38 years. The specific age distribution is as follows as shown in Table 1:

Table 1: The number and percentage of patients with GBS in each age group

Age (Y)	Number of cases	Percentage of the total (%)
Y≤20	19	20.7
20<Y≤40	32	34.8
40<Y≤60	43	46.7
Y>60	9	9.8

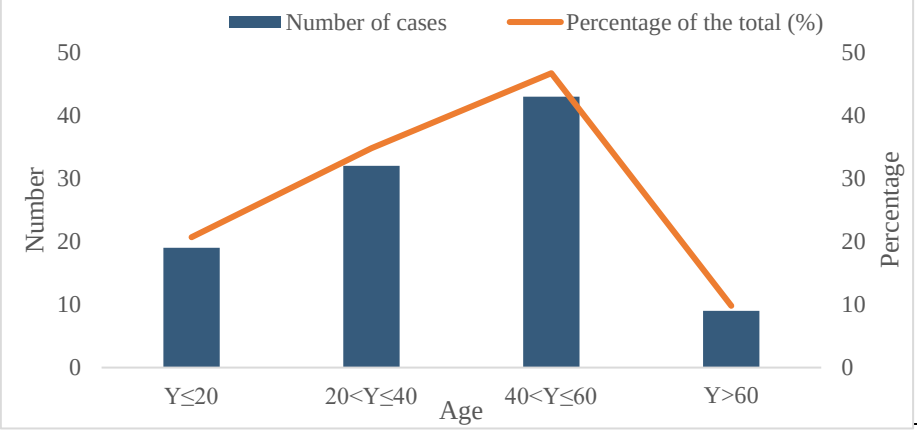

Figure 1: Analysis of the number and proportion of GBS patients in different age groups

From Figure 1, it can be found that most patients with GBS are between 46-60 years old, accounting for 46.7%, followed by 20-40 years old, accounting for 34.8%, and the least is older than 60 years old, accounting for 9.8%.

(3) The correlation between the levels of cytokines IL-33 and ST2 in GBS patients and the age of the patients

Using SPSS software, Spearman correlation coefficient analysis was used, and the P value was two-tailed.

Table 2: Correlation of IL-33 and ST2 levels with age in GBS patients (pg/ml)

Group	T1 (acute phase of onset)		T2 (recovery phase)	
	r	P	r	P
Plasma\IL-33	0.296	0.094	0.185	0.303
Plasma\ST2	-.294	0.097	-.223	0.212
CSF\IL-33	0.132	0.654	0.522	0.055
CSF\ST2	0.270	0.350	-.011	0.969

The results Table 2 showed that there was no correlation between the levels of IL-33 and ST2 and the age of GBS patients, whether in T1 or T2 stage.

(4) The effect of gender on the levels of cytokines IL-33 and ST2 in GBS patients

In order to detect whether the levels of IL-33 and ST2 in the plasma and cerebrospinal fluid of GBS patients in T1 stage are related to the gender of the patients, SPSS software was used for analysis, and the independent sample t test was used for analysis. The P value was two-tailed, and $P<0.05$ was considered statistically significant.

Table 3: Effect of gender of GBS patients on T1-phase IL-33 and ST2 levels (pg/ml)

Group	Male	Female
Plasma\IL-33	291.1±202.5	353.5±192.2
Plasma\ST2	197.8±111.4	183.1±83.68
CSF\IL-33	165.9±110.2	182.2±82.38
CSF\ST2	253.0±51.90	240.3±135.1

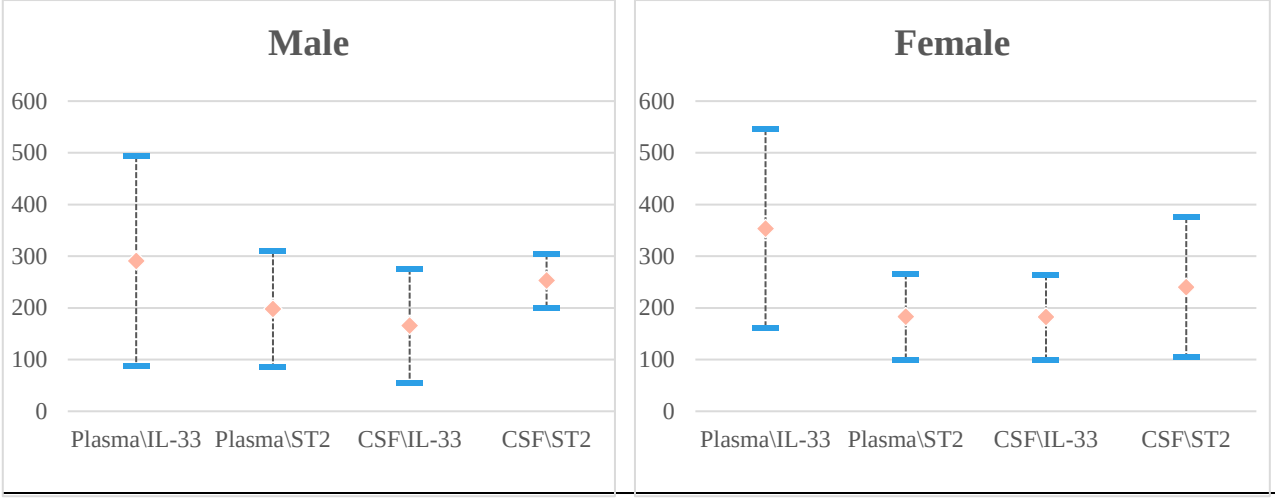

Figure 2: Gender analysis of IL-33 and ST2 levels in T1 phase of GBS patients

The results Table 3 Figure 2 and showed that gender of GBS patients had no effect on the levels of IL-33 and ST2 in plasma and cerebrospinal fluid in T1 stage.

