Relationship between Ag-NORs and T Lymphocyte Subsets in Children with Epilepsy

Hongping Zhong
Xiaoning Cheng
Peiqiang Yan

Hongping Zhong Department of Pediatrics, Yan'an University Affiliated Hospital, Yan'an 716000, Shaanxi, China, Xiaoning Cheng Department of Pediatrics, the Second Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine, Xianyang 712000, Shaanxi, China, Peiqiang Yan Department of Pediatrics, Hongze District People's Hospital, Huai'an 223000, Jiangsu, China, *Corresponding author: Peiqiang Yan (E-mail: Yanpeiqiang2000@163.com)*

With the acceleration of the pace of life, the number of children with epilepsy is increasing. This study was to investigate the relationship between Ag-NORs and T lymphocyte subsets in children with epilepsy. In the morning, 3ml of elbow vein blood was collected from epileptic children on an empty stomach. After standing at room temperature for 0.3-1 hour, the serum was centrifuged (2000 rpm, 4 minutes). 3 ml of mixed venous blood was collected in EDTA-K2 vacuum container. The sample was taken out within 5 hours after staining. 30 μ l of Itcite reagent and 30 μ l of anticoagulant were put into TruCount tube and mixed evenly. Then flow cytometry was used for detection, and facscomp software was used for automatic inspection and calibration. In the same way, the automatic analysis software muitiset was used to obtain 20000 white blood cells, count 8000-10000 lymphocytes automatically, and calculate CD4 + T lymphocytes, CD8 + T lymphocytes and CD4 + / CD8 + ratio. Ag-NORs detection: under aseptic conditions, 0.5ml anticoagulant blood was added into the medium flask containing rpm-1640 and incubated in the incubator at 37 °C for 72 hours. After mixing the cell suspension at room temperature, put it into a 10ml glass tube, heat it and dry it. Wait until the temperature of the water solution tank rises to 80-90 °C, and then put aluminum plate on it. The staining was placed under the microscope on the stage, and the image was adjusted to make the image analysis program effective. The ratio of nuclear area to nuclear silver staining area of 30 lymphocytes was counted. This ratio reflects the content of Ag-NORs in the nucleolar forming region of T lymphocytes. The ratio of Ag-NORs area to nuclear area was 0.32 ± 0.03, 0.38 ± 0.03 and 0.46 ± 0.03, respectively. This study is helpful to provide guidance for the treatment of epilepsy in children.

Keywords: Pediatric Epilepsy, T Lymphocyte Subsets, Ag-NORs Area, Nuclear Silver Staining Area
Tob Regul Sci.™ 2021;7(5-1): 3410-3417
DOI: doi.org/10.18001/TRS.7.5.1.117

With the progress of society and economic development, the level of medical care has been greatly improved, and the overall health of children in our country has also been greatly improved, but compared with developed countries, there is still a big gap. Epilepsy has a serious adverse effect on the growth and development of children. Moreover, the current misunderstanding of epilepsy and discrimination against patients with epilepsy in society can easily cause huge psychological pressure on sensitive children and even lead to serious psychological disorders. This is also one of the increasingly prominent problems in the treatment of pediatric epilepsy in recent years. Although due to the rapid development of medicine in modern times, the level of diagnosis and treatment of Chinese and Western medicine has been continuously improved, and most children can gradually recover, but there are still many complex problems that have not been resolved, and some refractory epilepsy seriously affect the physical and mental health of children. The family and society bring heavy burdens.

In children with epilepsy, the body's immune system will change. Under normal circumstances, the subtle balance between T lymphocyte subsets is a central link in maintaining the stability of the internal environment of the immune system. The body maintains a normal immune function state, which depends on the cooperation or mutual restriction between various immune cells. When the

internal environment changes, the content of Ag-NORs secreted by T lymphocytes will change significantly.

