

Targeting the Cytokine Network with Autologous Biological Therapy: Case-Based Evidence of Immune Modulation in Rheumatoid Arthritis

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Abstract

Cytokine imbalance is a defining feature of rheumatoid arthritis, where persistent immune activation sustains chronic synovial inflammation and systemic symptoms beyond articular involvement. Conventional therapies target broad inflammatory mediators but often lack precision in restoring immune homeostasis, leading either to insufficient response or overt suppression. This pilot study investigates whether low-dose administration of autologous cytokine fractions may induce targeted immune modulation in patients with rheumatoid arthritis. Nine subjects with confirmed diagnosis of active disease and laboratory profiles indicating cytokine dysregulation underwent peripheral blood collection to isolate plasma-derived cytokines via magnetic affinity extraction. The obtained extracts were processed to generate multicomponent bioactive fractions enriched in regulatory cytokines, then administered intramuscularly according to a standardized protocol. Serum cytokine levels, including interleukin 1 alpha and interleukin 6 as representative markers of chronic inflammation, were assessed at baseline and after six months of treatment. A consistent trend toward cytokine normalization was observed across the cohort. Interleukin 1 alpha showed a notable reduction, particularly among male patients, while interleukin 6 demonstrated a more pronounced decrease in females, suggesting sex-related variability in responsiveness. Clinical improvement was reported in parallel, with reduction of morning stiffness, fatigue and joint pain in subjects previously unresponsive to standard care. No adverse reactions or paradoxical flares were documented. The overall pattern supports the hypothesis that autologous cytokine fractions may act not as pharmacological substitutes but as regulatory signals capable of guiding immune recalibration. Rather than suppressing inflammation indiscriminately, this approach appears to promote a physiological rebalancing of cytokine dynamics, potentially enhancing tolerogenic pathways while limiting pathogenic activation. Although preliminary and observational in nature, these findings introduce autologous cytokine therapy as a plausible biological strategy for immune modulation in rheumatoid arthritis and encourage further controlled investigations to define its integration with conventional treatments.

Keywords: Cytokine; Inflammation; Immunomodulation; Cytokine Modulation; Biological Therapy; Autologous Therapy; Low-Dose Immunotherapy

Abbreviations

Ag: Antigen; Ab: Antibody; VAS: Visual Analogue Scale; RA: Rheumatoid Arthritis; SD: Standard Deviation; IL: Interleukin; TNF- α : Tumor Necrosis Factor-Alpha; TGF- β : Transforming Growth Factor-Beta; anti-CCP: Anti-Cyclic Citrullinated Peptide; RF: Rheumatoid Factor

Introduction

The immune system is an interconnected network of organs, tissues, cells, and molecules distributed throughout the body. Among its components, plasma plays a central role, as it contains approximately 90% of the elements involved in tissue reaction and regeneration, making it a true reservoir of biological factors with reparative or stimulatory functions.

At the core of the immune response lies the interaction between antigen (Ag) and antibody (Ab). The binding between the antigenic epitope and the variable region of the antibody is highly specific: each antibody binding site recognizes a specific antigenic determinant. Structurally, antibodies consist of Fab regions, responsible for antigen binding, and Fc regions, which mediate interactions with cells and components of the innate and adaptive immune systems, such as natural killer cells, phagocytes, and complement. The Fc regions are fundamental for the *in vivo* efficacy of passive immunotherapies, and monoclonal antibodies are employed precisely for their neutralizing capacity, selectivity for specific epitopes, and Fc-mediated effector functions [1].

In the context of allergies, the immune system misinterprets the allergen as a threat, activating mast cells and eosinophils and inducing excessive production of cytokines such as IL-4, IL-5, and IL-13, which leads to the release of histamine and other inflammatory mediators [2]. Allergen-specific immunotherapy, introduced by Leonard Noon in 1911, represents the only validated etiological treatment: it consists of the controlled and repeated administration of the allergen to induce immune tolerance, modulating rather than suppressing the immune response [3-5].

