TRANSFUSION INTERREGIONALE CRS
INTERREGIONALE BLUTSPENDE SRK

LE STOCKAGE DES PLAQUETTES A FROID

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STORAGE OF PLATELETS.

Platelet concentrates are stored at 22°C

Day 0 Day 7 Day 14

Today

PC at 22°C

- 5-7 days of storage: pressure on platelet supply
- Room temperature increases the risk of bacterial contamination (compared to cold storage) in absence of pathogen inactivation treatment (not the case in Switzerland)

New/additional practices?

PC at 4°C

Storage at 4°C

- Increase storage time and improve supply chain

  - What about platelet characteristics?
  - Decreased storage lesions ?
  - *In vivo* function beyond the platelet recirculation ?

COLD STORAGE.

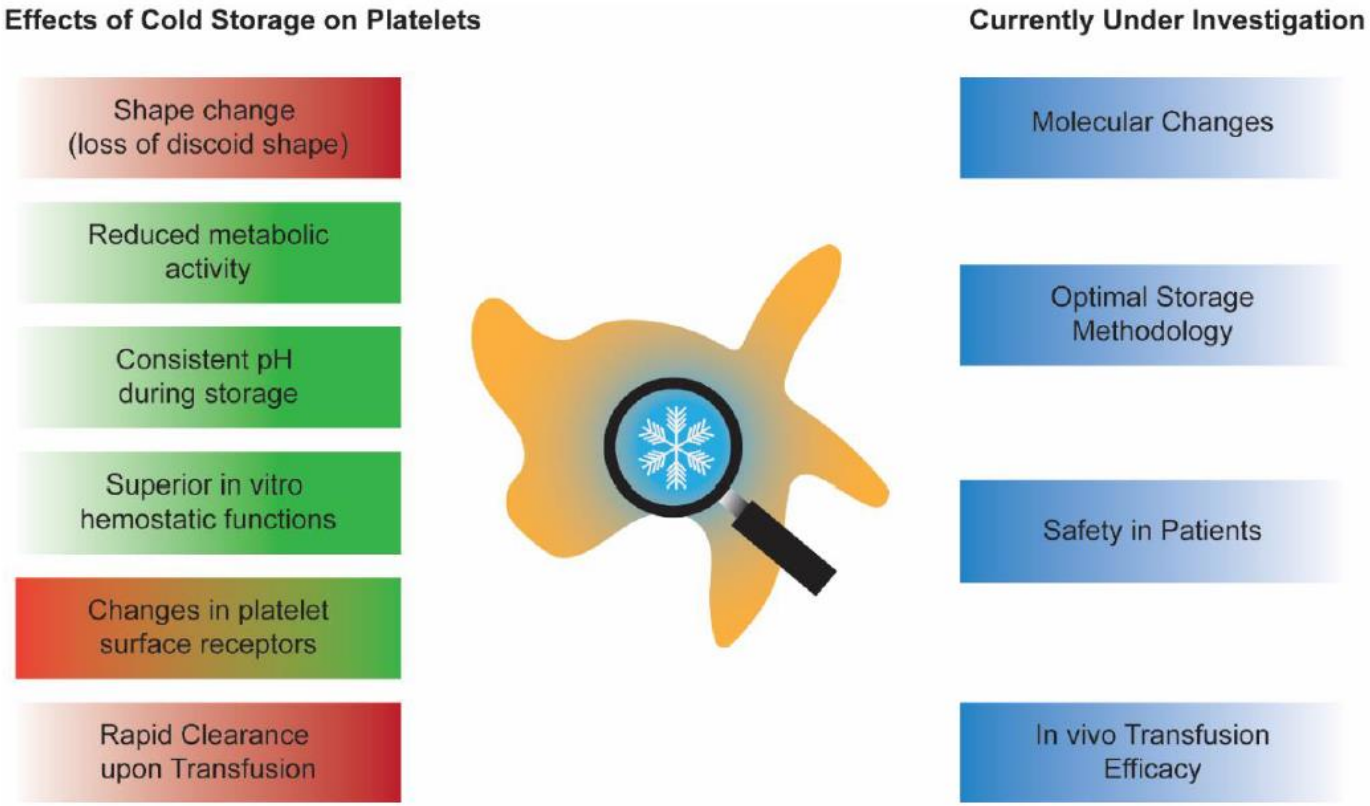

Figure 1. Summary of known effects of cold storage on platelets and aspects of cold-stored platelets currently under investigation. Deleterious effects of cold storage are shaded in red, whereas beneficial effects are shaded in green. The box shaded with red and green colors represents conflicting effects of cold storage on platelet surface receptors.

Zhao and Devine, *Int J Mol Sci*, 2022

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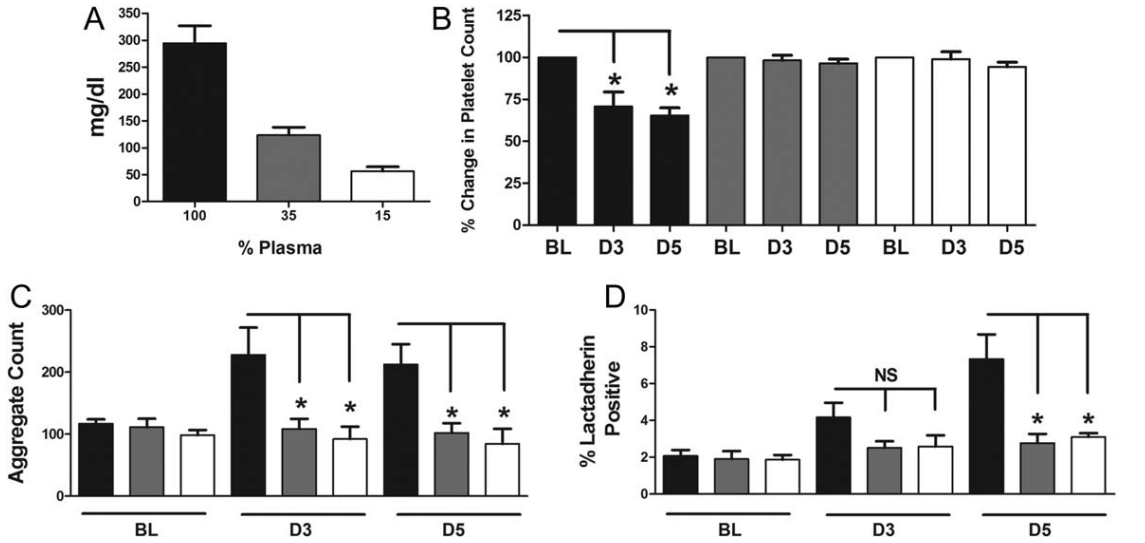
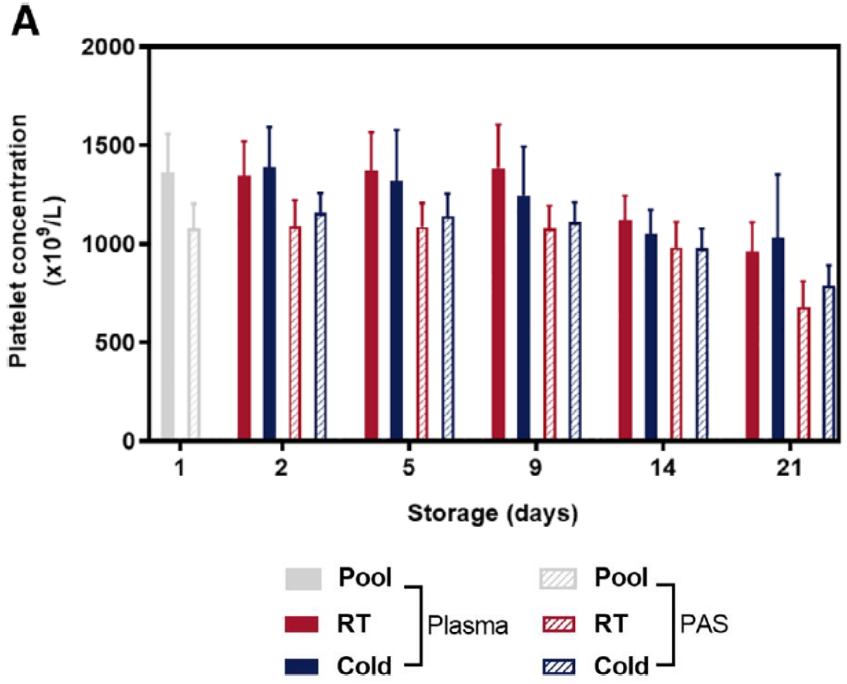
COLD STORAGE.

