PRELIMINARY RESULTS OF NONSPECIFIC IGY THERAPY IN CATS WITH FIV: DYNAMICS OF WBC AND SERUM PROTEINS ELECTROPHORESIS

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Abstract

The present study focus on using hyperimmune egg yolk nonspecific IgY in 8 FIV positive cats over a 5 months period (September 2015- February 2016), out of which 7 cats survived to the end of the treatment period. Blood samples were collected prior and post the IgY administration and were used for the determination of WBC count and the electrophoresis of serum proteins.

The aim of the study was to observe the impact of nonspecific IgY treatment on the immune system of FIV infected cats.

Preliminary data showed that adjuvant therapy with chicken egg yolk IgY of FIV infected felines has a positive impact and is free of adverse reactions.

Key words: *FIV, IgY, WBC, Serum proteins electrophoresis*

1. INTRODUCTION

1.1. FIV

Feline immunodeficiency virus (FIV) causes one of the most common infectious diseases of cats (Lee et al. 2002). FIV is a lentivirus (Mosallanejad et al. 2010) that belongs to the retroviridae family and causes immunosuppression in cats (Lee et al. 2002). FIV differs from feline leukemia virus (FeLV) and feline foamy virus (FFV) (Mosallanejad et al. 2012) and stands closely related to human immunodeficiency virus type 1(HIV-1) (Mosallanejad et al. 2012, Arjona et al. 2000), to the extent that it has been proposed as the best animal model with which to study the pathogenesis of HIV-1 and therapy for humans infected with the virus (Arjona et al. 2000). It was first reported in 1987 (Lee et al. 2002) and it has worldwide distribution in domestic cats (Lee et al. 2002, Mosallanejad et al 2012, Arjona et al. 2000). Seroprevalence of FIV ranges from1% of low-risk cats in the United States and Canada to 44% of sick pet cats in Japan (Lee et al. 2002). Five different subtypes have been recognized for FIV (subtypes A, B, C, D, and E). In natural settings, FIV is transmitted primarily by parenteral inoculation of virus present in saliva or blood, presumably by bite and fight wounds, accounting for the higher prevalence in male cats (Mosallanejad et al. 2012), but it is also transmitted in utero or through colostrum and milk (Arjona et al.2000).

The feline immunodeficiency virus (FIV) is unique among the non-primate lentiviruses in that in its natural host species, the domestic cat, it induces a disease similar to AIDS, characterised by a progressive depletion of CD4+ T lymphocytes. Consequently, an immunodeficiency syndrome develops (Shimojima et al. 2004) for months or even years (Arjona et al. 2000), characterised by wasting, neurological manifestations, chronic stomatitis and gingivitis and an increased incidence of lymphoma (Shimojima et al. 2004). Progression of the disease follows a pattern typical of that observed with primate lentiviruses, starting with a relatively short (weeks) acute phase denoted by increasing viral loads, febrile episodes, weight loss, lymphadenopathy and neutropenia. The acute phase is followed by an of t-protracted (years) asymptomatic phase denoted by relatively strong antiviral immune responses, lower viral titers, a gradual decline in CD4+ cells, and minimal clinical symptoms. The terminal phase is marked by immunologic decompensation, exacerbation of plasma viral load, and clinical symptoms of immunodeficiency with opportunistic infections. Lymphoid tissue

alterations include thymic depletion, lymphoid hyperplasia, plasmocytosis, and terminal lymphoid depletion. As the disease progresses, the decline in the number of CD4+ T cells continues, with ultimate increase in viral load in the later stages of the disease. Animals, if not euthanized, generally die of opportunistic infections (Elder et al. 2008).

1.2. Laboratory Abnormalities

Common abnormalities on the Complete Blood Count in cats infected with FIV include lymphopenia and neutropenia. Occasionally monocytopenia or leukocytosis occurs. The most common and significant abnormality on the chemistry panel in cats infected with FIV is hyperproteinemia, which results from increased γ-globulin concentrations and is a direct result of FIV infection (rather than the result of opportunistic infection) (Sykes 2014). Hypergammaglobulinemia primarily results from increases in IgG; it reflects polyclonal B-cell stimulation because the IgG produced is not FIV specific. It is a direct consequence of FIV infection because healthy FIV-positive SPF cats are also hypergammaglobulinemic (Greene 1998).

1.3. Current options of treatment for FIV

Despite the significant achievements and understanding of FIV biology, this virus is still a significant source of morbidity and mortality in felines and the treatment options for infected cats are ineffective, toxic, or cost-prohibited. The lack of effective treatment options leads most owners to opt for euthanizing or provide palliative care for FIV infected cats (Supeanu et al. 2015). FIV-related disease has been treated in various ways and with varying efficacy. The principal agents evaluated during the past 20 years include interferons (IFNs), other immunomodulators (eg, staphylococcal protein A, Serratia marcescens membrane/ribosome preparation, inactivated parapoxovis virus, and acemannan), and antiretroviral agents (eg, azidodideoxythymidine, dideoxycytidine, phosphomethoxyethyl-adenine, and suramin), either alone or in combination (de Mari et al. 2004).

1.4. IgY

Recently, the utilisation of Immunoglobulin Y (IgY) from egg chickens yolks immunized against certain pathogens has been the focus of attention in immunotherapy and immunodiagnosis, since IgY antibodies are the predominant serum immunoglobulin in birds, reptiles and amphibia and are transferred to confer passive immunity to embryos and neonates (Sudjarwo et al. 2014).

Three immunoglobulin classes have been shown to exist in chickens: IgA, IgM, and IgY (Kovacs-Nolan & Mine 2004). Immunoglobulin Y (IgY) is the major antibody produced by chickens (Gallus gallus domesticus) (Bentes et al. 2015) and makes up for about 75% of the total antibody population (Kovacs-Nolan & Mine 2004). It is continuously synthesised at a large scale, secreted into the blood and transferred to the egg yolk, where it accumulates (Bentes et al. 2015). Chicken IgY is the functional equivalent of IgG, the major serum antibody found in mammals (Kovacs-Nolan & Mine 2004), but it shows fundamental structural differences in comparison to the IgG molecule (e.g., higher molecular weight than that of mammalian IgG, the presence of an additional CH domain and two carbohydrate sidechains, a lower content of β-sheet structure, and more disordered structure compared with mammalian IgG). It also differs in proteolysis, temperature, pressure, and freezing stability. The IgY binds neither to proteins A and G, nor to the mammalian complement, and does not cross-react with mammalian immunoglobulins (Polanowski et al. 2012). In mammals, the transfer of maternal antibodies can take place after birth; however, in chickens, the maternal antibodies must be transferred to the developing embryo, to give acquired immunity to the chick. Serum IgY is selectively transferred to the yolk, which may contain from 5 to 25mg/ml of IgY.

