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Personalized approach to treating chronic hepatitis C in children

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Predictors of positive virological response to interferon therapy in children have not been established, which is why it is necessary to identify them and subsequently develop individualized treatment regimens yielding the best possible results. **Objective:** form personalized chronic hepatitis C treatment regimens in children on the basis of identification of virological response predictors and retrospective evaluation of the conducted interferon therapy efficiency. **Study participants:** 98 children of 3-18 years of age (mean age – 10.0±0.8 years) with chronic hepatitis C: 65 of them had HCV 1 genotype (66.0%), 33 – HCV 2-3 genotype (34.0%). We measured anthropometric parameters (weight, height), determined viral load level in blood serum (polymerase chain reaction (PCR)), performed HCV genotyping (before the therapy) and analyzed lymphocytic immunophenotype parameters of all children before the interferon therapy course and over time (4, 12, 24 and 48 weeks after the therapy initiation). **Results:** Analysis of the obtained results demonstrated that the recombinant IFNα-2a therapy efficiency improves if recombinant IL2 (roncoleukin) is added to the treatment regimen: double increase in the rate of achievement of the primary virological remission (PVR) and sustained virological response (SVR) ($p < 0.05$). Use of a pegylated IFNα-2b in combination with ribavirin did not yield any significant difference in comparison with treatment with recombinant IFNα-2a and recombinant IL2. Interferon therapy is more effective in children of at least 30 kg of body weight and 134 cm of height without physical developmental delay than in younger children (of smaller weight and height) at the moment of interferon therapy initiation ($p < 0.001$). Thus, anthropometric parameters of patients may serve as PVR predictors regardless of the HCV genotype at the treatment initiation. If lymphocytes ≥ 2,500/mcl, the PVR achievement rate is 85.0%; if lymphocytes ≤ 2,000/mcl – 5.0% ($p = 0.000$). If the total amount of lymphocytes is 2,000-2,500, the examination should be repeated 12 weeks after the therapy initiation: increase in the number of lymphocytes in comparison with the initial values by 10.0% or more is positive prognostic SVR predictor. **Conclusions:** It is necessary to take into consideration parametric data and the child's age at the moment of therapy initiation in order to schedule the launch of interferon therapy and choose a therapy regimen for chronic viral hepatitis C in children. Total amount of blood lymphocytes, child's body weight and height at the moment of treatment initiation are predictors of positive virological response to treatment.
Keywords: *chronic viral hepatitis C in children, child morbidity, interferon therapy, antiviral therapy, positive virological response predictors, primary virological remission, sustained virological response.*

INTRODUCTION

Efficacy of interferon therapy for chronic hepatitis (Hepatitis C Virus, HCV) in children varies from 0.0 to 90.0% depending on the applied treatment regimens and HCV genotype [1-14]. Fast virological response and early virological response are predictors of favorable interferon therapy

outcome in adults – sustained virological remission (SVR) is achieved in 89 and 70% of cases, respectively [15]. Predictors of positive virological response to interferon therapy in children (necessary to determine the need in and the period of interferon therapy in order to forecast efficacy thereof) have not been determined yet, which is why it is necessary to reveal them and develop individualized treatment regimens producing the best possible effect on their basis.

Objective: develop personalized chronic hepatitis C treatment regimens for children on the basis of the revealed virological response predictors and retrospective assessment of efficacy of the conducted interferon therapy.

PATIENTS AND METHODS

Study participants

The full-design study involved 98 children of 3-18 years of age (average age – 10.0 ± 0.8 years) with chronic hepatitis C (65 children with genotype HCV 1 [66.0%], 33 – with genotype HCV 2-3 [34.0%]) treated at the gastroenterology department with a hepatology group of the Scientific Center of Children's Health.

All the children underwent the following examination procedure before and during the interferon therapy (4, 12, 24 and 48 weeks after the treatment initiation):

- anthropometric measurement (weight, height);
- determination of the viral load in blood serum using polymerase chain reaction (PCR) and HCV genotyping assay (before the therapy initiation).

