



## RESEARCH ARTICLE

### PLASMA RICH IN LEUCOCYTE GROWTH FACTORS IN PATIENTS WITH CEREBRAL PALSY. CASE-CONTROL STUDY

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#### ABSTRACT

**Background:** There is no doubt that the platelet rich plasma is a common medical technique, that is known as regenerative medicine, through which local and systemic effect of known plasma growth factors occurs activation, cell proliferation and differentiation depending on recovered cell fraction in the final product obtained. Here, a case-control study presented to objectify the benefit of the systemic application of leukocyte plasma growth factors in cerebral palsy patients undergoing specific neurological rehabilitation programs.

**Material and Methods:** In a population of 50 Caucasian patients with age range between 5 and 15 years diagnosed with marked severe generalized spasticity and cerebral palsy, under the same program of neurorehabilitation; an intravenous injection of leukocyte rich plasma (25 ml) was administered in a group of 25 of them. Monitoring the cognitive development it was performed by Barthel scale, before and at 1, 2, 3,4,5 and 6 months after injection. The cell count leukocyte-platelet was determined by coulter type Beckman, as well as insulin-like-1 growth factor (IGF-1), platelet-derived growth factor (PDGF), vasculo-endothelial growth factor (VEGF) and transforming growth factor B (TGF-B) through specific kits of ELISA in patients before treatment, in the final product, as well as in both groups at 24 hours of the same, a month, 2,3,4,5 and 6 months after treatment. Specific descriptive statistics techniques were used as soon as the F-Fisher test for inferential statistical study of the results.

**Results:** No adverse effects were observed in patients with the exception of a small hematoma in the area of channeling venous access. There has been a clear improvement statistically significant at 2 month follow-up in cognitive sphere (memory, ability to perform more complex tasks, and the acquisition of new skills) clearly higher in the group of patients treated with plasma rich in leukocyte growth factors, ( $p = 0.013$ ), remaining stable from the 3rd month follow-up. Although at 24 h of therapy in the treatment group, serum levels of growth factors VEGF and TGF-B type increased 5-6 times as compared to baseline reference levels and the control group, statistically significant ( $p = 0.02$ ) was not obtained correlated with cognitive improvement during 6 months of clinical follow-up, because plasma levels of growth factors obtained were similar in both groups

**Conclusion:** We propose that this therapy is useful in these patients to take the neurostimulator and neuroregenerator power of endogenous growth factors derived from leukocytes, increasing the effect of neurorehabilitation and shortening of cognitive recovery without finding correlated with plasma levels of growth factors obtained during the study of the sample

#### INTRODUCTION

The use of plasma rich in growth factors in various fields of medicine especially orthopedics, dentistry, and general surgery has experienced an extraordinary development given the enormous capacity for regeneration, differentiation and chemotaxis that produce so-called growth factors, modulating angiogenesis, and cellular plasticity of injured tissues.

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Among them the best known are: Insulin-Like growth factor (IGF-1), transforming growth factor A or B (TGF-A B), vasculo-endothelial growth factor (VEGF), and platelet derived growth factor (PDGF). Through complex biochemical regulatory Feed-Back type mechanism that involve numerous cytokines, the injured cell has specific receptors for these proteins which have shown great power to involve in apoptotic and antiapoptotic mechanisms that regulate both their own life cycle and as cell differentiation. Also recent studies have objectified the possibility of improve levels of certain plasma growth factors depending on the enrichment in the final concentrate with platelet or mononuclear fraction.

However, there are other fields of application in medicine with new expectations, as is the neuroendocrinology and neurorehabilitation, where infused locally or systemically take ability to immunomodulation and chemotaxis on neuronal cells. Also it has been shown in patients with neurological degenerative diseases (eg, Alzheimer's disease, vascular encephalopathy, multiple sclerosis, ALS, and-anoxic hypoxic encephalopathy), the plasma levels of several growth factors are below baseline values, so that hypothesized that could interfere with the mechanism of cellular hypoxia, producing both a function of neuroprotection, regeneration and differentiation of neuronal tissue. The purpose of this case-control study is to objectify the possibility of improvement in cognitive sphere in patients with central neurological hypoxic-anoxic perinatal cerebral palsy, shortening the period of specific neurorehabilitation.

Society of Hematology and Hemotherapy regarding biochemical, hematological and serological before obtaining whole blood samples. As established in the scientific literature, were excluded from the study patients with tumor, infectious or previous hematologic diseases, in who the application of therapy is contraindicated.

**Structuring study arms:** a sample of 50 caucasian patients, affected of cerebral palsy with spasticity and severe cognitive impairment was selected according to the rules of the European Society of Neurology and Neuropediatrics. The range age was between 5 and 15 years, 20 of whom were men and 30 women. All patients were subjected at the same Neurorehabilitation treatment as specified in Table 1. The 50 patients were divided into 2 arms: arm 1 corresponding to 25 patients ( 10 women and 15 men) undergoing intravenous infusion of a single dose