Chicken eggs present an ideal alternative antibody source to mammals, as the IgY in the chickens' blood is transported to the egg where it accumulates in large quantities (Kovacs-Nolan & Mine 2004). The method of producing antibodies in hens is much less invasive, requiring only the collection of

eggs (Kovacs-Nolan & Mine 2004), rather than the collection of blood by venipuncture (Goldring et al. 2003), and is therefore less stressful on the animal, and sustained hightitres in chickens reduce the need for frequent injections. The animal care costs are also lower for the chicken compared to that for mammals. In the egg, IgY is stable for months, and once purified it may be stored for years in the cold. As the industrial scale automated collection and separation of eggs is currently carried out, the large-scale production of specific IgY for immunotherapeutic purposes is feasible (Kovacs-Nolan & Mine 2004). In contrast to mammalian IgG, IgY antibodies do not activate mammalian complement, do not cross-react with Fc receptors, with mammalian rheumatoid factor, or with human anti-mouse antibodies, thus eliminating false-positive results in serological assays. Also, chickens are able to develop a better response against mammalian antigens, due to the phylogenetic distance between mammals and birds (Sudjarwo et al. 2014).

2. MATERIALS AND METHODS

8 FIV positive cats were included in the study over a 5 months period (September 2015-February 2016), out of which 7 survived to the end of the treatment period of 10 days. The aim of the study was that of observing the impact of nonspecific IgY treatment on some of the components of the immune system of FIV infected cats: White Blood Cells and Serum Proteins, especially Gamma-proteins, mostly in respect to the life quality improvement. The study was performed according to the in force ethic procedures.

The IgY used is a polyclonal IgY, more exactly obtained from chickens inoculated with the following bacteria: *Acinetobacter baumannii*, *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Clostridium difficile-spori*, *Clostridium difficile-corpibacterieni*, *Clostridium difficile-anatoxina*, *Candida albicans*, *Helicobacter pylori*, *Streptococcus mutansand* is labelled as a food-supplement. The IgY was administered daily for 10 days, at a dosage of 10 mg, PO.

Symptomatic and supportive therapies implemented for side pathologies:

- 5 patients, due to the presence of bacterial infections, also received antibiotics therapy; of the five cases, 4 responded well to the antibiotics, while one case failed to improve its respiratory condition;
- 2 patients received supportive respiratory treatment for their upper respiratory chronic syndrome;
- 1 patient received antidiarrheal therapy for its chronic digestive disorders.

3. PATIENT SELECTION AND ANAMNESIS

Table 1. General info of the 7 cats included in the study that survived the 10 days treatment period

Patient nr.	**Age (Years)**	**Sex, Hormonal status**	**Clinical status**	**Supportive care**
1.	6	Male, Neutered	Chronic proliferative gingivitis	Steroidal anti-inflammatory therapy
2.	5	Female, Neutered	Bacterial dermatitis Uveitis	Antibiotics,topic treatment for uveitis, antiviral therapy
3.	14	Female, Neutered	Respiratory upper chronic disease	Antibiotics, liver and kidney failure treatment, supportive respiratory therapy
4.	9	Male, Neutered	Respiratory upper chronic disease	Antibiotics, supportive respiratory therapy
5.	6	Male, Neutered	Clinically healthy	No supportive care
6.	8	Male, Neutered	Periodontitis	Antibiotics
7.	12	Female, Stray	Respiratory upper chronic disease	Antibiotics, supportive respiratory therapy

4. DIAGNOSIS ASSAYS

Consequent the clinical and paraclinical examinations, the preliminary orientation diagnostics were based on quick tests (Fassisi FIV, FassisiFeLV/FIV). The tests used for disease confirmation were ELISA or Western Immunobloting.

5. CLINICAL AND PARACLINICAL ASSAYS

Blood samples were collected prior and post the IgY administration and were used for the determination of WBC count and the electrophoresis of serum proteins.

Table 2. Clinical manifestations (investigations and anamnesis) prior treatment of the 7 cats included in the study that survived the treatment period

Clinical sign	**Number of FIV patients**
Modified appetite	5
Weight loss	4
Respiratory upper chronic disease	4
Periodontitis	4
Fatigue	3
Lymphadenopaty	2
Fever	2
Diarrhea	1
Vomiting	1
Uveitis	1
Dermatitis	1

Table 3. Clinical manifestations for each patient before treatment

Note: Patient no. 5 was clinically healthy when it received the IgY treatment

Patient no.		**1**	**2**	**3**	**4**	**5**	**6**	**7**
Clinical sign	Modified appetite	X		X	X		X	X
	Weight loss	X		X			X	X
	Respiratory upper chronic disease			X	X		X	X
	Periodontitis	X		X			X	X
	Fatigue			X	X			X
	Lymphadenopaty	X					X	
	Fever				X		X	
	Diarrhea						X	
	Vomiting						X	
	Uveitis		X					
	Dermatitis		X					

6. RESULTS AND DISCUSSIONS

Chart 1. Evolution of Absolute Number of White Blood Cells before and after treatment