The children were examined at the research laboratory of viral safety of blood and blood component transfusions of the Hematology Research Center (Federal State Budgetary Institution) of the Ministry of Health of the Russian Federation (headed by PhD in Medicine T.A. Garanzha).

Lymphocyte immunophenotype parameters were analyzed at the centralized clinical diagnostic laboratory of the Scientific Center of Children's Health headed by MD, PhD E.L. Semikina.

The children undergoing interferon therapy were divided into 3 groups.

The 1st group involved 47 children (average age – 10.9 ± 0.5 years) undergoing treatment with recombinant interferon alpha-2a (IFNα-2a) administered subcutaneously in the dose of 3 mn IU 3 times per week. 35 of these children (75.0%) had genotype HCV1; 12 (25.0%) – genotype HCV 2-3.

The 2nd group involved 31 children (average age – 10.9 ± 0.7 years) undergoing treatment with recombinant IFNα-2a administered subcutaneously in the dose of 3 mn IU 3 times per week and recombinant interleukin 2 (IL2) in the dose of 0.2 mg/kg (not more than 1 mg) 2 times per week. 20 of these children (65.0%) had genotype HCV 1; 11 (35.0%) – genotype HCV 2-3.

The 3rd group involved 20 children (average age – 9.31 ± 0.9 years) undergoing treatment with pegylated interferon alpha-2b (IFNα-2b) in the dose of 60 mg/kg per week in combination with ribavirin in the dose of 15 mg/kg per day. 10 of these children (50.0%) had genotype HCV 1; 10 (50.0%) – genotypes HCV 2 and 3.

Therapy length in the children with genotype HCV 1 was 48 weeks, in the children with genotypes HCV 2 and 3 – 24 weeks.

We conducted virological response monitoring in accordance with recommendations of the European Association for the Study of the Liver (EASL) [16] in order to assess efficacy of antiviral therapy: determination of quantitative expression of HCV genes (RNA) in blood serum with PCR before the treatment and 4, 12, 24, 48 and 72 (in the event of genotype HCV 1) weeks after the therapy initiation.

- Fast virological response (FVR) was registered if HCV RNA was not detected (≤ 50 IU/ml) after 4 weeks of treatment.
- Early virological response (EVR) was registered if HCV RNA was not detected after 12 weeks of therapy, but was positive after 4 weeks.

● Delayed virological response (DVR) was registered if HCV RNA was not detected after 24 weeks of therapy, but was positive after 12 weeks and decreasing by more than 2 log 10 IU/ml.
● Partial virological response (PaVR) was registered if HCV RNA was lower by more than 2 log 10 IU/ml than in the beginning, but had been detected until the 24^{th} therapy week inclusive.
● Primary virological remission (PVR) was registered if HCV RNA was not detected in blood serum after the therapy completion.
● Sustained virological response was registered if HCV RNA had not been detected in blood serum for 6 months after the therapy completion.
● Virological breakthrough (VB) was registered if HCV RNA appeared in blood serum at any time during the treatment.
● Disease recurrence was registered if HCV RNA appeared in blood serum after the therapy completion.
In order to determine treatment response predictors, we considered the children with PVR "responding", the children without PVR in the setting of the conducted interferon therapy – "non-responding".

Statistical data manipulation

The data were statistically manipulated using parametric and non-parametric statistical methods by means of software package Statistica-6. Analysis of the obtained data included calculation of the following parameters: arithmetic mean of frequency distribution (M) and arithmetic mean error (m) (analysis of quantitative studies). Significance of the differences obtained in the compared groups was assessed using the Student's t-test. The differences between the compared groups were deemed statistically significant at $p < 0.05$. The significance of differences in the frequency of occurrence of one or another parameter between the compared groups was assessed using the chi-square goodness of fit test (χ^2) in the event of analysis of qualitative studies. The significance of differences and of the observed association were determined using χ^2 tables (one degree of freedom). The differences between the compared groups were deemed statistically significant at $p < 0.05$.