The state of immunity, whether specific or nonspecific, toward an antigen can be induced either by vaccine administration (active immunization) or by the introduction of exogenous antibodies (passive immunization). This principle of immunization is supported by extensive experimental evidence, both in animal models and clinical studies, demonstrating its capacity to induce immune memory and protection [6,7].

In allergology, some evidence suggests that the administration of microdoses of antigens may lead to a faster and better-tolerated desensitization compared to conventional high-dose protocols, with a lower incidence of systemic adverse events [8]. The use of microdoses of bioactive molecules - including antigens, hormones, antibodies, or cytokines - is being explored for its ability to interact with the immune system in a non-aggressive manner, respecting natural endogenous regulatory mechanisms and enhancing immunological tolerance. These approaches share the goal of rebalancing rather than suppressing the immune response, acting physiologically and in accordance with the self-regulatory properties of the immune system.

In light of these observations, an alternative therapeutic paradigm is emerging, in which cytokine administration may represent a viable strategy for the treatment of inflammatory conditions, minimizing the risk of side effects and promoting the restoration of immune tolerance [9,10].

Within the immune system, cytokines play a pivotal role. They are signaling molecules whose primary function is to activate, recruit, or inhibit the actions of immune cells. Each cytokine is capable of naturally triggering processes of self-regulation, repair, and regeneration of damaged tissues. Their release is tightly regulated: under homeostatic conditions, serum levels remain below threshold, thereby preventing unnecessary immune responses [11,12]. Alterations in this regulatory network underline numerous immune-mediated diseases, in which the imbalance of interleukins and cellular growth factors drives a shift toward a dysfunctional immune response [13]. Such dysregulation can manifest either as chronic hyperactivation or insufficient immune response two facets of the same pathogenic mechanism.

Conditions characterized by abnormal cytokine expression include systemic lupus erythematosus, multiple sclerosis, psoriasis, and chronic inflammatory bowel diseases. These observations have reinforced the paradigm that cytokines are not merely biomarkers of inflammation but true pathogenic drivers and therefore represent key therapeutic targets.

Among autoimmune disorders, rheumatoid arthritis (RA) represents one of the most extensively studied models of cytokine network dysregulation. It is characterized by the overexpression of TNF- α , IL-1 β , and IL-6, which promote synovial proliferation and cartilage destruction [13]. In parallel, a reduction in the regulatory activity mediated by IL-10 and TGF- β has been described, contributing to the loss of tolerance toward self-components [14]. The clinical efficacy of biological agents and cytokine-targeting drugs further confirms the central pathogenic role of cytokines in RA [15].

Within this perspective, the use of autologous cytokine fractions - extracted from the patient’s plasma and separated into their respective α and β subunits - has been proposed as a strategy to inhibit overexpressed cytokines or stimulate deficient ones, in accordance with the physiological logic of endogenous regulation. The use of autologous cytokine fragments represents a nonconventional approach: the patient’s own blood serves as the source of cytokines, which are purified and fractionated into α and β subunits for personalized therapeutic use. This approach offers advantages in terms of reduced immunogenicity, targeted response, and individual adaptability to the patient’s profile compared with recombinant molecules or standard monoclonal antibody therapies [16].

Aim of the Study

The present study aims to evaluate this approach in a cohort of patients with rheumatoid arthritis and documented cytokine imbalance, assessing the potential for directional modulation of the immune profile through the administration of personalized cytokine-based therapy.

Materials and Methods

Study design

The study involved the clinical and laboratory observation of nine patients (5 females, 4 males; aged 42-62 years, mean age 50 $\pm$ SD 6.44 years) diagnosed with rheumatoid arthritis, confirmed by seropositivity for anti-CCP antibodies and/or rheumatoid factor (Table 1).

Subject	Gender	Age	Anti-CCP antibody (U/mL)	RF (UI/mL)
1	F	44	42	34
2	F	45	23	32
3	M	54	39	44
4	M	56	19	14
5	M	42	98	41
6	F	47	43	12
7	M	62	21	35
8	M	51	29	32
9	F	49	54	9

Table 1: Gender, age, CCP antibody and RF of the 9 subjects of this study. The normal range for anti-CCP antibodies is < 20 U/mL. The normal range for rheumatoid factor (RF) is < 15 UI/mL.

