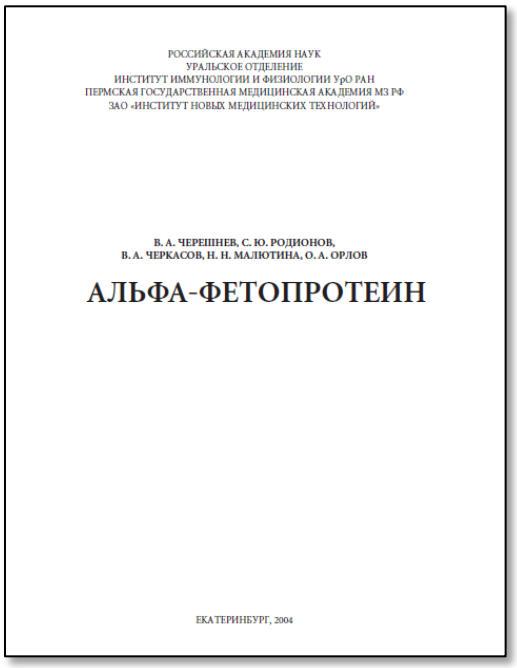
Double Blind, Randomized, Placebo-Controlled Study in Patients with Inflammatory Bowel Disease

Summary:

- **78 patients - 56 colitis, 22 Crohn's**
  - **AFP dosing: 4 µg/kg daily (28 colitis(IV) and 10 Crohn's(IM))**
  - **40 (28 colitis and 12 Crohn's) received placebo for 30 days**
  - **5-15 years duration with radiological evidence of disease**
- **After 30 days 100% of AFP patients had definite improvement in their symptoms**
- **All laboratory parameters** (Hemoglobin, albumin, IGA, IGM, decrease in CD71+ cells, etc.) normalized. 47% of patients gained weight of up to 12kg.
- 30% of Crohn's and 32% of colitis had complete restoration of normal bowel movements and no symptoms of disease.
- Endoscopy: improvement in the intestinal mucosa with reduction in the number of ulcerative lesions. 30% of Crohn's patients reduced steroid dose to half and 20% went off steroids. 32% of colitis patients were able to reduce steroid dose by 50-60% and 7% went off steroids completely.
- No changes were observed in placebo patients.


РОССИЙСКАЯ АКАДЕМИЯ НАУК
УРАЛЬСКОЕ ОТДЕЛЕНИЕ
ИНСТИТУТ ИММУНОЛОГИИ И ФИЗИОЛОГИИ УрО РАН
ПЕРМСКАЯ ГОСУДАРСТВЕННАЯ МЕДИЦИНСКАЯ АКАДЕМИЯ МЗ РФ
ЗАО «ИНСТИТУТ НОВЫХ МЕДИЦИНСКИХ ТЕХНОЛОГИЙ»

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ЕКАТЕРИНБУРГ, 2004

Alpha Fetoprotein, Chereshnev V. A., 2004

English Translation of Chapter 4 Follows Below

4.4. USE OF AFP MEDICATION AS PART OF THE TREATMENT OF NON-SPECIFIC ULCERATIVE COLITIS AND GRANULOMATOUS ENTERITIS (CROHN'S DISEASE)

Non-specific ulcerative colitis (NUC) and Crohn's disease involve damage to the mucosal and sub-mucosal layers of the large and small intestines due to autoimmune disease [128, 171]. We conducted a placebo-controlled, randomized clinical study of AFP in patients with non-specific inflammatory diseases of the bowel at three clinics: the Ekaterinburg Gastroenterology Center, the Proctology Department of the Kurgansk Regional Clinical Hospital, and the RAM-TAL Center for Integrative Medicine in Jerusalem (Israel).

A total of 78 patients aged 16-72 years were recruited into the clinical study. Of these, 56 had NUC and 22 had Crohn's disease. The patients in the main group (38 people) received parenteral AFP at a dose of 4 μg/kg of body weight for 30 days. In the second group (40 people), therapy was performed using "Placebo" (5 mg lyophilized rheopolyglucin) with the same frequency, duration and route of administration.

The primary objective of the clinical studies was to assess the safety and efficacy of AFP in the treatment of patients with non-specific ulcerative colitis and Crohn's disease.

In accordance with the AFP study objective, standard pre- and post-treatment study methods were used which were formalized into an approved clinical protocol. The patients were examined at the beginning of the study and on the second day following its completion. The examination assessed the condition of organs and systems within a range of physical findings (gender, body weight, age, respiratory rate, pulse, body temperature, condition of the skin and mucosa, etc.), laboratory findings (complete urinalysis; complete and biochemical blood tests; immunogram), specialized and instrumental indicators (ultrasound of the abdominal organs, electrocardiogram, sigmoidoscopy, colonoscopy, fistulography, irrigoscopy, and tissue histology of the large intestine [114, 128, 171, 402]).