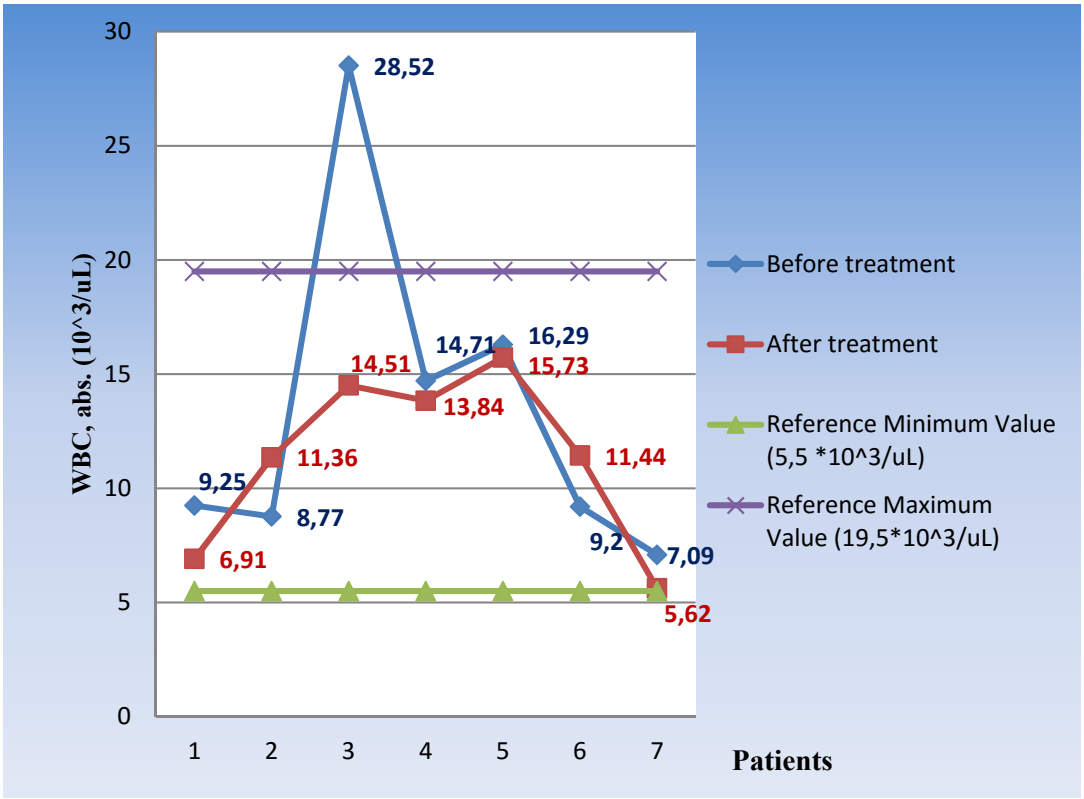


Chart 2. Evolution of Absolute Number of Neutrophils before and after treatment

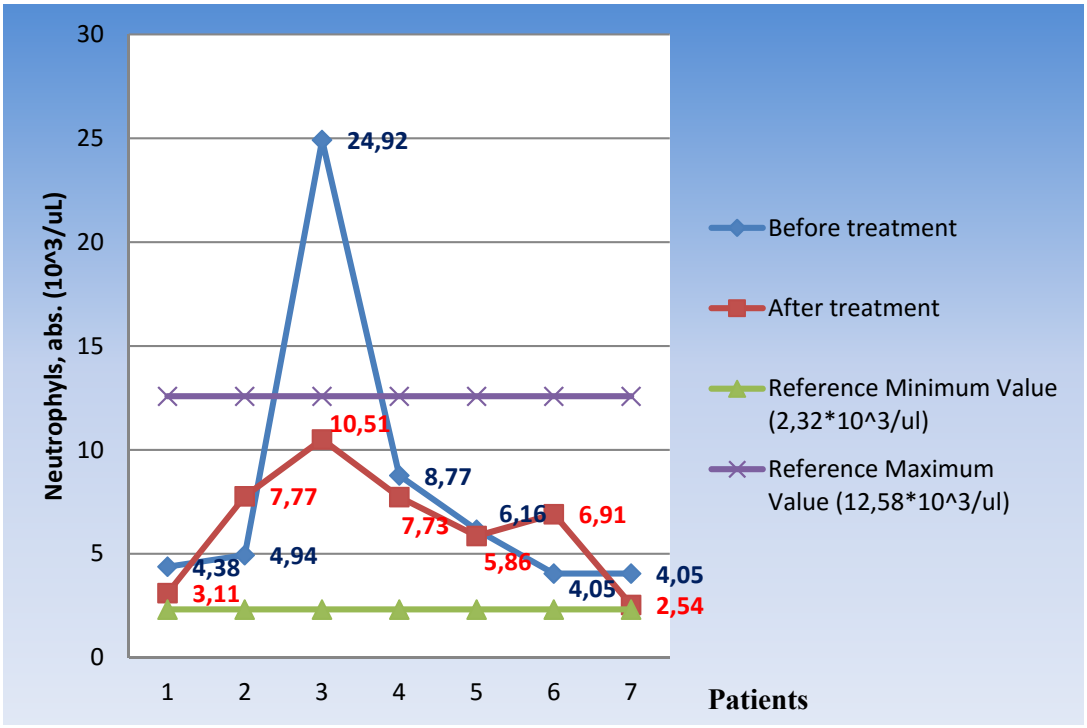


Chart 3. Evolution of Absolute Number of Lymphocytes before and after treatment

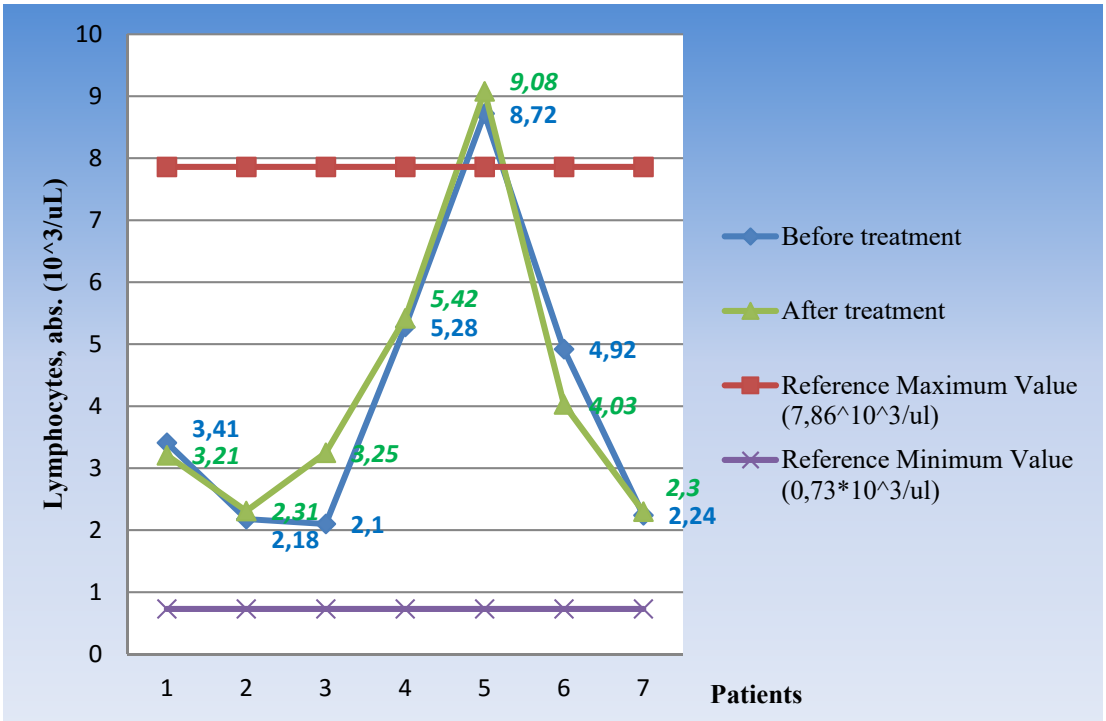


Chart 4. Evolution of Absolute Number of Monocytes before and after treatment