STUDY RESULTS AND DISCUSSION

Retrospective analysis of interferon therapy efficacy in group 1 demonstrated the following: fast virological response was registered in 2 cases out of 47 (4.3%); both patients had genotype HCV 2a (2 patients out of 12; 16.7%). Early virological response was registered in 1 case out of 47 (2.1%) in 1 patient with genotype HCV 1 (out of 35 [2.9%]). Delayed virological response was registered in 12 cases out of 47 (25.5%); 7 patients had genotype HCV 1 (20.0%), 5 patients – genotypes HCV 2 and 3 (41.7%). Partial virological response was registered in 12 cases out of 47 (25.5%); 11 patients had genotype HCV 1 (31.4%), 1 patient – genotype HCV 3a (8.3%). Primary virological remission was registered in 13 cases out of 47 (27.7%); 8 patients had genotype HCV 1 (22.9%), 5 patients – genotypes HCV 2 and 3 (41.7%). Sustained virological response was registered in 11 cases out of 47 (23.4%); 6 patients had genotype HCV 1 (out of 35 [17.1%]); 5 patients – genotypes HCV 2 and 3 (out of 12 [41.7%]). Virological breakthrough was registered in 5 cases out of 47 (10.6%); all the children had genotypes HCV 2 and 3 (41.7%). Disease recurrence was registered in 2 cases out of 47 (4.3%) in the children with genotype HCV 1 (5.7%).
In group 2, fast virological response was registered in 9 cases out of 31 (29.0%); 5 patients had genotype HCV 1 (out of 20 [25.0%]), 4 patients – genotypes 2 and 3 (out of 11 [36.4%]). Early virological response was registered in 7 cases out of 31 (22.6%); 1 patient had genotype HCV 1 (5.0%); 6 patients – genotypes HCV 2 and 3 (54.5%). Delayed virological response was registered in 1 case out of 31 (3.2%) in a patient with genotype HCV 1. Partial virological

response was registered in 3 cases out of 31 (9.7%) in patients with genotype HCV 1. Primary virological remission was registered in 17 cases out of 31 (54.8%); 7 patients had genotype HCV 1 (35.0%), 10 patients – genotypes HCV 2 and 3 (90.9%). Sustained virological response was registered in 15 cases out of 31 (48.4%); 5 patients had genotype HCV 1 (25.0%); 10 patients – genotypes HCV 2 and 3 (out of 11 [90.9%]). Virological breakthrough was registered in 2 cases out of 31 (6.5%) in patients with genotype HCV 1. Disease recurrence was registered in 2 cases out of 31 (6.5%); 1 patient had genotype HCV 1, the other – genotype HCV 3.

In group 3, fast virological response was registered in 6 cases out of 20 (30.0%); 2 patients had genotype HCV 1 (out of 10 [20.0%]), 4 patients – genotypes 2 and 3 (out of 10 [40.0%]). Early virological response was registered in 13 cases out of 20 (65.0%); 7 patients had genotype HCV 1 (70.0%); 6 patients – genotypes HCV 2 and 3 (60.0%). Delayed virological response was not registered in any of the children. Partial virological response was registered in 1 case out of 20 (5.0%) in 1 patient with genotype HCV 1 (10.0%). Primary virological remission was registered in 16 cases out of 20 (80.0%); 6 patients had genotype HCV 1 (60.0%), 10 patients – genotypes HCV 2 and 3 (100%). Sustained virological response was registered in 16 cases out of 20 (75.0%); 7 patients had genotype HCV 1 (70.0%); 9 patients – genotypes HCV 2 and 3 (90.0%). Two children (10.0%) did not respond to the therapy; both patients had genotype HCV 1 (20.0%). Virological breakthrough was registered in 2 cases out of 20 (10.0%); both patients had genotype HCV 1 (20.0%). Disease recurrence was registered in 1 child with genotype HCV 2 (10.0%). Comparative analysis of the response to interferon therapy is given in pic. 1.