PLASMA CONCENTRATION AND PLATELET COUNT



stored at 4°C in the presence of (■) 100, (▩) 35, and (□) 15% plasma (n = 4).

A-PC in Intersol



A-PC in plasma or SSP+

Getz *et al*, *Transfusion*, 2016; Johnson *et al*, *Transfusion*, 2021

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COLD STORAGE.

MORPHOLOGY

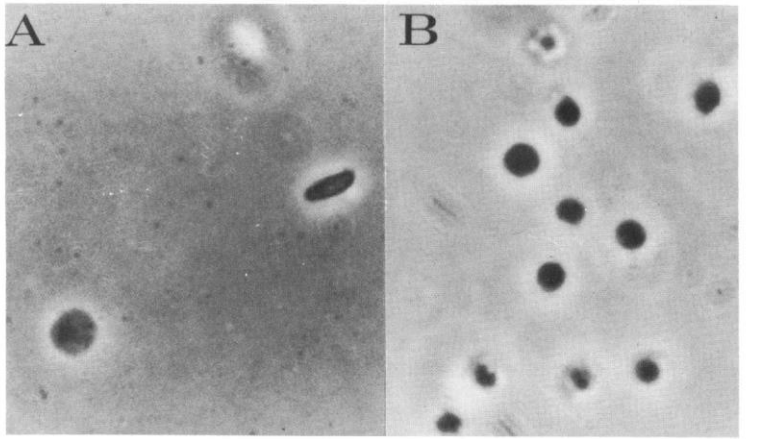
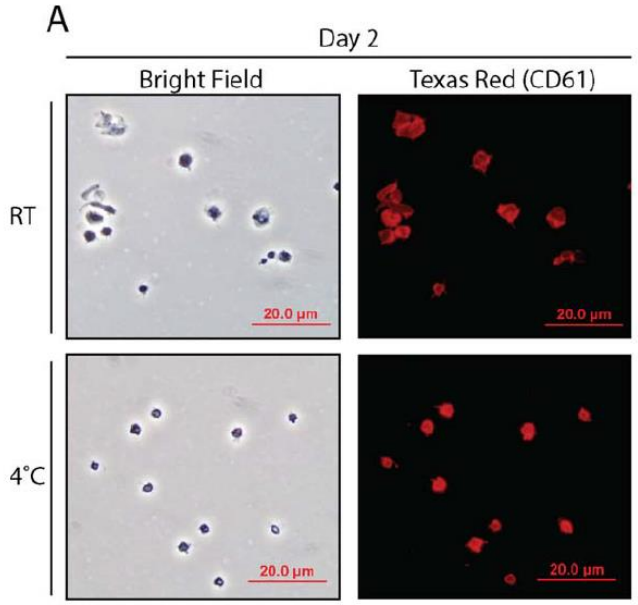
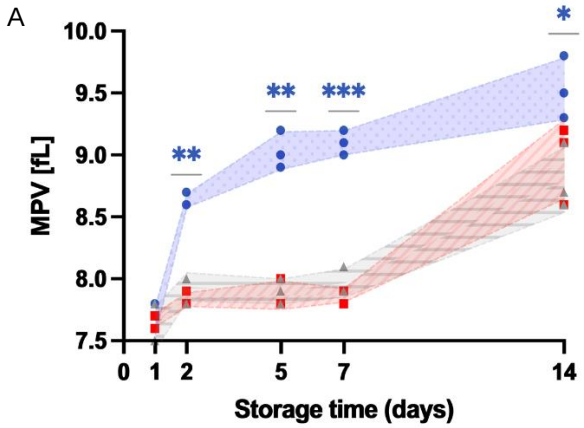


FIGURE 4. *Altered Platelet Morphology (Oil, Phase-Contrast Microscopy X1000) after Exposure to Cold Temperature (4°C).*
A shows the normal discoid configuration of fresh platelets with views of the cell's flat surface (left) and a cell oriented perpendicular to the microscopical slide (right). Platelets maintain this shape for at least 48 to 72 hours of storage at 22°C.
After exposure to 4°C (B) platelets rapidly become sphered. This change is irreversible after 18 hours' storage.[32]

Platelet-rich plasma



BC-PC in SSP+



BC-PC in SSP+

Loss of discoid shape
Increase of MPV
Absence of swirling in PC at 4°C

Murphy and Gardner, *NEJM*, 1969; Johnson *et al*, *Transfusion*, 2016; Muret *et al*, unpublished data