Patients with epilepsy have their own disorders of autoimmune function. Elliott J found that drug-resistant epilepsy has a negative impact on the quality of life and is related to the increase in morbidity and mortality and the high cost of the medical system. He looked for a cost-effectiveness analysis of the use of cannabis products to treat children with drug-resistant epilepsy to inform public health care payers about reimbursement decisions for such products. He will also look for cost-effectiveness analysis of other drug treatments for children with drug-resistant epilepsy, as well as estimates of medical resource usage, cost and utility, in order to solve this decision problem in the subsequent cost-effectiveness analysis. He will search the published grey literature for economic evaluation of cannabis-based products and other drugs for the treatment of childhood drug-resistant epilepsy, as well as research on resource utilization and effectiveness. He uses Drummond and CHEERS checklists for assessment. Although his research has a certain role in promoting the development of drug-resistant epilepsy, the research content is not comprehensive [1]. Suryaningtyas W introduced the development of the newly established Children's Epilepsy Surgery Center in Surabaya, Indonesia, its limitations and obstacles, lessons learned from early experience, and how to deal with these difficulties. He reviewed the medical records of all cases of epilepsy surgery performed at Sutongmo General Hospital in Surabaya from 2009 to 2016. Consult with the surgical team and family members before surgery. Between 2009 and 2016, he performed 19 epilepsy surgeries on children between 3 months and 16 years old. The actual candidates for surgery were 40 patients, but 21 patients refused to undergo surgery for several reasons. The most common reasons for surgery failure included 7 patients worried about unbearable complications, 5 patients out of economic considerations, and 9 patients had reservations about their interests. Although his research explained the experimental data, some of the content description was inaccurate [2]. Sacino M believes that Magnetic Resonance Guided Laser Interstitial Hyperthermia (MRgLITT) is a new technology that provides an effective minimally invasive treatment for the clinic. However, compared with traditional open surgery, there are few data on the cost. This article aims to explore the cost-effectiveness of MRgLITT in the treatment of epilepsy in children. He retrospectively analyzed the medical records of pediatric patients who received MRgLITT through the Visualase hyperthermia system from December 2013 to September 2017. He uses sensitivity analysis of 4 variables to evaluate the validity of the results. Although his sensitivity analysis of cost variables is robust, he lacks a specific description of the methods used [3]. Saengow V E believes that poor drug compliance can lead to poor seizure control in children with epilepsy. EAS includes a 20-minute educational program led by a pediatric neurologist, a pediatrician, and a pharmacist, using simple tools such as epilepsy diaries and epilepsy cards. He uses Morishy's Medication Compliance Scale to assess medication compliance. The comparison of medication compliance occurred before the subjects received the education program and during the 3-month and 6-month follow-up. A total of 108 patients were enrolled. Among them, the average age of the participants was 7 years and 6 months. Male accounted for 59.3%. Although EAS can improve the drug compliance of children with epilepsy, his research lacks innovation [4].

In this study, the Ag-NORs of T lymphocytes in the peripheral blood of children with epilepsy were detected, in order to discover the possible pathogenesis of pediatric epilepsy and the relationship between Ag-NORs in T lymphocytes and the clinical characteristics and biological behaviors of pediatric epilepsy, and explore its Significance in tumor diagnosis, differential diagnosis and disease monitoring. By comparing and analyzing whether the content of Ag-NORs in the nucleolar forming area of T lymphocytes is different between normal people and pediatric epilepsy patients, it is hoped that this system will provide scientific information for the auxiliary diagnosis and prognosis evaluation of pediatric epilepsy patients. in accordance with.

Ag-NORs IN PEDIATRIC EPILEPSY

Epilepsy

Epilepsy is a neurological disease characterized by abnormal discharge of brain cells and repeated attacks, which can lead to various brain dysfunctions

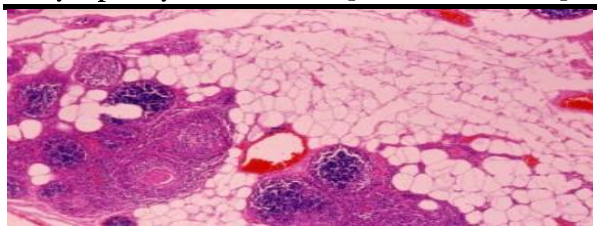
and abnormal behaviors. Benign childhood epilepsy with centrotemporal spikes (BECTS), also known as Rolandic epilepsy, but 20%-70% of patients have seizures, 8-10 years old is the peak period of onset. According to the 2017 International Anti-Epilepsy League classification, BECTS is classified as self-limited focal epilepsy, which is called self-limited epilepsy with central temporal spikes [5].