(5) Onset season

Every March is a season, and it is divided into three, four, and five springs in turn. The patient

cases and proportions are shown in the following table 4:

Table 4: The number of patients with GBS in each season and the percentage of the total

Disease season	Number of cases	Percentage of the total (%)
spring	31	33.69
summer	18	19.56
autumn	15	16.3
winter	28	30.43

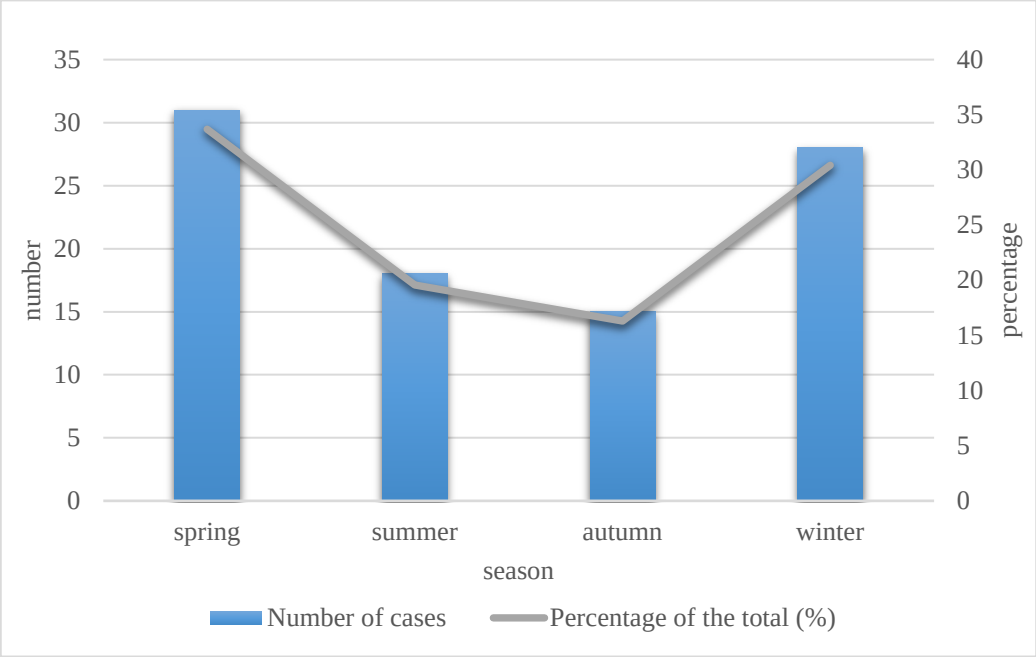

Figure 3: Analysis of the number and proportion of patients with GBS in each season

As can be seen from Figure 3, the incidence of GBS is mostly concentrated in winter and spring, accounting for 30.43% and 33.69%, respectively, followed by summer, accounting for 19.56%, and finally summer, accounting for 16.3%.

4.2 Comparison of IL-33 and ST2 Levels in Patients with Two Subtypes of GBS

In order to compare whether the secretion levels of IL-33 and ST2 in blood and cerebrospinal fluid were different between the two groups of GBS patients at T1 and T2 stages, the levels of IL-33 and ST2 were detected by ELISA and analyzed independently.

(1) Comparison of plasma levels of IL-33 and ST2 in patients with two subtypes in acute phase

Table 5: Comparison of IL-33 and ST2 levels in T1 stage of patients with two subtypes of GBS (pg/ml)

Group	AIDP	AMAN
Plasma\IL-33	306.3±198.5	343.4±205.3
Plasma\ST2	200.9±114.0	174.8±66.10
CSF\IL-33	181.0±81.61	168.4±111.8
CSF\ST2	217.5±62.10	303.7±145.8

The results in Table 5 show that there was no statistical difference in the levels of IL-33 or ST2 in both the AIDP group and the AMAN group in the two subtypes of GBS patients (P>0.05).

(2) Comparison of plasma levels of IL-33 and ST2 in patients with two subtypes during the recovery period

Table 6: Comparison of IL-33 and ST2 levels in T2 stage of patients with two subtypes of GBS (pg/ml)

Group	AIDP	AMAN
Plasma\IL-33	258.8±141.6	347.3±210.8
Plasma\ST2	173.1±92.66	170.4±119.9
CSF\IL-33	183.2±99.07	127.1±50.31
CSF\ST2	143.5±46.33	235.7±35.93**
**P<0.01 compared to AIDP.		

The results in Table 6 show that in terms of T2 level in GBS patients, compared with AIDP group, ST2 level in cerebrospinal fluid of AMAN patients was higher, and the difference was statistically significant (P<0.01). There was no significant difference in the levels of IL-33, ST2 and IL-33 (P>0.05).

5. Conclusions

In this study, in order to reveal whether IL-33 is involved in the pathogenesis of autoimmune disease GBS, ELISA was used to compare the levels of IL-33 and ST2 in plasma and cerebrospinal fluid of GBS patients with ONIDs, VE/VM and healthy individuals. differences in somatic expression to demonstrate the role of IL-33 and ST2 receptors in GBS pathogenesis and progression. Within two weeks of the onset of GBS, the protein content in the cerebrospinal fluid is significantly increased, which is a typical feature of GBS. Clinically, this feature is regarded as one of the most important indicators for the diagnosis of GBS. As an important means to detect and study peripheral nervous system diseases, immunological experimental methods are widely used in clinical work. ELISA is a vaccine based on the specific reaction of antigens and enzymes, combined with the efficient catalysis of enzymes in the substrate. Because of its high efficiency, accuracy and specificity, it is one of the most widely used immunological detection methods.

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