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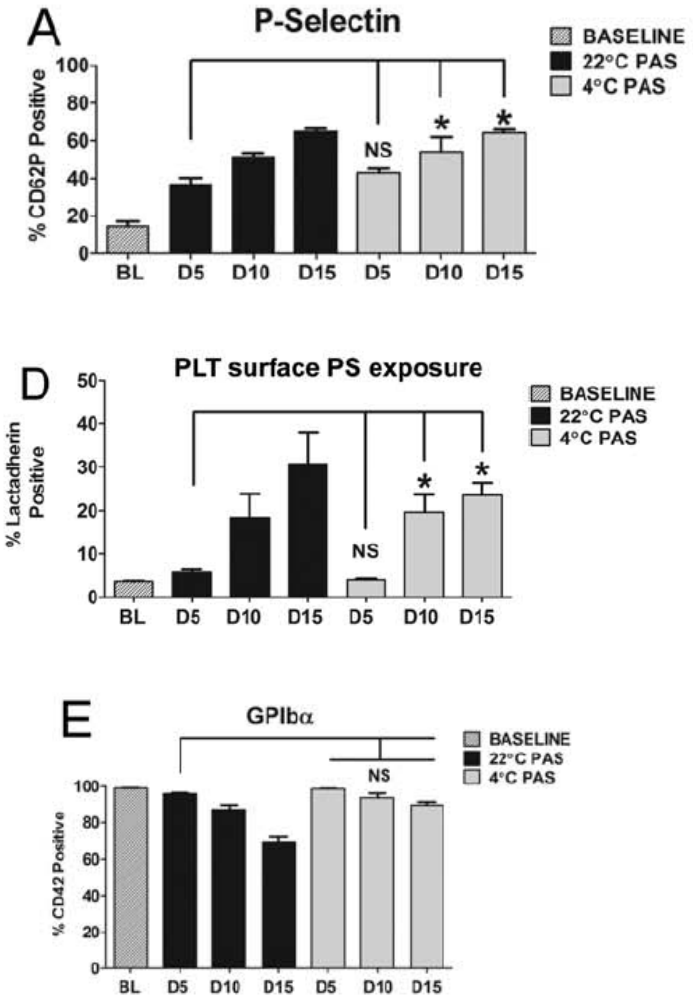
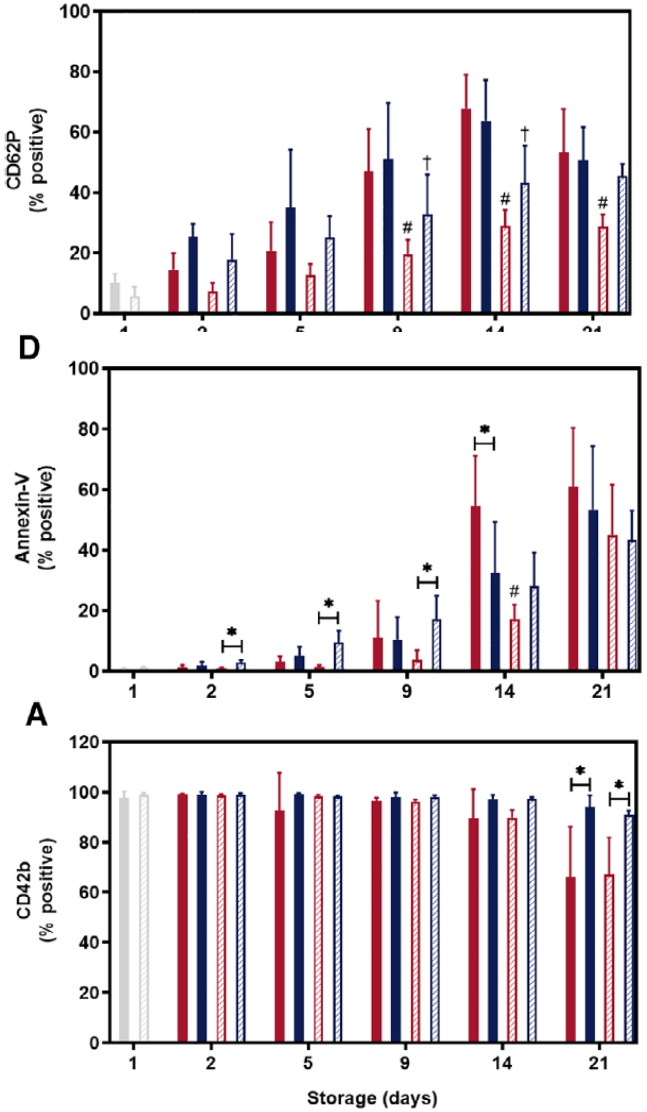
COLD STORAGE.

PHENOTYPE

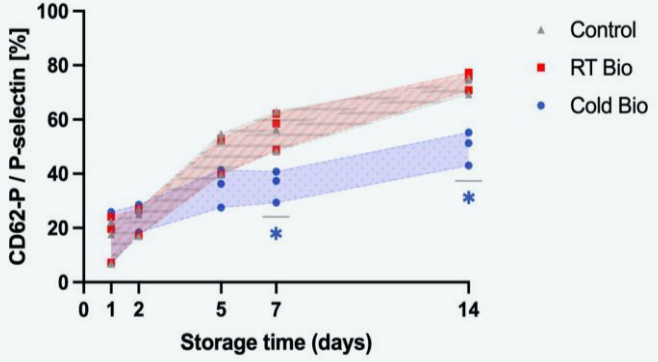


A-PC in Intersol



Experiment in Tyrode's buffer that contains Ca^{2+}

Lower platelet activation in absence of Ca^{2+}



A-PC in plasma or SSP+

Getz *et al*, *Transfusion*, 2016; Johnson *et al*, *Transfusion*, 2021; Muret *et al*, manucript in preparation