**Table 1. Techniques used specific neurorehabilitation**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>• * Bobath Concept: Motor learning in which normal movement pathways are provided through proposed functions and guided by the therapist.</li> <li>• * Affolter Method: tactile and kinesthetic by performing activities of daily life in order to make the patient interact with the environment stimulation. very useful application in vegetative states.</li> <li>• * Cognitive Therapeutic Exercise or Perfetti Method: The implication of cognitive processes (cortical elements) within a motor rehabilitation power greater capacity to process information and organize the movement.</li> <li>• * Vojta: A technique in which with specific stimuli and starting from a certain postures, repeated unchained motor reactions (patterns reflects locomotion) on the trunk and extremities.</li> <li>• * Grimaldi Technique: neuromuscular facilitation in which a transmission means of plastic is used to normalize muscle tone.</li> <li>• * Craniosacral Therapy: noninvasive technique by working on improving membranes fascias and body functions of the individual.</li> <li>• * Basal Stimulation: concept based on a personal approach in taking relevant importance the experiences and perceptions of the day. Very useful in cases where difficulties are communicative, mental, motor and sensory relations.</li> <li>• * Virtual Techniques: Using video consoles (wii, wii-feet) that stimulate coordination, balance, motor planning, etc .. ofreciendo a useful constant feedback in neurorehabilitation.</li> </ul> |
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## MATERIALS AND METHODS

**Ethical Considerations:** All patients or authorized representatives included in the study gave their oral consent as both signed written in a form where explained the purpose of the procedure, mechanism of realization and possible side effects associated with it.

**Selection criteria of the treatment group:** The group of treatment with growth factors was selected following the rules of analytical inclusion for autotransfusion of the Spanish

(25cc) of concentrate plasma growth factors enriched with leukocytes and arm 2 as a control group of 25 patients (10 women and 15 men) who simply followed the specific therapy of neurorehabilitation.

**Collection and processing blood samples:** All samples were obtained from whole blood by venipuncture with vacuum Vacutainer 20-22 G in needle 9 ml EDTA tubes. Pre- and after follow-up period of the study, rheological measurements were processed by Coulter type Beckman for determining cell count and specific ELISA kits for determining the following leukocyte- plasma growth factors: Insulin-Like growth factor

(IGF-1) , Transforming growth factor B (TGF-B), Vasculo-endothelial growth factor (VEGF) and Platelet derived growth factor (PDGF). Centrifugation of samples was conducted in 16 tubes and shaft angular centrifuge type CEMCON-2 with a radius axis of 5cm. Protocol for obtaining enriched mononuclear leukocyte growth factors- fraction by standardized Alcaraz *et al* (2015), consisting of a single centrifugation at 3500 rpm for 30 minutes at room temperature was used.

minimum and standard deviation) and inferential analysis of symmetry, correlation and statistical power by the F Fisher test, SPSS version 5 were used

**RESULTS**

No adverse effect was objectified, except small hematoma in the area of self-limiting venipuncture.

**Table 2. Barthel index**

| Barthel Index Activity                                                                                                                                                                                                                                                 | Score |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| FEEDING<br>0 = unable<br>5 = needs help cutting, spreading butter, etc., or requires modified diet<br>10 = independent                                                                                                                                                 |       |
| BATHING<br>0 = dependent<br>5 = independent (or in shower)                                                                                                                                                                                                             |       |
| GROOMING<br>0 = needs to help with personal care<br>5 = independent face/hair/teeth/shaving (implements provided)                                                                                                                                                      |       |
| DRESSING<br>0 = dependent<br>5 = needs help but can do about half unaided<br>10 = independent (including buttons, zips, laces, etc.)                                                                                                                                   |       |
| BOWELS<br>0 = incontinent (or needs to be given enemas)<br>5 = occasional accident<br>10 = continent                                                                                                                                                                   |       |
| BLADDER<br>0 = incontinent, or catheterized and unable to manage alone<br>5 = occasional accident<br>10 = continent                                                                                                                                                    |       |
| TOILET USE<br>0 = dependent<br>5 = needs some help, but can do something alone<br>10 = independent (on and off, dressing, wiping)                                                                                                                                      |       |
| TRANSFERS (BED TO CHAIR AND BACK)<br>0 = unable, no sitting balance<br>5 = major help (one or two people, physical), can sit<br>10 = minor help (verbal or physical)<br>15 = independent                                                                               |       |
| MOBILITY (ON LEVEL SURFACES)<br>0 = immobile or < 50 yards<br>5 = wheelchair independent, including corners, > 50 yards<br>10 = walks with help of one person (verbal or physical) > 50 yards<br>15 = independent (but may use any aid; for example, stick) > 50 yards |       |
| STAIRS<br>0 = unable<br>5 = needs help (verbal, physical, carrying aid)<br>10 = independent                                                                                                                                                                            |       |

Final concentrated product was performed aseptically under laminar flow hood B in 3.5 ml EDTA tubes. The application was realized in the treatment group by slow intravenous infusion at speed of 1 ml per second.

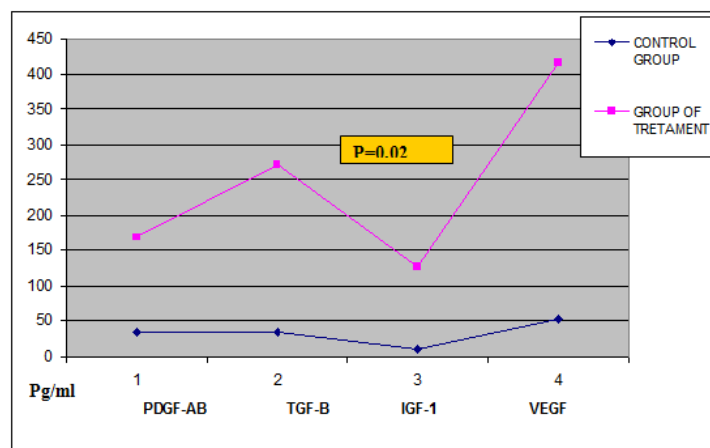
**Cognitive assessment of patients:** Barthel scale was used for cognitive assessment of patients with a number of items at score ranging from 0-15; could be seen in Table 2.

