Lavaggio delle sacche criopreservate e reinfusione

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Patients care during infusion of HPCs

- The presence of the **cryoprotectant** and **changes resulting from the freezing and thawing process** necessitate special precautions during and after the infusion of the HPC product into the patient.
- The responsibility of the infusing team is to avoid as much toxicity as possible for the patient without harming the viability of the PBPCs at the same time.
- **In the clinical practice**, the graft is often thawed bedside and immediately infused unmanipulated through a central line

Sauer-Heilborn et Al, Transfusion 2004;44:907-916

Section D - Quality management plan

- D8.4 Processing procedures shall be validated in the Processing Facility and documented to result in acceptable target cell viability and recovery.
  - D8.4.1 Published validated processes shall be verified within the Processing Facility prior to implementation.
  - D8.4.2 The Processing Facility shall use validated methods for preparation of cellular therapy products for administration.
  - D8.4.3 Cord blood units that have not been red cell reduced prior to cryopreservation shall be washed prior to administration.
  - **D8.4.4 Cord blood units that have been red cell reduced prior to cryopreservation should be diluted or washed prior to administration.**
  - D8.4.5 If the Processing Facility lacks experience with the type of cellular therapy product requested for a recipient, personnel shall obtain the manufacturer's instructions and follow these instructions to the extent possible.
  - D8.4.5.1 The Processing Facility should verify the processing procedures utilizing practice units similar to the cellular therapy product intended for administration when feasible.

Potential risks of infusion of unmanipulated thawed grafts (I)

Dimethyl sulfoxide (DMSO)

- It is the most popular cryoprotectant in the clinic
- It is a hygroscopic polar compound developed originally as solvent for chemicals.
- It is added at up to 10% to reduce intracellular ice formation and osmotic stress during freezing
- Serum $t_{1/2}$ of DMSO is 20 hours, less then 50% is excreted through the urines.
- A small portion is expired through the lungs for about 24 hours -> characteristic breath odor

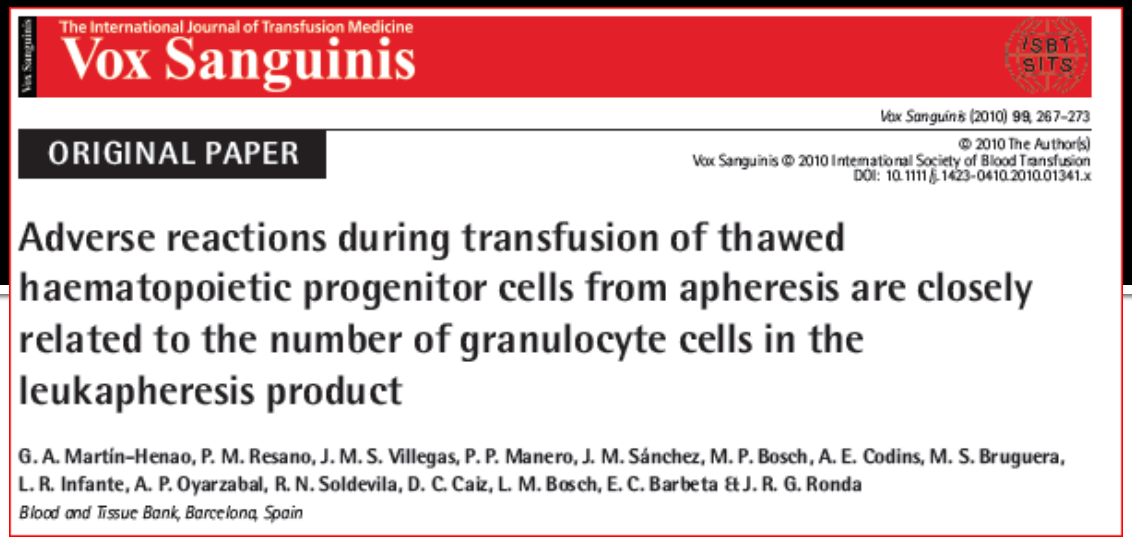
Potential risks of infusion of unmanipulated thawed grafts (II)

- Several side effects have been described during infusion of DMSO.
  - The most frequent symptoms range from mild to moderate severity and include:
    - gastrointestinal (nausea, vomiting, diarrhoea and abdominal cramps)
    - respiratory (cough, dyspnea)
    - Cardiovascular (hypotension, hypertension, bradycardia)
    - neurological
    - dermatological (skin flushing, rash)
    - anaphylaxis.

Potential risks of infusion of unmanipulated thawed grafts

- **Granulocytes** break down during the freezing process, due to their low osmotic tolerance
- **RBCs** undergo lysis when the product is thawed.

Thus, the thawed HPC product contains **granulocyte debris** (e.g., membrane fragments and enzymes), **RBC stroma**, and **free Hb** which may cause side effects when infused into the recipient.



The International Journal of Transfusion Medicine
Vox Sanguinis
ISBT SITS
Vox Sanguinis (2010) 99, 267–273

ORIGINAL PAPER

DOI: 10.1111/j.1423-0410.2010.01341.x

Adverse reactions during transfusion of thawed haematopoietic progenitor cells from apheresis are closely related to the number of granulocyte cells in the leukapheresis product

G. A. Martín-Henao, P. M. Resano, J. M. S. Villegas, P. P. Manero, J. M. Sánchez, M. P. Bosch, A. E. Codins, M. S. Bruguera, L. R. Infante, A. P. Oyarzabal, R. N. Soldevila, D. C. Caiz, L. M. Bosch, E. C. Barbeta & J. R. G. Ronda
Blood and Tissue Bank, Barcelona, Spain

- **24.8%** of adverse events, mostly moderate to severe

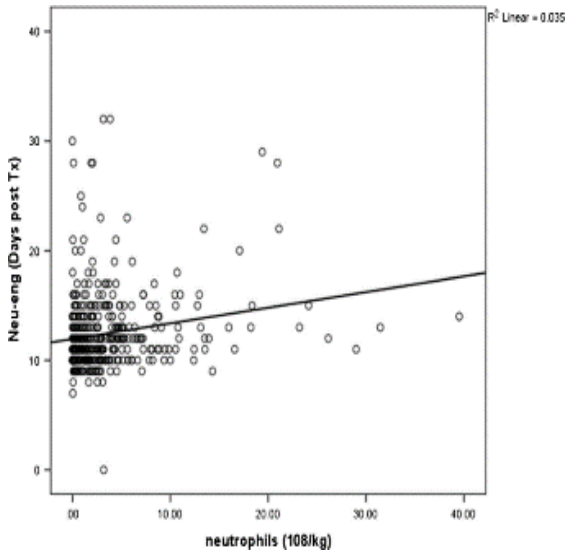
- ✓ The volume of DMSO/kg ($P < 0.001$),
- ✓ volume of red-blood-cells/kg ($P = 0.02$)
- ✓ number of nuclear cells (NCs)/kg ($P < 0.001$)
- ✓ number of granulocytes/kg ($P < 0.001$)