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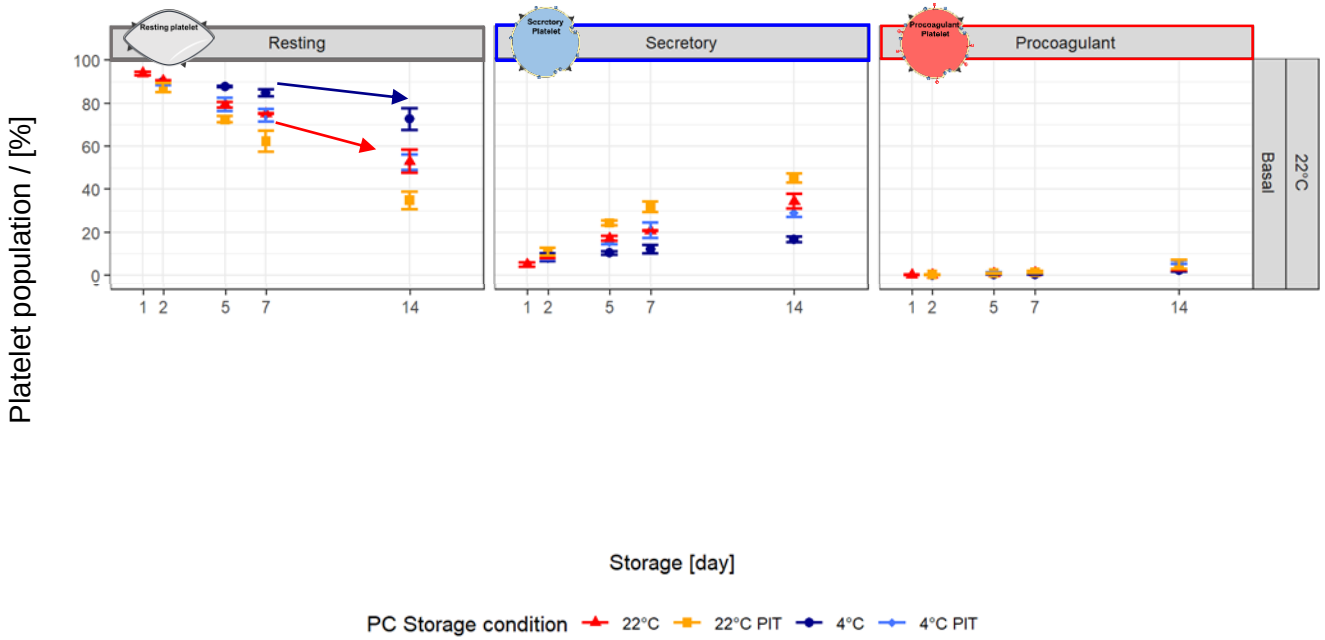
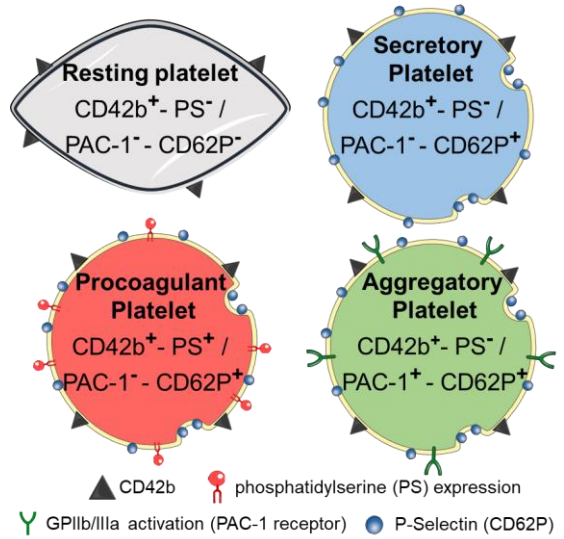
COLD STORAGE.

PHENOTYPE





4°C platelet maintained a resting platelet phenotype, compared to RT platelets.

PIT-PCs stored at 4°C are sensitive to calcium and magnesium supplemented buffer

Muret *et al*, manuscript in preparation

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COLD STORAGE.

AGGREGOMETRY

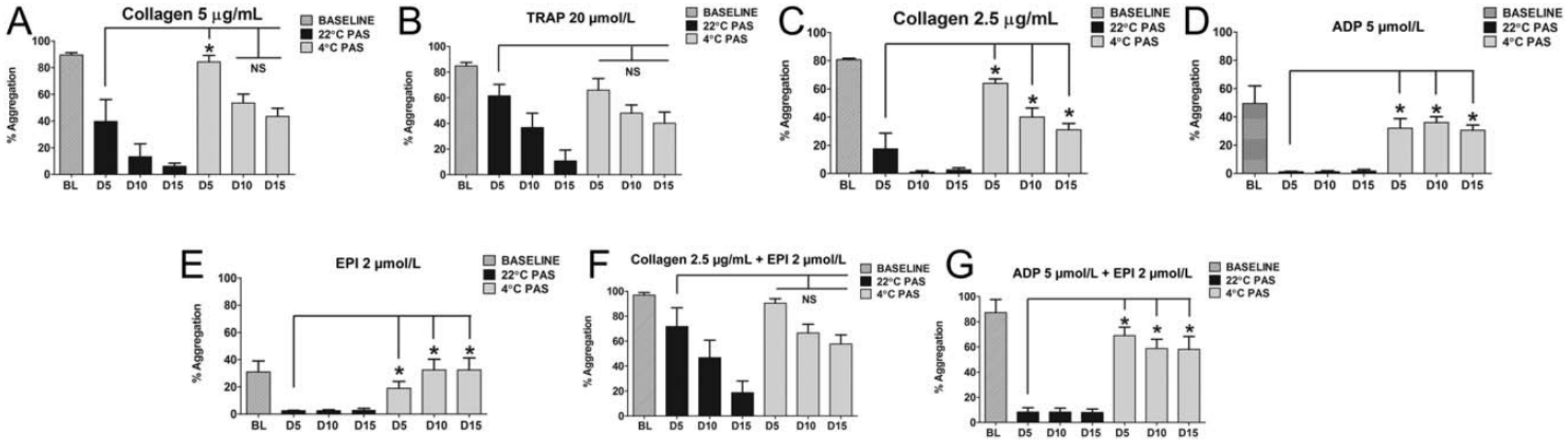


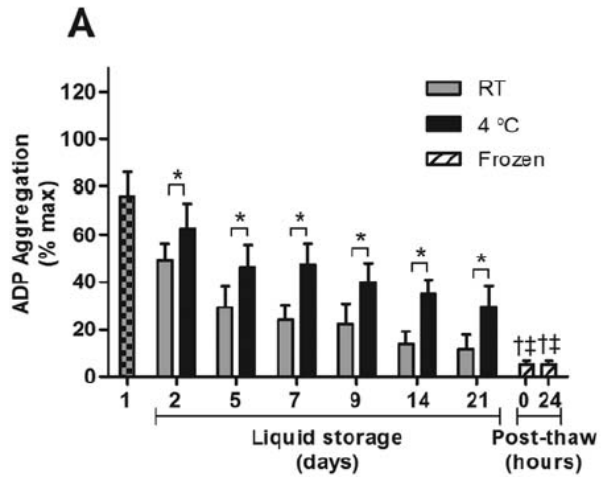
Fig. 7. PAS PLT maximal aggregation response to single and multiple agonists. The aggregation of 22 or 4°C PAS-stored PLTs was measured at the indicated time after addition of (A) 5 µg/mL collagen, (B) 20 µmol/L TRAP, (C) 2.5 µg/mL collagen, (D) 5 µmol/L ADP, (E) 2 µmol/L epinephrine, (F) 2.5 µg/mL collagen plus 2 µmol/L epinephrine, (G) 5 µmol/L ADP + 2 µmol/L epinephrine. *$p < 0.05$; NS = not significantly different.