**Statistical data processing:** For statistical and interpretation of the data obtained according to the variables studied, both technical descriptive statistics (mean, median, maximum,

In Tables 3-6 can objectify the data obtained in terms of rheological measurements in whole blood of 50 patients, in the 25 plasmas enriched with leukocytes and in 25 patients after 24 hours of treatment. In the graph 3, it is noted as cognitive test scores are very similar in both arms of the study, prior to treatment. After 24 h of infusion, concentrations of growth factors in the group of treated patients were 2 to 4 times higher for those factors type PDGF and IGF-1 and 5 to 7 times higher for factors type VEGF and TGF-B, with respect to their initial baseline values and the control group, statistically significant (p = 0.02), as can be seen in figure 1. However from first month follow-up until end of study, levels of growth factors

Table 3. Rheological values of the 50 patients studied:

|            | PDGF-AB<br>(10-50<br>pg/ml) | TGF-B1<br>(10-70<br>pg/ml) | IGF-1<br>(0,5-19,5<br>pg/ml) | Vegf<br>(15-85<br>pg/ml) | Platelets<br>(150.000-<br>350.000/mm3) | Leukocytes<br>(3.200-<br>9000/mm3) | Granulocytes<br>/mm3 | Mononuclears<br>/mm3 | CD<br>34 +<br>/mm3 |
|------------|-----------------------------|----------------------------|------------------------------|--------------------------|----------------------------------------|------------------------------------|----------------------|----------------------|--------------------|
| Patient 1  | 45                          | 60                         | 18                           | 80                       | 210000                                 | 7500                               | 4875                 | 1275                 | 0.9                |
| Patient 2  | 40                          | 25                         | 10                           | 45                       | 210000                                 | 6500                               | 3575                 | 1625                 | 0.3                |
| Patient 3  | 43                          | 55                         | 17                           | 80                       | 190000                                 | 6230                               | 3738                 | 1246                 | 0.4                |
| Patient 4  | 43                          | 67                         | 15                           | 75                       | 170000                                 | 7500                               | 4500                 | 1125                 | 0.5                |
| Patient 5  | 15                          | 25                         | 7                            | 30                       | 180000                                 | 8900                               | 5340                 | 1335                 | 0.3                |
| Patient 6  | 35                          | 24                         | 12                           | 40                       | 175000                                 | 8900                               | 5340                 | 1956                 | 0.2                |
| Patient 7  | 20                          | 15                         | 7                            | 30                       | 260000                                 | 7200                               | 4320                 | 1440                 | 0.2                |
| Patient 8  | 30                          | 20                         | 7                            | 35                       | 176000                                 | 7430                               | 4458                 | 1114                 | 0.4                |
| Patient 9  | 91                          | 60                         | 16                           | 75                       | 350000                                 | 7430                               | 4086                 | 1337                 | 0.7                |
| Patient 10 | 45                          | 55                         | 18                           | 70                       | 195000                                 | 9500                               | 5700                 | 1425                 | 0.7                |
| Patient 11 | 35                          | 20                         | 15                           | 40                       | 205000                                 | 8300                               | 4980                 | 1909                 | 0.2                |
| Patient 12 | 12                          | 15                         | 4                            | 25                       | 250000                                 | 8500                               | 5100                 | 1890                 | 0.1                |
| Patient 13 | 45                          | 60                         | 17                           | 75                       | 240000                                 | 8700                               | 5481                 | 1131                 | 0.7                |
| Patient 14 | 43                          | 55                         | 17                           | 70                       | 300000                                 | 7600                               | 4560                 | 1140                 | 0.4                |
| Patient 15 | 15                          | 55                         | 18                           | 70                       | 214907                                 | 7500                               | 4500                 | 1500                 | 0.2                |
| Patient 16 | 42                          | 67                         | 18                           | 87                       | 220659                                 | 7590                               | 4109                 | 1221                 | 0.8                |
| Patient 17 | 41                          | 21                         | 10                           | 44                       | 215401                                 | 6201                               | 3600                 | 1624                 | 0.5                |
| Patient 18 | 44                          | 53                         | 17                           | 88                       | 195793                                 | 6013                               | 3490                 | 1276                 | 0.8                |
| Patient 19 | 40                          | 66                         | 15                           | 74                       | 181098                                 | 7901                               | 4501                 | 1112                 | 0.2                |
| Patient 20 | 17                          | 22                         | 7                            | 32                       | 191209                                 | 8587                               | 5354                 | 1309                 | 0.6                |
| Patient 21 | 37                          | 23                         | 12                           | 43                       | 175397                                 | 8401                               | 5176                 | 1966                 | 0.6                |
| Patient 22 | 25                          | 17                         | 7                            | 31                       | 262981                                 | 7010                               | 4012                 | 1490                 | 0.6                |
| Patient 23 | 33                          | 22                         | 7                            | 33                       | 169127                                 | 7091                               | 4301                 | 1830                 | 0.5                |
| Patient 24 | 99                          | 68                         | 16                           | 77                       | 339129                                 | 7178                               | 4912                 | 1900                 | 0.9                |
| Patient 25 | 41                          | 52                         | 18                           | 76                       | 184091                                 | 9280                               | 5769                 | 1421                 | 0.8                |
| Patient 26 | 32                          | 21                         | 15                           | 40                       | 200436                                 | 8120                               | 4210                 | 1932                 | 0.4                |
| Patient 27 | 11                          | 13                         | 4                            | 23                       | 251465                                 | 8401                               | 5300                 | 1890                 | 0.2                |
| Patient 28 | 47                          | 69                         | 17                           | 72                       | 201154                                 | 8598                               | 5900                 | 1900                 | 0.5                |
| Patient 29 | 45                          | 51                         | 17                           | 79                       | 330012                                 | 7901                               | 4390                 | 1140                 | 0.4                |
| Patient 30 | 19                          | 57                         | 18                           | 75                       | 260123                                 | 7689                               | 4211                 | 1500                 | 0.5                |
| Patient 31 | 40                          | 62                         | 18                           | 82                       | 218013                                 | 7211                               | 4600                 | 1275                 | 0.8                |
| Patient 32 | 43                          | 21                         | 10                           | 43                       | 219032                                 | 6480                               | 3410                 | 1625                 | 0.3                |
| Patient 33 | 48                          | 52                         | 17                           | 81                       | 191913                                 | 6219                               | 3800                 | 1219                 | 0.7                |
| Patient 34 | 42                          | 62                         | 15                           | 73                       | 171934                                 | 7500                               | 4212                 | 1119                 | 0.6                |
| Patient 35 | 12                          | 28                         | 7                            | 31                       | 187091                                 | 8967                               | 5012                 | 1321                 | 0.8                |
| Patient 36 | 33                          | 22                         | 12                           | 42                       | 175708                                 | 8941                               | 5900                 | 1780                 | 0.6                |
| Patient 37 | 22                          | 18                         | 7                            | 39                       | 260000                                 | 7212                               | 4150                 | 1503                 | 0.9                |
| Patient 38 | 39                          | 27                         | 7                            | 33                       | 174814                                 | 7012                               | 4800                 | 1145                 | 0.5                |
| Patient 39 | 99                          | 67                         | 16                           | 76                       | 255060                                 | 7432                               | 4120                 | 1903                 | 0.6                |
| Patient 40 | 47                          | 51                         | 18                           | 71                       | 195000                                 | 9019                               | 5500                 | 1093                 | 0.5                |
| Patient 41 | 34                          | 29                         | 15                           | 44                       | 260124                                 | 8190                               | 4123                 | 1012                 | 0.4                |
| Patient 42 | 15                          | 14                         | 4                            | 22                       | 255098                                 | 8122                               | 5450                 | 1701                 | 0.5                |
| Patient 43 | 45                          | 65                         | 17                           | 78                       | 243981                                 | 8000                               | 5911                 | 1016                 | 0.8                |
| Patient 44 | 43                          | 51                         | 17                           | 79                       | 317321                                 | 7801                               | 4012                 | 1045                 | 0.5                |
| Patient 45 | 15                          | 55                         | 18                           | 71                       | 217877                                 | 7546                               | 4560                 | 1501                 | 0.3                |
| Patient 46 | 45                          | 69                         | 18                           | 89                       | 212066                                 | 7209                               | 4900                 | 1200                 | 0.8                |
| Patient 47 | 40                          | 27                         | 10                           | 45                       | 217912                                 | 6109                               | 3123                 | 1601                 | 0.4                |
| Patient 48 | 43                          | 53                         | 17                           | 89                       | 190543                                 | 6320                               | 3800                 | 1222                 | 0.5                |
| Patient 49 | 43                          | 60                         | 15                           | 74                       | 178913                                 | 7591                               | 4911                 | 1125                 | 0.4                |
| Patient 50 | 15                          | 20                         | 7                            | 33                       | 188912                                 | 8011                               | 5901                 | 1333                 | 0.5                |
| MAXIMUM    | 99                          | 69                         | 18                           | 89                       | 350000                                 | 9500                               | 5911                 | 1966                 | 0.9                |
| MINIMUM    | 11                          | 13                         | 4                            | 22                       | 169127                                 | 6013                               | 3123                 | 1012                 | 0.1                |
| AVERAGE    | 32,75                       | 36,09                      | 12,14                        | 53,03                    | 259563                                 | 7709                               | 4619                 | 1405                 | 0.5                |



