



BLOOD FAR FORWARD

THE COLD STORED PLATELETS PROGRAM

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Blood Far Forward

Three Primary Research Modules

1. Donor Performance and reinfusion - Donor safety research
2. Blood efficacy and safety- Blood Research
3. Training and educational requirements



Norwegian Naval
Special Operation
Commando



Norwegian Armed
Forces Medical Services



HELSE BERGEN
Haukeland universitetssjukehus



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DISCLAIMER

The opinions or assertions contained herein are the private views of the authors and are not to be regarded as official nor as reflecting the views of UiB or Norwegian Armed Forces Medical Service.



Timeline

Blood Far Forward Cold Stored Platelets Program

Cold stored platelets in whole blood

- In vitro comparison of fresh whole blood and reconstituted whole blood
- Effects of platelet sparing filtration of whole blood. Blood Far Forward 1-3 (ClinicalTrials Id: NCT01892670).
- Platelet Recovery And Survival After Whole Blood Treated with Mirasol Pathogen Reduction. Medic 1-2. (ClinicalTrials Id: NCT02330081)
- In vitro Platelet function in Whole Blood collected from a «Walking Blood Bank» (the Royal Norwegian Navy).
- Whole Blood in Civilian Air Ambulance, Bergen
- Leucoreduced whole blood for austere environments
- Leukoreduced cold stored whole blood in a Norwegian emergency helicopter service. An observational study on storage conditions and product quality (HEMS).
- Whole blood in hospital Massive Transfusion Packages.
- Local quality registry on massive transfusions/whole blood
- In vitro hemostatic function of cold-stored leukoreduced CPDA-1 whole blood

Cold stored platelet concentrates

- In vitro platelet function in apheresis platelets stored cold stored cold with agitation for up to 21 days.
- Cold stored apheresis platelets in PAS in treatment of post-operative bleeding in patients undergoing complex cardiothoracic surgery. (ClinicalTrials Id: NCT02495506) Part 1: Storage for up to 7 days.
- In vitro platelet function in apheresis platelets stored cold stored cold without agitation for up to 21 days.
- Effect of delayed cold storage of platelet concentrates in PAS.
- Cold stored apheresis platelets in PAS in treatment of post-operative bleeding in patients undergoing complex cardiothoracic surgery. (ClinicalTrials Id: NCT02495506) Part II: Storage for 7-14 days.

2012

2013

2014

2015

2016

2017

2018



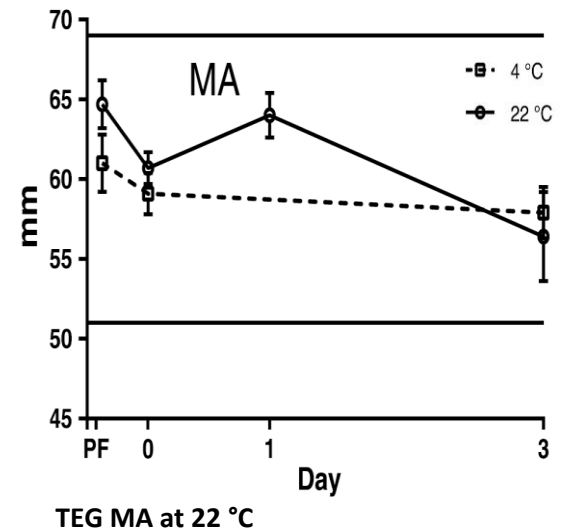
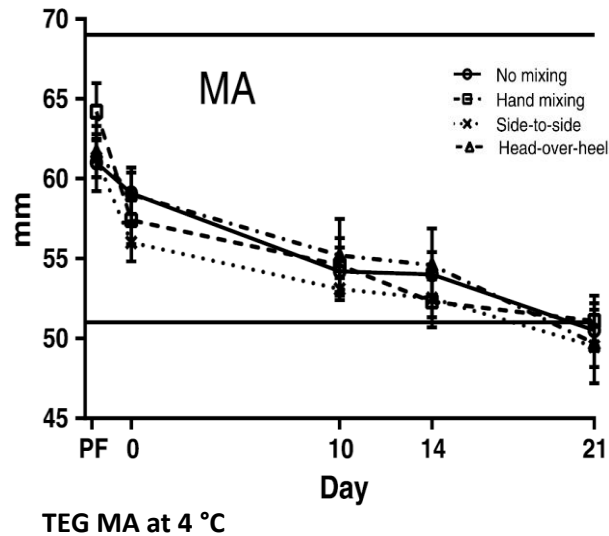
Leukoreduced whole blood for austere environments

-- filtration, agitation, temperature

Remaining hemostatic function within normal range:

- 21 days at 4 °C,
- 3 days at 22 °C or
- 2 hours at 32 °C

No benefit from agitation when cold stored





The MEDIC study

In vitro Characteristics and in vivo Platelet Quality of Whole Blood Treated with the **Mirasol® Pathogen Reduction Technology System**

		Treated STORED (N=13)	FRESH Control (N=13)
Platelet 24-hour recovery (%)	Mean (SD)	43.0 (9.6)	51.9 (12.2)
	Median	46.9	48.1
	95 % CI	37.2, 48.9	44.5, 59.3
Platelet survival time (hours)	Mean (SD)	157.1 (24.8)	192.7 (19.0)
	Median	156.6	192.3
	95 % CI	142.1, 172.1	181.2, 204.2



6,5 mL of radioactive labeled platelets in PPP ready for autologous reinfusion.

Conclusion:

The PRT treatment of whole blood and consecutive storage for 24 hours at room temperature did not make the platelets unsuitable for transfusion.



Coagulation function of stored whole blood is preserved for 14 days in austere conditions: A ROTEM feasibility study during a Norwegian antipiracy mission and comparison to equal ratio reconstituted blood

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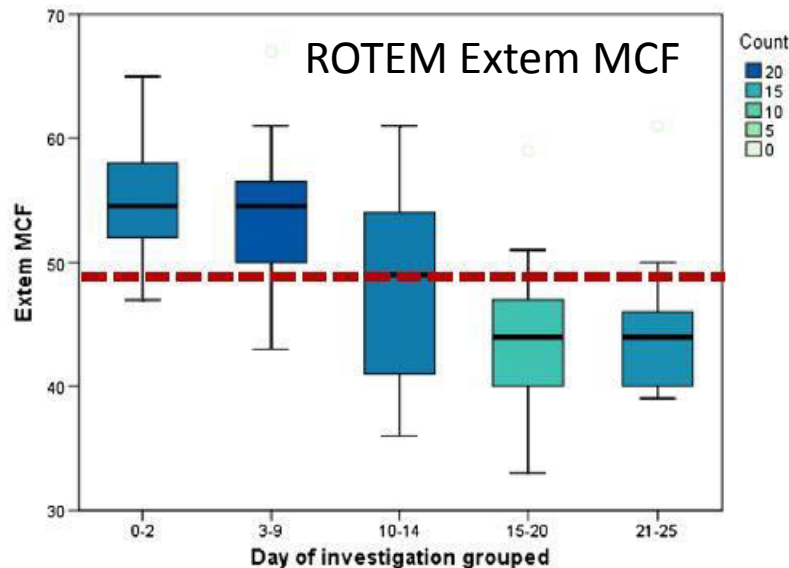


TABLE 2. Comparison of Rotational Thrombelastography Results of Stored Whole Blood from Start of Storage (Day 0–2) through the End of Storage

	Day of Investigation (Grouped)									
	0–2		3–9		10–14		15–20		21–25	
	n		n	n		n		n		
EXTEM										
MCF, mm	18	55.6 (4.7)	20	53.6 (5.7)	18	48.7 (7.5)*	13	44.2 (6.5)*	17	44.4 (5.4)*
α , degree	18	63.7 (6.6)	12	62.2 (8.9)	18	54.5 (14.4)*	13	51.3 (19.6)*	18	50.0 (17.8)*
INTEM										
CT, s	17	173 (12)	18	195 (15)*	16	207 (24)*	14	216 (17)*	17	269 (92)*
FIBTEM										
MCF, mm	17	12.5 (4.0)	15	13.1 (4.7)	17	11.4 (4.3)	12	13.3 (5.4)	16	12.8 (5.6)

* $p < 0.05$ when compared with Day 0 to 2 (independent-samples t test. SPSS version 22.0)

Results shown as mean (SD). Before analysis, tests warning screen errors, preliminary results, or sampling errors were removed.



TABLE 3. Comparison of Impedance Aggregometry From Equal Ratio RWB Versus Healthy Subject Fresh WB on the Day of Collection (Day 1) and at Day 5

	Impedance Aggregometry (AUC, U)				
	ADP	COL	TRAP	ASPI	RISTO
Published Reference Ranges for WB	36.0–101.0	24.0–79.0	75.0–137.0	42.0–100.0	27.0–124.0
Healthy Subject WB Day 1	44.8 (4.1)	35.8 (2.7)	83.4 (5.8)	37.3 (5.4)	43.9 (9.1)
Refrigerated Stored WB Day 5	30.8 (6.7)	17.6 (5.2)	43.3 (6.9)	31.9 (6.1)	30.9 (7.7)
Refrigerated WB Day 10	16.7 (4.6)	10.3 (3.2)	25.8 (4.1)	15.9 (3.7)	18.5 (3.0)
6:6:1 RWB with Day 5 Platelets*	0.08 (0.2)	0.00	4.7 (2.1)	0.00	0.00

*Stored at 22°C

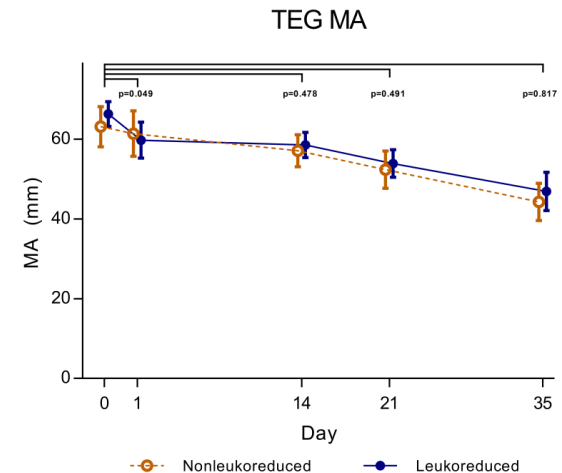
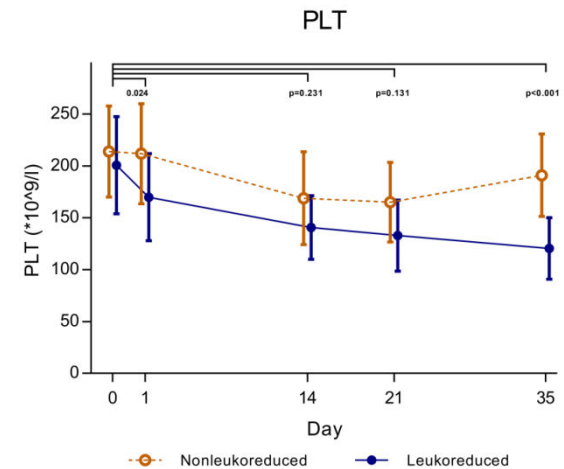
Average platelet count for 6:6:1 RWB samples was $95 (2) \times 10^3/\mu\text{L}$. For comparison, published reference ranges¹⁴ are shown in Row 1, and stored WB values from healthy subjects¹³ previously published by our laboratory are shown in Rows 2 to 4.

