

# CYTOFLUORIMETRIC ANALYSIS OF TWO BONE MARROW COLLECTION SYSTEMS AND THE IMPORTANCE OF CLINICAL RESULTS

Stefano Papini<sup>1</sup>, Cristina Borrelli<sup>1</sup>, Lella Petrella<sup>1</sup>, Mara Fanelli<sup>1</sup>, Maria Pia Petrilli<sup>1</sup>, Pietro Modugno<sup>1</sup>, Veronica Picone<sup>1</sup>, Andrea Mazzanti<sup>2</sup>, Pietro Ornati<sup>2</sup>, Enrico Maria Centritto<sup>1</sup> and Eugenio Caradonna<sup>1</sup>

Gemelli Molise-Campobasso<sup>1</sup>, IRCSS Maugeri - Pavia<sup>2</sup>

## AIM

We analyzed whether two different approaches for bone marrow aspiration (Harvest H vs. Aspire Medical incA) had an effect on the number of stem cells and the purity of bone marrow (BM) harvested.

## MATERIAL AND METHODS

Endpoint of study was occurrence of leg amputation after injection of stem cells obtained with two different sampling techniques for bone marrow aspiration (HvsA); 240 cc of BM are harvested and centrifuged with a standard needle for a final quantity of 40cc. Due to different construction of Marrow Cellution needle 40cc of BM were obtained and centrifugation was not necessary. We compared baseline characteristics of two pts group detect factors, beside technique used for bone marrow aspiration. Continuous variables were reported as median with interquartile range (IQR), and categorical variables were reported as proportion and percentages. Continuous variables were compared using the Mann-Whitney U test for independent samples and Wilcoxon signed rank test for paired samples, while categorical variables were compared using Chi square test. Statistical significance was set at  $p < 0.05$

## DISCUSSION AND CONCLUSION

Bone marrow aspirate which contain a complex mix of nucleated cells, platelets, and growth factors, is known to promote angiogenesis in ischemic tissue. Bone marrow harvesting and preparation is important for the different localization of the cells. Moreover manipulation of bone marrow, as with centrifugation impact the presence and number of stem cells. Increased number of red blood cells alter the function of stem cells. This innovative method (A) allows to harvest only 40cc of bone marrow at different level reducing the contamination of BM from peripheral blood and avoids. Cell population avoid the manipulation of centrifugation and are better preserved. This novel approach appears to improve clinical results.

## RESULTS

We included  $n=58$  patients in the H group and  $n=17$  patients in the A group. Major risk factors for artery disease and comorbidities were balanced in two groups. We analyzed following cell populations by flow cytometer (Beckman Coulter Navios) CD34, CD133, CD117 and CD309. HCT value was also assessed. There purity of BM with H was 73% before centrifugation and 88% after. Purity of BM with A was 93.4 % and is statistically superior to H both pre and post centrifugation. Cell lines CD133 of patients in the A group were significantly different in respect to ones of group H after centrifugation with both approaches (Mann-Whitney-U test CD133% H 0.10 (0.06-0.20); A 0.01-0.02 < 0.0001). Leg amputation was significantly more frequent patients in H group (20/58, 34.5%) as compared to the patients in the A group (1/17, 5.9%;  $p=0.0209$ ). Multivariable logistic regression analysis confirmed that the A technique was independently associated with a 88% reduced probability of leg amputation as compared to the H technique, also when corrected for the age of the patients (OR 0.12, 95% confidence interval: 0.006 to 0.7001,  $p=0.057$ ) (Table 1).

	Harvest (n=58)	Aspire (n=17)	p value
<b>Parameters</b>			
Age, median (IQR)	74 (63-80)	64 (53-70)	0.0049
<b>Risk factors</b>			
Smoke, n (%)	24 (41.4%)	6 (35.3%)	0.6524
Hypertension, n (%)	51 (87.9%)	15 (88.2%)	0.9729
<b>Comorbidities</b>			
Thromboangiitis obliterans, n (%)	10 (17.2%)	4 (23.5%)	0.5585
COPD, n (%)	45 (77.6%)	10 (58.8%)	0.1240
DM, n (%)	32 (55.2%)	11 (64.7%)	0.4846
CKD, n (%)	11 (19.0%)	6 (35.3%)	0.1573
CAD, n (%)	20 (34.5%)	5 (29.4%)	0.6965
<b>Clinical status</b>			
TcpO <sub>2</sub> (Pre), median (IQR)	12 (7 to 15)	12 (9 to 17)	0.2840
Ulcers, n (%)	17 (29.3%)	6 (35.3%)	0.6380
Necrosis, n (%)	25 (43.1%)	3 (17.6%)	0.0564
Gangrene, n (%)	7 (12.1%)	1 (5.9%)	0.4674
<b>Site of Ischemia</b>			
Inferior Right, n (%)	17 (29.3%)	5 (29.4%)	0.9936
Inferior Left, n (%)	17 (29.3%)	7 (41.2%)	0.3564
Inferior non-specified, n (%)	24 (41.4%)	5 (29.4%)	0.3729