As we see in the group of patients treated with plasma leukocyte enriched levels of growth factors VEGF and TGF-B increased 5 to 7 times, while the growth factors PDGF and IGF-1 did between two and four times more compared to baseline figures of this group and relative to the control arm.

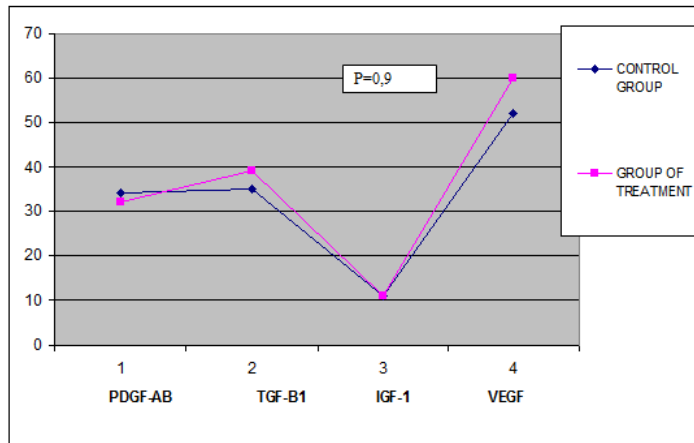
Figure 1. Rheological mean levels of Leukocyte growth factors in both study groups at 24 h of treatment