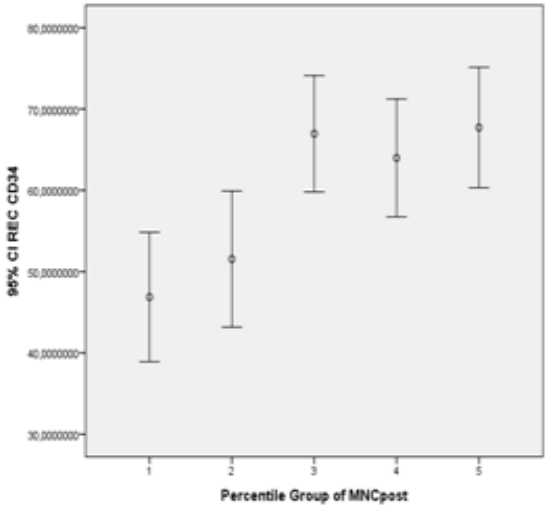
  in the infused graft were significant in the **univariate** analysis for the occurrence of ARs.

- The amount of granulocytes/kg remained significant in the **multivariate** analysis

Impact of the graft quality on the clinical outcome: $CD34^+$ content and PMN contamination

Evaluation of 446 consecutive patients who underwent autologous transplantation in one centre between 2001 and 2012. The impact of pre-transplant and collection factors together with CD34(+) dosing ranges on engraftment, hospital length of stay (LOS) and survival endpoints were assessed in order to identify factors which might be optimized to improve outcomes for patients undergoing autologous transplantation using HPC-A





total cell count (TNC), mononuclear cell count (MNC),

HPC-A: haemopoietic progenitor cells-apheresis

- Time to platelet engraftment was significantly delayed in those receiving low versus medium or high CD34+ doses.
- **Increasing neutrophil contamination of HPC-A was strongly associated with slower neutrophil recovery**

Lijun Bai *et al.* Ann Hematol (2014) 93:1655–1664

Recovery of viable CD34+ cells is proportional both to TNC and MNC content of the frozen product

Urbani *et al.* EBMT2015
Manuscript submitted

Delayed recovery after autologous peripheral hematopoietic cell transplantation: potential effect of a high number of total nucleated cells in the graft

The numeration of total nucleated cells and granulocytes should be considered as a possible quality control variable of PHSCs submitted for cryopreservation.

Hélène Trébéden-Negre, Michelle Rosenzwajg, Marie-Laure Tanguy, François Lefrere, Nabih Azar, Farhad Heshmati, Ramdane Belhocine, Jean-Paul Vernant, David Klatzmann, and Françoise Norol

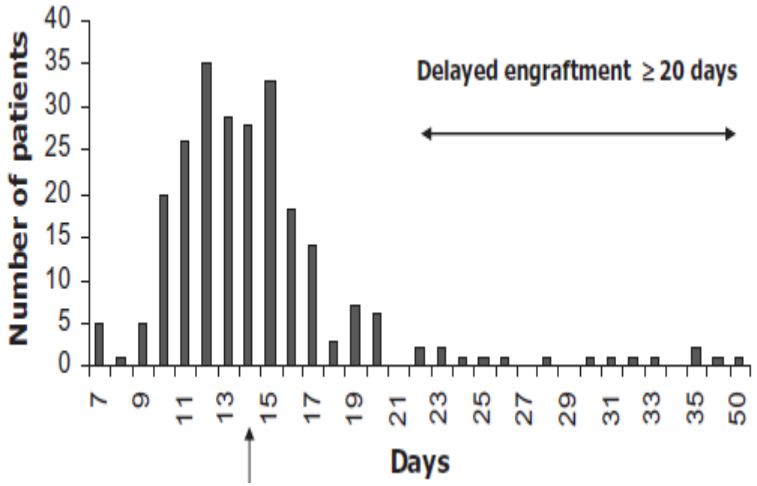


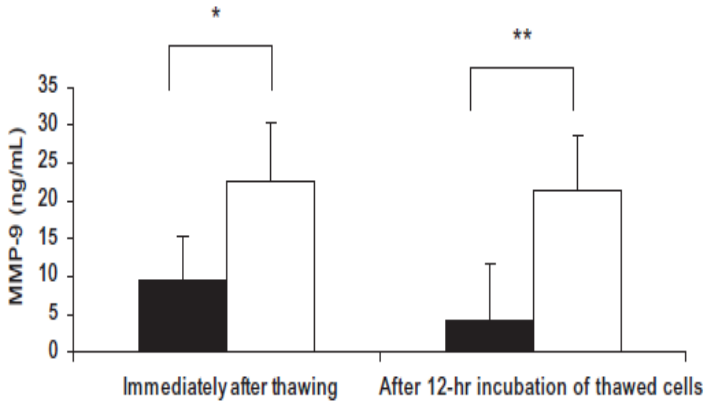
TABLE 3. Predicitive factors of delayed engraftment (>20 days) in patients receiving sufficient numbers of CD34+ cells*

Variable	p value	OR estimate	95% Wald confidence limits
Age	0.04	1.062	1.002, 1.126
TNCs ($\times 10^8$/kg)	0.0044	1.108	1.032, 1.188

* Multivariate analysis by a forward logistic regression model allowed to define predictive factors for delayed engraftment.