6:6:1 RWB, reconstituted WB created from equal ratio components; ASPI, arachidonic acid; AUC, area under the curve; COL, collagen; RISTO, ristocetin; TRAP, thrombin receptor activating peptide; U, arbitrary units.



In vitro hemostatic function of cold stored leukoreduced CPDA-1 whole blood

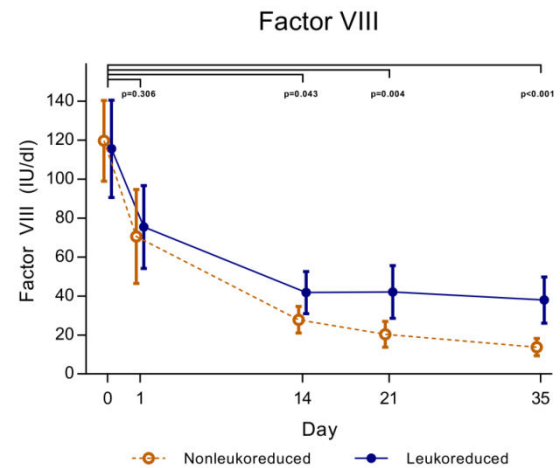
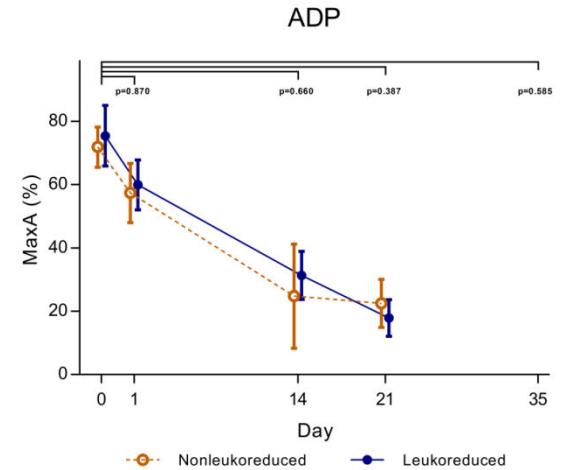
- Some loss of platelets in filtration. No significant differences during storage.
- Gradual decrease in TEG MA in both groups (below normal range on day 35). No difference between groups except on day 1.
- N= 8 per group





In vitro hemostatic function of cold-stored leukoreduced CPDA-1 whole blood

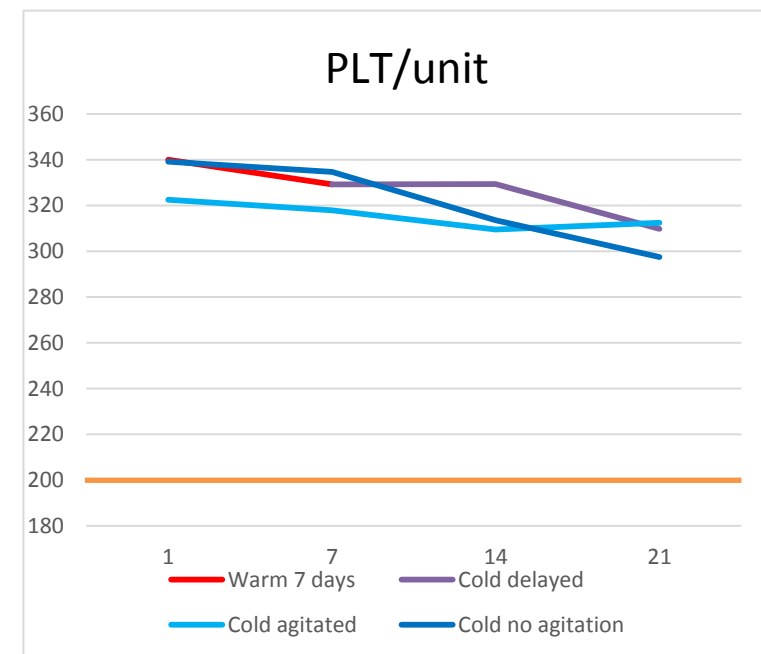
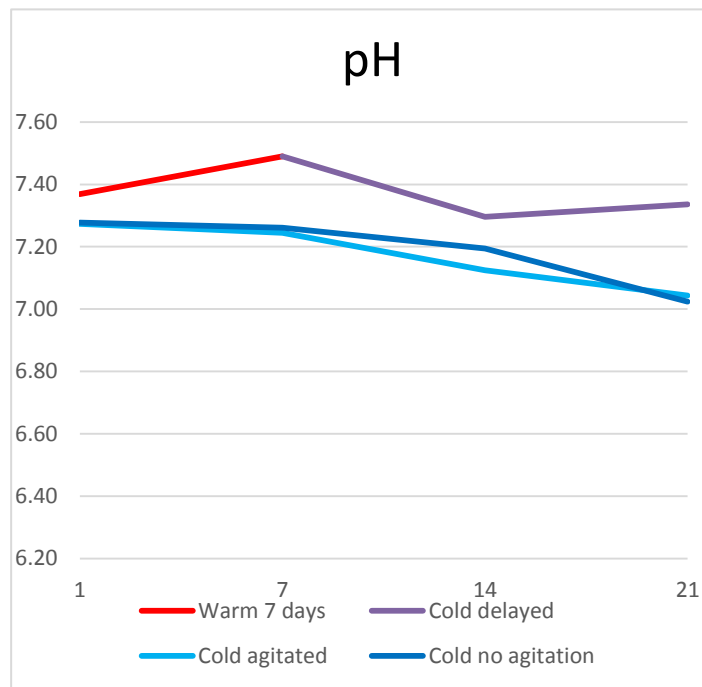
- Loss of most ADP-induced light transmission aggregometry response by day 21. No difference between groups.
- Factor VIII is better preserved in leukoreduced group.





In vitro quality and platelet function of continuous and delayed cold stored apheresis platelet concentrates in PAS

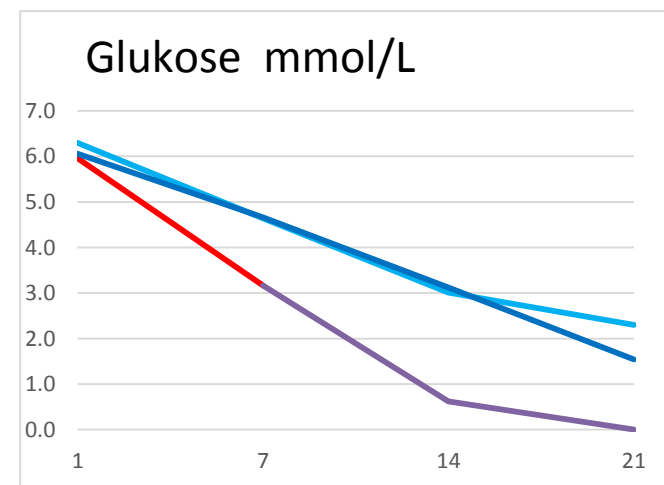
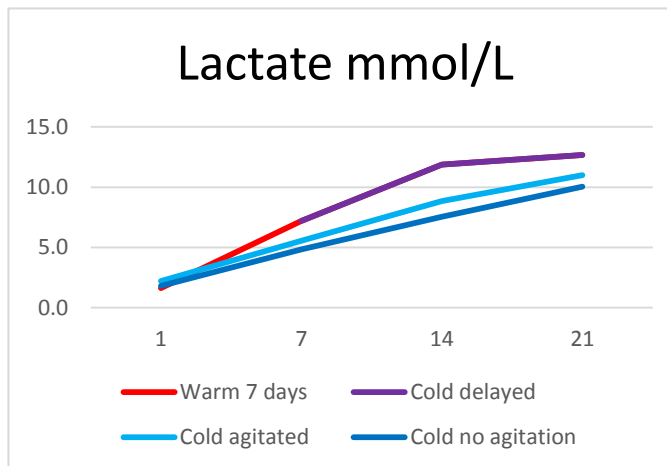
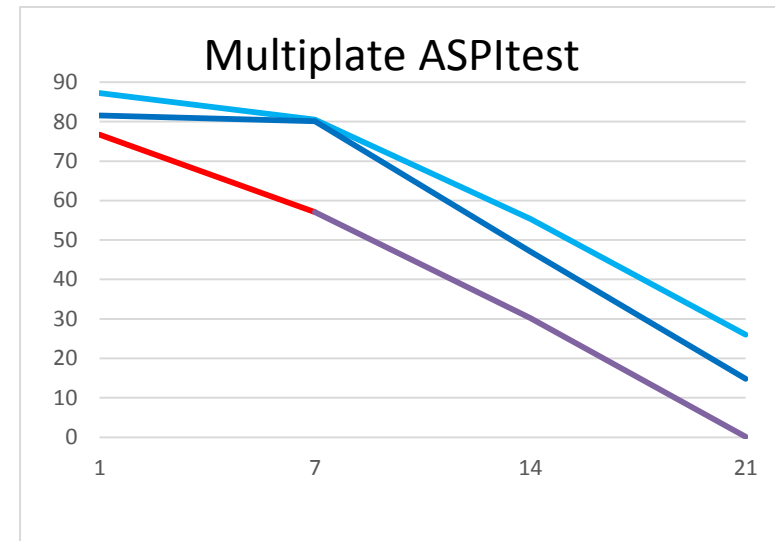
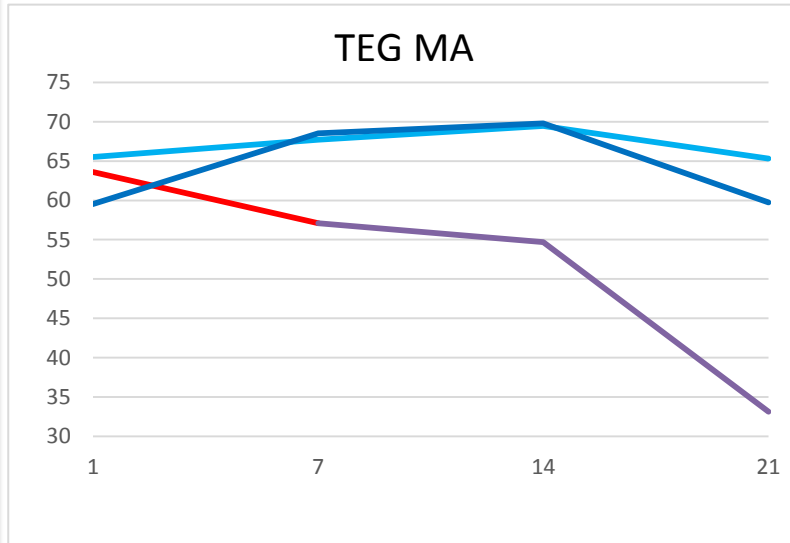
- Cold-stored platelet concentrates meet the EU quality requirements on pH and platelet content



Braathen H, Sivertsen J, Lunde THF, Strandenes G, Hervig T, Kristoffersen EK, Apelseth TO. Quality control of cold-stored apheresis platelet concentrates, ISBT Copenhagen 19th June 2017 3C-S12-04. Manuscript to be submitted: Braathen H, Sivertsen J, Lunde THF, Kristoffersen EK, Assmus J, Hervig TA, Strandenes G, Apelseth TO



Platelet function and metabolism



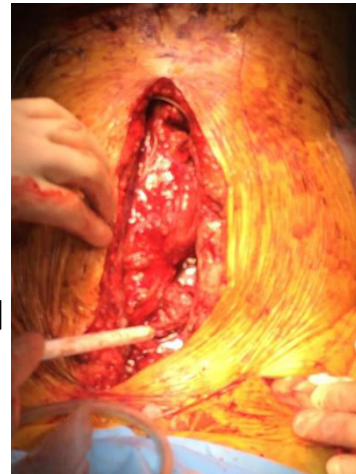


Cold stored platelets control bleeding after complex cardiothoracic surgery: A randomized non-inferiority clinical trial.

