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STUDY PROTOCOL FOR JOINT PRP KIT SYSTEM VALIDATION

1. Introduction

The aim of the study is to test some parameters related to the platelet rich plasma product (PRP) obtained by JOINT PRP KIT system. The system is composed by a complete kit of individually sterile accessories for collection of 20 mL of anticoagulated venous blood (10% of sodium citrate) in a patented separator device and a certified medical device centrifuge (DM class II) for PRP concentration (ONE STEP). The final platelet concentrate is enriched in leukocytes, so the resulting product will be a Leuco-PRP (L-PRP).

2. Design of study

The study was carried out at a single center (Laboratorio Preparazione Emocomponenti e Medicina Rigenerativa – SC Medicina Trasfusionale AO SS Antonio e Biagio - Alessandria) in according with laboratory procedures.
In each test 20 mL of blood was used, obtained from a pool of donors with the same blood type. The samples were divided into three groups based on the platelet concentration (PLT) per mm^3:

group A: 150.000 - 180.000
group B: 180.000 - 210.000
group C: > 210,000

3. Study parameters

The assessed parameters will allow to determine the equivalence efficiency of the preparation method, even if the samples have different basal cell parameters.
For each group, three tests were performed in parallel and the results were compared in single and average value.
The parameters taken into consideration are:
- platelets concentration per mm^3
- leukocytes concentration per mm^3
- Interleukin-1 ra release (semi-quantitative dosage pg/mL)

The method efficiency was assessed according with the platelets concentration per mm^3 in the final product (PRP) as referred in the Ministerial Decree of 02 November 2015. Compared to the Indications for Use was also assessed the ease-of-use and checked the possibility of carrying out, through the system, a procedure that respects the safety conditions, in order to obtain a safe final product.

4. Platelets and white blood cells count

Platelets and white blood cells count was performed, using a hematology analyzer (Micros Horiba Technology), for each sample in both whole blood (20 mL) and final products: PRP (2 mL) and PPP (6 / 7 mL).

5. Interleukin-1 receptor antagonist (IL-1ra)

Rationale: the inflammation is due to the presence of inflammatory cytokines generally produced by a trauma occurred in local site or from other pathologies. Interleukin-1 (IL-1) is the most abundant inflammatory cytokine present in serum and biological fluids in case of trauma or injury. The IL-1 ra protein is the natural receptor antagonist of interleukin 1 (classified as a primary inflammatory cytokine) and is mainly produced by polymorphonuclear cells. Its anti-inflammatory action is expressed through or the direct binding of its soluble form (IL-1RIs and IL-1RIIs) to the IL-1, preventing the binding of IL-1 with target cells, or going to compete with IL-1 for the direct receptor binding on target cells. Because IL-1 is a cytokine with high inflammatory activity, also performed at low concentrations, is necessary a high concentration of IL-1 ra in order to inhibits its action; in fact, the binding affinity of IL-1 ra with cells receptor is lower than the IL-1 ones and therefore, to obtain an anti-inflammatory effect, the IL-ra concentration must be high. The presence of leukocytes into this final PRP product, in particular the monocyte-macrophage and lymphocyte component (mononuclear cells - PMN), increase the IL-1 ra concentration. Normally, in the humoral environment, it is present in low quantities (normal value in plasma: 105-1193 pg/mL), however its quantity increases as the concentration of mononuclear cells (PMN) increases.
Therefore IL-1 ra (pg/mL) was studied as probable anti-inflammatory activity index of PRP produced with this system.

6. Study sample size

The blood samples were divided in the previously defined groups (A, B, C) according to platelet count and 3 different samples per group were analyzed; they were processed according to the instructions for use of JOINT-PRP KIT 20 mL and centrifuged in the dedicated centrifuge Gyrozen MD 416 - LabTech with a preset program (3200 RPM for 5 min). For IL-1ra dosage, aliquots of PRP (0.5 mL) and supernatant plasma (0.5 mL) were taken from each processed sample. The quantitative test used was the immunoassay Elisa Quantikine Human IL-1 ra/IL-1 F3 (R&D systems – Bio-Techne USA).