TABLE 4. Proteolytic enzymes and proinflammatory cytokines quantification in the graft supernatant, immediately after thawing, according to the engraftment kinetics*

	Mean engraftment (days)		
	≤20 (n = 16)	>20 (n = 16)	p value
IL1β	9.35 ± 9.98	37.48 ± 33.72	0.02
IL-6	7.58 ± 13.81	32.66 ± 28.14	0.02
IL-8	531.9 ± 505	546.72 ± 269	NS
Elastase	936 ± 368	1712 ± 421	0.002
MMP-9	9.42 ± 5.97	22.61 ± 7.72	0.01



Reducing the infusion toxicity
1. DMSO @ lower concentrations

Autologous peripheral blood progenitor cells cryopreserved with 5 and 10 percent dimethyl sulfoxide alone give comparable hematopoietic reconstitution after transplantation

Çiğdem A. Akkök, Knut Liseth, Ingerid Nesthus, Turid Løkeland, Kari Tefre, Øystein Bruserud, and Jenny F. Abrahamsen

TRANSFUSION 2008;48:877-883

- During a 6-year period, 103 patients were transplanted with autologous PBPCs, cryopreserved with either **10% (48 pts)** or **5% (55 pts)** DMSO, respectively, in two consecutive cohorts.
- Median interval between collection and HSCT was 32 days (18-168)
- **No significant difference in median time to PMN and PLT engraftment was demonstrated** in the 2 groups, as well as transfusion requirements and duration of days admitted to hospital
- Cryopreservation with 5% DMSO alone followed by short-term storage in nitrogen is a simple, standardized, and safe
- **Data about DMSO 5% long-term storage are missing**

Reducing the infusion toxicity
2. Cell washing I

Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution

(histocompatibility/transplantation/hematopoiesis/stem cells/blood banking)

PABLO RUBINSTEIN*†, LUDY DOBRILA*, RICHARD E. ROSENFIELD*, JOHN W. ADAMSON‡, GIOVANNI MIGLIACCIO‡, ANNA RITA MIGLIACCIO‡, PATRICIA E. TAYLOR§, AND CLADD E. STEVENS§

PNAS 1995;92:10119-10122

- "Immediately after being thawed, each PCB unit is diluted with an equal volume of a solution containing 2.5% (wt/vol) human albumin and 5% (wt/vol) Dextran 40 in isotonic salt solution, with continuous mixing, and then centrifuged at 400 x g for 10 min. The supernatant is removed, and the sedimented cells are resuspended slowly in fresh albumin/dextran solution to a volume appropriate for infusion to patients or, in these experiments, to the volume originally collected."

Table 3. Leukocytes and hematopoietic progenitors in PCB units, before freezing and after thawing in the presence or absence of cryoprotectant

	Mean cell count/unit, $\times 10^{-6}$			
		Thawed		
Cell type	Fresh	+ DMSO	− DMSO	P*
Total leukocytes	944 ± 73.1	883 ± 70.7	867 ± 78.4	NS
Viable leukocytes	895 ± 70.4	315 ± 40.2	543 ± 71.9	<0.0001
Neutrophils	478 ± 36.0	109 ± 21.5	146 ± 28.2	0.013
Lymphocytes	267 ± 45.8	156 ± 33.6	264 ± 47.4	<0.0001
Progenitors	1.10 ± 0.28	0.68 ± 0.29	1.29 ± 0.33	0.0004

"Hyperosmolarity (10% DMSO 1.25 M) and osmotic shock upon a brusque reduction of osmolality may be responsible."

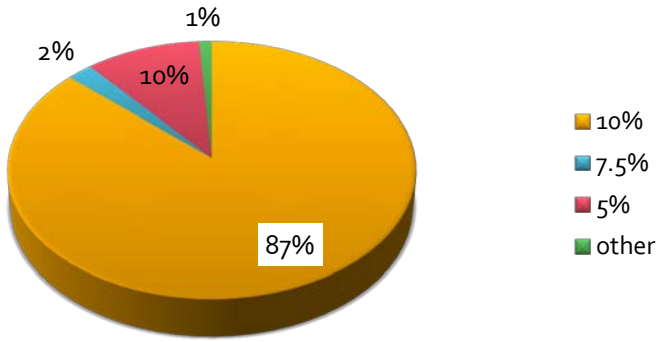
Variation in dimethyl sulfoxide use in stem cell transplantation: a survey of EBMT centres

P Windrum[1], TCM Morris[1], MB Drake[1], D Niederwieser[2] and T Ruutu[3], on behalf of the EBMT Chronic Leukaemia Working Party Complications Subcommittee

Bone Marrow Transplantation (2005) 36, 601–603

- ✓A questionnaire was mailed to 444 EBMT centres involved in auto HSCT
- ✓The study was completed in December 2003
- ✓Replies were received from 97 centres (22%), 2 no auto for about 34,000 transplants
- ✓Of the 95 responding centres, 57 had seen DMSO toxicity (60%)
- ✓The mean incidence per centre of DMSO toxicity was 2.1%.
- ✓10/95 centers washed their product

[DMSO] n=90 Centers



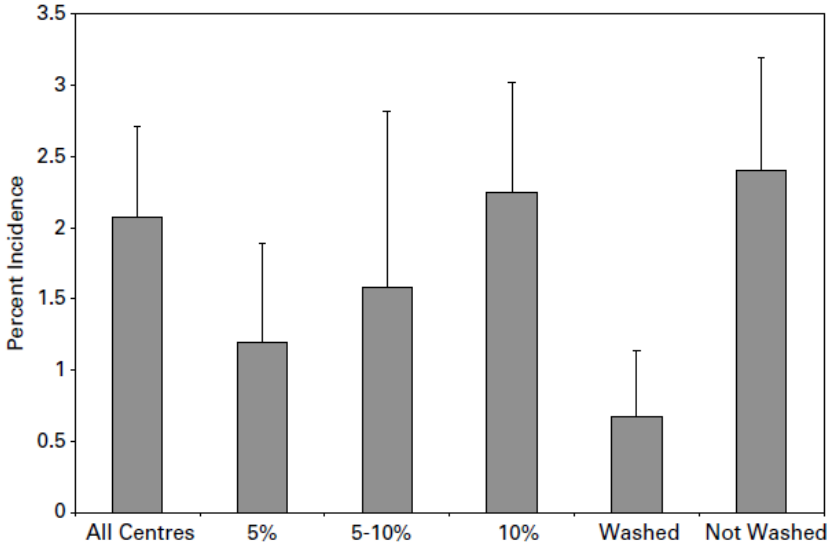


Figure 1 Mean centre incidence of DMSO toxicity by DMSO reduction strategy. Error bars show standard errors.