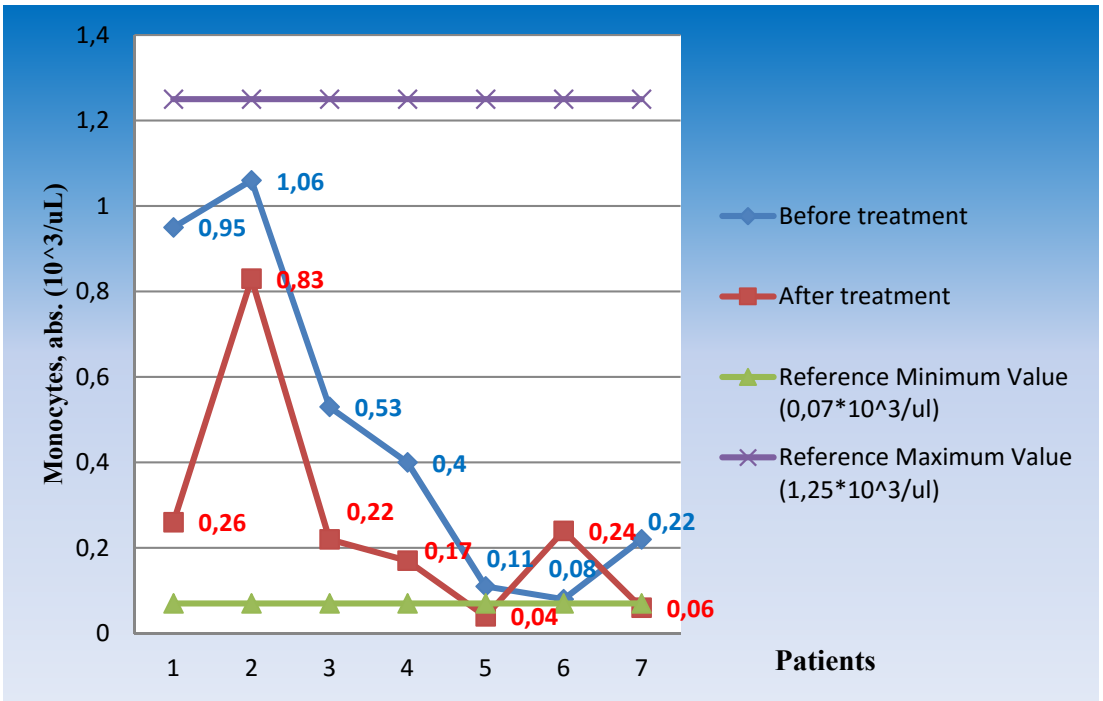


Chart 5. Evolution of Absolute Number of Eosinophils before and after treatment

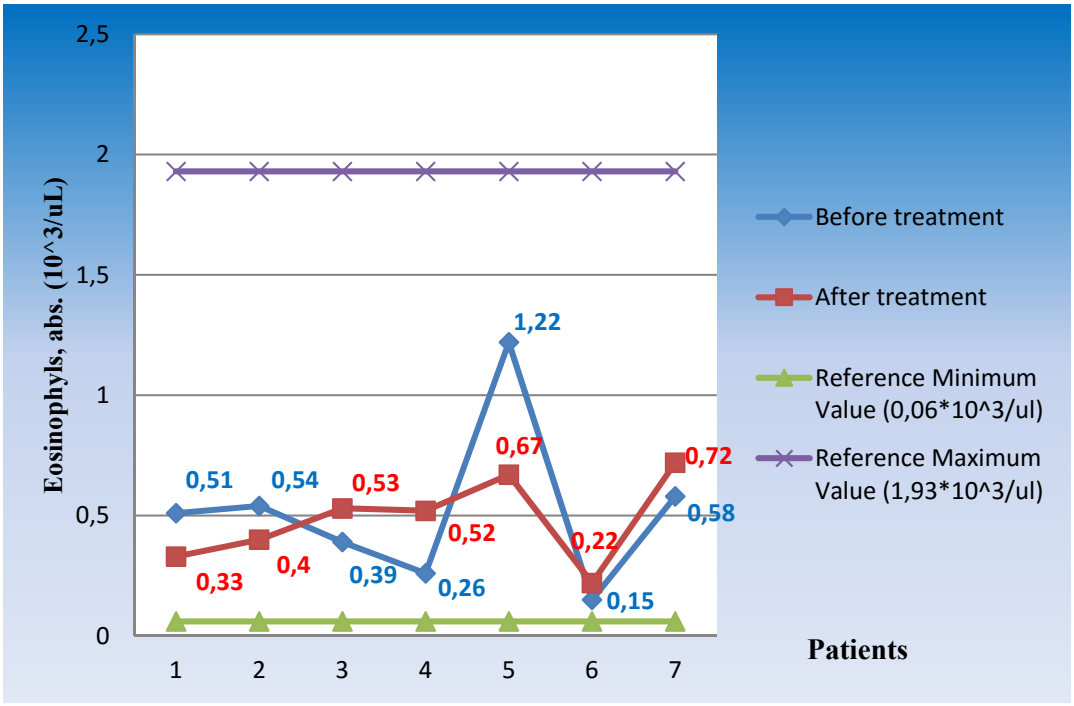


Chart 6. Evolution of Absolute Number of Basophils before and after treatment

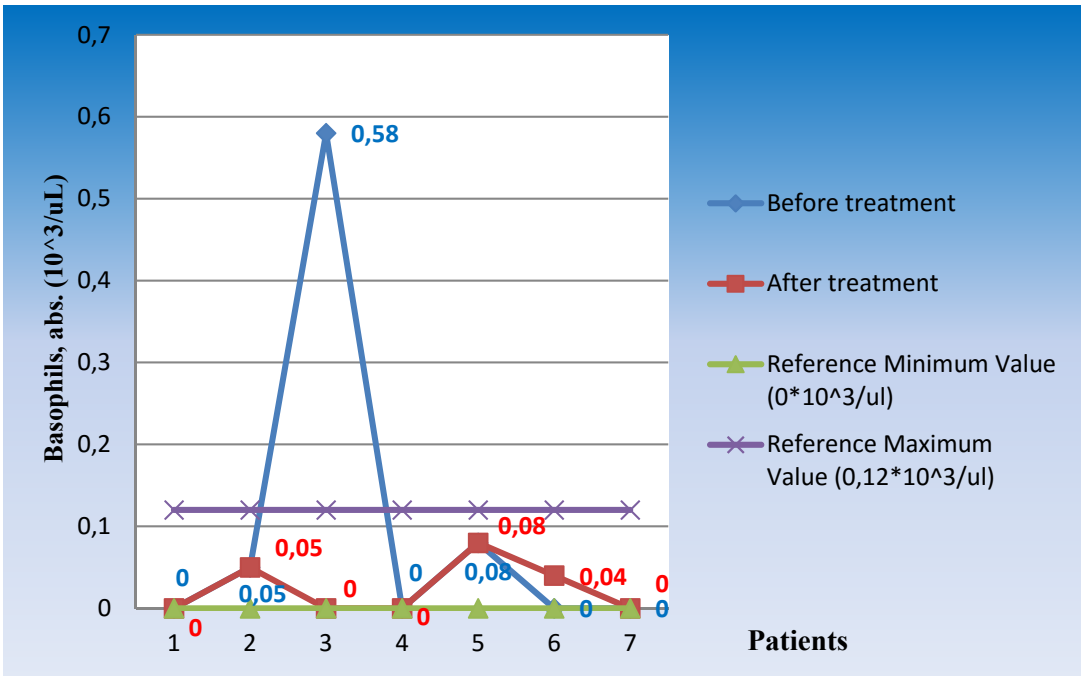


Chart 7. Evolution of Absolute Value of Total Proteins before and after treatment