The initial screening assessment (T0) was performed prior to the initiation of cytokine therapy. At this point, serum levels of IL-1 α , IL-2, IL-4, IL-6, and IL-8 were analyzed, in addition to a symptom evaluation conducted using the Visual Analogue Scale (VAS).

The VAS is a tool used to measure pain intensity, consisting of a 10-cm line on which the patient marks a point corresponding to their perceived level of pain. The endpoints of the line represent “no pain” (0) and “worst possible pain” (10). The distance in millimeters from the starting point (0) indicates the perceived pain intensity.

The subsequent assessment (T1) was conducted at the end of the cytokine administration cycle, which lasted six months. Table 2 reports the cytokine measurement values obtained at both time points.

	Subject	1	2	3	4	5	6	7	8	9
T0	IL-1 α (pg/mL)	440	380	410	390	480	320	290	380	390
	IL-2 (U/mL)	46	39	-	38	-	40	50	54	52
	IL-4 (U/mL)	120	-	150	165	140	-	-	-	155
	IL-6 (pg/mL)	-	180	140	120	-	170	140	-	110
	IL-8 (pg/mL)	-	1020	-	-	-	-	-	-	-
T1	IL-1 α (pg/mL)	190	220	120	100	200	280	190	110	190
	IL-2 (pg/mL)	22	12	-	22	-	29	31	32	38
	IL-4 (pg/mL)	99	-	102	88	110	-	-	-	80
	IL-6 (pg/mL)	-	90	110	80	-	120	105	-	75
	IL-8 (pg/mL)	-	720	-	-	-	-	-	-	-
	Symptoms (T0)	6	5	7	2	4	7	6	6	5
	Symptoms (T1)	4	3	5	2	4	3	2	4	1

Table 2: The cytokine measurement values in the 9 subjects at T0 and T1.

Blood collection and extraction procedure

The extraction process of autologous cytokines is divided into several phases. First, blood collection is performed, followed by plasma separation through decantation and subsequent plasma extraction. For each cytokine targeted for isolation, the following steps are repeated: suspension of the sample in solution and addition of specific magnetic targets for the cytokine of interest, magnetic separation of cytokines, removal of the magnetic targets, molecular chain disruption of cytokines via centrifugation, separation of the resulting molecular chains, dilution of the sample, and final stabilization and preservation of the obtained cytokine fraction.

Cytokine extraction: Phase 1

The skin was disinfected with cotton soaked in antiseptic solution. Venipuncture was performed with a sterile 10 mL syringe and a 20G/0.9 mm needle, collecting 10 cc of blood. Smaller-gauge needles were avoided to prevent cytokine damage.

The sample was left to decant vertically for 5-48 hours at room temperature (15-25°C), or in a temperature- controlled device for the same period. Decantation was chosen instead of centrifugation, since the latter would irreversibly damage cytokines, compromising the final product.

After decantation, the supernatant plasma (~5 cc) was collected with a micropipette under sterile conditions. Cytokines of interest are in the intermediate layer between plasma and serum. Collecting 1-2 mL of serum ensured retrieval of cytokines without interfering with the activity of magnetic beads. Without this step, extraction of the intermediate cytokine-rich fraction would be incomplete.

Cytokine extraction: Phase 2

Plasma obtained by decantation was suspended in 0.9% NaCl solution containing magnetic microspheres coated with specific ligands for the target cytokine.

For separation, a secondary magnet from the kit was placed against the container wall. The magnet attracted the cytokines bound to the microspheres. The supernatant was removed with a micropipette, isolating the pellet containing cytokine-bound microspheres.

The bound cytokines were then eluted in 0.9% NaCl solution to release them from the microspheres. A magnet was again applied to attract the beads, leaving cytokines free in solution. The cytokine-containing supernatant was collected and centrifuged at 400g for 5 minutes to break cytokine molecular chains.