In the course of the AFP clinical study, several subgroups of patients were identified:

1. Patients with Crohn's disease (10 people), who received therapies including sulfanilamide, prednisolone, vitamins, and agents to normalize intestinal microflora, as well as intramuscular AFP at 4 μg/kg of weight per day for 30 days.

2. Patients with non-specific ulcerative colitis (28 people) who received therapies including vitamins (B1, B6, C), ε-aminocaproic acid, 1% solution of CaCL2, 10% solution of albumin, corticosteroids, antihistamines, sulfanilamide, as well as 30 days of intravenous (drip) AFP (4 μg/kg in 10 ml saline solution once daily).

In the corresponding comparator groups (Crohn's disease – 12 people; non-specific ulcerative colitis – 28 people), similar combinations of therapy were performed with "Placebo", using lyophilized rheopolyglucin as a substitute for AFP. In the control groups, "Placebo" was administered with the same regimen.

All patients were treated with the indicated regimen using identical ampoules containing 75 μg of AFP and 5 mg of rheopolyglucin with authenticity certifications. The results were entered into the patient charts. No subjects were excluded from the study for any reason. All study subjects received written information on the medication before beginning treatment, as well as a written voluntary consent form for participation in the AFP studies.

All patients in the study were placed on regular schedules for clinical observation (5-15 years). To assess the efficacy of AFP, a customary set of laboratory and instrumental parameters was employed and implemented through a program of scheduled examinations and specialized treatments for each given category of patients. During the recruitment stage of the study, persons were included who, in the opinion of the coloproctologists, were in need of operative correction or colectomy. The patients had received various pathogenetic medications throughout the course of their illnesses, but had seen no improvement; and in more than half of the cases the disease had progressed.

Common to all patients was their long-term, chronic recurrent disease, accompanied by pain in various parts of the abdomen caused by the inflammatory process and intestinal impairment. Overall, the changes in the patients' conditions were toxic-allergic in nature. Among these were dystrophic processes in the internal parenchymatous organs, blood abnormalities (anemia, leukocytosis, hypoproteinemia, dysproteinemia, decrease in the number of microelements in the blood, elevated erythrocyte sedimentation rates, and increased clotting), as well as non-specific joint damage in the extremities and spine, damage to the eyes in the form of keratosis, and granulomatous processes of the skin.

X-ray images of the bowel with Crohn's disease or non-specific ulcerative colitis were indistinguishable in their diversity, and showed dramatic, uneven narrowing of the affected areas of the intestine with uneven wall contours and an absence of haustration. In relief, the mucosal lining was either dentate with spiked projections or honeycombed. During endoscopic examination, the lumen of the intestine usually showed swelling of the mucosa, hyperemia, small hemorrhages, and in many there was fibrous exudate. The affected area of the bowel appeared deformed due to the thickening of the walls and narrowing of the lumen. One characteristic sign among all the patients was the presence of longitudinal, narrow, deep ulcerative defects with transverse ulcerative fissures limiting the intact portions of the mucosal lining (appearing like cobblestones). In histologies, the microscopic presentation corresponded to a non-specific inflammatory process, with several particular features: inflammatory-cellular infiltrate spread throughout all the layers of the intestinal wall, but appearing mostly in the submucosal layer with a pronounced productive granulomatous process.

Both the long-term therapeutic catamnesis of patients who did not experience any significant changes in the course of their disease and the overall condition of the patients should be noted. Three of the patients formed long-term, functioning fistulas as a result of spontaneous perforations of the colon.

A comparative analysis of the treatment results in patients with Crohn's disease and NUC in the AFP and placebo groups revealed the following trends. One hundred percent of patients received AFP as part of therapy for Crohn's disease or non-specific ulcerative colitis, 100% of the patients subjectively noted definite improvement in their overall condition. Positive changes in the condition of patients in the primary group occurred approximately during treatment days 10-14. By the end of the course of therapy, compensation or subcompensation was achieved in terms of practically all laboratory parameters (Table 4.4.1), integrated into an overall statistical analysis by their proximity to a pathogenetic presentation of NUC or Crohn's disease, and also by their similarity to a set of symptoms and the severity of the particular disease [128, 171]. It was determined that using AFP in a complex therapeutic regimen reduces inflammatory-proliferative processes in the mucosal lining of the intestine by decreasing levels of IgA and IgM, as well as CD71+-cells, characteristic of leukocyte activation (fig. 4.4.1, 4.4.2). The improvement in laboratory values in this group of patients correlated with the clinical observations of the patients' conditions.