These data indicate that efficacy of the therapy with recombinant IFNα-2a increases if recombinant IL2 (roncoleukin) is added to the treatment regimen; the PVR and SVR rates double ($p < 0.05$). Use of pegylated IFNα-2b in combination with ribavirin was not significantly different from the treatment with recombinant IFNα-2a and recombinant IL2.

Comparison of efficacy of the therapy with recombinant IFNα-2a or the combination of pegylated IFNα-2b and ribavirin demonstrated a significantly higher achievement of the SVR and the PVR when the latter therapy regimen was used ($p < 0.001$).

Comparison of the obtained results with the data of the systemic review of the worldwide studies of HCV interferon therapy efficacy in children demonstrates that the SVR achievement frequency when IFNα-2a monotherapy was used is comparable with the world data on the IFNα treatment efficacy, whereas the SVR achievement frequency when recombinant IFNα-2a and recombinant IL2 are used is as high as the pegylated IFNα-2b treatment efficacy and slightly lower than when the combination of pegylated IFNα-2b and ribavirin is used ($p = 0.111$).

Given that the SVR both in children and adults depends on the HCV genotype [15], we analyzed treatment efficacy in the children infected with different HCV genotypes (pics. 2 and 3).

The presented data demonstrate that the SVR was achieved in 17.1% of cases of infection with genotype HCV 1 when recombinant IFNα-2a monotherapy was used; inclusion of recombinant interleukin-2 in the regimen contributed to the SVR rate increase up to 25.0%; the highest SVR achievement rate (70.0%) was observed in the group of children treated with pegylated IFNα-2b and ribavirin.

The SVR was achieved in 41.7% of cases of infection with genotype HCV 2 or 3 when recombinant IFNα-2a was used; use of the combination of recombinant IFNα-2a and recombinant IL2 contributed to the more than double SVR rate increase (up to 90.9%). The children undergoing treatment with pegylated IFNα-2b and ribavirin also had a high SVR achievement rate (90.0%).

Comparison of anthropometric data of the children with the results of virological monitoring of the response to treatment demonstrated direct correlation between treatment efficacy and the child's body weight and height at the moment of interferon therapy initiation (pics. 4 and 5).

The presented data demonstrate that the PVR was significantly less often achieved in the children with height of 125.3 ± 7.3 cm and body weight of 25.9 ± 4.3 kg undergoing treatment

with recombinant IFNα-2a and recombinant IL2 than in the children with height of 149.1 ± 4.1 cm and body weight of 42.6 ± 4.3 kg ($p < 0.05$).
The PVR was significantly less often achieved in the children with height of 113.2 ± 6.8 cm and body weight of 19.4 ± 2.0 kg undergoing treatment with pegylated IFNα-2b and ribavirin than in the children with height of 142.2 ± 8.4 cm and body weight of 38.1 ± 5.3 kg ($p < 0.05$).
No significant differences in dependence of virological response on height ($p = 0.228$) and body weight ($p = 0.185$) were observed in the event of treatment with recombinant IFNα-2a.
Thus, the PVR was significantly more often achieved in the children with body weight of 33.0-47.0 kg and height of 134.0-153.0 at the moment of therapy initiation undergoing treatment with either the combination of recombinant IFNα-2a and recombinant IL2 or the combination of pegylated IFNα-2b and ribavirin. 26 (81.3%) out of 32 (63%) patients (out of 56 children from both groups) with the PVR had body weight ≥ 30 kg and height ≥ 134 cm, whereas neither of the 24 children, who had not achieved the PVR, had body weight ≥ 30 kg and height ≥ 134 cm, although deviations of anthropometric parameters from the age norms did not exceed 1 percentile. This indicates that interferon therapy in children with body weight of at least 30 kg and height of at least 134 cm at the moment of interferon therapy initiation without physical development delay was more efficient than in the younger children (with lower body weight and height) ($p < 0.001$); anthropometric parameters of the patients at the moment of treatment initiation may serve as PVR predictors for determining the need in and the period of HCV interferon therapy regardless of the HCV genotype.
Analysis of interferon therapy efficacy dependence on the absolute blood lymphocyte count demonstrated that the primary virological remission was achieved in 85.0% of cases when the level of lymphocytes was ≥ 2,500/mcl and in 5.0% of cases when the level of lymphocytes was ≤ 2,000/mcl ($p = 0.000$). Absolute lymphocyte count within the range from 2,000 to 2,500 is an indication for repeated examination 12 weeks after the therapy initiation: increase in the lymphocyte count by 10% or more in comparison with the initial value is a positive prognostic predictor of the SVR. Dynamic pattern of the absolute lymphocyte count is given in pics. 6-8.
Thus, such parameter as the absolute lymphocyte count may be a predictor of positive response to interferon therapy and may be used for determining whether it is reasonable to carry the therapy on after 12 weeks of treatment.