Methods and devices for thawed HSC washing

Methods or Devices	Mechanism
Manual centrifugation	Centrifugation
CytoMate	Filtration by spinning membrane
Sepax S-100 / Sepax 2	Steps of dilution and centrifugation using a rotating syringe
Cobe 2991	Centrifugation in a variable-volume rotor
Microfluidic method	Diffusion-based extraction in microfluidic channels
Dialysis through hollow-fiber dialyzer	Dialysis across semi-permeable hollow fiber membranes
Dilution-filtration through hollow-fiber dialyzer	Controlled dilution and controlled filtration through semi-permeable hollow fiber membranes

Shu Z et Al, BMT 2013

WASHING THE THAWED GRAFT

CONS

- Sub-optimal graft washing might result in loss of progenitor cells, compromising the engraftment potential
- Time and materials consuming

PROS

- Removal of **all** the toxic components of the graft (DMSO, RBC stroma, free Hb, PMNs debris)
- All the process is carried out in a controlled environment
- The product is stable for hours and doesn't need to be infused immediately

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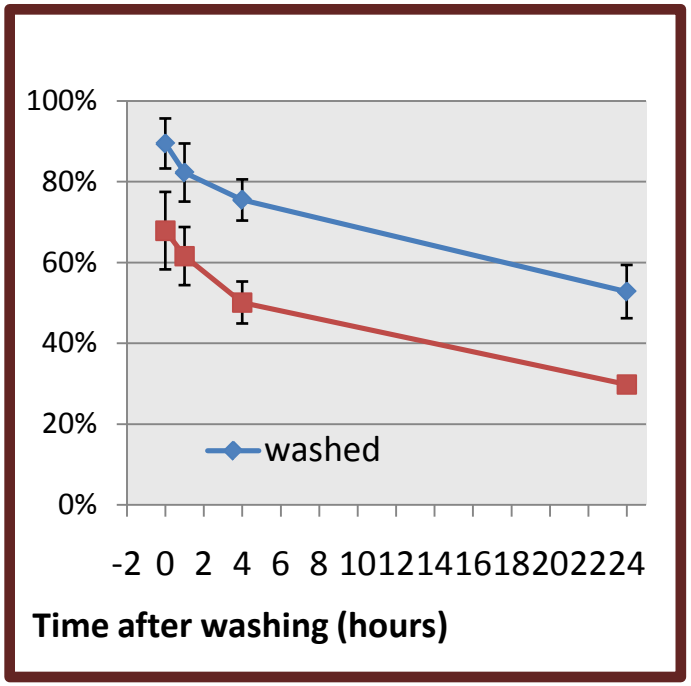
FULLY AUTOMATED WASHING OF CRYOPRESERVED PBSC IN A MULTICENTER STUDY

PRE-CLINICAL STUDY

- A pre-clinical study was carried out to assess recovery, viability and stability of thawed PBSC
- Ten PBSC samples/centre were thawed, characterized and washed by SmartWash system, according to a shared protocol
- Higher viable $CD34^+$ recovery was shown in washed samples at all time points

Recovery of viable CD34+



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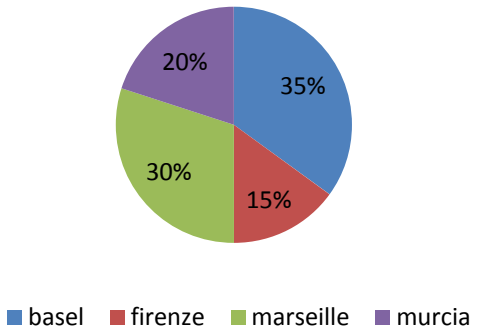
FULLY AUTOMATED WASHING OF CRYOPRESERVED PBSC IN A MULTICENTER STUDY

Center distribution of thawed viable CD34⁺ <70% n=20

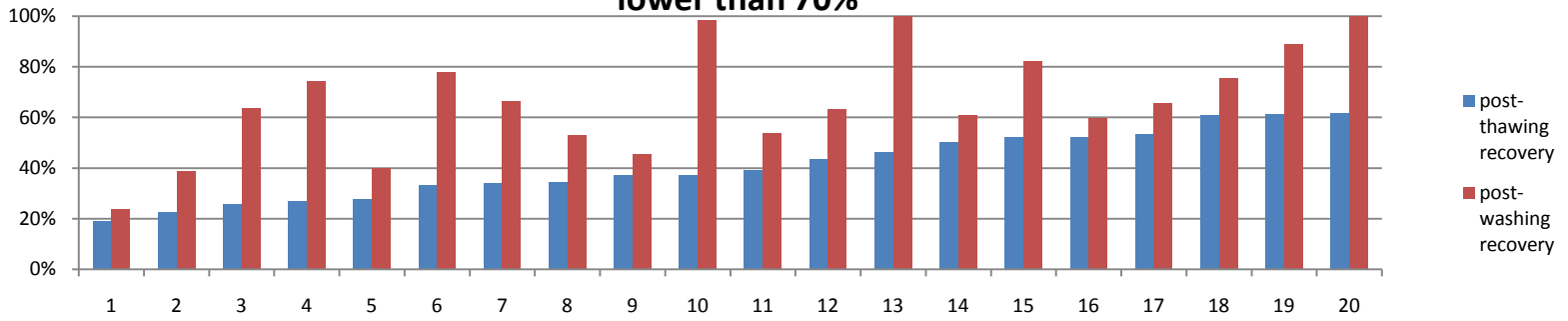


PRE-CLINICAL STUDY

- **Inter-laboratory variability showed no statistically significant differences, even though cellular composition of the apheresis was heterogeneous**
- **Clinical grade hydroxyethyl starch 6% (130 kDa) was validated and used as washing solution.**

WASHING IMPROVES LOW AFTER-THAWING RECOVERY

Impact of washing on 20 samples showing a post-thawing viable CD34+ recovery lower than 70%



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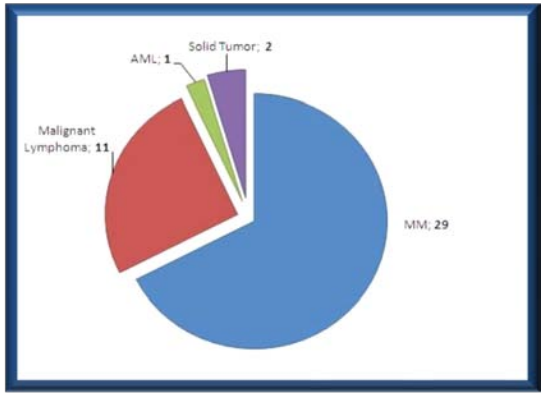
Centre régional de lutte contre le cancer Provence-Alpes-Côte d'Azur