After centrifugation, the sample rested for 20-30 minutes, followed by differential centrifugation at 4500g for 10 minutes to separate α helices and β sheets. β sheets deposited at the bottom, while α helices remained suspended in a thin layer at the surface. The final sample was diluted 1:1000 in 0.9% NaCl solution for therapeutic use. It was stabilized for 24 hours at -25°C and stored at 0-5°C until administration, with a maximum shelf life of 6 months.

This protocol was repeated for each cytokine of interest. Separation of α and β chains enabled selective use: α subunits for suppression/modulation, β subunits for stimulation.

The choice of chains for administration was based on quantitative analysis of each patient's cytokine profile, determined in the laboratory.

Administration and follow-up

The autologous multicytokine therapy, formulated on an individual basis and strictly personalized for each patient, was administered via intramuscular or subcutaneous injection at peripheral sites. The frequency and dosage were determined according to each patient's baseline cytokine profile and therapeutic goal (inhibition or stimulation). The preparations contained isolated molecular fragments of autologous cytokines, obtained through the separation of α or β subunits from peripheral plasma, following the protocol described in section blood collection and extraction procedure.

The follow-up was structured to include a 6-month evaluation (T1) at the end of the administration cycle, aimed at assessing the trend of the cytokine parameters under investigation.

All cytokine measurements were performed using high-sensitivity chemiluminescent immunoassays. Data were compared longitudinally with baseline values (T0), using the same certified laboratory for all time points.

No adverse events or systemic reactions to the therapy were reported. Data were analyzed in parallel to evaluate correlations between cytokine dynamics and subjective clinical response.

Results and Discussion

The study included the longitudinal observation of nine patients with rheumatoid arthritis (RA) (5 females, 4 males; mean age 50 ± 6.44 years) treated with personalized administration of autologous cytokine fragments in addition to standard pharmacological therapy. Cytokine profiles and symptom assessments were performed at baseline (T0) and at the end of the administration cycle (T1), which

lasted six months. The data demonstrated consistent directional modulation of the cytokine network and symptomatic improvement in all subjects. No adverse events related to the treatment were reported.

Comparative analysis of serum interleukin levels (IL-1 α , IL-2, IL-4, IL-6, and IL-8) revealed a generalized trend toward reduction of pro-inflammatory cytokines (Figure 1), consistent with the therapeutic goal of downregulation in conditions of overexpression.

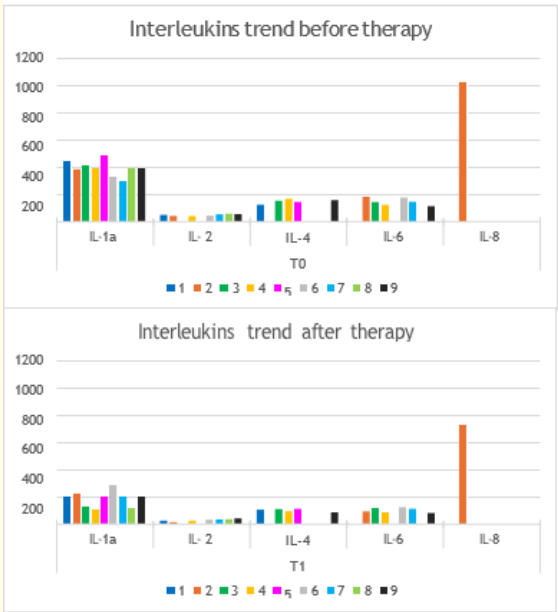


Figure 1: The graph provides an overview of the interleukin levels measured at T0 and T1 in the nine study subjects.

We describe below the specific results obtained for each individual interleukin analyzed.

A reduction in serum levels was observed in all nine patients (Figure 2). Baseline values (T0), ranging from 290 to 480 pg/mL, decreased at follow-up (T1) to a range of 100 to 280 pg/mL. Subject 5 showed the largest absolute change (from 480 to 200 pg/mL), whereas Subject 4 reached the lowest final value (100 pg/mL).