**Table 4. Average levels of growth factors in leukocytes enriched plasmas obtained**

|            | PDGF-AB<br>(10-50<br>pg/ml) | TGF-B1<br>(10-70<br>pg/ml) | IGF-1<br>(0,5-19,5<br>pg/ml) | VEGF<br>(15-85<br>pg/ml) | Platelets<br>(150.000-<br>350.000/mm3) | Leukocytes<br>(3.200-<br>9000/mm3) | Granulocyte<br>/mm3 | Mononuclears<br>/mm3 | CD<br>34 +<br>/mm3 |
|------------|-----------------------------|----------------------------|------------------------------|--------------------------|----------------------------------------|------------------------------------|---------------------|----------------------|--------------------|
| Patient 1  | 296                         | 450                        | 250                          | 575                      | 500000                                 | 21000                              | 3150                | 18270                | 240                |
| Patient 2  | 270                         | 300                        | 150                          | 545                      | 600000                                 | 22000                              | 4400                | 16500                | 180                |
| Patient 3  | 190                         | 370                        | 200                          | 590                      | 500000                                 | 21000                              | 3150                | 16800                | 270                |
| Patient 4  | 250                         | 480                        | 190                          | 540                      | 400000                                 | 20000                              | 4000                | 17400                | 210                |
| Patient 5  | 150                         | 365                        | 110                          | 460                      | 600000                                 | 21000                              | 4200                | 14700                | 170                |
| Patient 6  | 160                         | 370                        | 160                          | 530                      | 500000                                 | 24000                              | 6000                | 19200                | 175                |
| Patient 7  | 200                         | 390                        | 120                          | 470                      | 400000                                 | 21500                              | 4515                | 15910                | 170                |
| Patient 8  | 150                         | 350                        | 105                          | 390                      | 700000                                 | 21500                              | 3440                | 12900                | 120                |
| Patient 9  | 253                         | 520                        | 277                          | 590                      | 600000                                 | 21500                              | 4085                | 18705                | 215                |
| Patient 10 | 220                         | 470                        | 210                          | 590                      | 700000                                 | 24000                              | 3600                | 20400                | 200                |
| Patient 11 | 150                         | 370                        | 160                          | 480                      | 690000                                 | 23000                              | 4600                | 17940                | 150                |
| Patient 12 | 190                         | 350                        | 190                          | 320                      | 500000                                 | 20000                              | 4000                | 12000                | 70                 |
| Patient 13 | 280                         | 420                        | 230                          | 570                      | 710000                                 | 22000                              | 3300                | 18700                | 200                |
| Patient 14 | 250                         | 420                        | 199                          | 570                      | 650000                                 | 22000                              | 4400                | 16500                | 185                |
| Patient 15 | 245                         | 430                        | 190                          | 590                      | 570000                                 | 23000                              | 4370                | 19550                | 200                |
| Patient 16 | 280                         | 459                        | 253                          | 590                      | 620000                                 | 21000                              | 3100                | 18500                | 240                |
| Patient 17 | 270                         | 380                        | 153                          | 580                      | 660000                                 | 22600                              | 4500                | 16700                | 180                |
| Patient 18 | 250                         | 390                        | 290                          | 570                      | 710000                                 | 27000                              | 3000                | 16500                | 270                |
| Patient 19 | 230                         | 490                        | 170                          | 590                      | 630000                                 | 20900                              | 4900                | 17900                | 210                |
| Patient 20 | 220                         | 390                        | 100                          | 499                      | 700000                                 | 21530                              | 4100                | 14500                | 259                |
| Patient 21 | 130                         | 380                        | 190                          | 580                      | 610000                                 | 24070                              | 6400                | 19900                | 300                |
| Patient 22 | 210                         | 290                        | 100                          | 489                      | 730000                                 | 26700                              | 4400                | 15900                | 350                |
| Patient 23 | 230                         | 400                        | 103                          | 391                      | 630000                                 | 25000                              | 3500                | 12480                | 270                |
| Patient 24 | 240                         | 590                        | 240                          | 570                      | 730000                                 | 22700                              | 4001                | 18400                | 200                |
| Patient 25 | 200                         | 490                        | 200                          | 510                      | 670000                                 | 23000                              | 3670                | 20900                | 290                |
| MAXIMUM    | 296                         | 590                        | 290                          | 590                      | 730000                                 | 27000                              | 6400                | 20900                |                    |
| MINIMUM    | 130                         | 290                        | 100                          | 320                      | 400000                                 | 20000                              | 3000                | 12000                |                    |
| AVERAGE    | 215,39                      | 407,19                     | 172,82                       | 521,32                   | 604147                                 | 22411                              | 4038                | 16913                |                    |

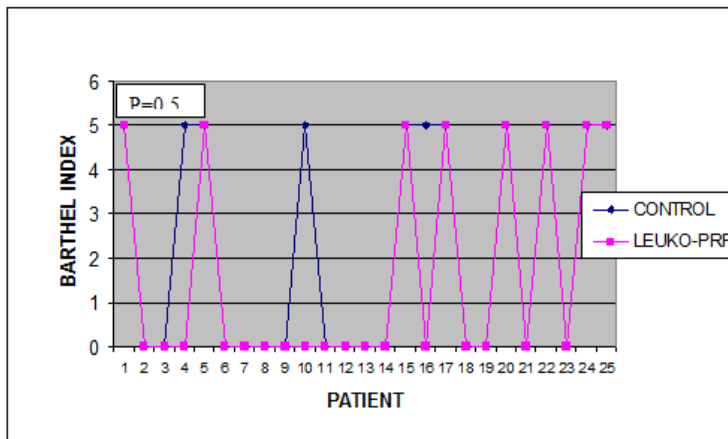
**Table 5. Average levels of growth factors in the group of patients undergoing infusion of leukocyte growth factors at 24h of treatment**