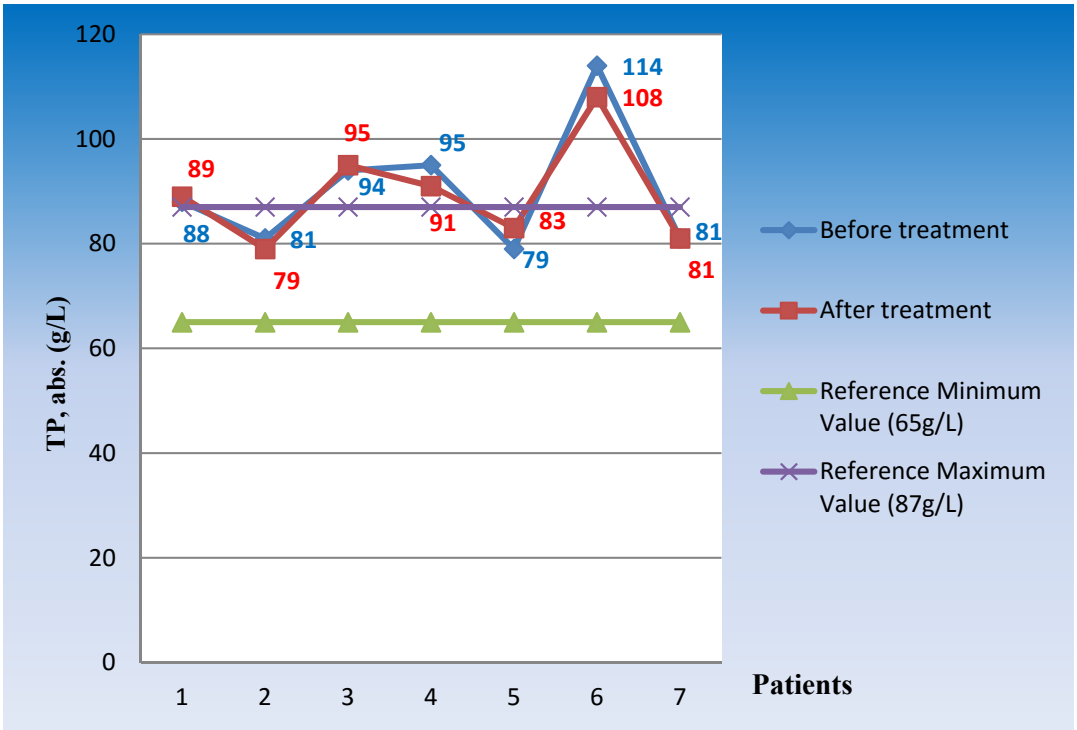


Chart 8. Evolution of Absolute Value of Albumin before and after treatment

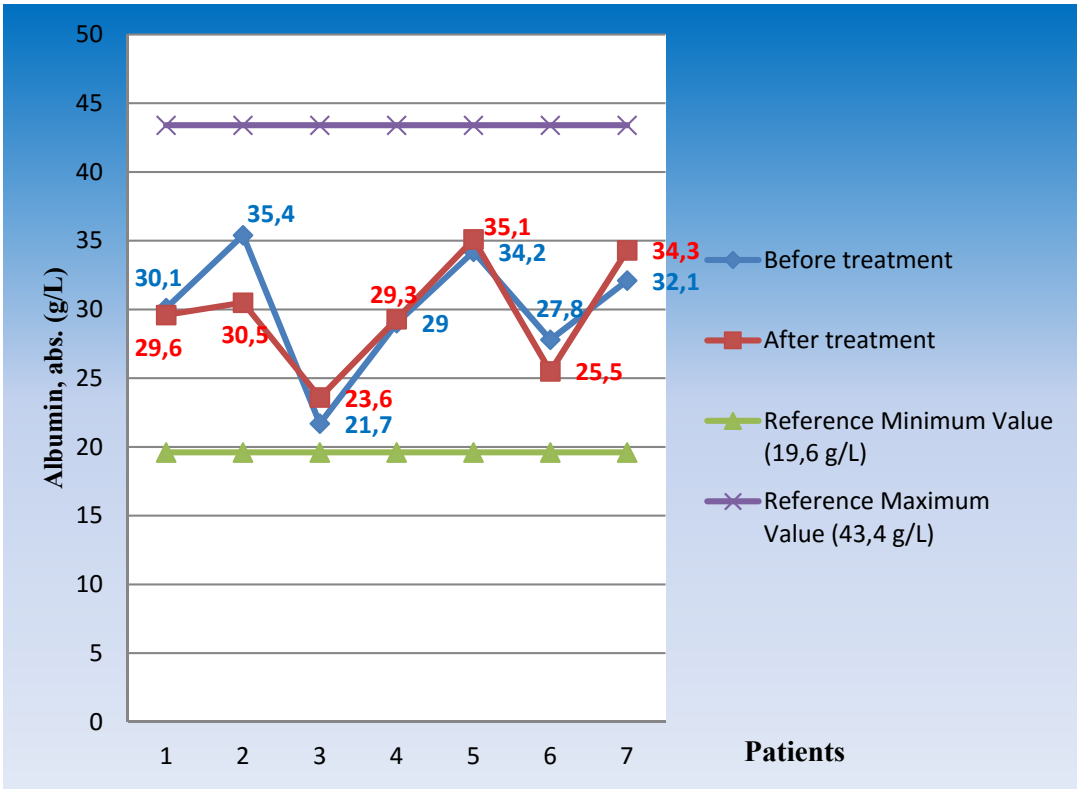


Chart 9. Evolution of Absolute Value of Alpha-globulin before and after treatment