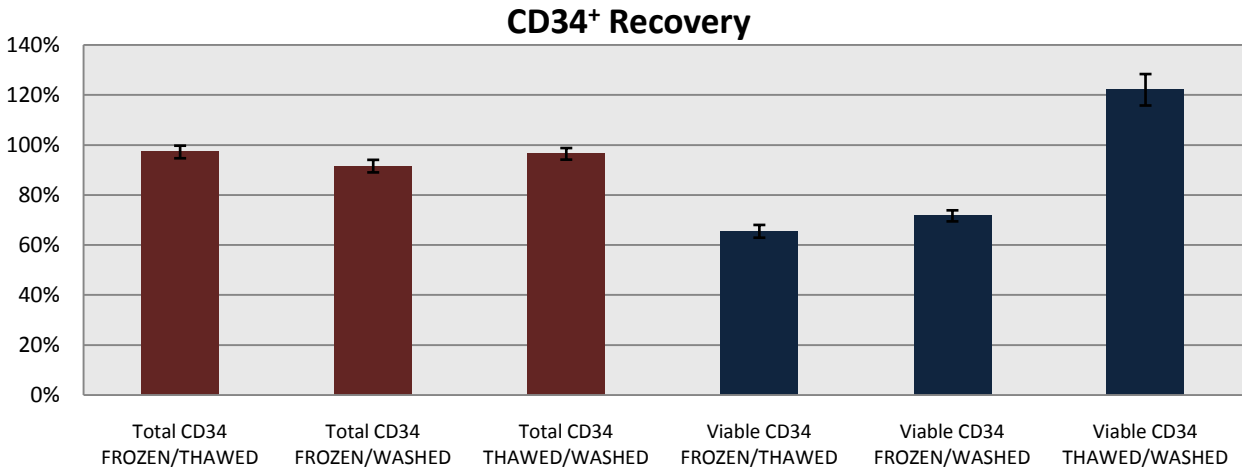
Servicio Murciano de Salud
Arrixaca
Hospital Universitario "Virgen de la Arrixaca"
UNIDAD DE TPH – TERAPIA CELULAR

FULLY AUTOMATED WASHING OF CRYOPRESERVED PBSC IN A MULTICENTER STUDY

CLINICAL STUDY n=43	
Age at HSCT (median, range)	59 (19-71)
Gender (m/f)	20/23
$CD34^{+}x10^{6}$/kg infused (median, range)	3.52 (0.61-20.8)



Diagnosis



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Servicio Murciano de Salud — Arrixaca — Hospital Universitario "Virgen de la Arrixaca"

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FULLY AUTOMATED WASHING OF CRYOPRESERVED PBSC IN A MULTICENTER STUDY

INFUSION-RELATED TOXICITY n=43

TOXICITY SCORE	INFUSION RELATED ADVERSE EVENTS
0	NONE
1	Throat irritation; Thrill; Flashing lights; Nausea; Pruritus; Vertigo; Light bradycardia (HR>40/min); Chest pain.
2	Vomiting; Severe bradycardia (HR<40/min); Flushes; Tremor; Confusion; Abdominal pain; Headache.
3	Bronchospasm; Vision loss.
4	Loss of consciousness; Seizure; Cardiac arrest

Toxicity scale (NCI modified)

TOXICITY SCORE	INFUSION-RELATED ADVERSE EVENTS (n)
1	1
2	0
3	0
4	0

Toxicity reported n=43

ENGRAFTMENT n=43

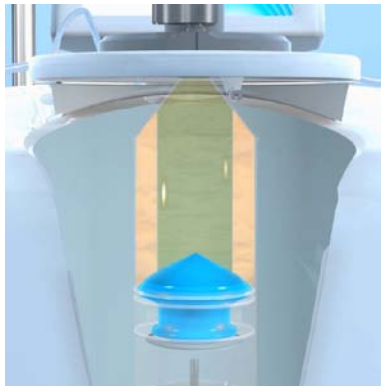
	Days to PMN $0.5*10^9/L$	Days to Platelets $20*10^9/L$
Median (range)	12 (9-19)	12 (7-30)