|            | PDGF-<br>AB<br>(10-50<br>pg/ml) | TGF-B1<br>(10-70<br>pg/ml) | IGF-1<br>(0,5-<br>19,5<br>pg/ml) | VEGF<br>(15-85<br>pg/ml) | PLATELETS<br>(150.000-<br>350.000/mm3) | Leukocytes<br>(3.200-<br>9000/mm3) | Granulocytes<br>/mm3 | Mononuclears<br>/mm3 | CD<br>34 +<br>/mm3 |
|------------|---------------------------------|----------------------------|----------------------------------|--------------------------|----------------------------------------|------------------------------------|----------------------|----------------------|--------------------|
| Patient 1  | 200                             | 420                        | 220                              | 530                      | 200436                                 | 8120                               | 4210                 | 1932                 | 0.4                |
| Patient 2  | 230                             | 270                        | 130                              | 510                      | 251465                                 | 8401                               | 5300                 | 1890                 | 0.2                |
| Patient 3  | 120                             | 320                        | 180                              | 520                      | 201154                                 | 8598                               | 5900                 | 1900                 | 0.5                |
| Patient 4  | 200                             | 440                        | 150                              | 500                      | 330012                                 | 7901                               | 4390                 | 1140                 | 0.4                |
| Patient 5  | 110                             | 325                        | 100                              | 420                      | 260123                                 | 7689                               | 4211                 | 1500                 | 0.5                |
| Patient 6  | 110                             | 340                        | 140                              | 510                      | 218013                                 | 7211                               | 4600                 | 1275                 | 0.8                |
| Patient 7  | 160                             | 360                        | 100                              | 430                      | 219032                                 | 6480                               | 3410                 | 1625                 | 0.3                |
| Patient 8  | 110                             | 320                        | 99                               | 350                      | 191913                                 | 6219                               | 3800                 | 1219                 | 0.7                |
| Patient 9  | 203                             | 500                        | 250                              | 530                      | 171934                                 | 7500                               | 4212                 | 1119                 | 0.6                |
| Patient 10 | 190                             | 420                        | 200                              | 560                      | 187091                                 | 8967                               | 5012                 | 1321                 | 0.8                |
| Patient 11 | 110                             | 350                        | 130                              | 440                      | 175708                                 | 8941                               | 5900                 | 1780                 | 0.6                |
| Patient 12 | 120                             | 300                        | 170                              | 300                      | 260000                                 | 7212                               | 4150                 | 1503                 | 0.9                |
| Patient 13 | 220                             | 400                        | 200                              | 520                      | 174814                                 | 7012                               | 4800                 | 1145                 | 0.5                |
| Patient 14 | 210                             | 410                        | 180                              | 550                      | 255060                                 | 7432                               | 4120                 | 1903                 | 0.6                |
| Patient 15 | 215                             | 400                        | 170                              | 540                      | 195000                                 | 9019                               | 5500                 | 1093                 | 0.5                |
| Patient 16 | 220                             | 409                        | 240                              | 540                      | 260124                                 | 8190                               | 4123                 | 1012                 | 0.4                |
| Patient 17 | 220                             | 350                        | 130                              | 530                      | 255098                                 | 8122                               | 5450                 | 1701                 | 0.5                |
| Patient 18 | 230                             | 360                        | 250                              | 530                      | 243981                                 | 8000                               | 5911                 | 1016                 | 0.8                |
| Patient 19 | 200                             | 450                        | 140                              | 540                      | 317321                                 | 7801                               | 4012                 | 1045                 | 0.5                |
| Patient 20 | 190                             | 350                        | 90                               | 445                      | 217877                                 | 7546                               | 4560                 | 1501                 | 0.3                |
| Patient 21 | 100                             | 330                        | 150                              | 550                      | 212066                                 | 7209                               | 4900                 | 1200                 | 0.8                |
| Patient 22 | 180                             | 270                        | 98                               | 440                      | 217912                                 | 6109                               | 3123                 | 1601                 | 0.4                |
| Patient 23 | 190                             | 350                        | 99                               | 340                      | 190543                                 | 6320                               | 3800                 | 1222                 | 0.5                |
| Patient 24 | 190                             | 520                        | 210                              | 530                      | 178913                                 | 7591                               | 4911                 | 1125                 | 0.4                |
| Patient 25 | 170                             | 430                        | 180                              | 500                      | 188912                                 | 8011                               | 5901                 | 1333                 | 0.5                |
| MAXIMUM    | 230                             | 450                        | 200                              | 505                      | 290000                                 | 8900                               | 4000                 | 2000                 | 20                 |
| MINIMUM    | 100                             | 120                        | 55                               | 200                      | 190000                                 | 3950                               | 3100                 | 1600                 | 5                  |
| AVERAGE    | 169,74                          | 272,7                      | 127,3                            | 415,45                   | 223602                                 | 7638                               | 3629                 | 1768                 | 11                 |



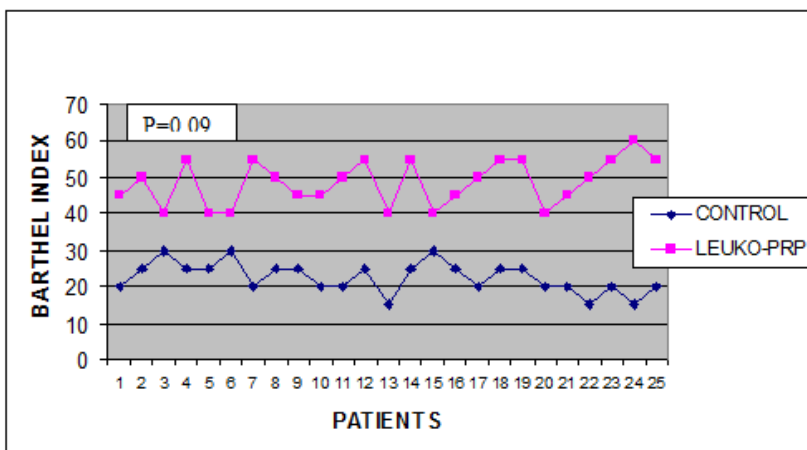
As we can see in the two study groups, levels of growth factors are substantially similar from the first month follow-up until the end of the study period, indicating that these proteins have a large plasma lability.