Although BECTS can be relieved spontaneously after puberty, and the prognosis is good, in recent years, a number of clinical studies and neuroimaging studies have found that children with BECTS have abnormal brain structure and function and are often accompanied by cognitive function problems. It affects patients to varying degrees. Children's studies and future career development. Due to its "benign, self-limiting" characteristics, there are controversies about whether it is treated or not and the choice of treatment methods. Understanding its pathogenesis, accurately locating the epileptogenic focus and its impact on cognitive function will help the diagnosis, evaluation, treatment of BECTS and the improvement of the quality of life of children [6-7]. The epileptic zone of epilepsy refers to the minimum resection of the cortex to achieve postoperative epilepsy control. The preoperative diagnosis requires a comprehensive evaluation of clinical seizure symptoms, neuroelectrophysiological examination and imaging examination results, so as to achieve postoperative epilepsy control. The prognosis of epilepsy surgery is closely related to the extent of the epileptic zone evaluated before surgery, especially in patients with MR-negative epilepsy. The pathological types of epileptic areas include cortical dysplasia, embryonic or congenital craniocerebral malformations, temporal lobe hippocampal lesions, neuroepithelial tumors, vascular malformations, infections, inflammations and glial scars, etc. The imaging findings are not the same, and even some minor changes in the cortex have negative results from conventional MR [8-9].

Ag-NORs

T lymphocytes are the main effector cells of cellular immunity. Many studies have confirmed that the immune function of T cells is low after the onset of stroke patients through flow cytometry, enzyme labeling and other methods. Nucleolar organizer regions associated proteins (nucleolar organizer regions associated proteins, Ag- NORs, also known as nucleolar forming region argyrophilin) is a protein that regulates rRNA transcription, which is essential for ribosome formation and intracellular protein synthesis, and can reflect the functional status of cells [10]. Therefore, detecting the Ag-NORs content of T lymphocytes in peripheral blood and understanding the proliferation activity of T lymphocytes is one of the reliable methods to reflect the status of the body's cellular immune function after stroke. In addition, the expression activity of Ag-NORs of T lymphocytes in children with Henoch-Schonlein purpura is significantly reduced, which affects the proliferation of T lymphocytes and weakens immune activity; the lymphocyte glucocorticoid receptor GCR may be reduced by expression. In addition, T lymph in children with epilepsy the expression activity of Ag-NORs of cells is significantly reduced, which affects the proliferation of T lymphocytes and weakens the immune activity; the lymphocyte glucocorticoid receptor GCR may reduce the endogenous glucocorticoid GC on the function of TH cells and other inflammatory mediators through the decreased expression. The inhibition of cells is involved in the pathogenesis of HSP [11-12].

Figure 1.
T lymphocyte subsets (http://alturl.com/kz9op)



T Lymphocytes

T lymphocytes are abbreviated as T cells, lymphoid stem cells derived from bone marrow, which develop and differentiate in the thymus. T cells all express CD3 molecules. T cells obtain diverse TCR expression in the thymus, and after positive and negative selection, they differentiate into CD4+T cells and CD8+T cells, namely helper T lymphocytes (Th) and cytotoxic T lymphocytes (CTL). Mature T cells only express CD4 or CD8+ molecules [13]. The initial CD4+ T cells (ThO) that are not stimulated by the antigen are regulated by the nature of the antigen and cytokines to differentiate into different lineages, such as Th1,

Th2, Th3, Th17, etc., and then participate in the anti-infection (especially anti-cell Infection of internal pathogens), delayed-type hypersensitivity, and auxiliary B cell activation [14]. CD8+T cells play their main functions by secreting granzyme, lymphotoxin, perforin and other substances to kill target cells, or by inducing apoptosis of target cells through Fas/FasL pathway, such as intracellular parasitic pathogen-infected cells or tumor cells. CD8+T cells are not damaged in the process of killing target cells and can kill multiple target cells continuously. CD8+ T cells participate in the immune rejection after organ transplantation [15-16]. The T lymphocyte subsets are shown in Figure 1.