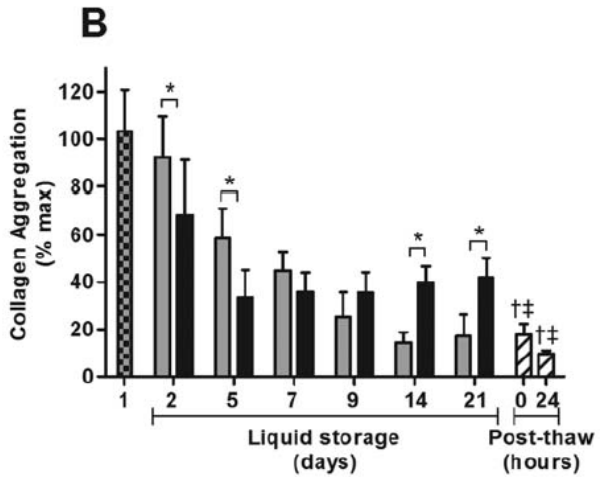
Higher response to ADP and collagen with cold platelets

Getz *et al*, *Transfusion*, 2016

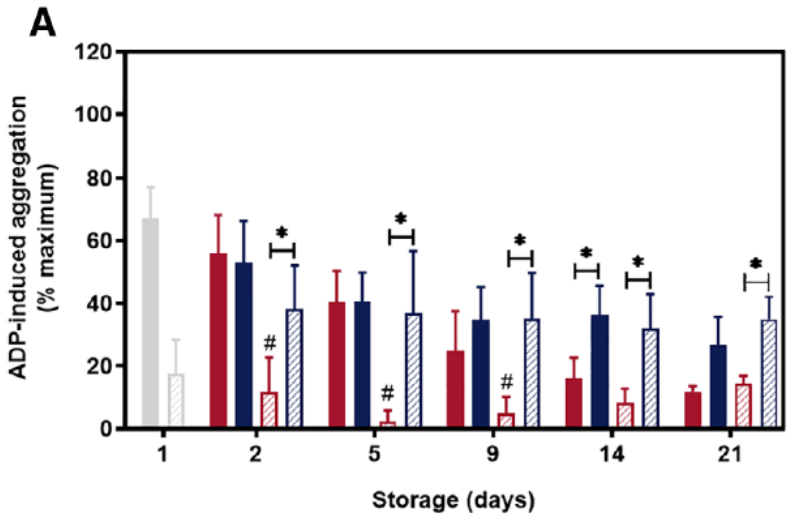
COLD STORAGE.

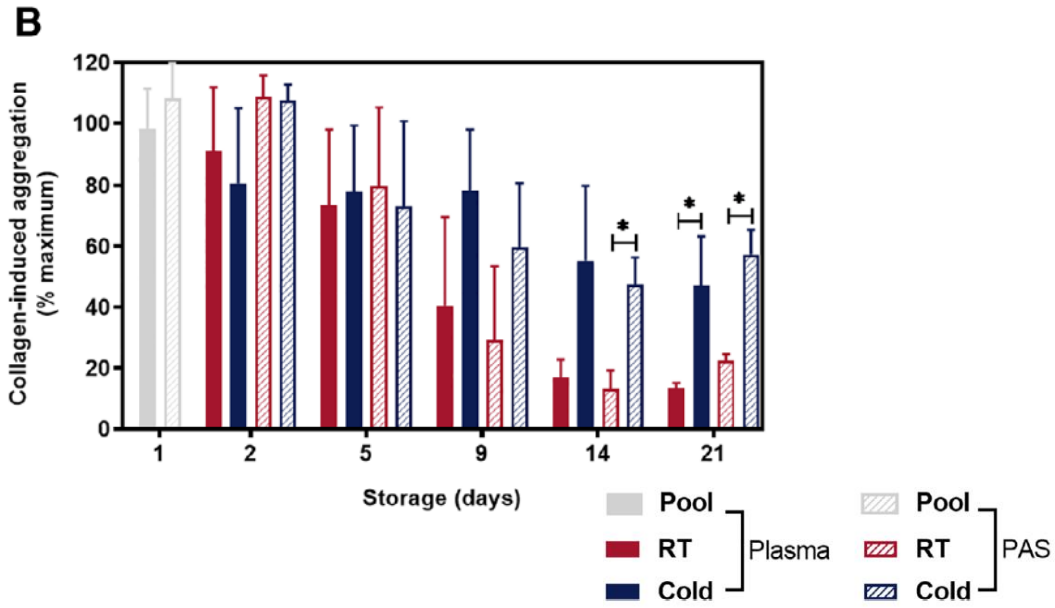
AGGREGOMETRY





Higher response to ADP with cold platelets
Collagen: lower response at the beginning of the storage