**Figure 2. Levels rheological factors means Leukocyte growth in both study groups from the first month of treatment until the end of the study period**



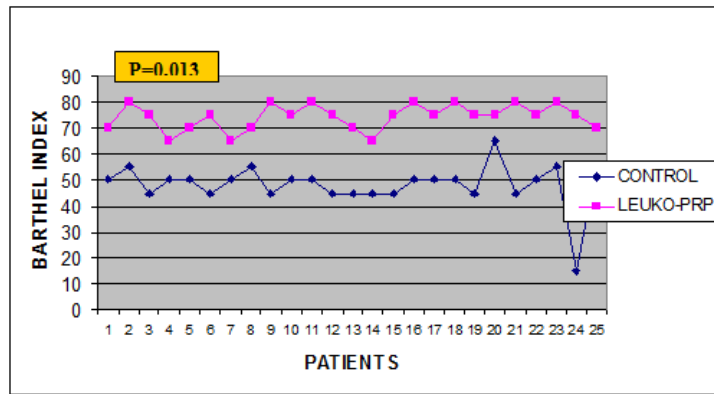
We can objectify as pre-treatment in the 2 study arms cognitive test scores are similar.

**Figure 3. Average Rating Barthel Scale in the two study groups before treatment**



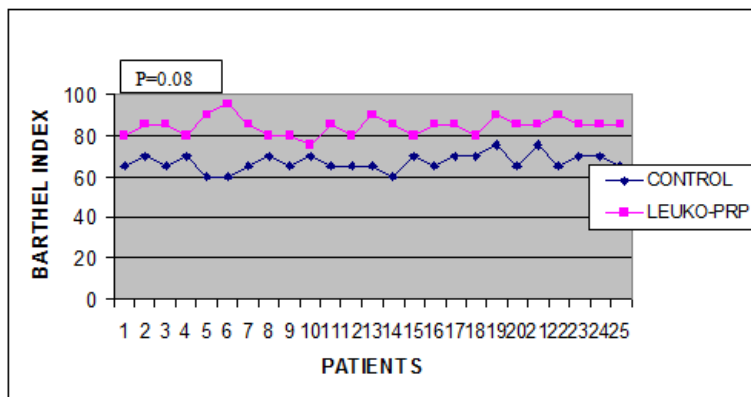
We can see how in the treatment group cognitive test scores are slightly higher compared to the control group.

**Figure 4. Average Rating Scale Barthel in the two study groups at month follow-up**



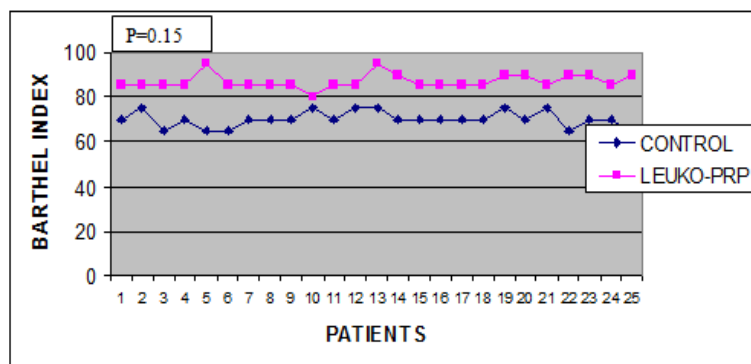
In this graph we see as the difference is accentuated on the cognitive test rating in favor of the treatment group, a statistically significant difference.

Figure 5. Average Rating Scale Barthel in the two study groups at 2 months follow-up



In Figure 6 we see how the cognitive test scores in the control group approach to the treatment arm, keeping the scores of the group of stable treatment.

Figure 6. Average Rating Scale Barthel in the two study groups at 3 months follow-up



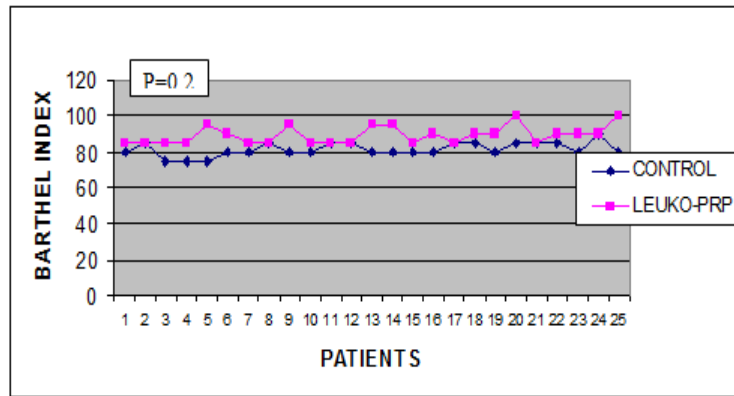
In Figure 7, the values of the control group are still approaching the processing arm following stable in the latter

Figure 7. Average Rating Scale Barthel in the two study groups at 4 months follow-up.

were stable and similar in both study groups, there being no correlation with cognitive evolution, as shown in Figure 2. Cognitive improvement is most evident in the group of patients treated with leukocyte factors growth, statistically significant at the 2nd month after treatment ( $P = 0.013$ ), objectified in Figures 4 and 5, remained stable in this group. From the 3rd month follow-up the total scores of cognitive test in control group approach to the treated leukocyte rich plasma arm, being practically matching at 5 months follow up. Figures 6-9.