T lymphocytes are abbreviated as T cells, which are lymphoid stem cells derived from bone marrow, which develop and differentiate in the thymus. After T cells undergo positive and negative selection in the thymus, they differentiate into CD4T cells and CD8*T cells, namely helper T lymphocytes (Th) and cytotoxic T lymphocytes (CTL) [17-18]. The initial CD4+ T cells (ThO) that are not stimulated by the antigen are regulated by the nature of the antigen and cytokines to differentiate into different lineages, such as Th1, Th2, Th3, Th17, etc., and then participate in anti-infection and delayed-type hypersensitivity Response, helper B cell activation, etc.; the main function of CTL is to kill target cells by secreting granzyme, lymphotoxin, perforin and other substances, or induce target cell apoptosis through the Fas/FasL pathway, such as cells infected by intracellular parasitic pathogens or Tumor cells [19-20].

PEDIATRIC EPILEPSY EXPERIMENT

Research Objects and Main Reagents

Research object

A collection of children with BECTS diagnosed in our hospital from April 2019 to December 2019 who did not use anti-epileptic drugs. All children were treated by pediatric physicians and above with a reference to the 2010 International League Against Epilepsy (ILAE). Epilepsy classification standards were developed for diagnosis, and healthy volunteers were recruited at the same time. This study was reviewed and approved by the Medical Ethics Committee of our hospital, and the subjects and guardians signed an informed consent form.

Intelligence test: On the day of MRI examination, children with BECTS were tested by a specialist using the Chinese Webster Children's Intelligence Scale (C-WISC) (aged 6-16 years).

Main reagents:

(1) Culture medium: 25% calf serum 100ml, penicillin and tetracycline 0.5% each.
(2) 1640 culture medium 10.4g.
(3) Plant cell agglutinin (PSA) 0.4mg/ml, PH 7.2.
(4) Silver stain solution: A solution is 50% AgNO3 aqueous solution, and B solution is 3% formic acid-2% gelatin.
(5) Fixing solution: methanol/glacial acetic acid=3/1.
(6) Hypotonic liquid: 0.07 mol/l NaCl.
(7) Stop solution: colchicine 12.5μg/ml.

Specimen Collection

The IL-10 of children with epilepsy was collected within 72 hours of onset, and blood samples were collected on days 6-8 and days 14-15. Ag-NORS samples were collected within 72 hours and 14-15 days after the onset of disease. Blood samples were collected once in a healthy control group. In the morning on an empty stomach, 3ml of cubital venous blood was drawn. After standing at room temperature for 0.3-1 hour, the serum was separated by centrifugation (2000 rpm, 4 minutes). After separation, the serum was immediately stored in a refrigerator at -40°C. Take 0.3-0.5ml and inject it into Ag-NORs culture medium under aseptic operation and shake it evenly. After incubating at 37.5°C for 72 hours, detect the level of Ag-NORs.

Detection of Ag-NORs and T Lymphocyte Subsets

Detection of T lymphocyte subsets by flow cytometry

Collect 3ml of mixed venous blood in EDTA-K2 vacuum container, take out the sample within 5h after staining treatment, put 30μL each of LTCITE reagent and anticoagulant in TruCOUNT tube, mix well, and react for 30 minutes at room temperature. After a few minutes, add 450μL of red blood cell lysate, mix well for 15 minutes at room temperature, and then perform flow cytometry detection, FACScomp software automatically checks the calibration. Then use the MuitiSet automatic analysis software in the same way to obtain 20,000 white blood cells, and automatically count 8,000-10,000 lymphocytes, and calculate the CD4+ T

lymphocytes, CD8+ T lymphocytes, and the ratio of CD4+/CD8+.