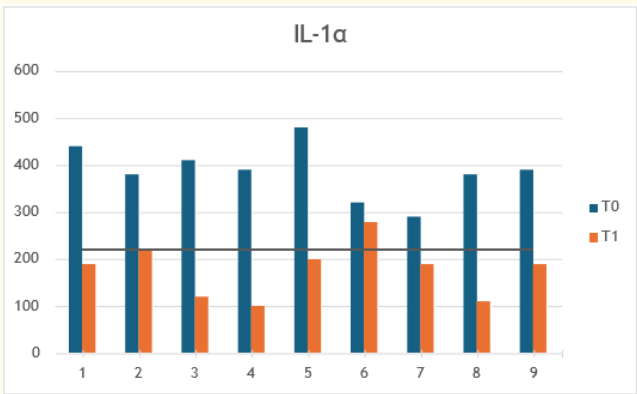


Figure 2: Trend of IL-1 α across the two measurements (T0 and T1) in the nine subjects under study. The highlighted black line represents the normal reference value for IL-1 α , corresponding to 220 pg/mL.

The dynamics of IL-1 α showed sex-specific differences. At baseline (T0), both male and female groups exhibited comparable levels, with a slight tendency toward higher values in females. At the end of the therapeutic cycle (T1), males showed a more pronounced reduction in IL-1 α , decreasing from a mean of 390 pg/mL to 144 pg/mL, suggesting a response more oriented toward downregulation of the pro-inflammatory profile. In females, the reduction was more gradual, while still maintaining a trend toward normalization. This pattern may reflect differences in receptor sensitivity or baseline activation of the inflammatory axis between sexes, as previously observed in autoimmune contexts with female predominance (Figure 3).

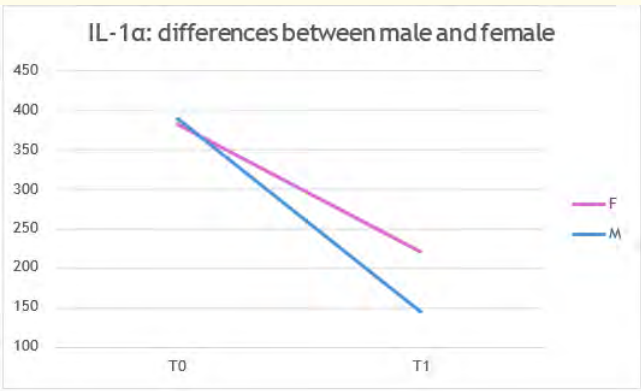


Figure 3: Serum IL-1 α levels in males and females at T0 and T1. A more pronounced reduction is observed in the male group compared to the female group.

In the seven subjects in whom IL-2 was measured, a marked decrease in levels was observed (Figure 4). Patients with baseline overexpression (T0, range 38-54 U/mL) showed a reduction to values ranging between 12 and 38 U/mL at T1.

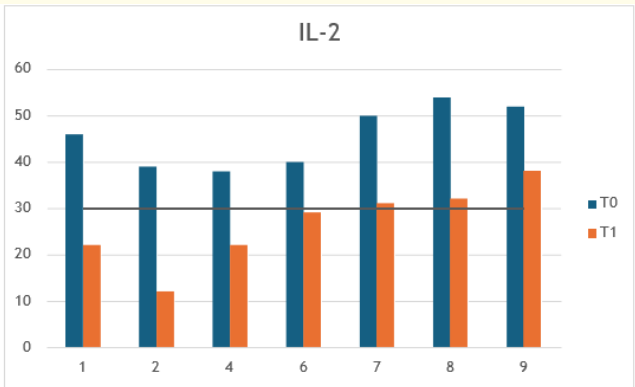


Figure 4: Trend of IL-2 across the two measurements (T0 and T1) in the nine study subjects. The highlighted black line represents the normal reference value for IL-2, corresponding to 30 U/mL.

In all five subjects with altered IL-4 levels, values decreased after therapy. Notably, Subject 4 decreased from 165 to 88 U/mL, while Subject 9 decreased from 155 to 80 U/mL (Figure 5).