- Effect on bleeding, platelet-aggregation, viscoelastic essays and risk



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Materials and methods

Study site: Haukeland University Hospital, Bergen, Norway

Study design:

- Two-armed randomized clinical pilot study

Study arms:

- Leukoreduced Single-donor platelets stored in 65% PASIIM / 35% plasma) with agitation for 7 days
 - Arm 1: Storage at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ (RT-PC)
 - Arm 2: Storage at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ (4C-PC)

Research question:

Are cold-stored single-donor platelets (4C-PC) equally effective to conventional room temperature stored platelets (RT-PC) in treatment of postoperative bleeding in patients undergoing complex cardiothoracic surgery?



Study objectives

Primary:

- Compare *in vitro* coagulation responses by change in platelet aggregation as measured by impedance aggregometry (Multiplate™)

Secondary:

- Compare postoperative immediate blood loss (until day 1 after surgery)
- Compare total blood use
- Describe immediate and short term complications



Study population

Inclusion criteria:

- i. Patients undergoing cardiothoracic surgery
- ii. Expected extra corporal circulation (ECC) time > 120 minutes
- iii. Dual platelet inhibition

Sample size planned: 20+20 platelet transfused patients

Patient enrolment prior to surgery.



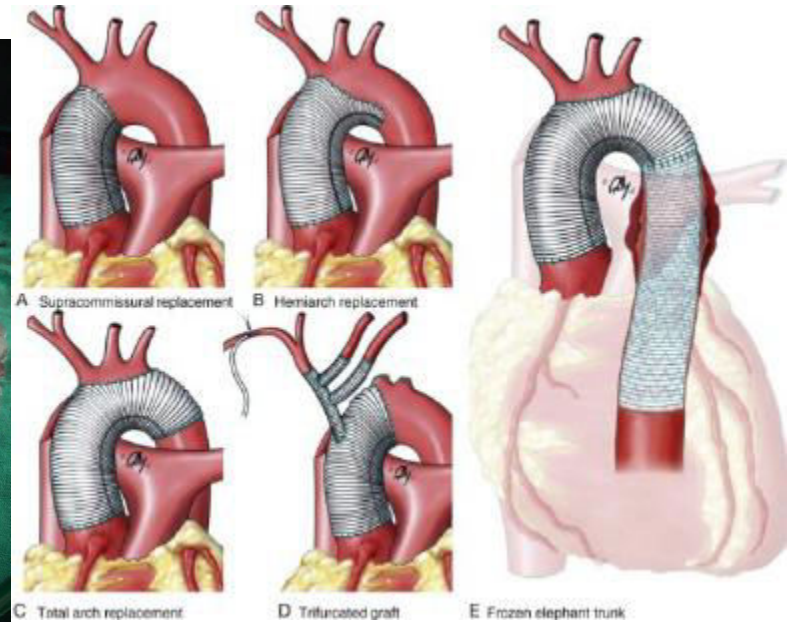
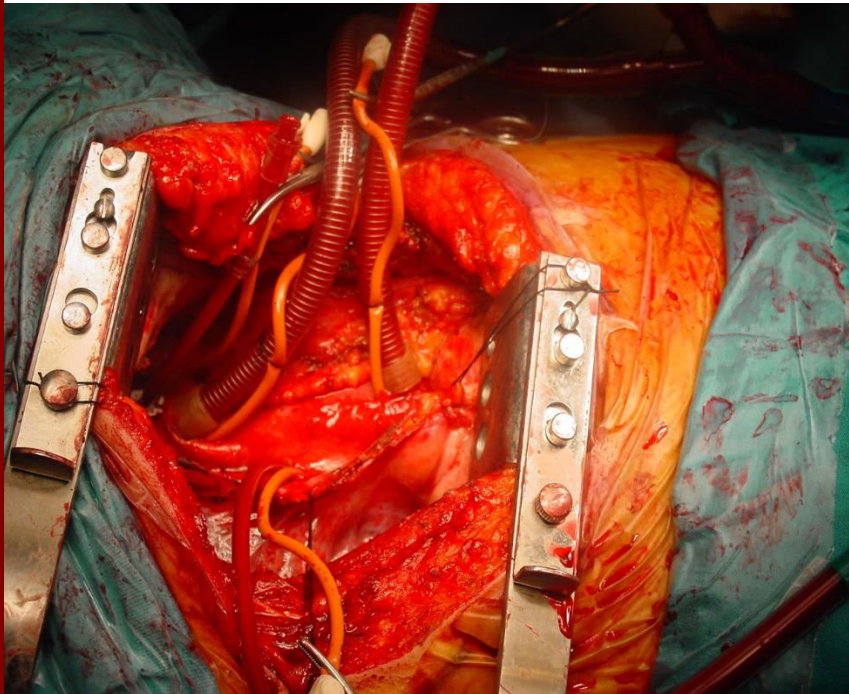
CRITERIA FOR PLATELET TRANSFUSION

- **SURGEON AND ANESTHETIST MAKE THE DECISION TOGETHER**
- **BASED ON VISUAL BLEEDING/MICROVASCULAR BLEED/PROBLEM WITH GAINING SURGICAL HEMOSTASIS («EYEBALLING»)**