AgNORs detection

1) Cultivation: Under aseptic conditions, add 0.5ml of anticoagulant blood to a culture flask containing RPM-1640, and incubate for 72 hours in an incubator at 37°C.
2) Preparation: After mixing the cultured cell suspension at room temperature, put it into a 10ml glass test tube, separate and remove the supernatant, and then add 5ml of low permeability solution and mix well. Add 0.5ml of fixative, mix at 1000RPM/separation center for 10 minutes, remove the supernatant, add 5ml of fixative to mix, discard the supernatant after centrifugation, and repeat the process. Add 0.3ml of fixing solution to the precipitate, mix well, drip 3-5 drops on the pre-cooled glass plate, and dry after heating.
3) Silver dyeing: Wait until the temperature of the aqueous solution tank rises to 80-90°C, and then put an aluminum plate on it. After heating the aluminum plate, drop a drop of cells on the sliding glass, and add the pigment solutions A4 and B2.
4) Image analysis: Place the staining under the microscope on the stage, adjust the image to make the image analysis program effective, and count the ratio of the nuclear area of 30 lymphocytes to the nuclear silver staining area. This ratio reflects the content of AgNors in the nucleolar formation area of T lymphocytes.

Statistical Processing

The final statistical processing of the data is determined by SPSS21.0 software. The chi-square distribution is used to compare component data, and the variance test is used to compare count data. The rank total test is used for normally distributed count data. The statistical validity of this study was set as $P<0.05$, and the statistical reasoning level of 5% was evaluated as meaningful.

RESULTS AND DISCUSSION

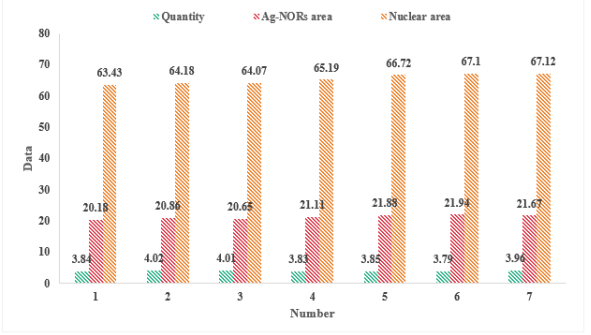
Table 1 shows the comparison of the levels of peripheral blood lymphocyte subsets between the experimental group and the control group. The percentage of CD3 cells in the peripheral blood of the experimental group was not significantly different from that of the control group, P=0.759 (P>0.05), the percentage of CD4+ cells, and the ratio of CD4+/CD8+ were significantly higher than those in the 20 control group. The P values were: P=0, P =0.005 (P<0.01). The percentage of CD8+ cells was significantly lower than that of the control group, P=0.001 (P<0.01).

Table 1.
Comparison of the levels of peripheral blood lymphocyte subsets between the experimental group and the control group

Group	Number of cases	$CD3^+$(%)	$CD4^+$(%)	$CD8^+$(%)	$CD4^+/CD8^+$
Test group	48	69.25±7.71	39.06±7.14	25.13±6.52	1.72±0.78
Control group	20	68.70±3.11	30.50±3.50	29.60±3.63	1.09±0.20
P	\	0.76	0.00	0.001	0.005