In 18 (47.3%) patients in the primary clinical groups, increases in body weight were recorded, and one of the severely ill patients put on 12 kg. In the placebo groups, the weight trends before and after treatment did not change.

A significant decrease in the frequency of dyspeptic disorders such as bloody diarrhea was seen during therapy which included AFP in patients with non-specific ulcerative colitis and Crohn's disease. We achieved complete stool normalization (quantitatively and qualitatively) by the end of treatment in 30% of Crohn's disease patients and in 32.1% of NUC patients.

Table 4.4.1

Changes in laboratory values for patients with NUC and Crohn's disease in the AFP and placebo groups as a result of treatment ($M \pm m$)

Value	AFP Group (n = 38)		Placebo group (n = 40)	
	Before treatment	After treatment	Before treatment	After treatment
Complete blood count				
Hemoglobin, g/l	82.3 ± 12.4	129.5 ± 8*	96.6 ± 11.8	96.1 ± 12.4
Erythrocytes x 10_{12}/l	2.8 ± 0.7	3.9 ± 0.7*	2.4 ± 1.1	2.8 ± 0.8
Leukocytes x 10_{12}/l	13.6 ± 2.7	8.2 ± 3.4	12.5 ± 3.3	9.9 ± 1.6
Banded neutrophils, %	9.7 ± 4.3	4.1 ± 2.2*	11.6 ± 3.3	9.4 ± 1.5
Segmented neutrophils, %	50 ± 9.4	31 ± 3.7*	49 ± 5.1	55.5 ± 5.2
Lymphocytes, %	20.2 ± 4.3	31 ± 2.2*	22.9 ± 4.6	24.7 ± 3.1
Eosinophils, %	4.2 ± 1.4	2.1 ± 1.7	5.1 ± 0.6	4.5 ± 1.2
Monocytes, %	9.3 ± 1.1	6.3 ± 0.9	7.2 ± 2.1	11.3 ± 0.7
Thrombocytes x 10_9/l	177.5 ± 38.9	189.7 ± 26.1	205.3 ± 32.7	210.6 ± 21.2
ESR, mm/hr	33.7 ± 12.1	16.4 ± 7.2*	46.3 ± 14.3	39.8 ± 11.2

End of table 4.4.1

Value	AFP Group (n = 38)		Placebo group (n = 40)	
	Before treatment	**After treatment**	**Before treatment**	**After treatment**
Biochemical blood count				
Total protein, mg%	53.5 ± 8.6	73.3 ± 2.2*	61.1 ± 12.1	58.4 ± 5.5
Albubin, mg%	32.7 ± 11.6	45.2 ± 6.4	34.7 ± 7.1	36.9 ± 5.4
Total bilirubin, mg%	1.5 ± 0.9	1.9 ± 3.6	1.8 ± 0.4	1.9 ± 0.3
AST, mmol/l	0.35 ± 0.06	0.47 ± 0.09	0.25 ± 0.02	0.46 ± 0.09
ALT, mmol/l	0.37 ± 0.04	0.32 ± 0.06	0.23 ± 0.08	0.56 ± 0.05
BUN, mg%	43.5 ± 6.9	7.8 ± 3.5*	37.2 ± 4.2	41.6 ± 13.5
Urea, mg%	5.2 ± 1.1	1.6 ± 0.9*	4.4 ± 2.1	4.1 ± 1.3
Creatinine, mg%	1.2 ± 0.4	1.5 ± 0.15	0.9 ± 0.3	1.4 ± 0.3
K_+, mg%	10.2 ± 3.7	16.1 ± 2.4*	7.7 ± 2.1	9.4 ± 1.6
Na_+, mg%	260.3 ± 40.9	352.6 ± 25.7	310.1 ± 19.3	280.4 ± 31.4
Mg_{2+}, mg%	2.3 ± 0.6	3.9 ± 0.7	1.9 ± 0.7	2.9 ± 1.4
Immunological blood test				
$CD3_+$, %	46.3 ± 7.3	49.7 ± 8.7	40.2 ± 9.4	45.4 ± 9.7
$CD11\beta_+$, %	6.6 ± 3.2	25.6 ± 5.3*	9.8 ± 3.5	7.8 ± 4.1
$CD22_+$, %	13.7 ± 3.3	9.4 ± 2.7	18.3 ± 3.1	10.4 ± 7.9
HLA-DR_+, %	19.8 ± 4.4	16.3 ± 3.7	19.6 ± 5.4	22.4 ± 4.7
$CD71_+$, %	6.2 ± 2.4	0*	4.1 ± 1.5	5.3 ± 2.6
IgA, g/l	5.5 ± 2.1	1.4 ± 0.5*	7.8 ± 1.1	8.4 ± 1.3
IgM, g/l	4.6 ± 1.3	1.1 ± 0.9*	3.3 ± 1.2	6.9 ± 0.9

*Statistical significance before and after treatment (p<0.05).