Automated washing

Sterile connection

HES slow dilution

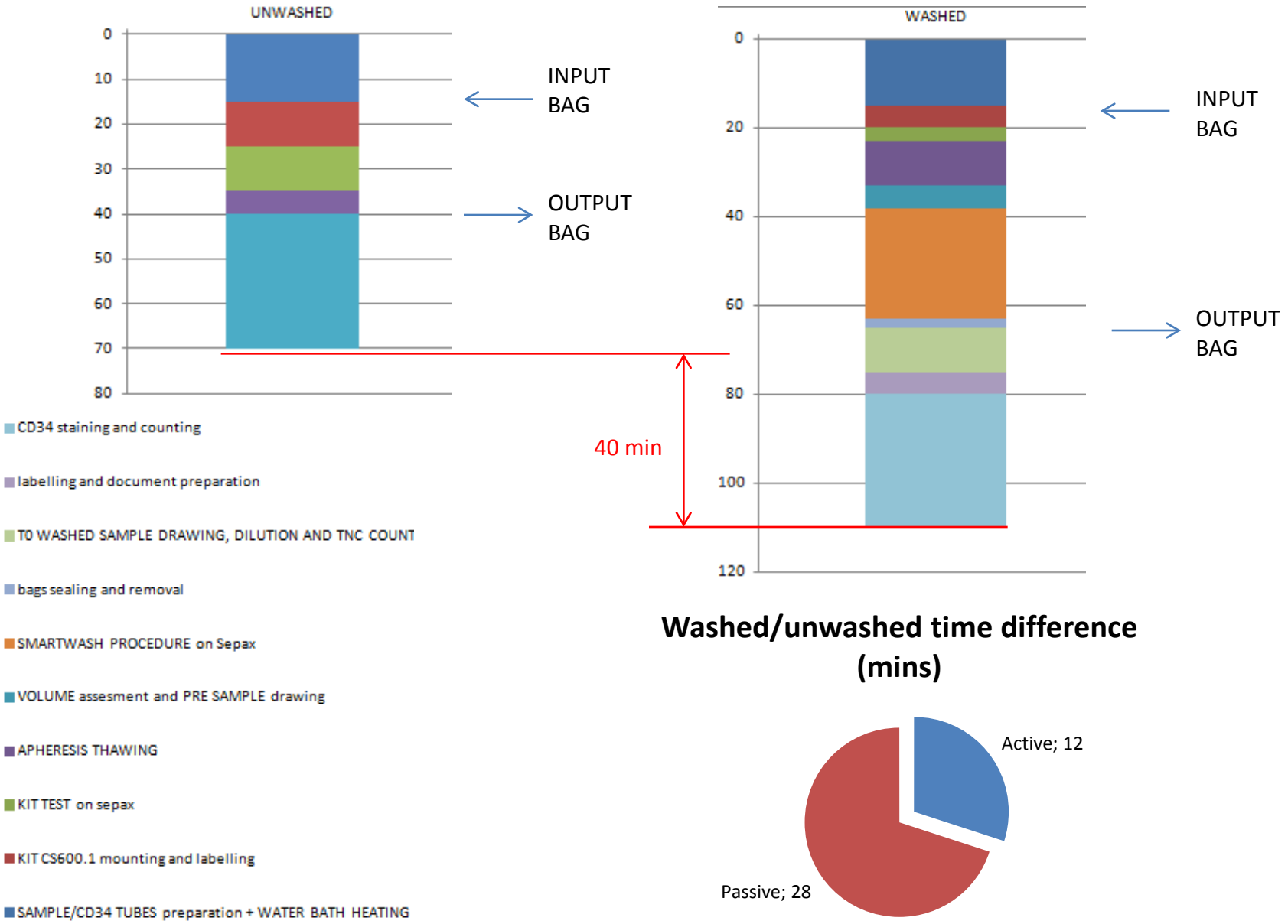
Chamber filling



sedimentation

Extraction of washed cells

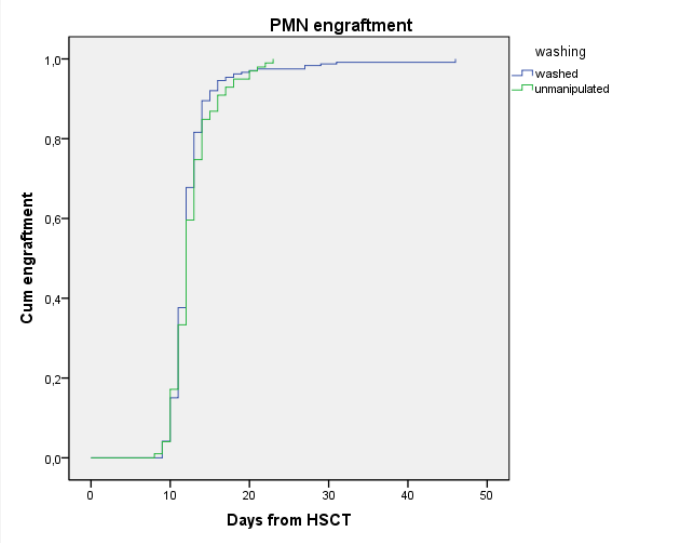
WORKLOAD

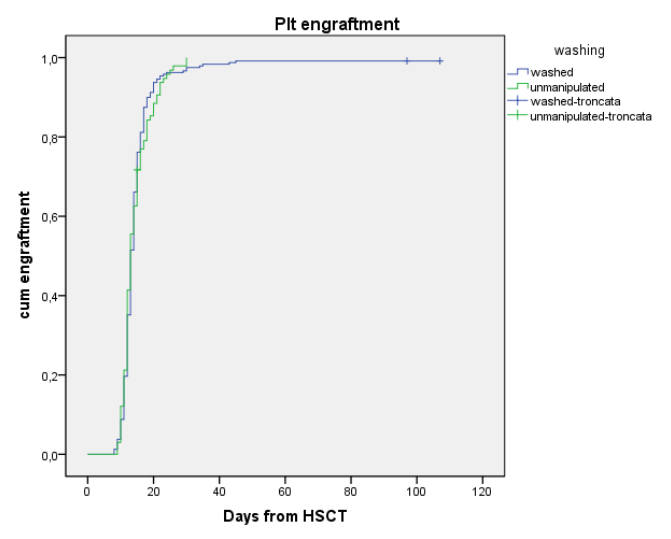


Washed/unwashed time difference (mins)

Washing the thawed PBSC graft: single centre experience I

	Washed	Umanip.	p
N	239	98	
Viable CD34+	4.4±2.3	2.5±2	<0.05
CD34+ viability	80.1±19.4	54.1±29	ns
CD34+ recovery	73.1±23.7	49±30	ns
PMN engraft	12 (9-46)	12 (8-23)	ns
Plt engraft	13 (8-107)	13 (9-30)	ns