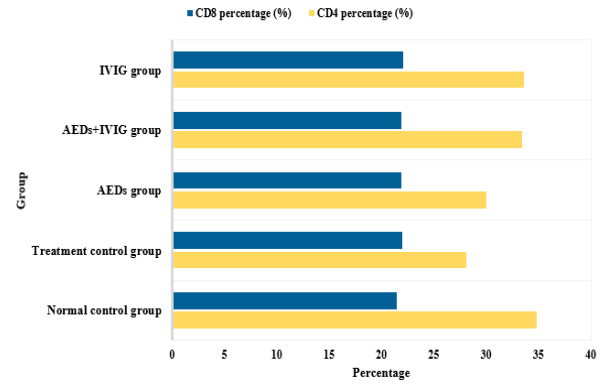
The characteristics of Ag-NORs in pediatric epilepsy are shown in Figure 2. The numbers of pediatric epilepsy were 3.97±0.65, 5.66±0.68, 6.67±0.90; the ratio of Ag-NORs area to nuclear area was 0.32±0.03, 0.38±0.03, 0.46±0.03; in terms of morphological factors they are 0.40±0.01, 0.31±0.03, 0.23±0.02, respectively. After statistical processing, the difference between different levels of pediatric epilepsy is very significant (P≤0.01). In terms of granular types, grade I, II, and III pediatric epilepsy are mainly intranucleolar type, intranucleolar type and aggregation type, and aggregation type respectively. This feature is obviously different from the diffuse and mixed types of lung adenocarcinoma. These parameters also fully indicate that as the malignant degree of epilepsy in children increases, the various characteristics of Ag-NORs also change accordingly, and vice versa.

Figure 2.
Ag-NORs characteristics of epilepsy in children



The essence of cell division and proliferation is DNA replication. However, tumor cells have the ability to proliferate indefinitely due to obstacles in the regulation and growth control system. To maintain this ability, a large amount of nucleic acid needs to be synthesized. DNA content determination can directly reflect the changes in the level of nucleic acid metabolism in the cell. Therefore, DNA image analysis can directly and objectively reflect the proliferation status of tumor cells. The average DNA content of the nucleus of various organs and tissues of normal people is a relatively constant diploid value. The comparison of the percentages of CD4 and CD8 in peripheral blood lymphocytes of children in each group is shown in Figure 3. The CD4 expression in the

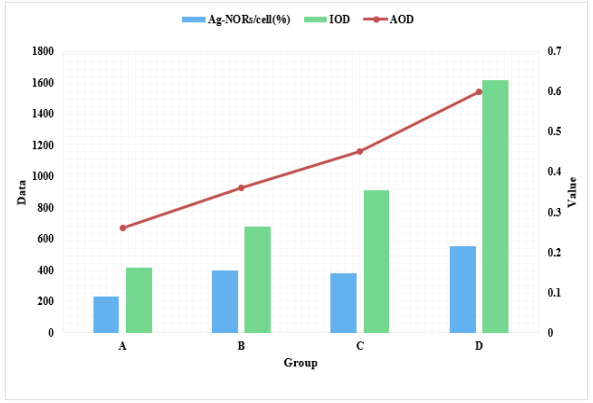
Figure 3.
Comparison of the percentages of CD4 and CD8 in peripheral blood lymphocytes of children in each group



treatment control group and the AEDs group was lower than that in the normal control group, and the CD4/CD8 ratio decreased, P<0.05, the difference was statistically significant; the comparison of the CD4 value between the treatment control group and the normal control group was t=-3.021, P<0.05, CD4/CD8 ratio comparison t-2.213, P<0.05, AEDs group and normal control group CD4 comparison t=2.462, P<0.05, CD4/CD8 ratio comparison t=2.957, P<0.05. The IVIG group and the AEDs+IVIG group can up-regulate the expression of CD4, and the CD4/CD8 ratio increases, and the difference is statistically significant; the CD4 comparison between the AEDs+IVIG group and the treatment control group is t=2.78, P<0.05, the CD4/CD8 ratio comparison t =3.01, P<0.05; Comparison of CD4 between IVIG group and treatment control group t=2.431, P<0.05, comparison of CD4/CD8 ratio t=-2.852, P<0.05.