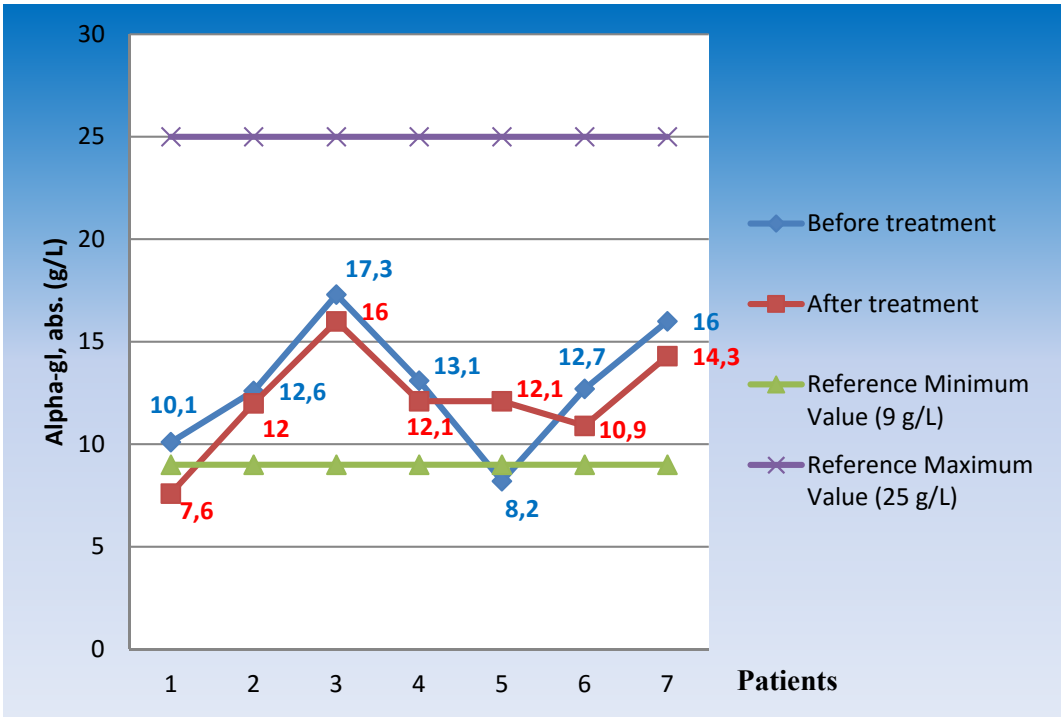


Chart 10. Evolution of Absolute Value of Beta-globulin before and after

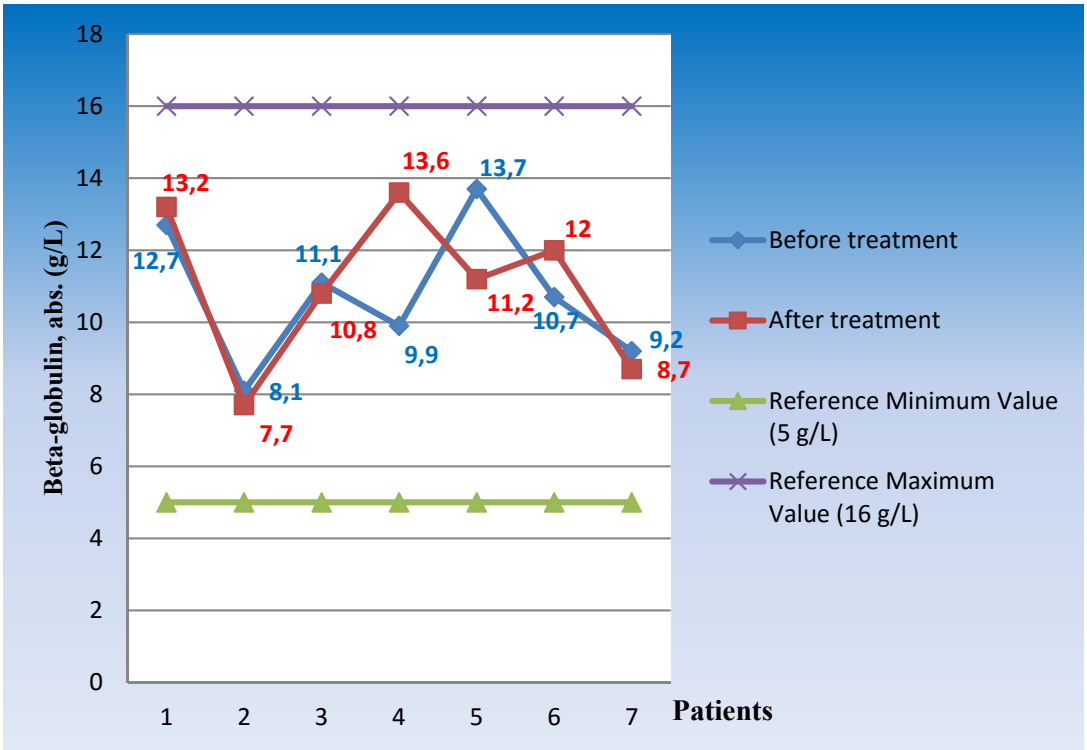


Chart 11. Evolution of Absolute Value of Gamma-globulin before and after treatment