Johnson *et al*, *Transfusion*, 2016 and *Transfusion*, 2021

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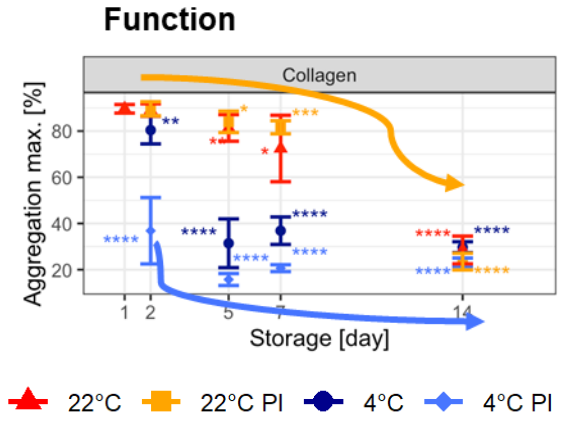
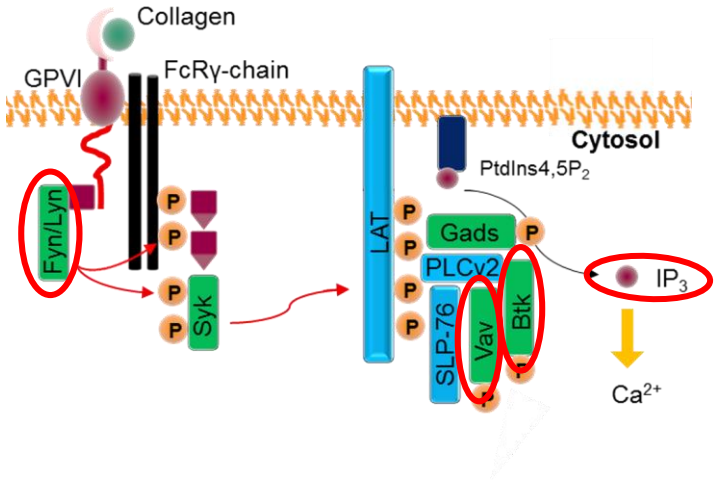
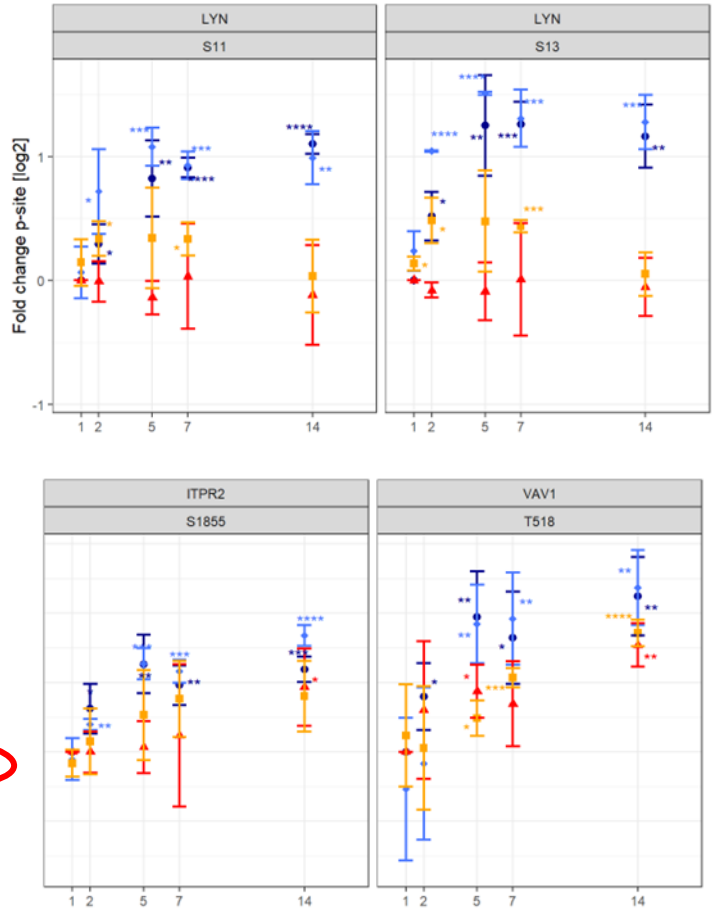
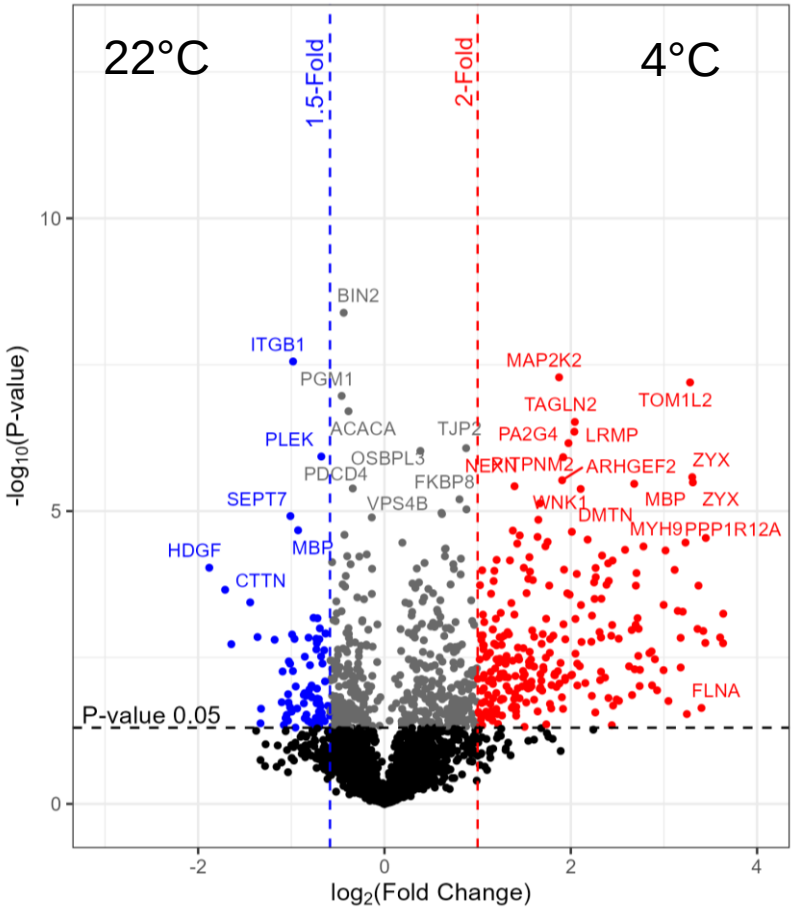
COLD STORAGE.

AGGREGOMETRY AND PROTEIN ACTIVITY









Upregulation of phosphoproteins in cold-stored platelets, involved in several platelet functions

Regulation of **phosphoproteins** in function of temperature (day 5, no Intercept)

Muret *et al*, manuscript in preparation

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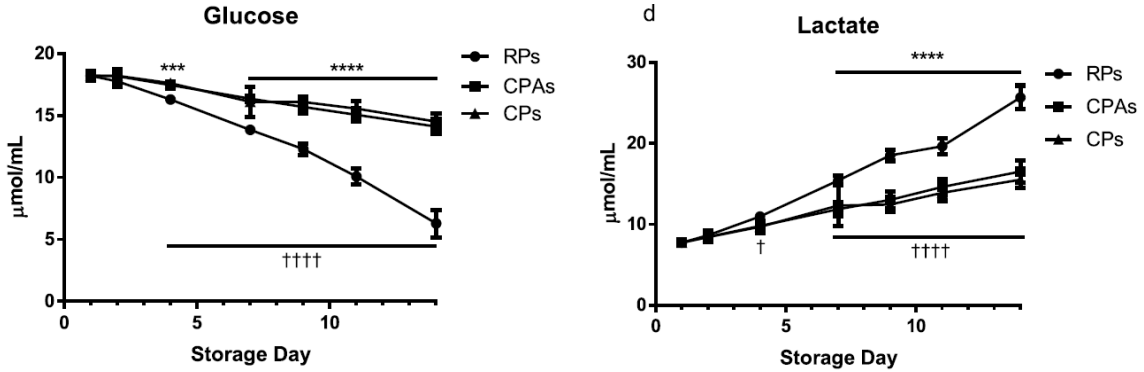
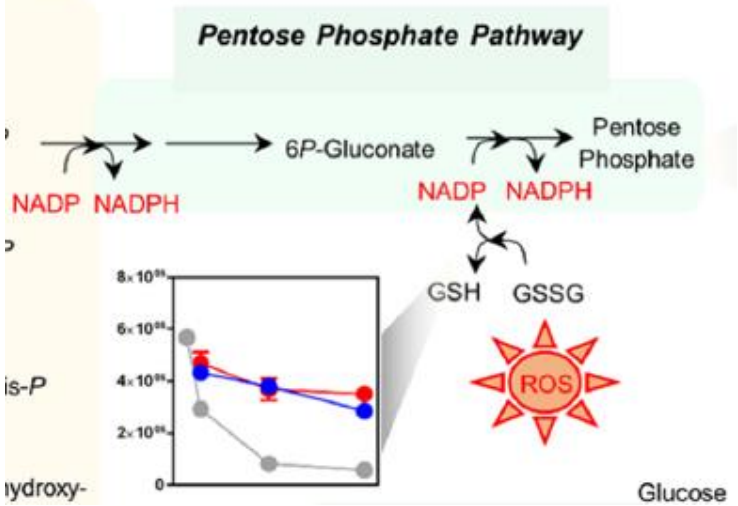
COLD STORAGE.