Comment should be made regarding one female patient with NUC (acute form) who was in a life-threatening situation associated with her excessive – up to 18 times a day – hemorrhagic diarrhea which could not be stopped with therapeutic measures. The use of AFP enabled a partial remission (stool 3 times a day) and control of the patient's overall condition.

Among the more interesting observations among patients with Crohn's disease was the partial, and in 20% of patients, complete recovery of the configuration of previously deformed wrist joints, as well as their functional ability (restored full range of motion). By the end of treatment, granulomatous skin processes had practically disappeared in all patients with Crohn's disease.

X-ray studies in patients with NUC and Crohn's disease have established that the contours of the lumen of the large intestine became more uniform, there were fewer incidents of reduced diameter in the intestinal lumen, and the passage of the intestinal contents through the intestinal tube was accelerated. Endoscopic monitoring of the intestinal mucosa noted a decrease in edema, hyperemia, and the amount of fibrous exudate in the lumen.

The number of ulcerative defects was significantly reduced, and as a result the surface of the mucosal lining of the colon was smoother. Histological tests showed a decrease in inflammatory-proliferative processes and in leukocyte-macrophage infiltration.

It is noteworthy that with the use of AFP as part of therapy, patients felt a distinct improvement in their well-being for the first time in years, which allowed us to reduce corticosteroid dose by 2-fold in 30% of the patients with Crohn's disease, and to completely discontinue it in 20% of patients. It was also possible to reduce corticosteroid dose by 2-3 fold in 32.1% of patients with non-specific ulcerative colitis, and to discontinue it in 7.1% of patients. No significant results could be achieved in the control group receiving "Placebo", despite the active use of contemporary medicinal therapies.

An analysis of the efficacy of AFP as part of the treatment for non-specific ulcerative colitis and Crohn's disease revealed a profound positive effect on the course and near-term outcome of the diseases. A positive effect from the use of AFP, resulting in a decrease in related qualitative and quantitative symptoms, was subjectively noted by 100% of patients. These positive changes were verified objectively using biochemical, hematologic and immunologic tests, as well as by X-ray and endoscopic studies.

Thus, no complications or adverse events were registered with the daily use of AFP at a dose of 4 μg/kg for 30 days by intramuscular or intravenous administration to patients with non-specific ulcerative colitis or Crohn's disease. Statistically significant clinical and para-clinical data was obtained for judging the safety and efficacy of including AFP in the set of therapeutic measures used to treat inflammatory bowel diseases.