The count of Ag-NORs, the quantitative detection of AOD and IOD in each group are shown

Figure 4.
Quantitative detection of Ag-NORs count, AOD and IOD in each group



in Figure 4. The results showed that the Ag-NORs count, AOD and IOD were not statistically different between the MF group and the CD30+CTCL group, but there were significant differences among the other groups. It can be seen in the HE tissue slice: the lymphocyte infiltration in the normal group is more diffuse, the cell morphology is regular, and it is round or similar. In some CTCL specimens, it can be seen that tumor cells have no infiltration in the epidermis, but only in the dermis, and some CTCL's typical epidermal phenomenon can be seen, that is, tumor cells invade the epidermal cells, the tumor cells have obvious atypia, and the nuclear morphology is irregular and deep dye. Anti-CD3, CD45RO, LCA, CD68, CD30, and CD20 are positively located on the cell membrane. After DAB is developed, the brown-yellow substance is arranged in a circle or a round shape to surround the nucleus. The hematoxylin counter-stained cell nucleus is blue, and the negative control is the cell nucleus. No brown material deposits around.

CONCLUSION

The number, size and intensity of NORs are clearly shown in the transport activity of ribosomal RNA. This reflects the correlation between transcription activity and protein synthesis status and cell proliferation activity. Therefore, the immune activity of T lymphocytes can indirectly

respond to the immune function of the whole body through the expression of Ag-NORs in the nucleolar formation region of T lymphocytes. The KL type immune analysis system uses real-time image collection, display, and image analysis technology to quantitatively analyze the research, instead of traditional camera photography and artificial microscope visual counting methods, to obtain real-time immunity in the T lymphocyte nucleolus formation area functional transcriptional activity changes. The theory realizes all the intelligence, making the test results more accurate, objective and convenient. The results of this study show that the I.S% value of children with epilepsy is significantly lower than that of normal people, and the detection of Ag-NORs is very important for determining children with epilepsy and other diseases. It is also of universal significance for the diagnosis and evaluation of the prognosis of tumor patients and tuberculosis patients. And the use of the KL image analysis system is easily accepted by patients due to the small amount of blood collected. At the same time, because of its fast, stable, accurate, and unmanned experiment, the results are intuitive and superior to other currently used immunoassay methods. Based on the above advantages Ag-NORs can be a meaningful method to detect the immune function of T lymphocytes in children with epilepsy.