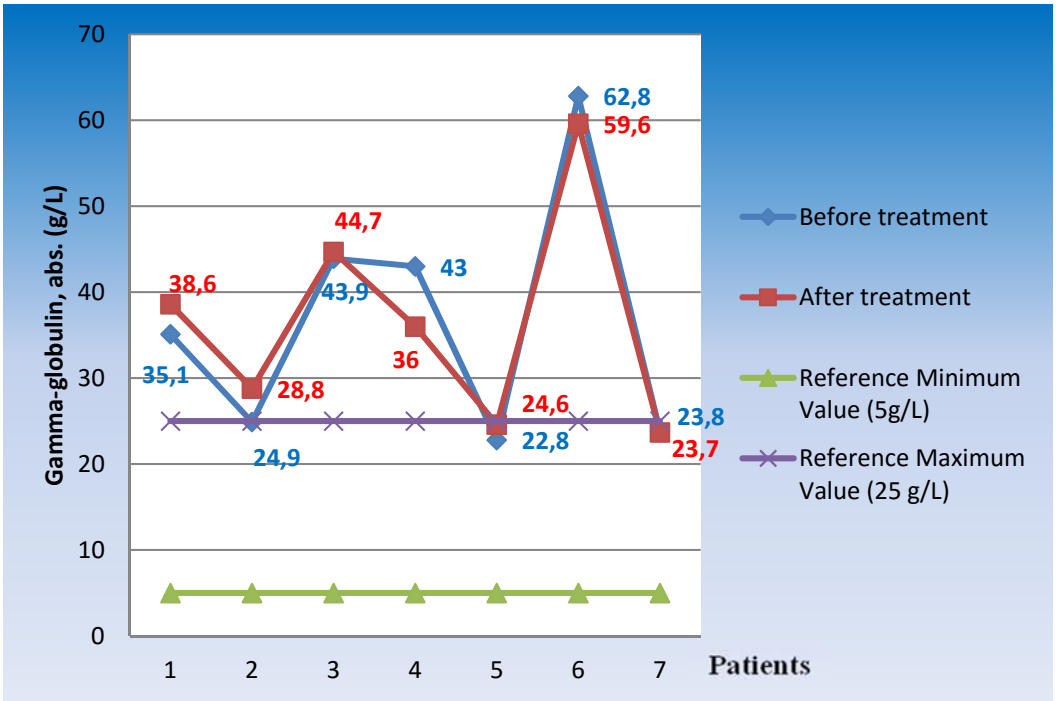


Chart 12. Evolution of Albumin/Globulin Ratio before and after treatment

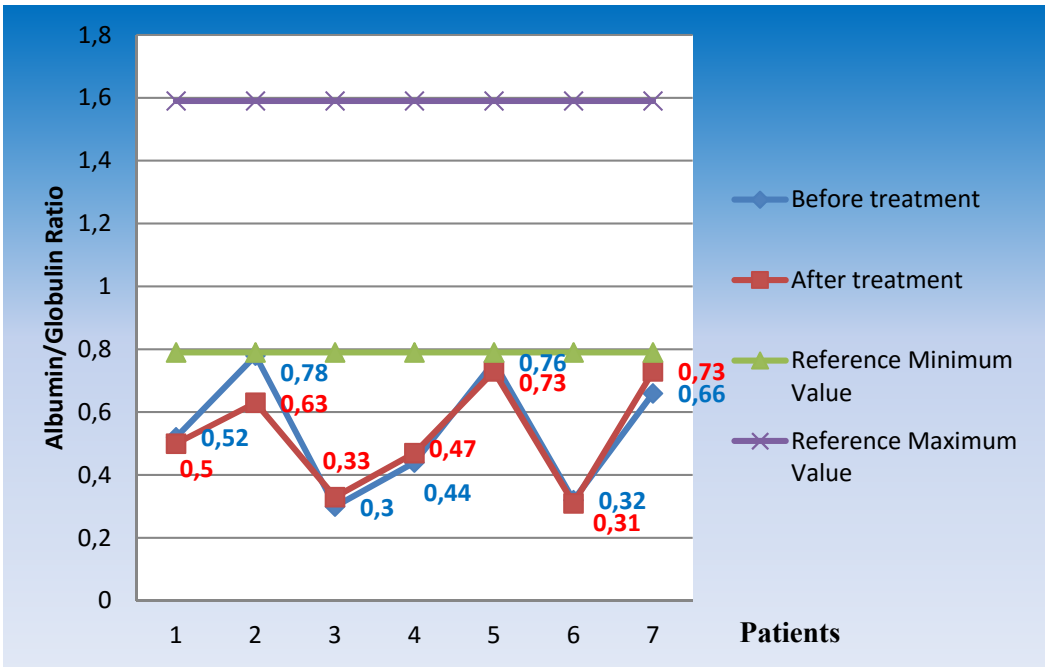


Table 4. Results of the WBC counts before IgY treatment (no. of patients)

Parameter	Increased	Decreased	Physiological
White Blood Cells	1	0	6
Neutrophils%	1	1	5
Lymphocytes%	2	1	4
Monocytes%	1	2	4
Eosinophils%	0	0	7
Basophils%	1	0	6
Neutrophils#	1	0	6
Lymphocytes#	1	0	6
Monocytes#	0	0	7
Eosinophils#	0	0	7
Basophils#	1	0	6

Table 5. Results of the WBC counts after IgY treatment (no. of patients)

Parameter	Increased	Decreased	Physiological
White Blood Cells	0	0	7
Neutrophils%	0	1	6
Lymphocytes%	2	0	5
Monocytes%	0	1	6
Eosinophils%	1	0	6
Basophils%	0	0	7
Neutrophils#	0	0	7
Lymphocytes#	1	0	6
Monocytes#	0	2	5
Eosinophils#	0	0	7
Basophils#	0	0	7