METABOLOMICS

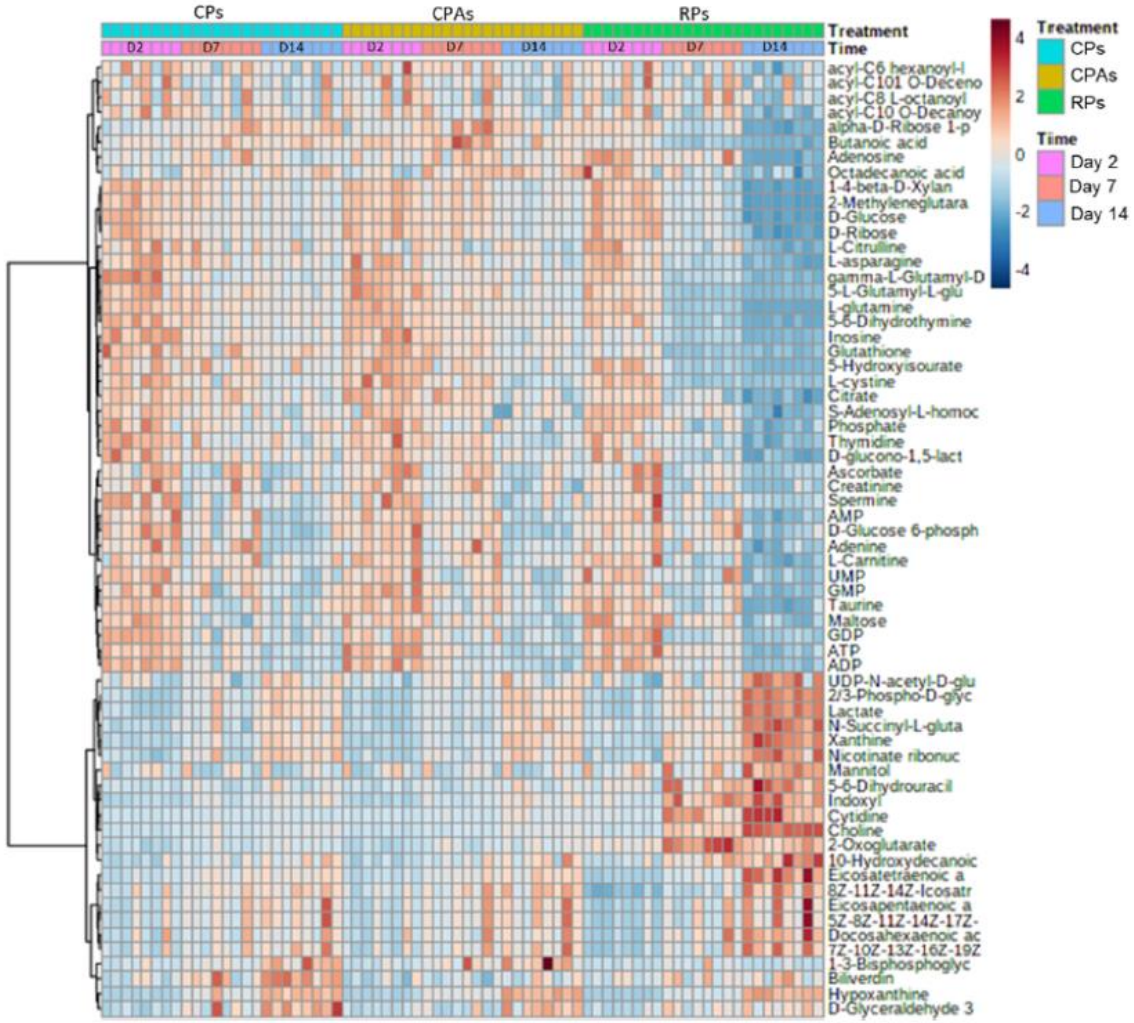


Lower glycolysis rate at 4°C



Lower oxidative stress

PC at 4°C | 22°C



Zhao *et al*, *J prot res*, 2021

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COLD STORAGE.

SUMMARY

Cold storage compared to room temperature

- Reduced glycolysis and lower oxidative stress
- Loss of discoid shape and swirling
- Higher response to ADP and lower response to collagen (at the beginning of the storage, potentially due to post-translational modifications of proteins)
- Regarding surface markers and platelet phenotypes
  - Exposure of phosphatidylserine (annexin-V +) and activation of αIIbβIII (PAC-1+)
  - Increase CD62P expression after pathogen inactivation
- To be taken with caution because of sample preparation

- Compliant with blood bank specifications (except temperature!) in Switzerland (*in vitro*)

AN OLD STORY.

THE NEW ENGLAND JOURNAL OF MEDICINE May 15, 1969

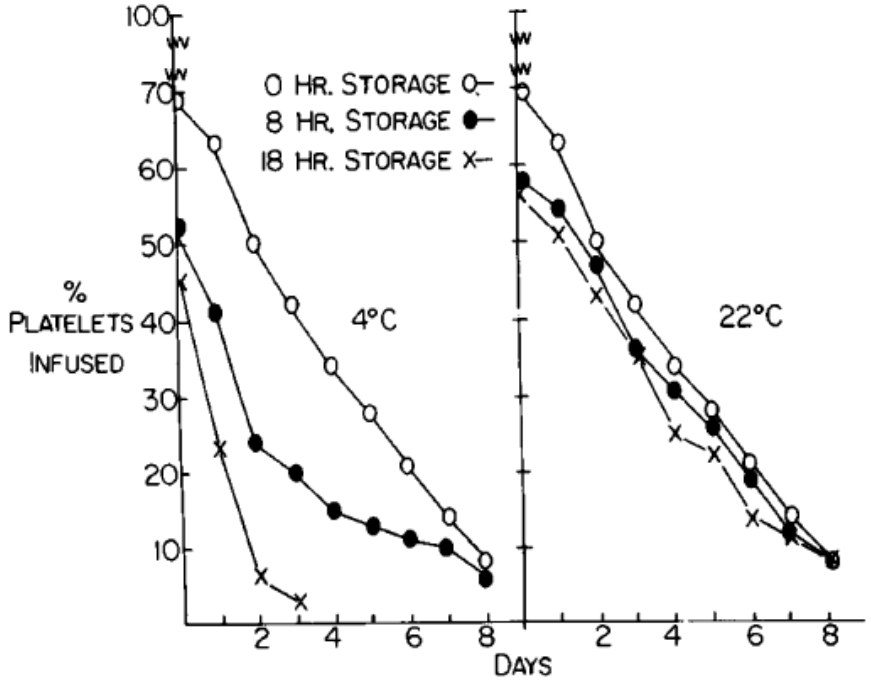
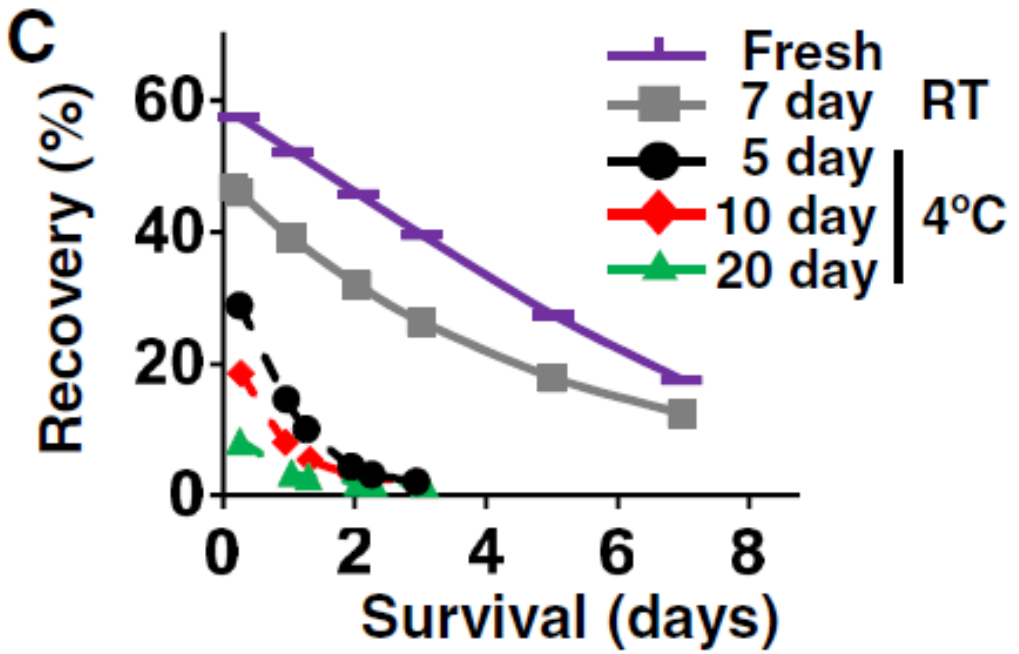