Most common type of surgery requiring platelet transfusion in our study



Might require deep hypothermia and circulatory arrest



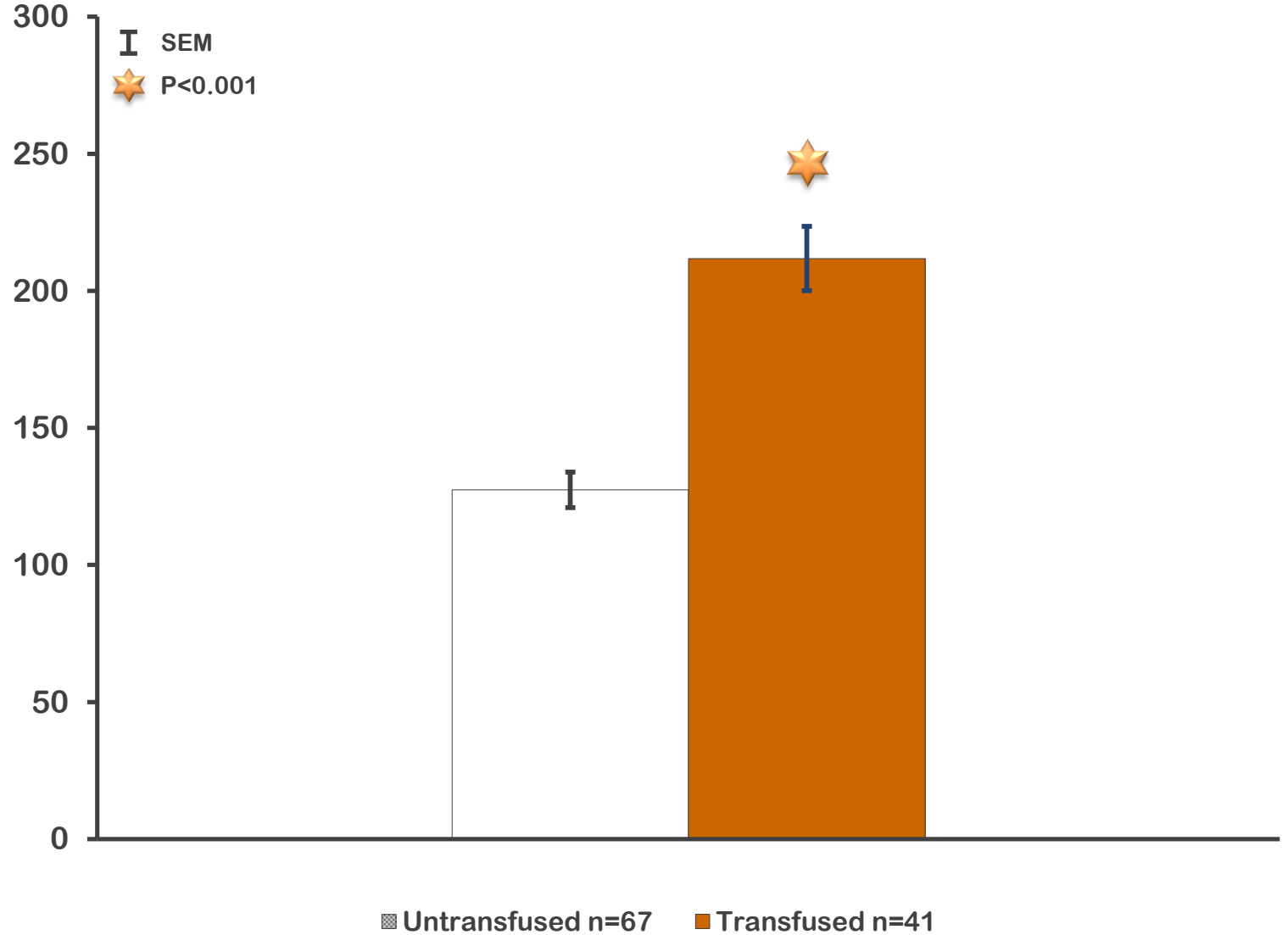
RESULTS – COMPARING PLATELET TRANSFUSED VERSUS NOT TRANSFUSED

**Number of patients enrolled and not
transfused platelets: 67**

**Number of patients enrolled
transfused platelets: 41**

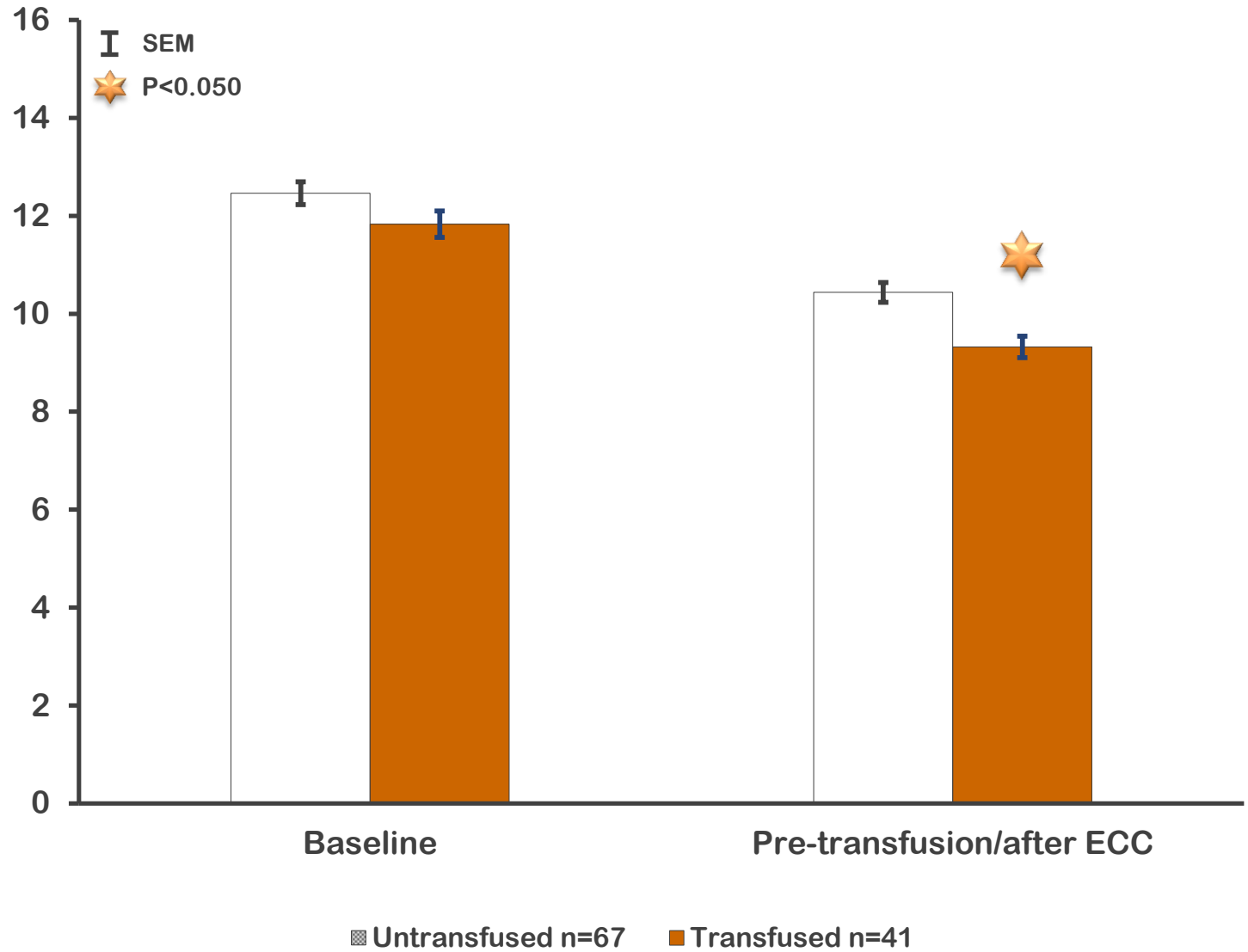


ECC duration (minutes)





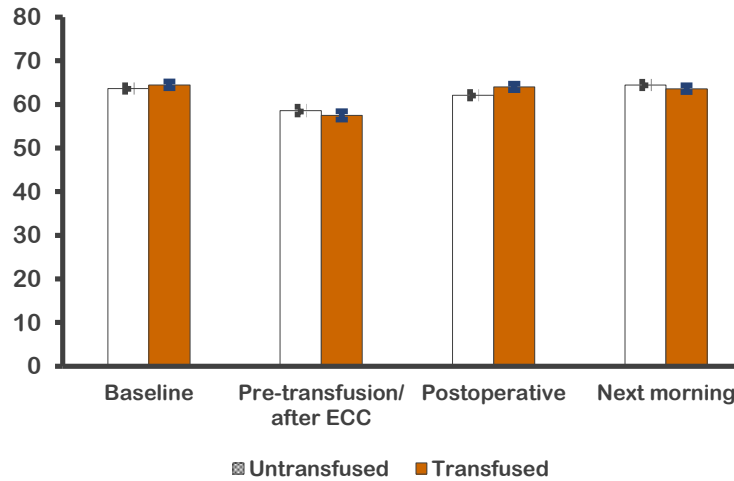
Hemoglobin (g/dl)



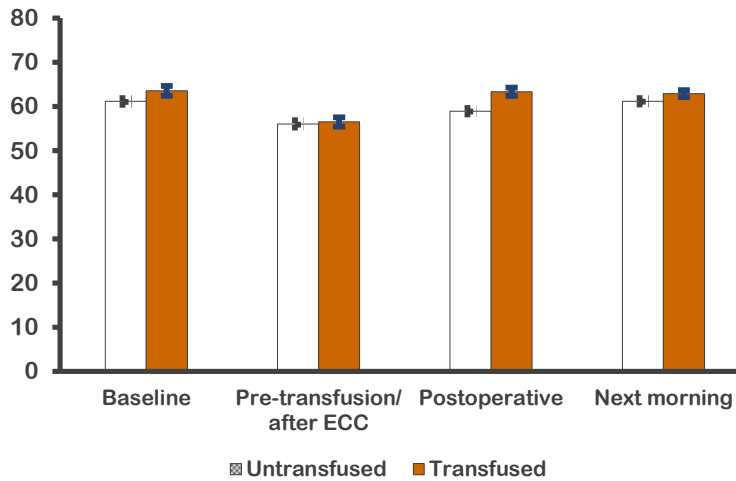


I SEM

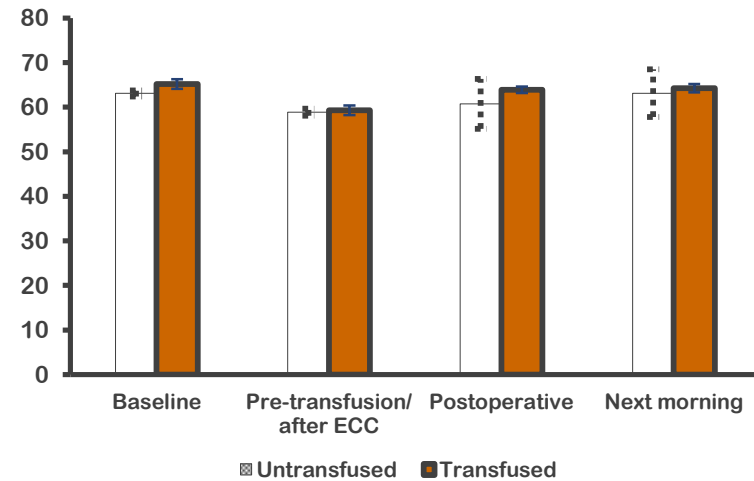
TEG heparinase MA (mm)



ROTEM in-tem MCF (mm)

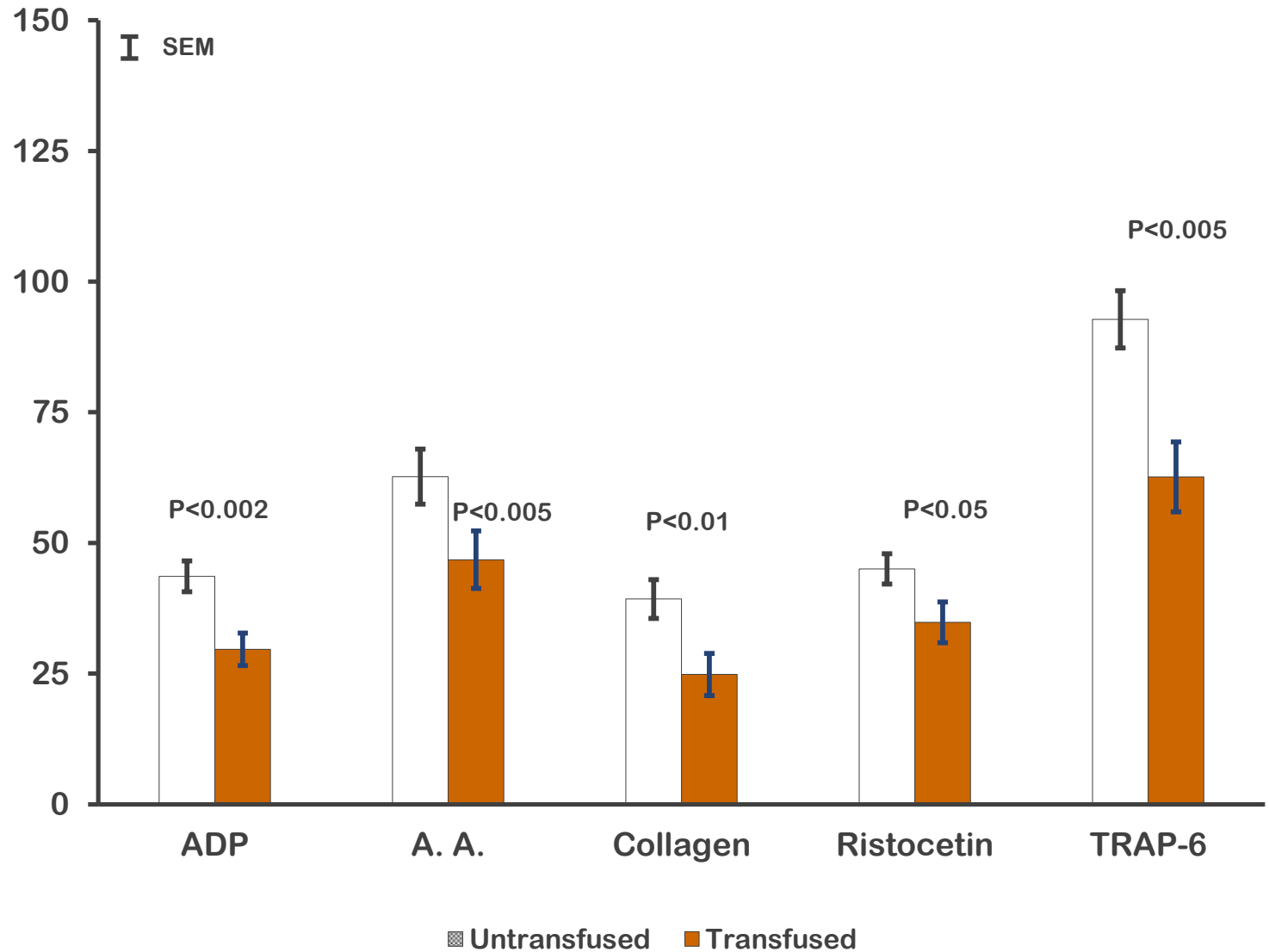


ROTEM ex-tem MCF (mm)