Table 6. Evolution of the feline WBC counts before and after IgY treatment (no. of patients)

Parameter	Raised	Diminished	Maintained
White Blood Cells	2	5	0
Neutrophils%	2	5	0
Lymphocytes%	5	2	0
Monocytes%	1	6	0
Eosinophils%	4	3	0
Basophils%	1	2	4
Neutrophils#	2	5	0
Lymphocytes#	5	2	0
Monocytes#	1	6	0
Eosinophils#	4	3	0
Basophils#	1	1	5

Table 7. Results of the electrophoresis of serum proteins before IgY treatment (no. of patients)

Parameter	Increased	Decreased	Physiological
Total protein (g/L)	4	0	3
Albumin (%)	0	3	4
Albumin, abs. (g/L)	0	0	7
Alpha-globulin (%)	0	2	5
Alpha-globulin, abs. (g L)	0	1	6
Beta-globulin (%)	0	0	7
Beta-globulin, abs. (g/ L)	0	0	7
Gamma-globulin (%)	7	0	0
Gamma-globulin, abs. (g/ L)	4	0	3
Albumin/globulin ratio	0	7	0

Table 8. Results of the electrophoresis of serum proteins after IgY treatment (no. of patients)

Parameter	Increased	Decreased	Physiological
Total protein (g/ L)	4	0	3
Albumin (%)	0	4	3
Albumin, abs. (g/ L)	0	0	7
Alpha-globulin (%)	0	3	4
Alpha-globulin, abs. (g/ L)	0	1	6
Beta-globulin (%)	0	0	7
Beta-globulin, abs. (g/ L)	0	0	7
Gamma-globulin (%)	7	0	0
Gamma-globulin, abs. (g/ L)	6	0	2
Albumin/globulin ratio	0	7	0

Table 9. Evolution of the serum proteins before and after IgY treatment (determined by electrophoresis) (no. of patients)

Parameter	Raised	Diminished	Maintained
Total protein (g/ L)	3	3	1
Albumin (%)	3	4	0
Albumin, abs. (g/ L)	4	3	0
Alpha-globulin (%)	3	4	0
Alpha-globulin, abs. (g/ L)	2	6	0
Beta-globulin (%)	3	3	1
Beta-globulin, abs. (g/ L)	3	3	1
Gamma-globulin (%)	4	2	1
Gamma-globulin, abs. (g/ L)	4	2	1
Albumin/globulin ratio	4	3	0

Table 10. Clinical manifestations which have improved after treatment for each patient

"↗" = improved "→" = mentained

Patient no.		**1**	**2**	**3**	**4**	**5**	**6**	**7**
Clinical sign	Modified appetite	↗	—	→	↗	—	↗	↗
	Weight loss	↗	—	→	—	—	↗	→
	Respiratory upper chronic disease	—	—	→	↗	—	→	↗
	Periodontitis	↗	—	→	—	—	↗	→
	Fatigue	—	—	→	↗	—	—	→
	Lymphadenopaty	↗	—	—	—	—	↗	—
	Fever	—	—	—	↗	—	↗	—
	Diarrhea	—	—	—	—	—	→	—
	Vomiting	—	—	—	—	—	→	—
	Uveitis	—	↗	—	—	—	—	—
	Dermatitis	—	↗	—	—	—	—	—

Table 11. Clinical manifestations which have improved after treatment for all patients

Clinical sign	**Number of FIV patients**
Modified appetite	4/5
Weight loss	2/4
Respiratory upper chronic disease	2/4
Periodontitis	2/4
Fatigue	1/3
Lymphadenopaty	2/2
Fever	2/2
Diarrhea	0/1
Vomiting	0/1
Uveitis	1/1
Dermatitis	1/1

Searching the literature, we can find a series of articles quoting the occasional presence of leukocytosis in FIV positive cats. For the present study, a single patient showed leukocytosis prior the treatment, due to the presence of neutrophilia and basophilia, which improved consequent the treatment. The rest of the study patients were characterized by the total leukocyte number being within physiological parameters.

The same literature confirms the fact that FIV positive cats often show neutropenia. In contrast, for the case of the present study, none of the tested cats tested positive for this pathology. However, one cat showed neutrophilia, that normalized consequent the administration of the treatment and 6 positive FIV patients had normal values for neutrophils, both prior and after treatment.

In most cases FIV positive cats are characterized by lymphopenia. In the present study, none of the patients showed this condition. Six patients had the lymphocytes values within normal parameters, both prior and after the treatment, whereas one cat that had lymphocytosis maintained its ascending values following the treatment.

The monocytes and eosinophils were within physiological parameters, both prior and after the treatment, in all tested animals.

Except for a single patient that was characterized as being clinically healthy, the rest of the group showed a various clinical symptomatology that was associated with the WBC and serum proteins values.

The patients accepted in the study had no signs of changes of the white blood count, that are specific to the FIV infection (except for one patient that was assessed as having leukocytosis), a fact that these modifications only stand as findings and are unfit for being used as compiling a certainty diagnosis. These abnormalities are rather determined by the symptomatology of opportunistic secondary clinical infections.

In respect to the serum proteins, 5 out of 7 patients showed elevated gamma-globulins levels that remained high until the end of the treatment with IgY. The other 2 patients showed gamma-globulins levels within physiological parameters.

All patients assessed with opportunistic infections showed hypergammaglobulinemia, but this is more characteristic to FIV, when considering the fact that the clinically healthy patient had high gamma-globulins values. The elevated values of these gamma-globulins determined high values of serum proteins in 4 patients and the albumins/globulins ratio was lower than the physiological limits for all patients, both prior and after the treatment.

In all patients, the absolute counts of albumins, alpha-globulins and beta-globulins were within physiological parameters, both prior and after the treatment. Their variations do not seem to be under the influence of the IgY treatment.

CONCLUSIONS

Preliminary data showed that administering chicken egg yolk IgY to FIV infected felines has no side effects. The WBC count seem to evolve toward the median average values, but maybe, the upgrade of the posology or longer observation period, could add relevant information. The albumin and the globulin level as well as their ratio, also seem to evolve toward the phisyological values. Larger number of subjects, expressing WBC count and biochemical blood values in the range of "characteristic to FIV positive cats" will allow to evaluate of the egg yolk IgY.

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