REFERENCES

1. Elliott, J., Mccoy, B., & Clifford, T. (2019) "Cost-effectiveness of Cannabinoids for Pediatric Drug-resistant Epilepsy: Protocol for A Systematic Review of Economic Evaluations", Systematic Reviews, 8(1), pp.1-5.
2. Suryaningtyas, W., Gunawan, P. I., & Subianto, H. (2020) "Lesson learned from Early Experience in Pediatric epilepsy Surgery Service in Surabaya, Indonesia", Neurology Asia, 25(1), pp.89-90.
3. Sacino, M., Huang, S., & Alexander, H. (2020) "An Initial Cost-Effectiveness Analysis of Magnetic Resonance-Guided Laser Interstitial Thermal Therapy in Pediatric Epilepsy Surgery", Pediatric Neurosurgery, 55(3), pp.1-8.
4. Saengow, V. E., Pavarojkit, P., &Honsing, S. (2019) "Educational Program to Improve Drug Adherence in Pediatric Epilepsy Patients and Caregivers in Limited Resource Area", Journal of Pediatric Epilepsy, 08(02), pp.044-048.
5. Liu, ., Xing, X. Y., & He, D. C. (2020) "Effect of Moxibustion on Clinical Symptoms, Peripheral Inflammatory Indexes and T Lymphocyte Subsets in COVID-19 Patients", Zhongguo Zhen Jiu = Chinese Acupuncture & Moxibustion, 40(12), pp.1271-1275.
6. El-Mazary, A. (2020) "The Effect of Intensive Phototherapy Treatment on CD4 and CD8 T-Lymphocyte Subsets in Neonatal Jaundice", Annals of Neonatology Journal, 2(1), pp.27-36.
7. Yao, Y. H., Zhao, W, F., & Gan, J, H. (2020) "Clinical Characteristics and Distribution of Peripheral T Lymphocyte Subsets in Patients with Schistosomal Hepatic Diseases in Suzhou City", Chinese Journal of Schistosomiasis Control, 32(2), pp.154-158.
8. Baltadzhiev, I., & Pavlov, P. (2020) "Assessment of the Percentages of T-lymphocyte Subsets in the Peripheral Blood of Mediterranean Spotted Fever Patients", Trakia Journal of Sciences, 18(2), pp.111-119.
9. Zhou, Y., Jiang, D., & Chu, X. (2021) "Prognostic Value of Peripheral Blood T Lymphocyte Subsets in Clear Cell Renal Cell Carcinoma", Translational Andrology and Urology, 10(1), pp.326-335.
10. Geng, T. R., Han, Y., & Qiu, Z. F. (2020) "Characteristics and Prognostic Value of Peripheral Blood T Lymphocyte Subsets in Patients with Severe Influenza", Zhonghua Nei Ke Za Zhi [Chinese Journal of Internal Medicine], 59(3), pp.200-206.
11. Ding, J., Yan, G. U., & Zhao, S, S. (2019) "Correlation of Peripheral Blood T Lymphocyte Subsets with Prognosis of Elderly Patients with Newly Diagnosed Multiple Myeloma", Zhongguo Shi Yan Xue Ye Xue Za Zhi / Zhongguo Bing Li Sheng Li Xue Hui = Journal of Experimental Hematology / Chinese Association of Pathophysiology, 27(6), pp.1876-1880.
12. Ma, Y., Yang, Y., & Yu, H. (2019) "Expressions of T Lymphocyte Subsets in the Peripheral Blood of Patients with Precancerous Lesions and Gastric Cancer", Acta Medica Mediterranea, 35(1), pp.165-168.
13. Chereshnev, V. A., Saidakova, E. V., & Korolevskaya, L. B. (2019) "Regulatory T-lymphocyte Subsets in Patients with HIV-infection Receiving Highly Active Antiretroviral Therapy", Bulletin of Siberian Medicine, 18(1), pp.247-256.
14. Xiao, T. X., Zhang, G. N., & Qiu, Z, F. (2019) "The Analysis of Peripheral Blood T Lymphocyte Subsets in Patients with Colorectal Cancer: a Single Center Cross-section Study", Zhonghua Nei Ke Za Zhi Chinese Journal of Internal Medicine, 58(6), pp.453-455.
15. Wha, B., Min, L. A., & Jw, C. (2019) "Effects of Dietary Lipid Sources on Growth Performance, Nutrient Digestibility, Blood T Lymphocyte Subsets, and Cardiac Antioxidant Status of Broilers - Science Direct", Animal Nutrition, 5(1), pp.68-73.
16. Marashly, A., Koop, J., & Loman, M. (2020) "Multiple Hippocampal Transections for Refractory Pediatric Mesial Temporal Lobe Epilepsy: Seizure and Neuropsychological Outcomes", Journal of Neurosurgery Pediatrics, 26(4), pp.1-10.
17. Alexander, H., Cobourn, K., & Fayed, I. (2020) "Magnetic Resonance-Guided Laser Interstitial Thermal Therapy for the Treatment of Nonlesional Insular Epilepsy in Pediatric Patients: Technical Considerations", Pediatric Neurosurgery, 55(3), pp.1-8.
18. Gulati, S., Shruthi, N. M., & Panda, P. K. (2020) "Telephone-based Follow-up of Children with Epilepsy: Comparison of Accuracy between A Specialty Nurse and A Pediatric Neurology Fellow", Seizure, 83(12), pp.98-103.
19. Rakhmanina, O. A., Levitina, E. V., & Khramova, E. B. (2020) "Comparative Analysis of Zonisamide and Topiramate Use in Treatment of Pediatric Epilepsy in

Real Clinical Practice: the Results of A Retrospective Study and Related Clinical Cases", Russian Journal of Child Neurology, 14(4), pp.17-25.

20. Lesko, R., Benova, B., & Jezdik, P. (2020) "The Clinical Utility of Intraoperative Electrocorticography in Pediatric Epilepsy Surgical Strategy and Planning", Journal of Neurosurgery Pediatrics, 26(5), pp.1-10.