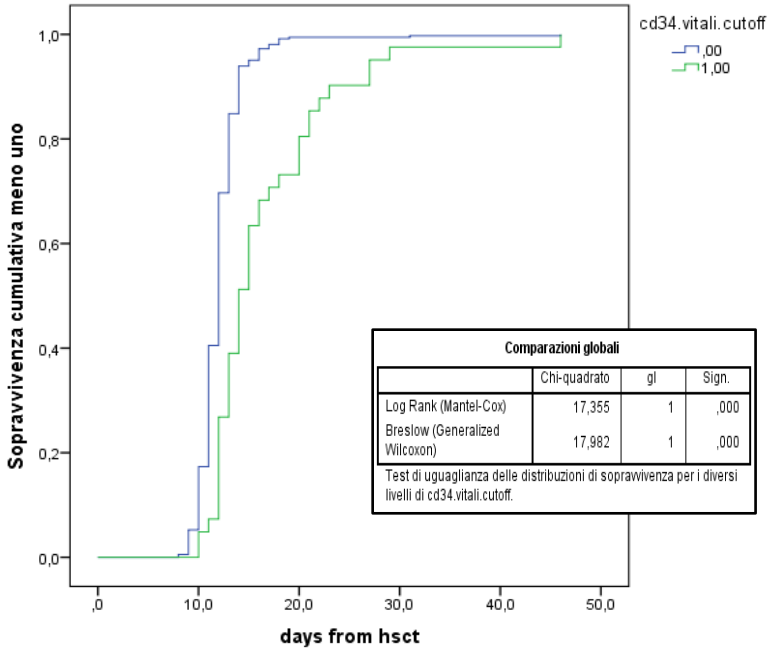


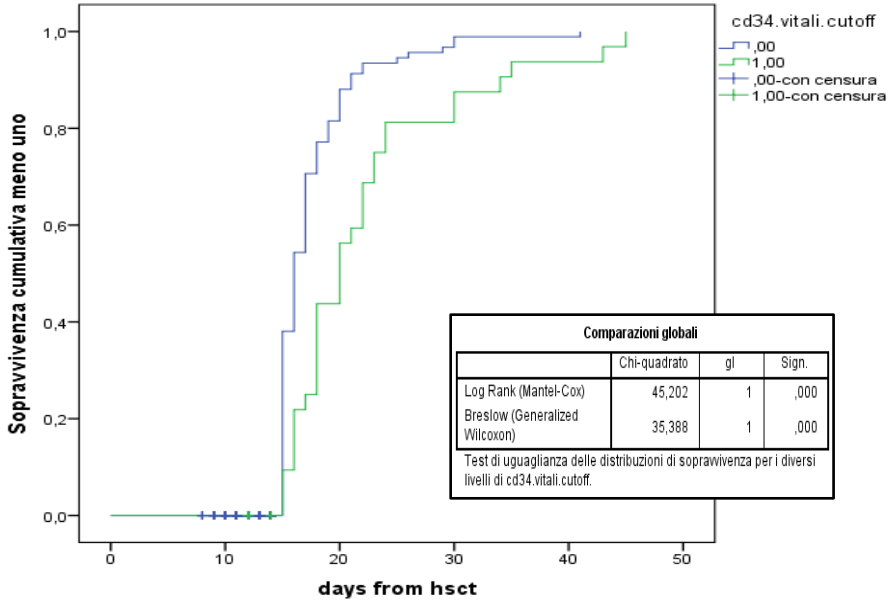


Washing the thawed PBSC graft: single centre experience II

n	314
Period	5/2012- 12/2016
Transplant number (1^{st}, 2^{nd})	280, 34
$CD34^{+}$ cells count	ISHAGE-Modified
Diagnosis	
MM 166 NHL 60 HDG 36 MS 34 AL 17 CLL 1	
Infusion-related toxicity (0, 1, 2)	303, 9, 2

Washing the thawed PBSC graft: single centre experience III





	Media	Dev. St	Mediana	Min	Max	10°percentile
CD34x10^6/kg	3,86	2,33	3,38	0,12	13,91	1,27
Vitalità % CD34	75	24	86.5	4	99	30
Recupero CD34	66	26,83	75,67	3,25	123	26,4

