# EXPERIMENTAL METHODS TO CLINICAL PRACTICE

# Change in the Titers of Serum IgG-Class Autoantibodies to Some Nervous Tissue Proteins in Patients with Life-Threatening Arrhythmias

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 6, pp. 677-679, June, 1998
Original article submitted January 18, 1997

Titers of autoantibodies to nerve growth factor, water-soluble astrocytic protein S-100, glial fibrillary acidic protein, and MP-65 membrane protein of the nervous cell superfamily of integrins was determined by solid-phase immunoenzyme assay in healthy children and in children with the Romano-Ward and sick sinus syndromes. Immunoreactivity to these antigens was either increased or decreased in 20% of sick children in comparison with that of healthy children of the same age.

**Key Words:** *arrhythmias; autoantibodies; nerve growth factor; neurospecific proteins*

Disturbances in neurogenic regulations of cardiac rhythm play a key role in pathophysiology of life-threatening arrhythmias in children: the Romano-Ward syndrome (prolongation of the *QT* interval) and in the sick sinus syndrome [9]. These disturbances may be related to dysfunction and partial underdevelopment of cerebral and peripheral structures responsible for the rhythm control [3]. Moderation of functional activity in the rhythm-controlling structures could result from disturbances in the development of these subdivisions during the early ontogenesis. The latter may be caused by alternations in the system of neurotrophic factors (such as nerve growth factor, NGF [14]), and of other proteins, which were recently shown to play an important role in the embryo- and fetogenesis. These proteins are not the classical neurotrophic factors, including, specifically, a water soluble astrocytic protein S-100, a glial fibrillary acidic protein (GFAP), and nervous cell membrane protein MP-65 of integrin superfamily [1,11].

The blood of healthy subjects contains natural (physiological) antibodies to these proteins, which can also bind a number of biologically active proteins [7,10]. This property motivated us to study the titres of the respective autoantibodies in the serum of children suffering from the abovementioned diseases.

## MATERIALS AND METHODS

This work compares the titers of relative immunoreactivity in the serum of psychical, somatic, and neurological healthy children at the age of 3-14 years ($n$=80) and in the serum of 39 children of the same age suffering from the life-threatening arrhythmias: 25 patients with sick sinus syndrome and 14 patients with the Romano-Ward syndrome.

Blood samples for analysis (50-100 μl) were drawn from the finger in the course of prophylactic medical examination of healthy children and during clinical or laboratory examination of patients treated

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0007-4888/98/0006-0601$20.00 ©1999 Kluwer Academic/Plenum Publishers

at the Center for Arrhythmia Diagnosis and Treatment.

The Romano-Ward syndrome was diagnosed on the basis of sustained prolongation of the *QT* interval in the ECG (to more than 440 msec), loss of consciousness, or presyncopal states in the anamnesis, as well as according to precedents of this disease in the family [9]. The sick sinus syndrome was diagnosed on the basis of persistent ECG transformations in the form of sinus bradycardia of 50 or less beats per minute, pauses longer than 2 sec as revealed by Holter monitoring of the sino-atrial block, nodal escape, and the subnodal subordinate rhythms against the background depression of the sinus rhythm [8].

The program of clinical instrumental examination included anamnestic and genealogical analyses, general examination in pediatric clinic, 12 standard leads of ECG with the help of a Mingofon-7 device in clino- and ortho- positions. Echocardiography was performed in the 2-dimension mode with an SSD-720 Aloka apparatus. Holter monitoring was carried out with a Memoport-C Hellige apparatus. The disturbances of rhythm and conductance were analyzed, and the heart beat pulsograms and histograms were plotted [5]. We also performed complex evaluation of the autonomic nervous system [4].

Serum immunoreactivity (SIR) was assessed by solid phase immunoenzyme assay [2]. The following antigens were determined: S-100, GFAP, MP-65, and NGF. Isolation and properties of these proteins were described elsewhere [1].