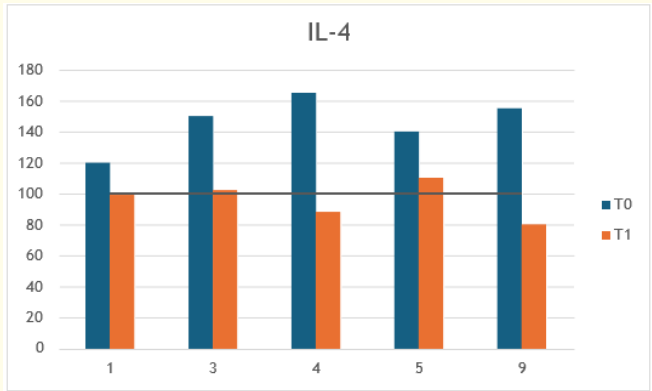


Figure 5: Trend of IL-4 across the two measurements (T0 and T1) in the nine study subjects. The highlighted black line represents the normal reference value for IL-4, corresponding to 100 U/mL.

IL-6, analyzed in the seven patients showing altered levels, was reduced in all cases studied (Figure 6). Baseline levels ranged from 110 to 180 pg/mL and decreased to 75-120 pg/mL at T1, with Subject 2 showing a substantial change from 180 to 90 pg/mL.

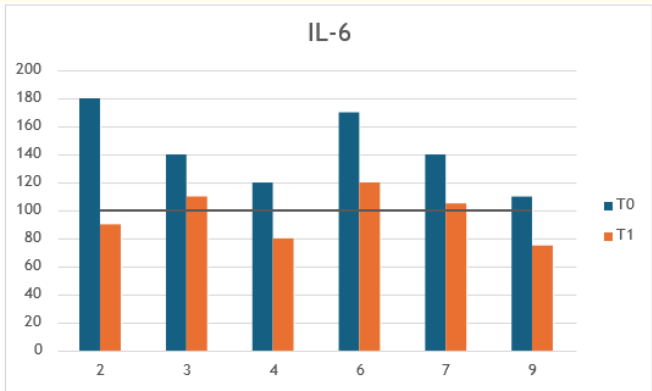


Figure 6: Trend of IL-6 across the two measurements (T0 and T1) in the nine study subjects. The highlighted black line represents the normal reference value for IL-6, corresponding to 100 pg/mL.

IL-6, a key marker of systemic inflammation, exhibited an opposite pattern compared to IL-1 α . In male subjects, the decrease between T0 and T1 was more limited, whereas in females a more pronounced reduction was observed, from a mean of 153.33 pg/mL to 95 pg/mL, suggesting a more favorable response to treatment in terms of chronic inflammation modulation. Interestingly, in some female patients, normalization of IL-6 was accompanied by a more evident subjective clinical improvement, supporting the hypothesis of a closer association between IL-6 and perceived symptoms in females (Figure 7).

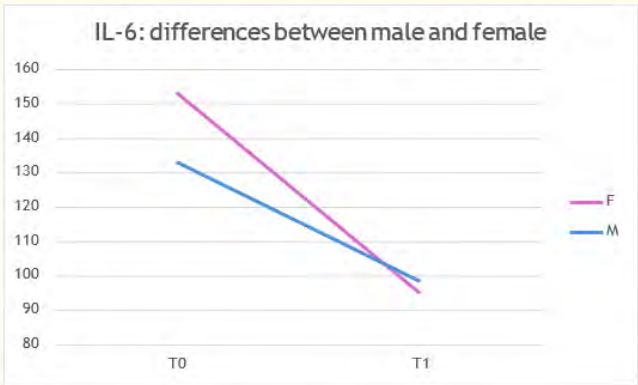


Figure 7: Changes in serum IL-6 levels in males and females at T0 and T1. The female group shows a more pronounced reduction compared to the male group.

IL-8, measured only in Subject 2, showed a marked decrease from 1020 pg/mL at T0 to 720 pg/mL at T1 (Figure 8).