CONCLUSIONS

It is necessary to take into consideration parametric data and age of the child at the moment of therapy initiation when determining the time of interferon therapy initiation and selecting the treatment regimen for chronic hepatitis C in children.
Absolute blood lymphocyte count, child's body weight and height at the moment of treatment initiation are predictors of the positive virological response to treatment.

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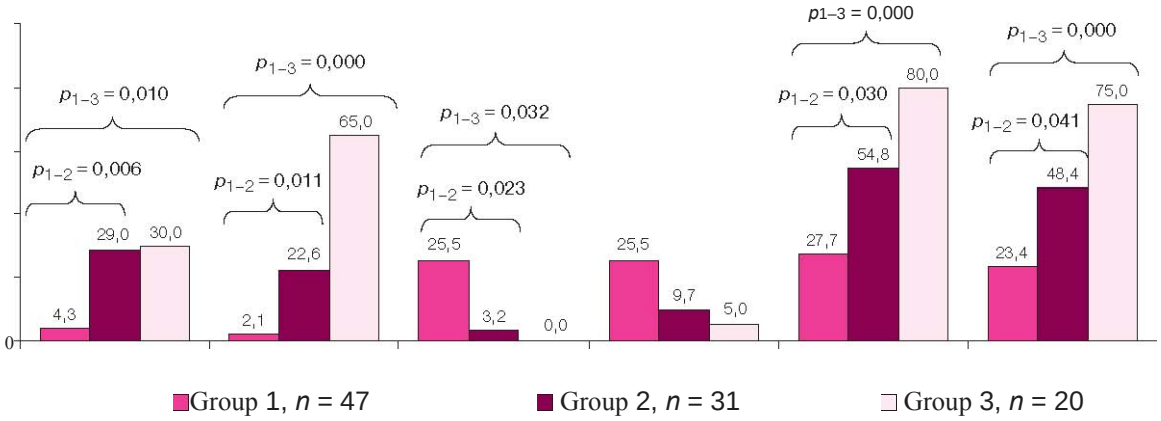
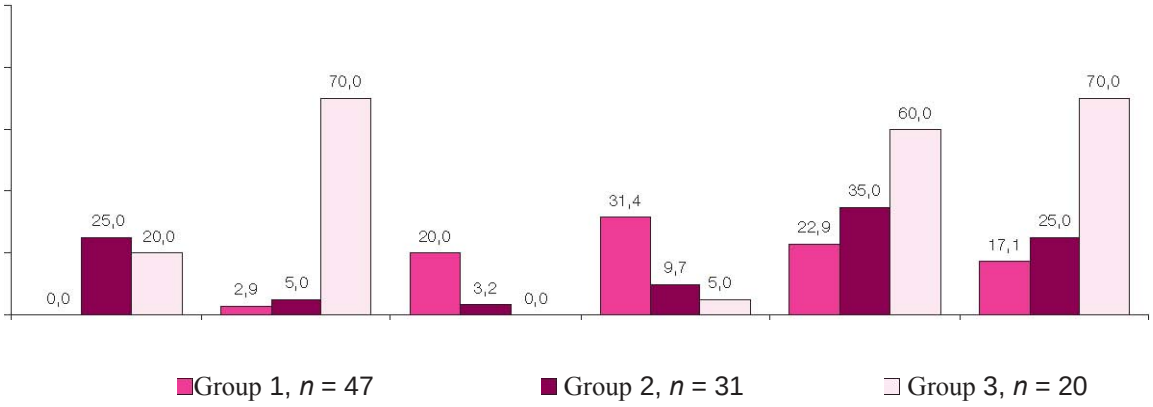
Pic. 1. Interferon therapy efficacy for chronic viral hepatitis C in children according to the virological monitoring employing various treatment regimens