Multiplate after ECC (AUC)





RESULTS:
COMPARING TRANSFUSION OF
4C Plt versus TRANSFUSION OF
RT Plt



Results: patient demographics

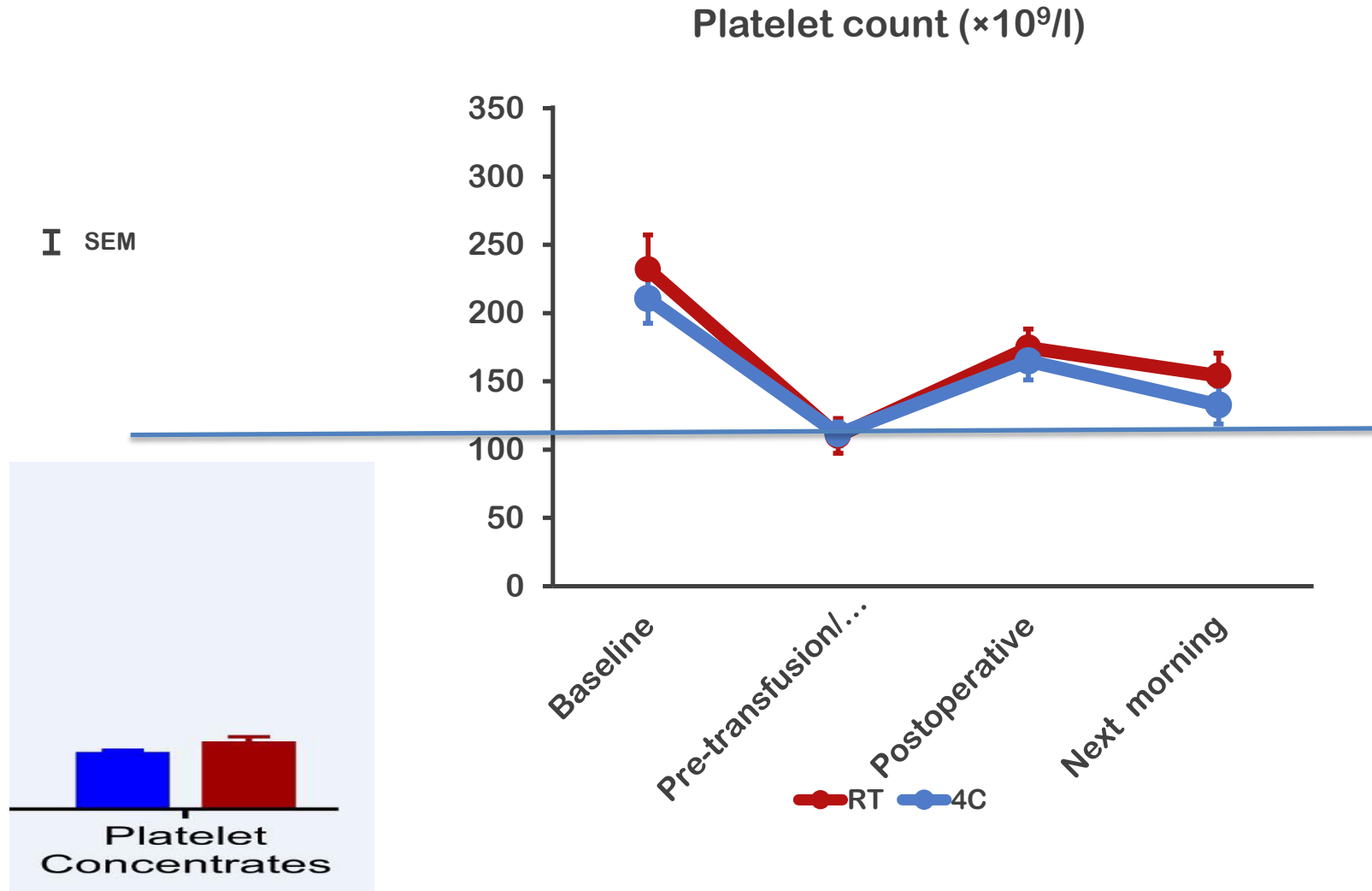
	4 °C (N=20)	22 °C (N=21)	P-value
Age	57 ± 3	64 ± 2	0.087
Gender (female)	7 (35%)	6 (29%)	0.658
Weight (kg)	82 ± 5	85 ± 3	0.589
Cardiopulmonary bypass (CPB) time (min)	201 ± 14	222 ± 18	0.392
Aortic clamp time (min)	125 ± 12	124 ± 10	0.959
EuroScore	29.4 ± 5.4	20.7 ± 4.7	0.233
Ejection fraction (EF)	55.0 ± 1.6	55.5 ± 1.9	0.836
Primary diagnosis			0.325
Aortic arch surgery	9 (45%)	10 (48%)	
Endocarditis	4 (20%)	4 (19%)	
Other	7 (35%)	7 (33%)	
Anti-platelet drug(s)	8 (40%)	11 (52%)	0.427

*Results reported as mean ± SEM or number (percentage) per group . Independent Samples T-Test. And Chi-Square Test. SPSS version 24.0. p<0.05 considered significant.