7. Blood cells count

	BASELINE		L-PRP				Plasma	
	PLT $10^3/mm^3$	WBC $10^3/mm^3$	PLT* $10^3/mm^3$	*PLT recovery conc fold*	WBC $10^3/mm^3$	*WBCrecovery conc fold*	PLT $10^3/mm^3$	WBC $10^3/mm^3$
group A	160	5,6	1034	6,4	8,3	1,5	70	0,4
	168	5,4	1079	6,4	11,3	2,1	66	0,3
	166	3,9	700	4,2	14	3,5	137	0,4
group B	186	5,0	1615	8,6	13	2,3	118	0.1
	186	4,7	1040	5,6	12,6	2,7	148	0,1
	197	4,6	914	4,6	12,1	2,6	122	0,1
group C	296	3,7	1486	5	17	4,5	97	0,1
	257	5,4	1146	4,5	19	3,5	170	0,2
	251	5,0	1123	4,5	16	3,2	118	0

***** Benchmark value of Italian DM 02.11.2015: 800 -1.200 per $10^3/mm^3$

8. IL-1 ra dosage

	WBC x $10^3/mm^3$	PMN % *	PRP IL-1 ra pg/mL	Plasma IL-1 ra pg/mL	V.N.
group A	8,3	**50 %**	**3691**	**401**	**105 - 1193 pg/mL**
	11,3		**3738**	**423**	
	14		**4075**	**596**	
group B	13	**61%**	**3514**	**391**	
	12,6		**3625**	**457**	
	12,1		**3611**	**426**	
group C	17	**61%**	**3681**	**402**	
	19		**3758**	**487**	
	16		**3622**	**418**	

* Benchmark value

9. Conclusions

The system has demonstrated a good platelet recovery performance (average concentration equal to 5.4 times the baseline), 99% of the tests satisfy the requirements of current DM about platelets concentration (DM 02 November 2015: 800 -1,200 for 10^3 / mm^3). Platelets are recovered quickly and easily from buffy coat isolation after centrifugation, this also include the collection of leukocytes (on average 13.7 x 10^3 / mm^3, an average concentration equal to 2.4 times the basaline) with greater presence of mononuclear cells (61% lymphocytes-monocytes)
compared to baseline, turning this product into a Leuco-PRP (L-PRP), according with current literature classifications. The JOINT PRP KIT product is mainly intended to be used for musculoskeletal pathologies with an anti-inflammatory and regenerative purpose. In addition to the right platelet concentration, leukocytes provide several inflammation antagonist molecules in the final product, in particular the mononuclear component releases an abundant quantity of IL-1 ra (at least 6 times greater than the plasma value). This exogenous quantity of autologous IL-1 ra administered in local site could strongly prevent the excess of IL-1 produced in a damaged tissue. The presence of molecules with both anti-inflammatory (leukocytes) and repairing/regenerative (platelets) activity have a 360 ° biological action to induce lesions healing at local level. The JOINT PRP KIT system is certified as class II medical device (preparation kit and centrifuge), provides all the components necessary for L-PRP preparation from the autologous venous blood extraction to the product administration to the patient. It is valid, easy to use and fulfill all the required features. The whole procedure of blood collection and separation/extraction of L-PRP is quick and simple (about 15 minutes), the preparation requires only one single centrifugation (*one step*). All the components within the kit are in single sterile packaging to guarantee an executive procedure as aseptic as possible; being the system in some passage an open system, the preparation in a sterile field is required to have the maximum safety conditions in order to get a *safe* product. The separator is a patented device, the separation occurs by hand, thus susceptible to different variables: patient's hematocrit and operator's manual skills; this variability is however reduced by the standardized final volume of 2 ml L-PRP.
The kit showed to be suitable for the purpose, as well as the product analized, resulting in compliance with the required standards and the further molecular investigations conducted has also enhanced its possible anti-inflammatory effect.

Laura Mazzucco Alessandria 24.06.2020