Serum immunoreactivity in healthy children and in patients was assessed relatively to the standard serum assumed to have a 100% SIR.

## RESULTS

Autoantibodies (IgG) against NGF, S-100, GFAP, and MP-65 antigens were detected in the blood of healthy children and of children with life-threatening arrhythmias.

Statistical analysis of serum autoantibody titers in healthy children using the $\chi^2$ [6] test showed that their distribution is normal ($p<0.05$). The parameters of the distributions are given in Table 1.

In order to carry out a comparative study of SIR in healthy and sick children, the normal value of reaction was determined, i.e., the interval of SIR values in the control group limited by $M\pm2\sigma$ boundaries (Table 1). In the following, sera with SIR lower than $M-2\sigma$ were termed as hypoactive (against particular antigen), while those with SIR higher than $M+2\sigma$ was considered as hyperactive. Table 2 shows the percent of healthy subjects with SIR higher or lower than the normal boundaries: no more than 5% of the total number. The number of hypo- and hyperactive sera being roughly equal.

The SIR data in the group of children with cardiac arrhythmias demonstrated pronounced (by 4-9 times for different antigens) increase in the number of patients with SIR lower or higher than the limits of normal reaction (Table 2). This feature was most expressed for membrane antigen MP-65.

SIR for MP-65 and FGAP were significantly different from the control in children with life-threatening arrhythmias ($p<0.05$). In these children, the hyperactive shift was more pronounced than the hypoactive one. Two other tested antigens, NGF and S-100, demonstrated virtually identical increase in the proportion of hyper- and hypoactive sera.

Our findings testify to bilateral changes in the titers of IgG autoantibodies against all examined antigens of nervous tissue in children with life-threatening arrhythmias. A significant increment in the titers of autoantibodies against GFAP and MP-65 was revealed. Based on these findings, one can suppose the involvement of autoimmune reactions in pathological processes in life-threatening arrhythmias.

Unfortunately, the role of neurospecific peptides in the development and function of nervous system remains unclear, which precludes comprehensive interpretation of our data.

Our study revealed changes in SIR against NGF in patients with cardiac rhythm disturbances, which was manifested in increased percent of sick children

**TABLE 1.** SIR in Healthy Children Against Nervous Tissue Antigens (% to the standard serum, $M\pm\sigma$)

| Antigens | SIR | Normal reaction[1] |
|---|---|---|
| NGF | 109±19 | 71-147 |
| S-100 | 108±19 | 70-146 |
| GFAP | 110±16 | 78-142 |
| MP-65 | 103±17 | 69-137 |

**Note.** [1]Interval of SIR titres in control, limited by 2s boundaries around the mean value.

**TABLE 2.** Percentage of Examined Children with SIR Index outward the Boundaries of Normal Reaction

| Antigens | Control | Patients with life-threatening arrhythmias |
|---|---|---|
| NGF | 3.0 | 17.2 (40; 60) |
| S-100 | 3.2 | 13.8 (50; 50) |
| GFAP | 4.3 | 17.2 (20; 80) |
| MP-65 | 3.2 | 27.4 (12; 88) |

**Note.** The percent of hypo- and hyperimmune sera (above or below of the normal) is given in parentheses.

who had SIR outward the boundaries of "normal reaction". According to modern views, NGF is an important factor determining the development and organization of central and peripheral aspects of nervous system. It provides directed growth and branching of nerve terminals, formation of cell-to-cell contacts during embryonic and postnatal development, and supports the activity of mature neurons [12,13]. It cannot be excluded that changes in the titers of autoantibodies to NGF may affect the content of NGF, thereby affecting the development of functional systems responsible for cardiac rhythm regulation.

The reasons for SIR variations in children with life-threatening arrhythmias remain unclear. These changes may result from some immunological disturbances in these patients — both primary and secondary that were provoked by sensibilization.

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