EUROSCORE=European system for Cardiac Operative Risk Evaluation

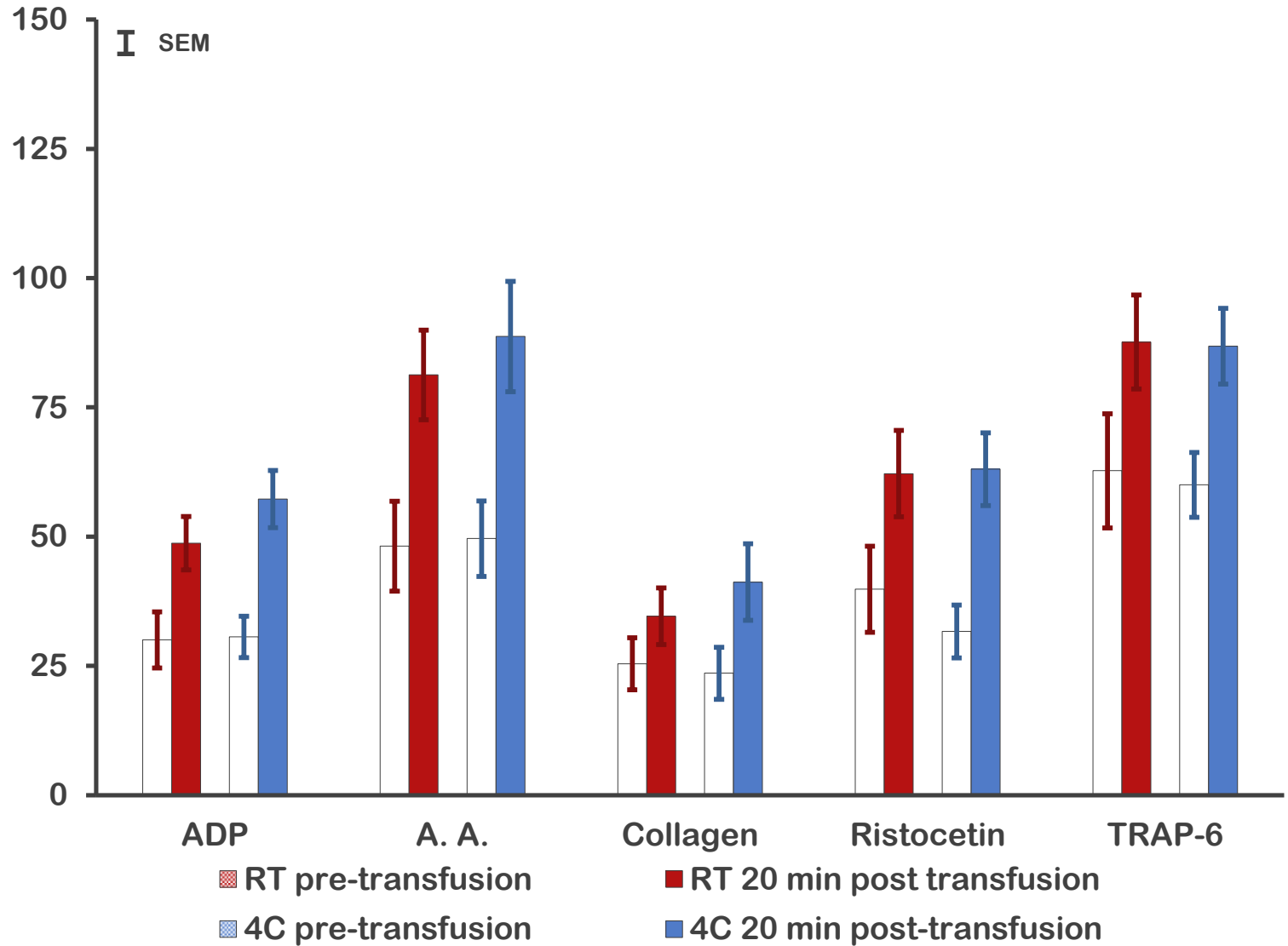


Platelet transfusions and total platelet counts



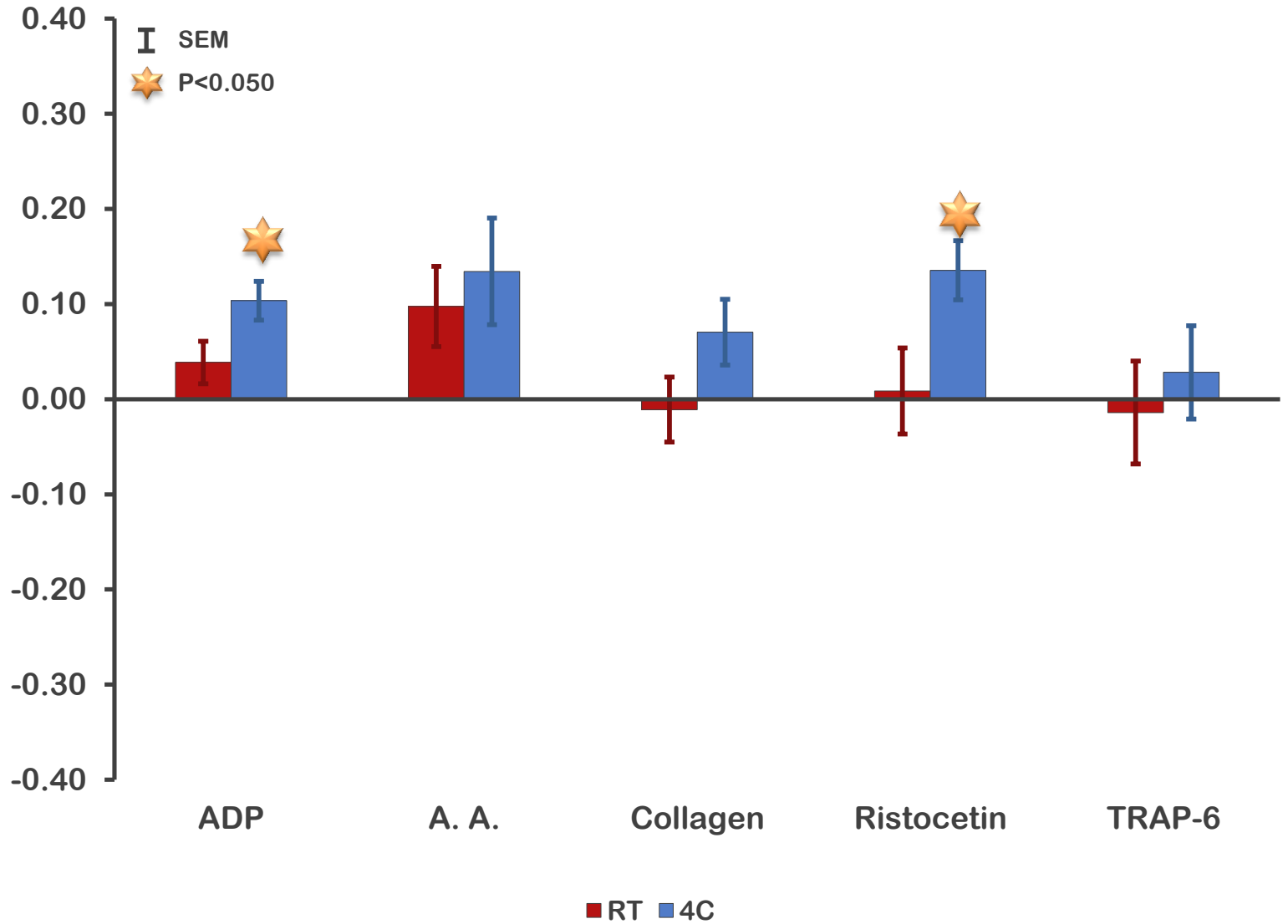


Multiplate before and after transfusion (AUC)





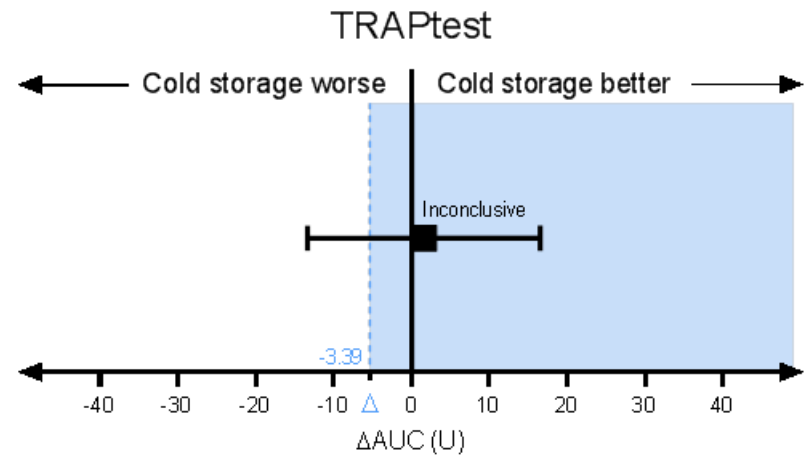
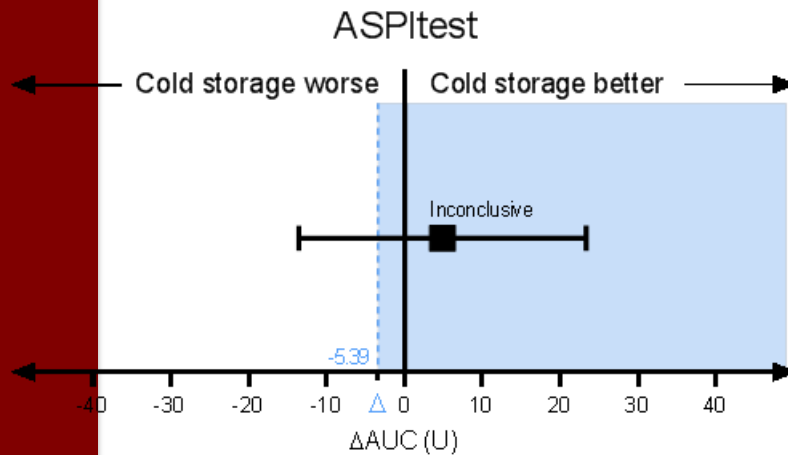
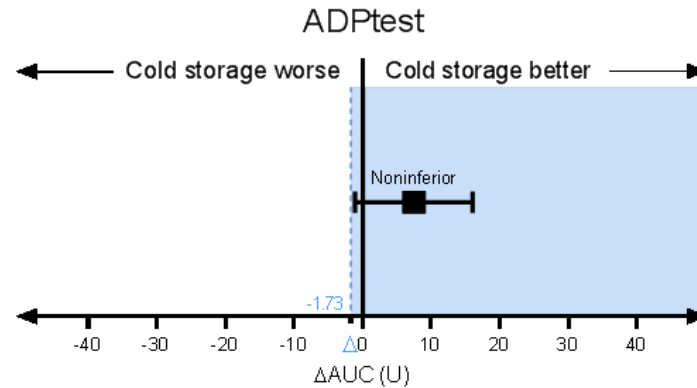
Change in Multiplate/TPK ratio following transfusion





Non-inferiority analyses

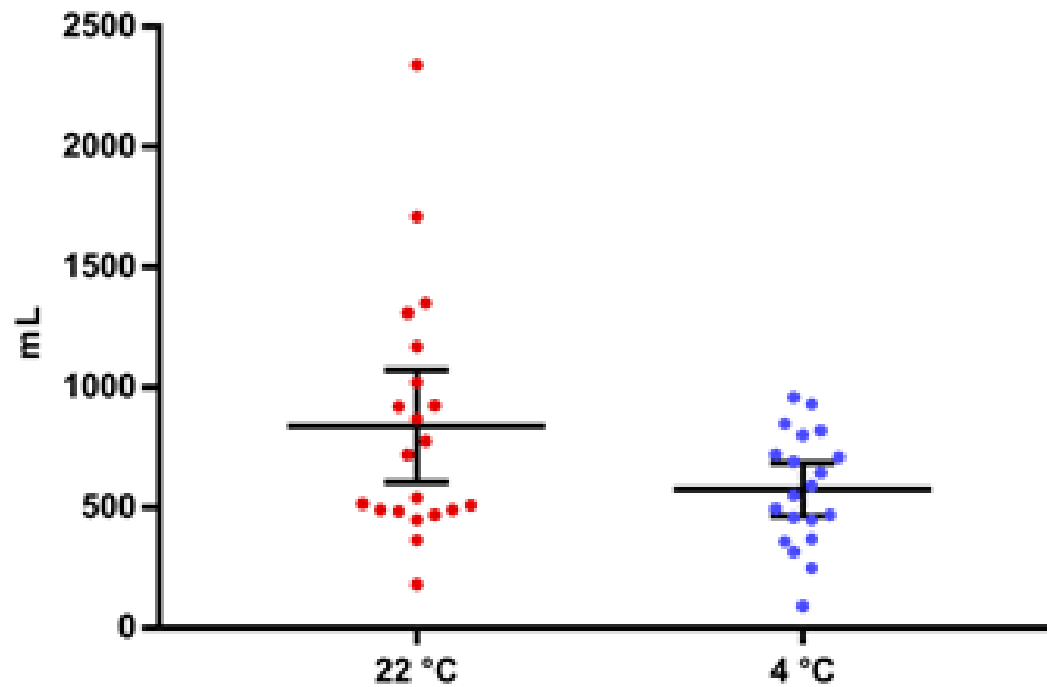
Multitplate aggregometry





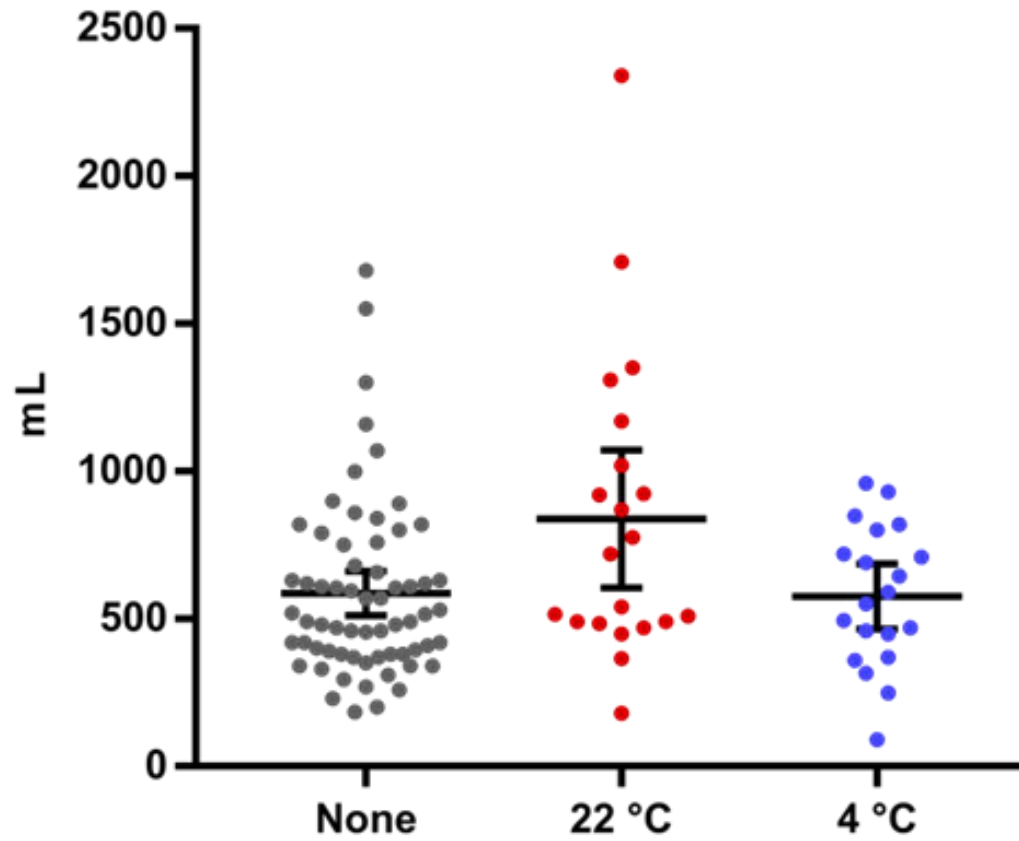
Cold platelets in PAS: Effect on bleeding