Impact of thawed graft quality on engraftment

Cut-off
WBC 14 days
PLT 17 days

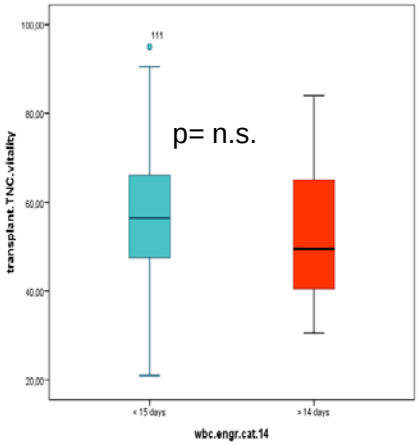
WBC

- Engraftment < 15 days
- Engraftment > 14 days

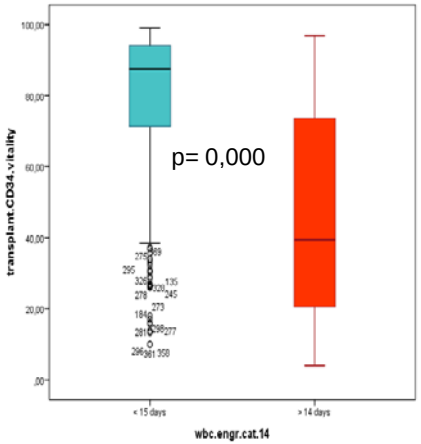
PLT

- Engraftment < 17 days
- Engraftment > 16 days

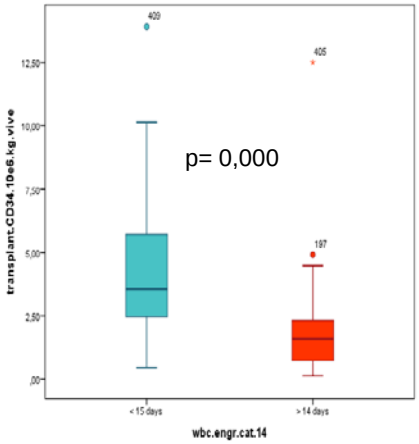
Vitalità TNC

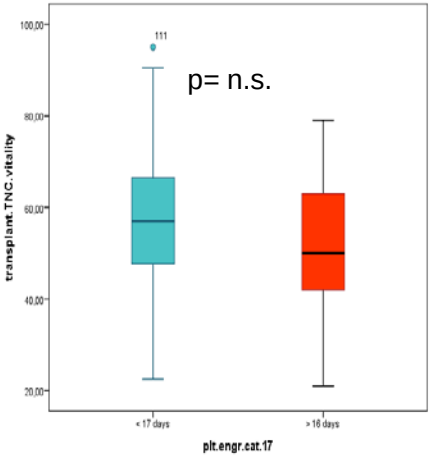


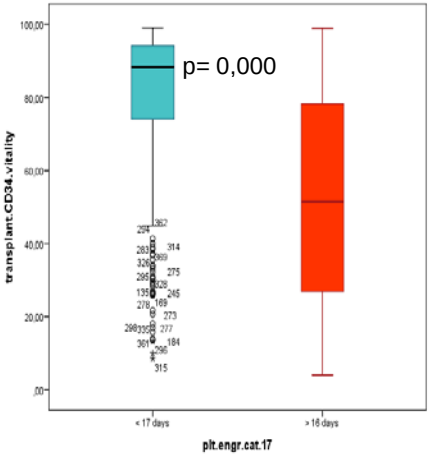
Vitalità VD34+

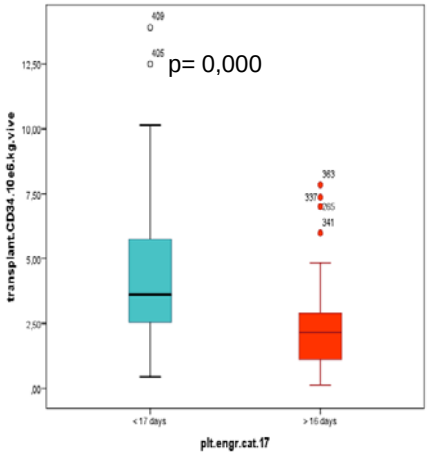


CD34+*10^6/kg





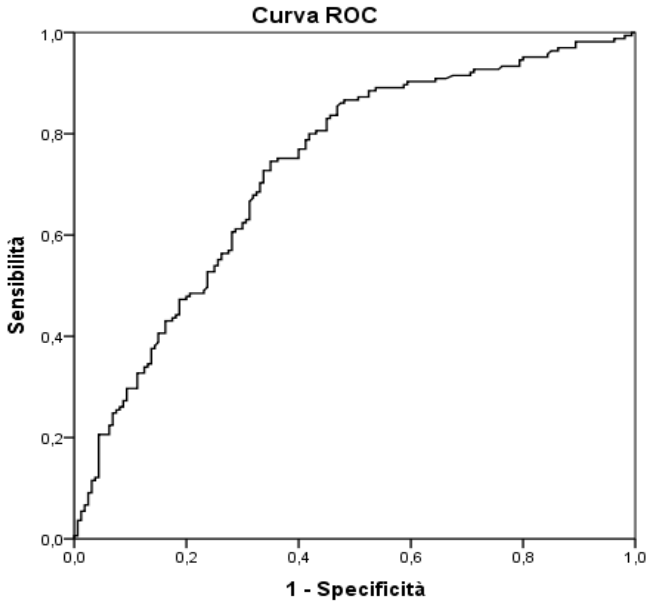




Impact of TNC on viability at thawing

Caratteristiche del GRAFT:

WBC 10^6/ml Cut-off e vitalità delle CD34 al momento dello scongelamento



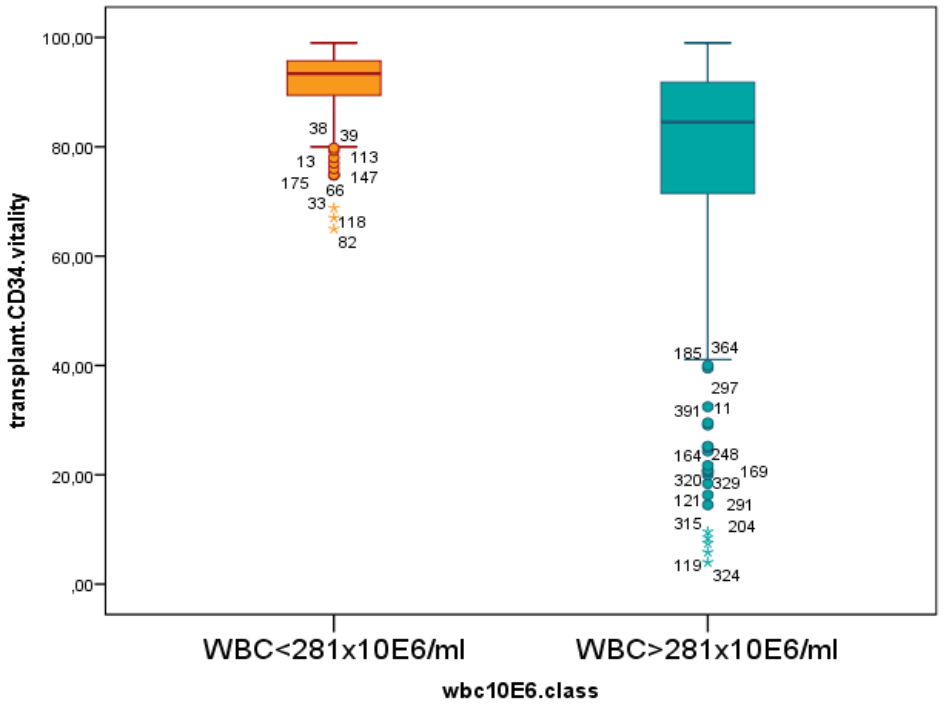
Area sotto la curva

Variabile/i del risultato del test: freezing.wbc10E6.ml.post

Ad area	Errore std[a]	Sign. asintotica[b]	Intervallo di confidenza asintotica 95%	
			Limite inferiore	Limite superiore
,730	,028	,000	,675	,785

Youniden-index (cutoff)

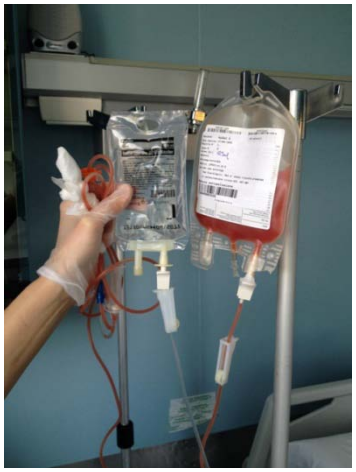
WBC **281x10^6/ml**



GRAFT PREPARATION AND INFUSION

•**Thawing, washing, sampling and labelling is entirely carried out in the processing lab**

Line filling and sampling



Bag washing

Infusion pump

Stop-cock/Syringe

•**The graft is transferred to the BMT Unit under controlled conditions**

•**The infusion procedure is entirely managed by the nursing staff**

•**No pre-medication is administered**

The correlation between the granulocyte content in infused stem cells and side effects of the infusion

Blood Transfus 2011;9:346

Elie Richa

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	No side effects median (range)	Any side effects, median (range)	p value
Granulocytes collected x10⁹/L	20.7 (0.2, 204.0)	36.3 (0.0, 440.0)	0.0149
Granulocytes infused x10⁹/L	12.5 (0.0, 76.4)	27.4 (0.0, 440.0)	0.0001
	No side effects, median (range)	**Major side effects, median (range)**	**p value**
Granulocytes collected x10⁹/L	24.0 (0.0, 292.0)	50.8 (2.7, 440.0)	0.0011
Granulocytes infused x10⁹/L	15.4 (0.0, 292.0)	47.2 (1.4, 440.0)	0.0041

We conclude that although there was a significant association between the amount of granulocytes and side effects of HPSC infusion, **there were no deaths or side effects necessitating the withholding of the infusion** and no correlations with the type of disease.

These results do not support the need to establish protocols to reduce the mount of granulocytes when collecting HPSC.

CELL WASHING: CONCLUSIONS I

- Graft washing is a clinical opportunity to improve both safety and logistics of the infusion process
- This option must be available whenever any infusion-related toxicity must be avoided or for outpatient procedures (DLI)
- The process needs a careful and exhaustive validation to provide an adequate safety profile

CELL WASHING: CONCLUSIONS II

- PBSC graft washed by the SmartWash system is stable and clinically feasible for several hours after thawing
- Frequency of infusion-related side effects is negligible
- Benefits for clinical staff include a reduced clinical burden compared to thawed products at the bedside

CELLULAR THERAPY AND TRANSFUSION MEDICINE UNIT

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