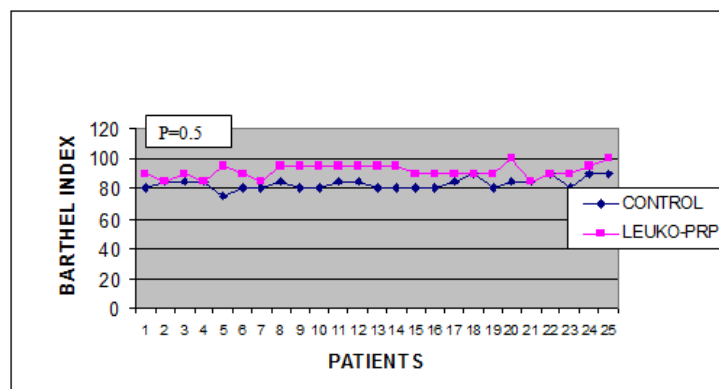
## DISCUSSION

The evolution of regenerative medicine in various clinical areas revolutionizes the field of tissue repair, providing an instrument for treatment which is economical, easy to use, no side effects, and less invasive (1, 3). However, scientific and social requirements make it necessary to design appropriate clinical trials to establish treatment protocols for each particular medical application (1, 2).



In Figure 8 we see how both cognitive test values in the control group as in the treatment arm are similar, remaining stable

Figure 8. Average Rating Scale Barthel in the two study groups at 5 months follow-up



In the latter graph cognitive test values practically coincident both are groups while retaining stability.

Figure 9. Average Rating Scale Barthel in the two study groups at 6 months follow-up

Today, medical areas with stronger scientific evidence to use plasma growth factors are dentistry (to repair the dental alveolar bed) and traumatology (arthropathy, tendinopathy, ligament injuries, and meniscopathy), with proper design randomized clinical trials in phase I-II (1, 4). But the empirical use in many diseases and medical specialties sometimes exceeds the capacity to produce sufficient scientific evidence power for use. An important fact to comment, as previously demonstrated by other authors is the great capacity of these proteins to spread through the tissues and the short half-life objectified once achieved therapeutic plasma levels that do not usually exceed 48-72h (11-12), which shows that the actuation mechanism is complex, it is believed that activating pathways or biochemical cascades through numerous chemokines or cytokines that involve in the inflammatory processes both specific tissue, such as migration, proliferation and differentiation of precursors cell maturation in different states and angiogenesis phenomena would produce increased tissue oxygenation with the consequent increase in cell survival and protection thereof. Some more promising medical fields for the use of this biotechnology are neurology, neuroendocrinology and neurorehabilitation. A few months ago was published the first clinical case of cognitive improvement supported by cerebral PET in a 5 years old child with severe cerebral palsy who was applied by intravenous infusion a plasma concentrate growth factors-enriched with buffy-coat mononuclear fraction (10).

Several authors hypothesized neuroregenerative phenomena, antiapoptotic, immunomodulatory and neurotrophic effects that would produce these autologous plasma growth factors on neuronal tissue, making this a feasible therapy from a medical point of view, to be applied in neurological diseases with neurodegenerative profile or hypoxic -anoxic, such as Alzheimer's disease, brain-stroke, spinal cord injury, and cerebral palsy (5, 6). Spontaneous remission of the signs and symptoms of cerebral palsy is rare due to the large number of neuronal glial mass and degenerate secondary to the effects of hypoxia in the evolution of the disease (9). Effects of neurostimulation, neurodegeneration and neuroprotection have been observed in these patients treated with synthetic growth hormone (HGF), which causes functional improvement, especially in the cognitive domain (eg, memory, language, ability to perform complex tasks, and acquisition of new skills). In these patients, the neuronal degenerative effect has been accompanied by a qualitative and quantitative marked decrease in plasma growth factors such as HGF-IGF-1-VEGD, PDGF, and TGF-B (7, 8), regulated by the hypothalamic axis pituitary, which produce a neuroprotective effect, due to neurotropic and chemotaxis phenomena, cell differentiation, and neuroplasticity in neuronal tissue. Furthermore, these substances have the ability to stimulate the so-called gray areas corresponding to those neural tissues found in hibernation as a result of lesional hypoxic or anoxic effect. However, treatment with synthetic growth hormone is costly, not only from the economic standpoint but also from the clinical point of view. The



possibility of using autologous plasma growth factors, locally or systemically in a single dose, to achieve a therapeutic effect in the medium-long period of time clinical outcome produced by growth factors without oncogenic side effects (5, 7), makes it attractive for use in these patients significantly reducing economic and clinical treatment cost.

**Limitations of the study:** This study has the following limitations: first the small sample size does not have sufficient statistical power could be obtained when interpreting the collected data. Secondly not known in which moment of study levels of growth factors stabilize in the treatment group. It is important to correlate with the clinical effect that might produce. This work is complex due to their plasma lability. However would be interesting to investigate when the peak plasma concentration level begin to decline until reaching baseline concentration in the patient. It could help to define the specific functionality of each growth factors studied in order to determine the clinical effect observed to design properly structured scientific studies and randomized clinical trials.

### Conclusion

We propose that this therapy is useful in patients with hypoxic-anoxic cerebral palsy to take advantage of the neurostimulator and neuroregenerator power of endogenous growth factors derived from leukocytes, increasing the effect of neurorehabilitation and shortening period of cognitive recovery and economical costs, with out side effects compared to conventional neurorehabilitation.

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