Chest Drain Output



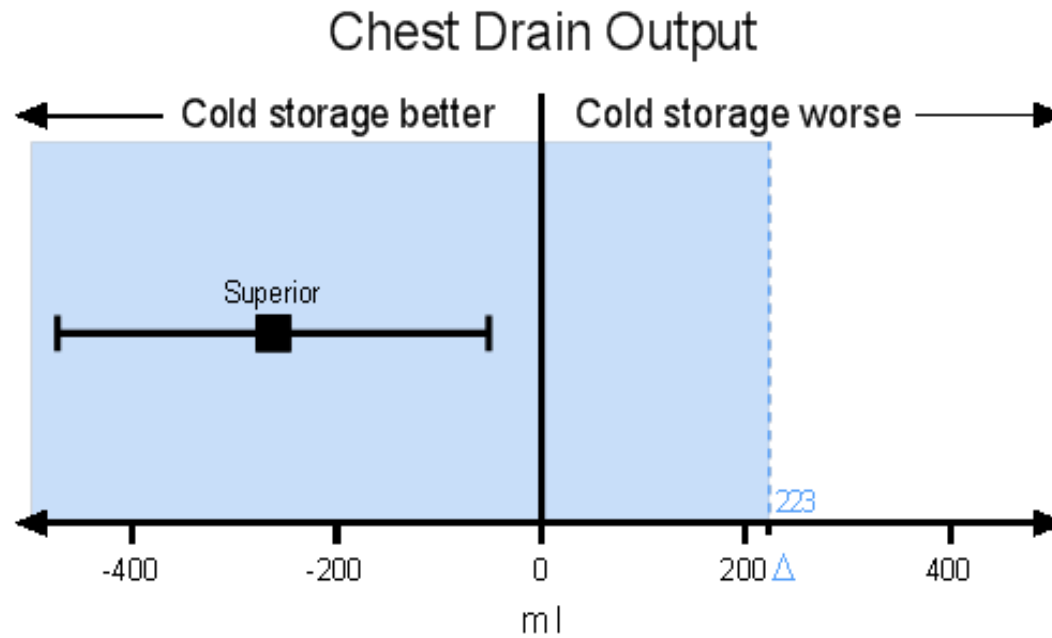


Chest Drain Output



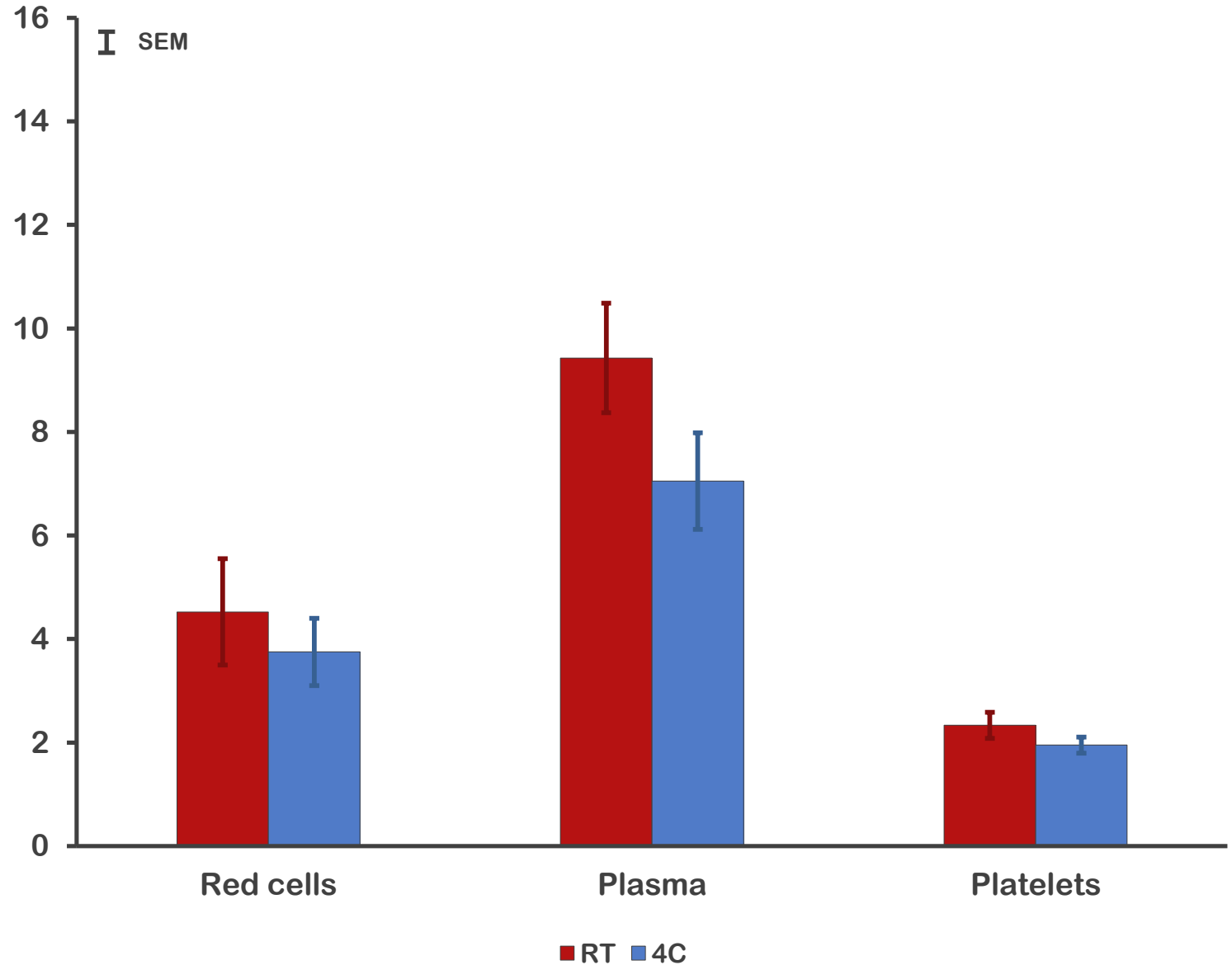


Non-inferiority Chest drain output





Blood component use (units)





Postoperative complications

- **No difference in mortality**

22 °C : 3 (14%) deaths during hospital stay (N=21)

4 °C: 2 (10%) deaths during hospital stay (N=20)

- **No difference in number of thromboembolic episodes during study period (28 days)**

22 °C : 7/21 (33%)

4 °C: 5/20 (25%)

- **No difference in length of stay ICU (days)**

22 °C : 6.1 ± 8.7

4 °C: 8.1 ± 9.2



CONCLUSION

Clinically important outcome: Lower bleeding in CSP, similar blood product use.

«Eyeballing» seems to be equally predictive for platelet transfusion compared to aggregation



Effect and safety of platelets stored cold for 7-14 days

Study site: Haukeland University Hospital, Bergen, Norway

Study design: Pilot prospective observational study

Study arm:

- **Leukoreduced Single-donor platelets stored in 65% PASIIM / 35% plasma) without agitation for 7-14 days**

Research question:

What is the effect and safety of single-donor platelets stored for 7-14 days in treatment of post-operative bleeding in patients undergoing complex cardiothoracic surgery?



Study population

Inclusion criteria:

- i. Patients undergoing cardiothoracic surgery
- ii. Expected extra corporal circulation (ECC) time > 120 minutes
- iii. Dual platelet inhibition

Sample size planned: 10 platelet transfused patients

Patient enrolment prior to surgery.



Study outcomes

1. Describe postoperative immediate blood loss (until day 1 after surgery)
2. Describe *in vitro* coagulation responses by change in platelet aggregation as measured by impedance aggregometry (Multiplate™)
3. Compare total blood use
4. Describe immediate and short term complications



Preliminary results

A total of 19 patients included:

- 8 patients received platelet transfusions
- 6 patients with no transfusion
- 1 mixed transfusion
- 1 intercurrent death (before transfusion)
- 3 excluded due to ECMO

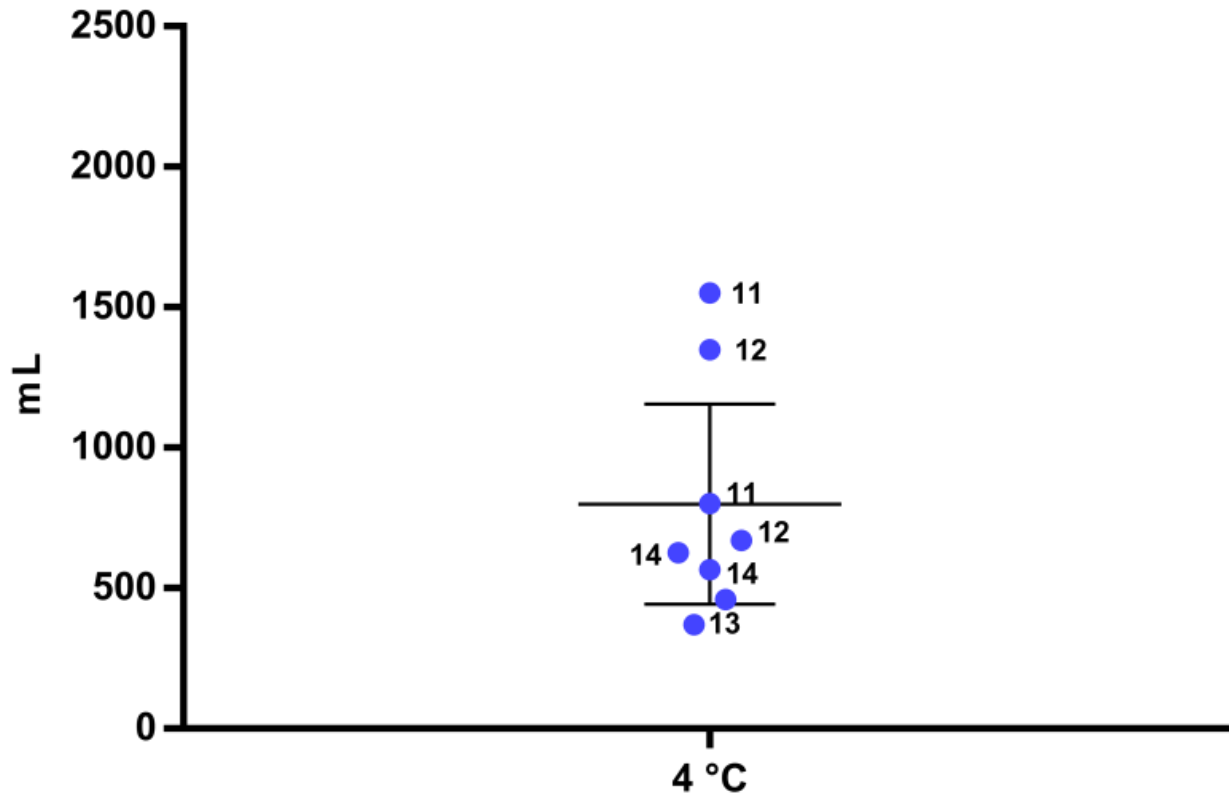
Patients with platelet transfusion: Demographics (N=8)