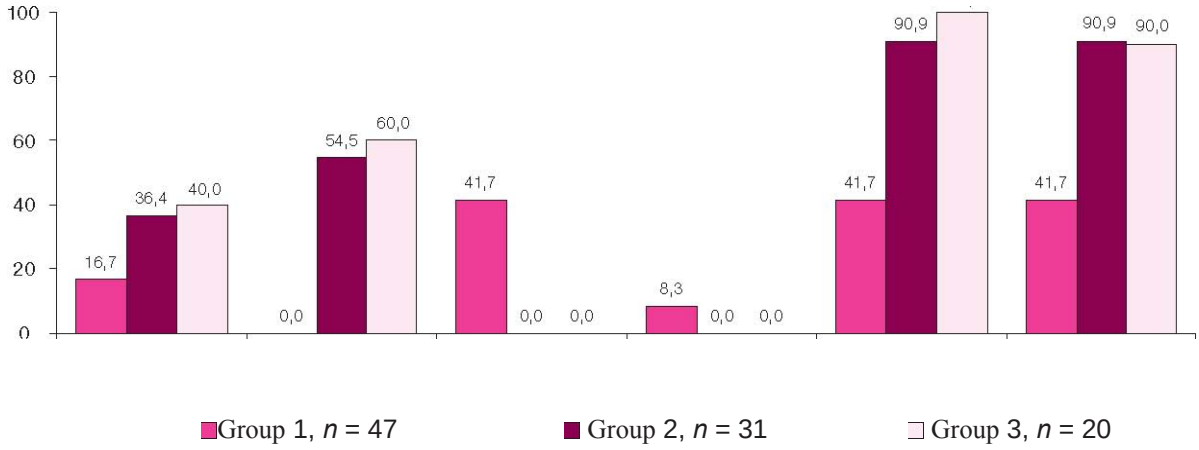


Note. * - p_{1-2} (comparison of groups 1 and 2); p_{1-3} (comparison of groups 1 and 3). Hereinafter, FVR – fast virological response, EVR – early virological response, DVR – delayed virological response, PaVR – partial virological response, PVR – primary virological remission, SVR – sustained virological remission.