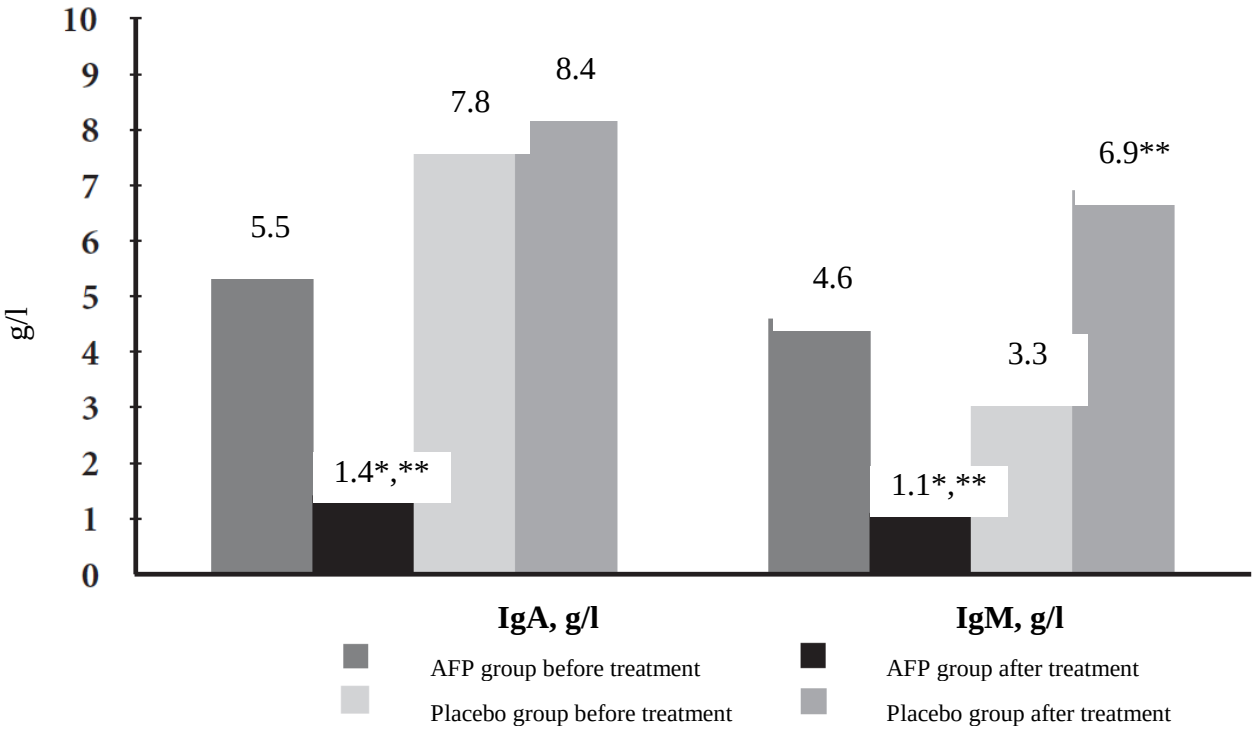

Fig. 4.4.1 **Effect of AFP on the levels of IgA and IgM in patients with non-specific ulcerative colitis**

*Significance compared to placebo group.
**Comparison before and after treatment with p <0.05.

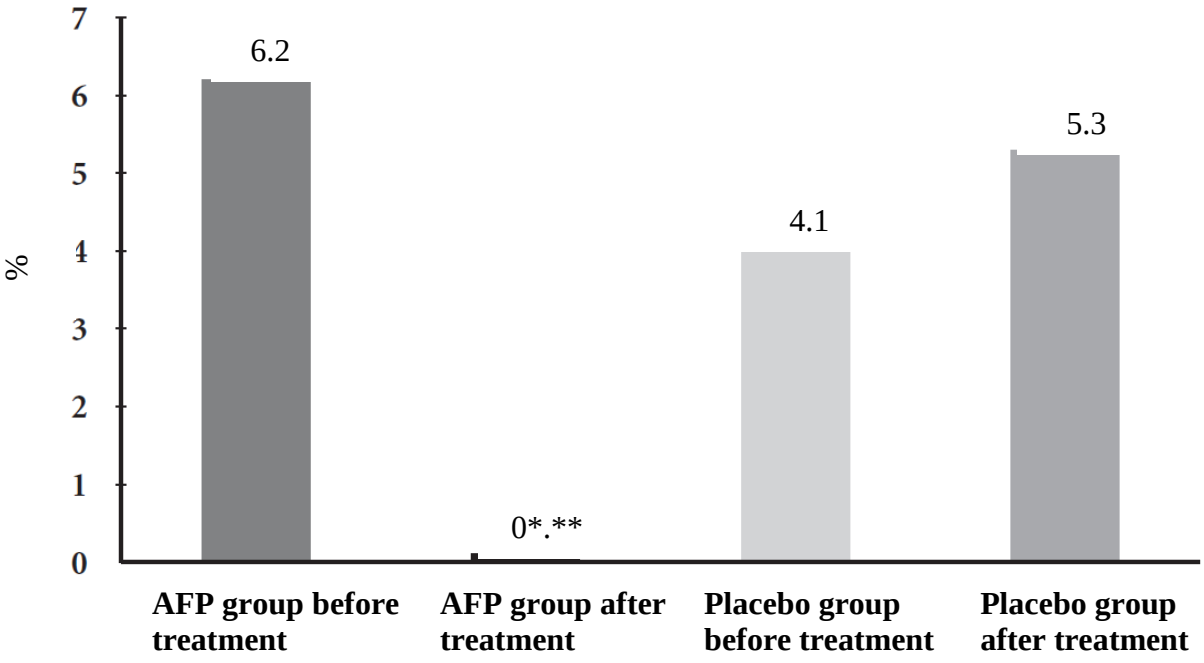

Fig. 4.4.2 **Effect of AFP on the level of CD 71+-cells in patients with non-specific ulcerative colitis**
*Significance compared to placebo group.
**Comparison before and after treatment with p <0.05.