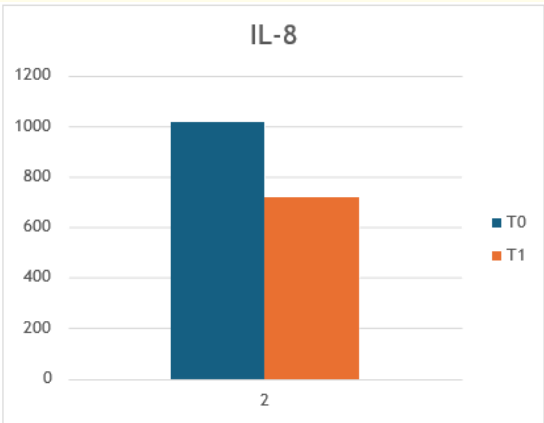


Figure 8: Trend of IL-8 across the two measurements (T0 and T1) in the nine study subjects.

Parallel to cytokine modulation, all patients reported significant clinical improvement, quantified through a symptom assessment scale. The mean symptom score for the entire cohort decreased from approximately 5.44 (T0) to 3.22 (T1) (Figure 9). Improvement was observed in all subjects, with notable changes in Subject 9 (from 5 to 1) and Subject 7 (from 6 to 2). These data suggest a correlation between the reduction of cytokine levels and subjective clinical response.

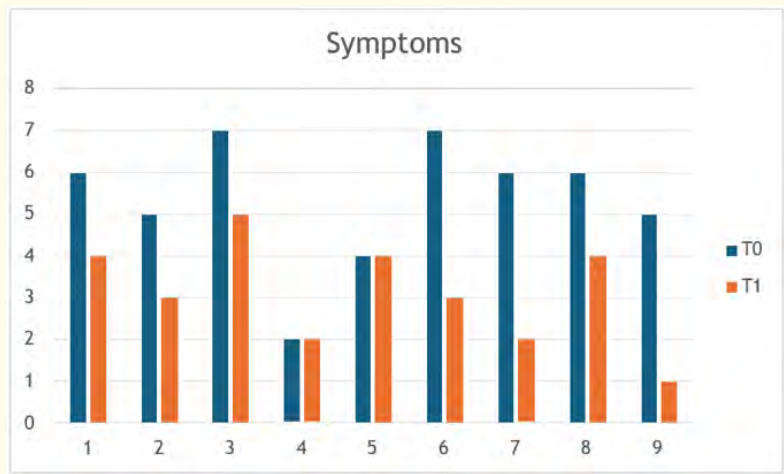


Figure 9: Trend of symptom scores before (T0) and after treatment (T1) in the nine study subjects.

The nine patients were divided into two subgroups based on concomitant standard biological therapy: the first four subjects were treated with Brentuximab (Figure 10), and the remaining five were treated with Infliximab (Figure 11).

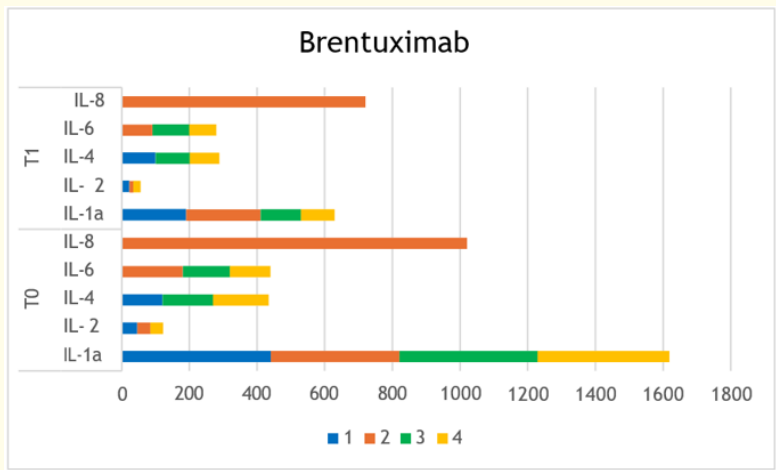


Figure 10: Interleukin trends in the first four subjects receiving Brentuximab. Analysis in this subgroup showed consistent downregulation of all measured cytokines (IL-1 α , IL-2, IL-4, IL-6, and IL-8) between T0 and T1. Symptomatic improvement was evident in all four patients.