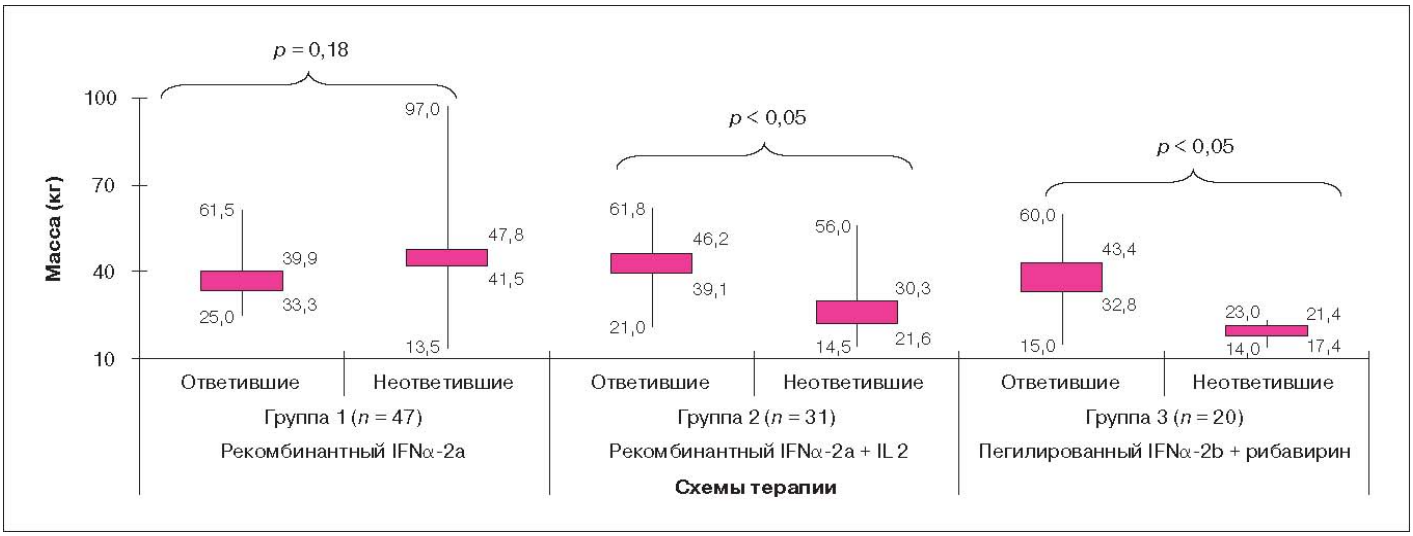
Pic. 2. Interferon therapy efficacy for chronic viral hepatitis C in children according to the virological monitoring employing various treatment regimens in patients with genotype HCV 1



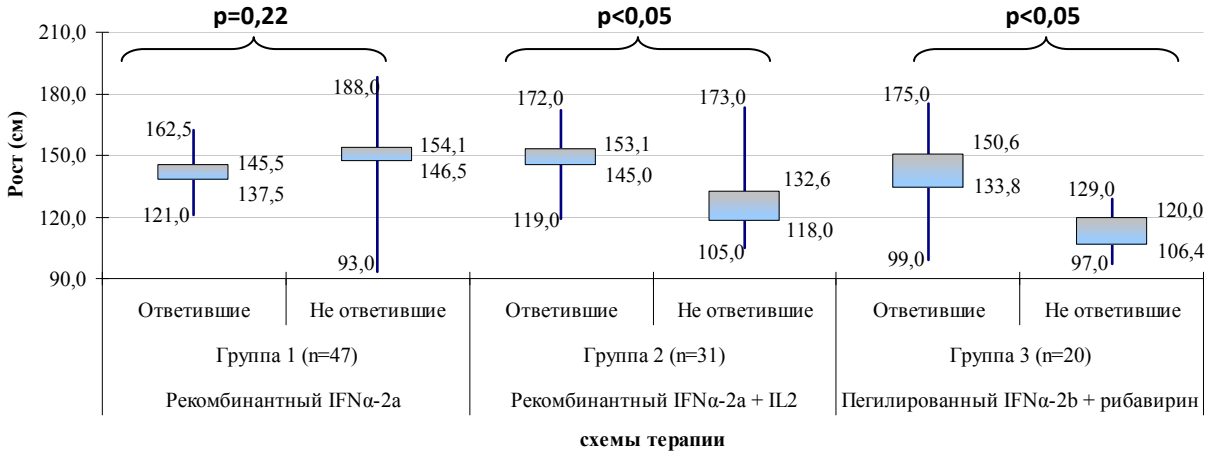
Pic. 3. Interferon therapy efficacy for chronic viral hepatitis C in children according to the virological monitoring employing various treatment regimens in patients with genotypes HCV 2 and 3



Pic. 4. Virological response depending on the child's body weight at the moment of therapy initiation