	Mean	95,0% CI for mean
Age	63	55-71
BMI	27,2	24,5-29,8
ECC Time	204	114-293



Platelets stored cold for 7-14 days – Effect on bleeding

Chest Drain Output

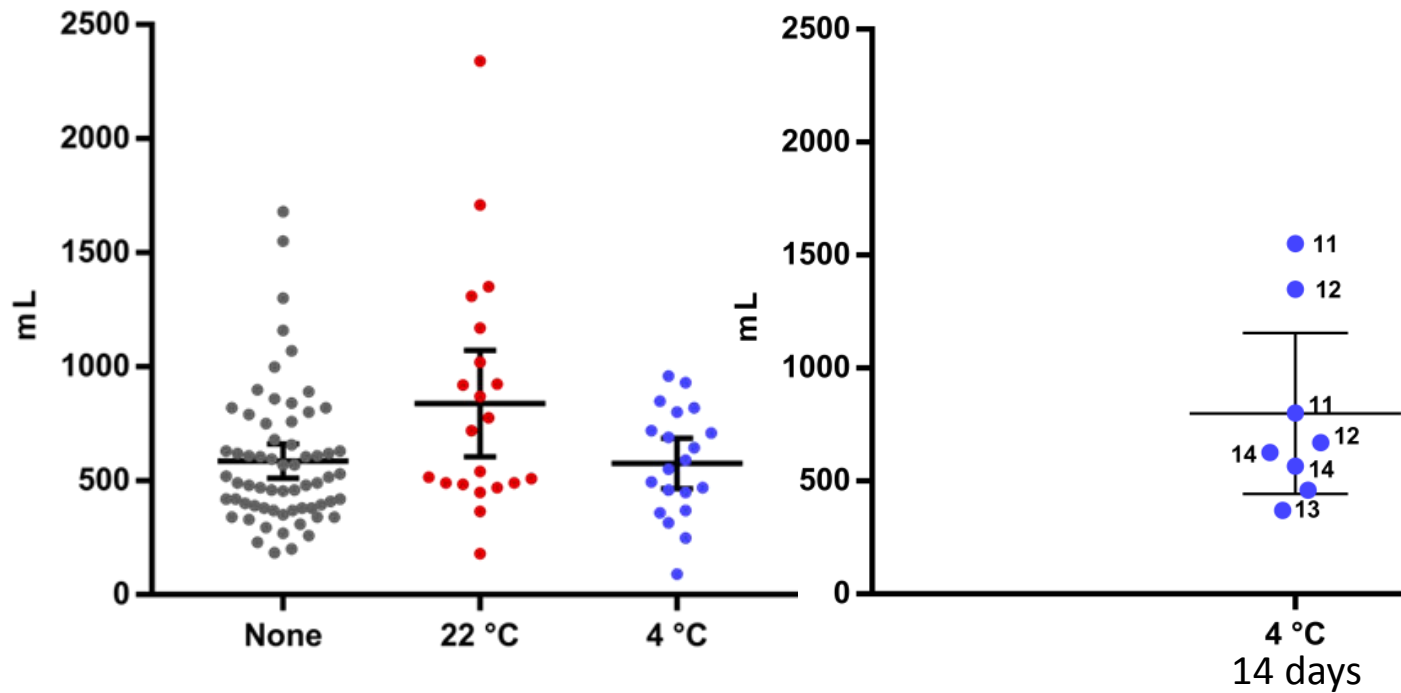




Chest drain output

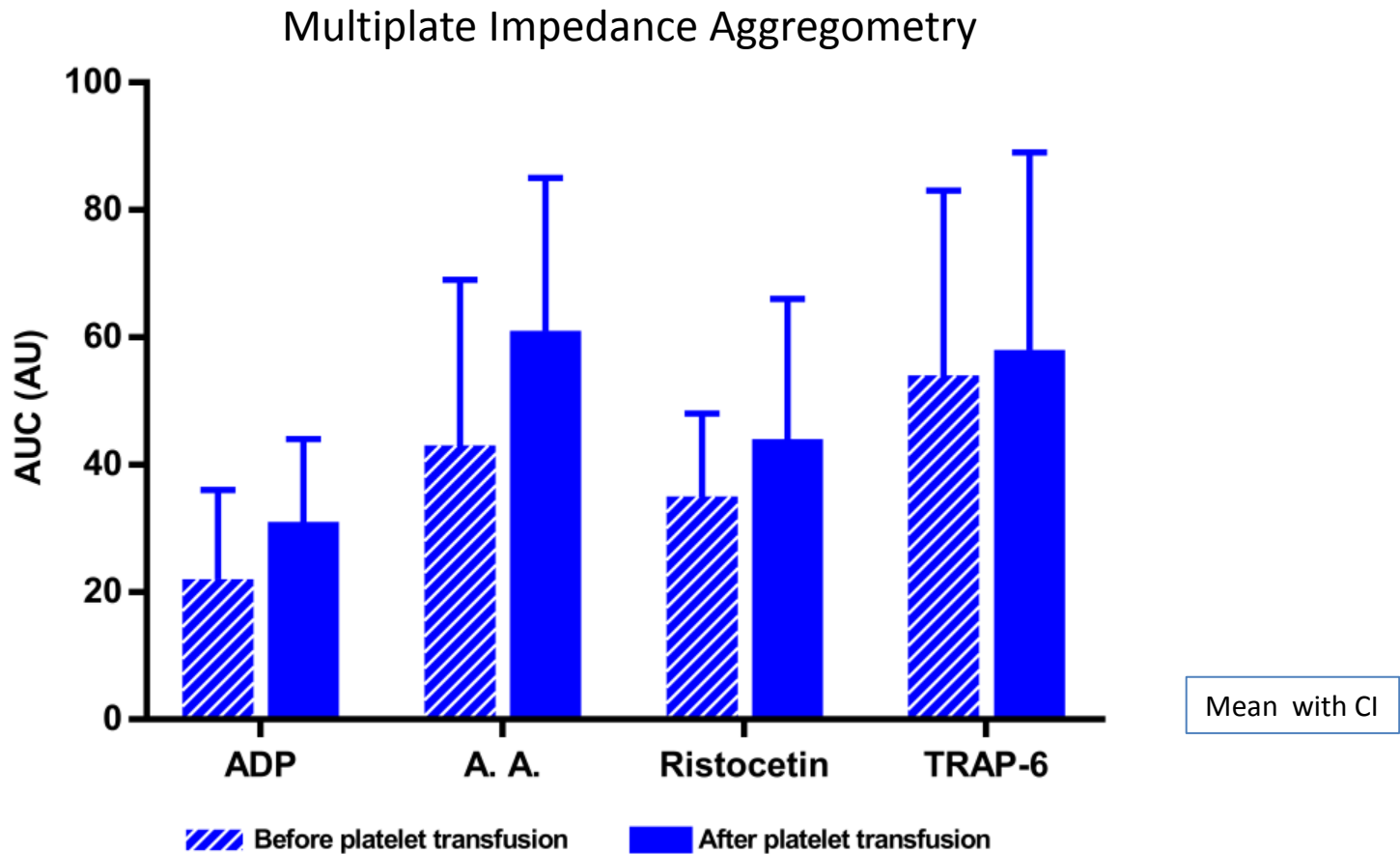
Randomized controlled trial:
7 days storage:

Observational study
14 days cold storage:





Platelets stored cold for 7-14 days - Effect on platelet aggregation

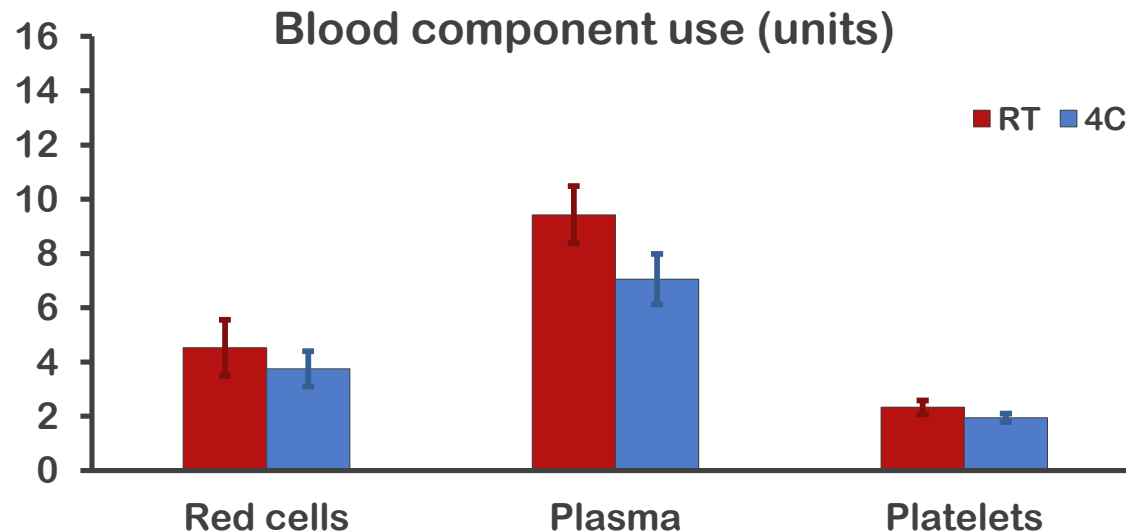




Total blood use

Cold stored platelets 7-14 days (N=8)

	Mean	95,0% CI for Mean
Platelet units	1,5	1,1-1,9
Platelet Age	12	11-13
RBC units	3,6	2,4-4,9
Plasma units	8,6	6,6-10,6





CONCLUSION

In vitro and in vivo studies indicate that cold stored platelets may be beneficial in life threatening bleeding.

Cold storage may enable extended storage time:

- improved availability of prehospital transfusion
- allow shipment
- allow for an inventory of platelet concentrates in smaller hospitals

Further clinical studies are encouraged.



Acknowledgements

The Blood Far Forward group in Bergen:

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Advisors, debaters and critics in
the THOR Network



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*Department of immunology and transfusion medicine, Department of Anaesthesia and Intensive Care,
Section of Cardiothoracic Surgery, Department of Health Disease, and Laboratory of Clinical
Biochemistry, Haukeland University Hospital, Bergen, Norway
Institute of Clinical Science, School of Medicine and Dentistry, University of Bergen, Norway
Norwegian Naval Special Operations Commando
Norwegian Armed Forces Medical Services*



“
DO. OR DO NOT.
THERE IS NO TRY.

-Yoda