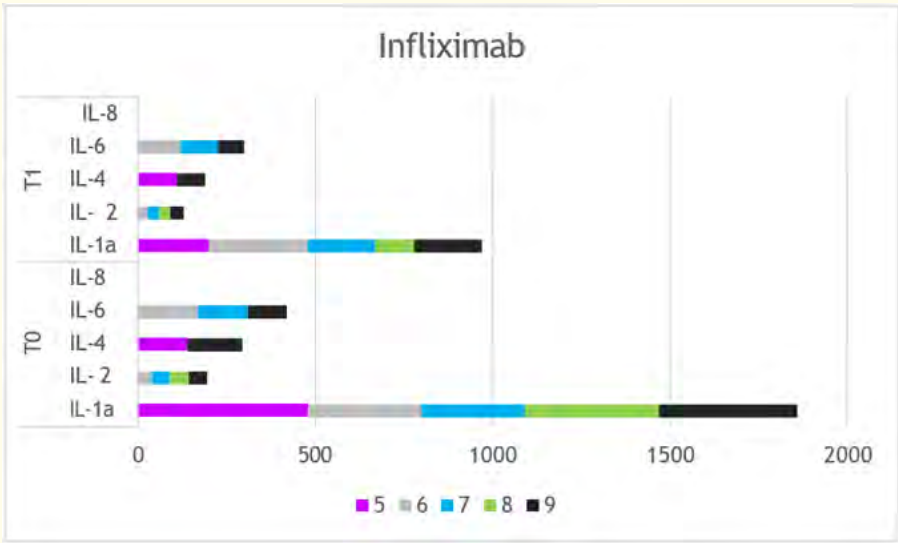


Figure 11: Interleukin trends in the five subjects receiving Infliximab. Analysis in this subgroup showed favorable cytokine modulation, with reductions in IL-1 α , IL-2, IL-4, and IL-6 levels. Significant clinical improvement was also observed in all patients in this group.

These preliminary results indicate that autologous cytokine fragment therapy was associated with downregulation of the inflammatory profile and clinical improvement in RA patients, both as monotherapy (as suggested by preliminary cases) and in combination with standard biological therapies.

Conclusion

The present study aimed to evaluate the efficacy and safety of an innovative and personalized therapeutic approach based on the administration of microdoses of autologous cytokine fragments in patients with rheumatoid arthritis (RA). The rationale for this strategy is based on the hypothesis that the α subunit of interleukins may exert an inhibitory or downregulatory effect. The goal is to intervene selectively, restoring homeostasis within the cytokine network.

The clinical and laboratory data obtained from this series of nine RA patients with documented cytokine imbalance show promising results. The most notable observation is the consistency in downregulation of the pro-inflammatory cytokines most implicated in RA pathogenesis, such as IL-1 α , IL-2, IL-4, and IL-6. All patients demonstrated a significant reduction in serum levels of these cytokines between baseline (T0) and follow-up (T1), consistent with the therapeutic goal of downregulating overexpressed cytokines.

Parallel to cytokine modulation, significant improvement in clinical symptoms was observed in all subjects. This finding supports the hypothesis of a correlation between selective intervention on the immune axis and tangible clinical benefit, in line with the principle that clinical response is proportional to the individual cytokine profile.

A crucial aspect of this approach is treatment safety and tolerability. No adverse events or systemic reactions related to the therapy were reported. The use of autologous molecules, purified and fractionated from the patient’s own plasma, reduces immunogenicity and the risk of systemic side effects commonly associated with standard biological therapies. Administration in microdoses, in accordance with natural endogenous regulatory mechanisms, allows for finer control of the dose-effect relationship.

Although the data derive from a limited patient cohort, the results support the hypothesis that microdoses of fractionated autologous cytokines may represent a safe and effective alternative for modulating the immune axis, paving the way for larger protocols in the immunological field. Further studies will be necessary to consolidate these findings and strengthen scientific evidence.

Conflict of Interest

The authors declare no conflict of interest.

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