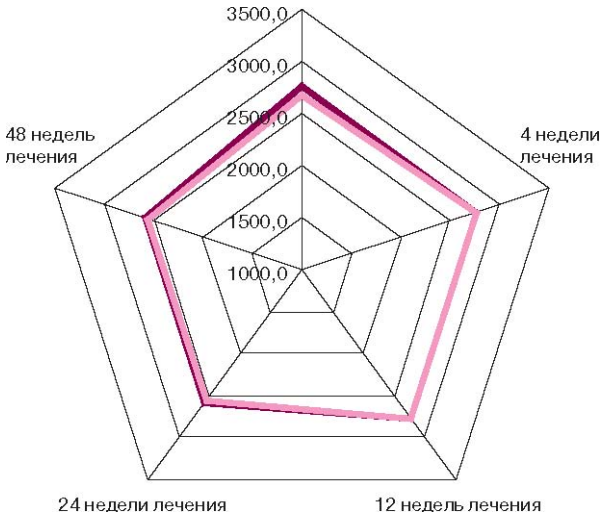


Pic. 5. Virological response depending on the child's height at the moment of therapy initiation

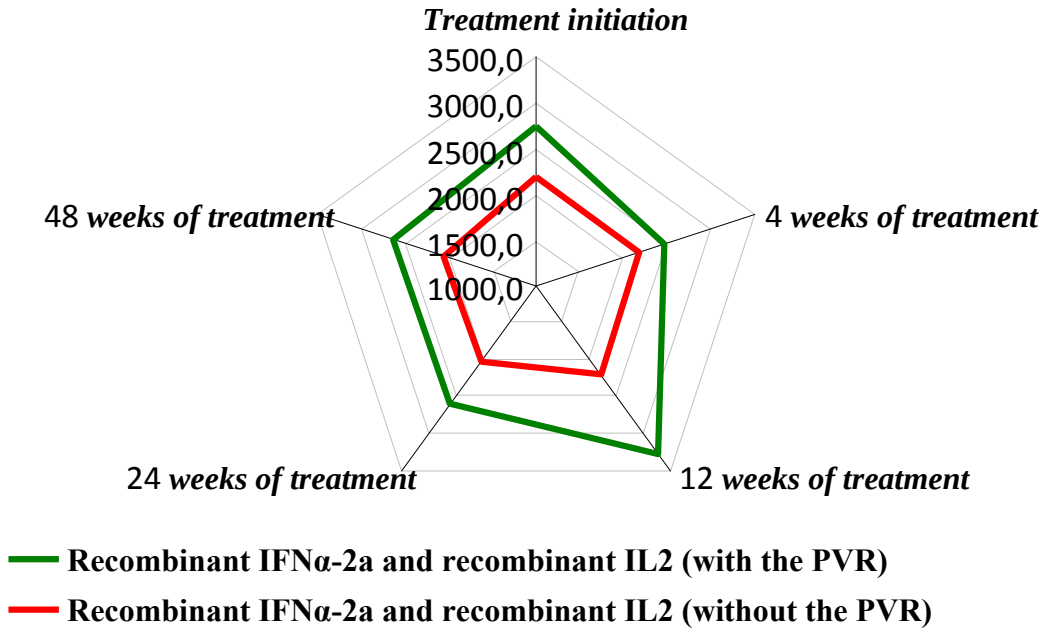


Рост (см)	*Height (cm)*
Ответившие	*Responding*
Не ответившие	*Non-responding*
Группа 1 (n = 47)	*Group 1 (n = 47)*
Группа 2 (n = 31)	*Group 2 (n = 31)*
Группа 3 (n = 20)	*Group 3 (n = 20)*
Рекомбинантный IFNα-2a	*Recombinant IFNα-2a*
Рекомбинантный IFNα-2a + IL2	*Recombinant IFNα-2a + IL2*
Пегилированный IFNα-2b + рибавирин	*Pegylated IFNα-2b + ribavirin*
схемы терапии	*Therapy regimens*

Pic. 6. Absolute lymphocyte count at the moment of treatment initiation and in the follow-up in the setting of recombinant interferon IFNα-2a therapy



Pic. 7. Absolute lymphocyte count at the moment of treatment initiation and in the follow-up in the setting of therapy involving recombinant IFNα-2a and recombinant IL2



Pic. 8. Absolute lymphocyte count at the moment of treatment initiation and in the follow-up in the setting of therapy involving pegylated IFNα-2a and recombinant IL2