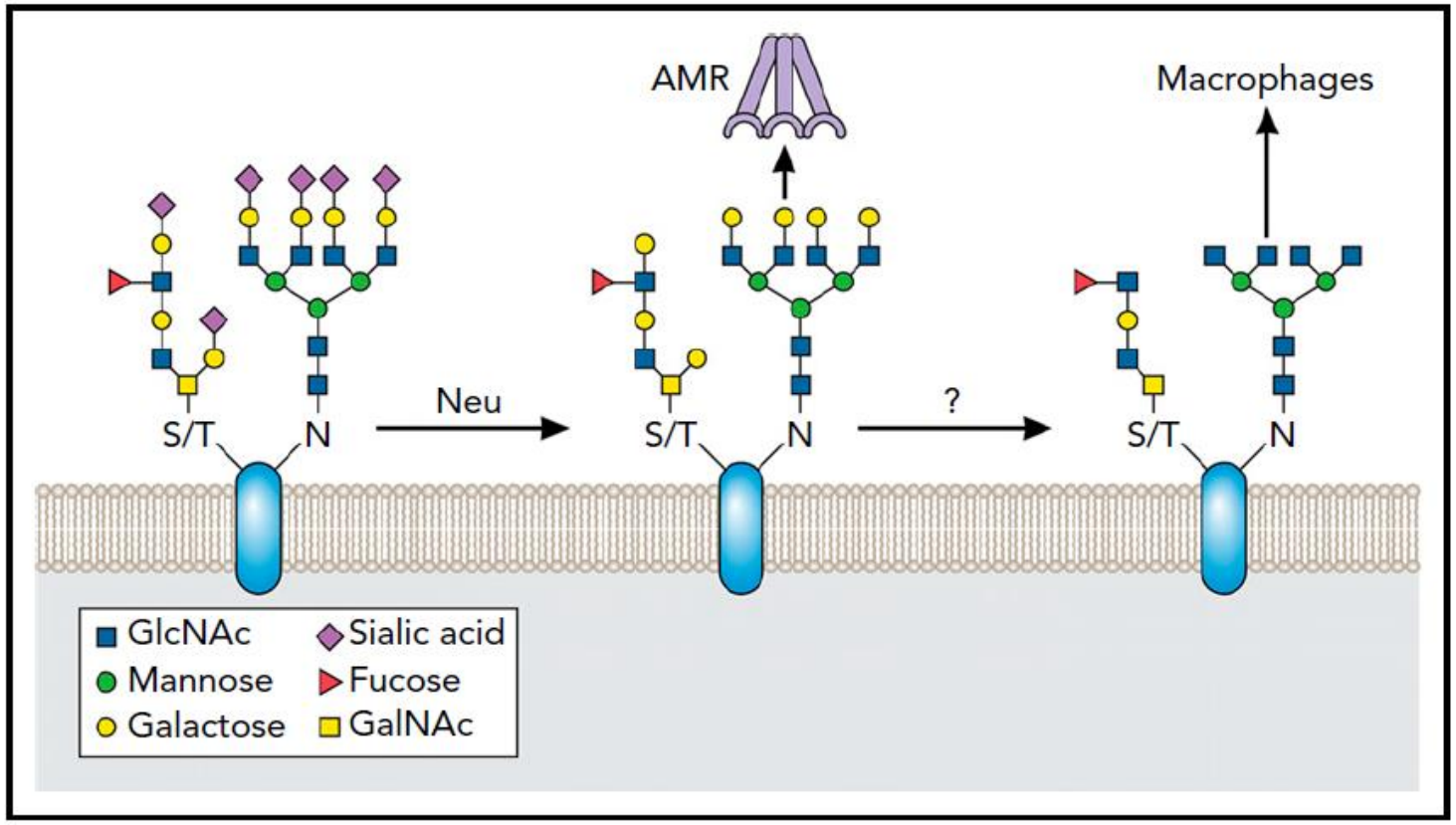
FIGURE 1. ^{51}Cr-*Labeled Platelet Life-spans after PRP Storage at 4°C and 22°C.*



Murphy and Gardner, *NEJM*, 1969; Stolla *et al*, *Transfusion*, 2020

RAPID CLEARANCE.

Figure 3. Protein desialylation as a clear-me sign in platelets. Over the platelet lifespan, surface glycoproteins lose the terminal sialic acid residues in their glycans, a process associated with clearance. Neuraminidases are glycoside hydrolases that can remove terminal sialic acid from glycans. Neuraminidases are found in platelets, which present neuraminidase on their surface downstream of GPIb-IX complex signaling. In many glycans, desialylation leads to exposure of the penultimate galactose residues on glycans. These can in turn be recognized by the AMR. Further deglycosylation leads to exposed GlcNAc residues, which may be recognized by other carbohydrate receptors and potentially mediate their uptake by macrophages.



The exposed β-gal on the platelet surface can be recognized by the Ashwell-Morell receptor (AMR), a multimeric endocytic receptor complex also known as the asialoglycoprotein receptor, on the surface of hepatocytes and/or liver macrophages (Kupffer cells), inducing the clearance of the platelet from circulation

Quach *et al*, *Blood*, 2018

RAPID CLEARANCE.

Quach *et al*, *Blood*, 2018

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CLINICAL IMPACT.

PLATELET LIFE SPAN

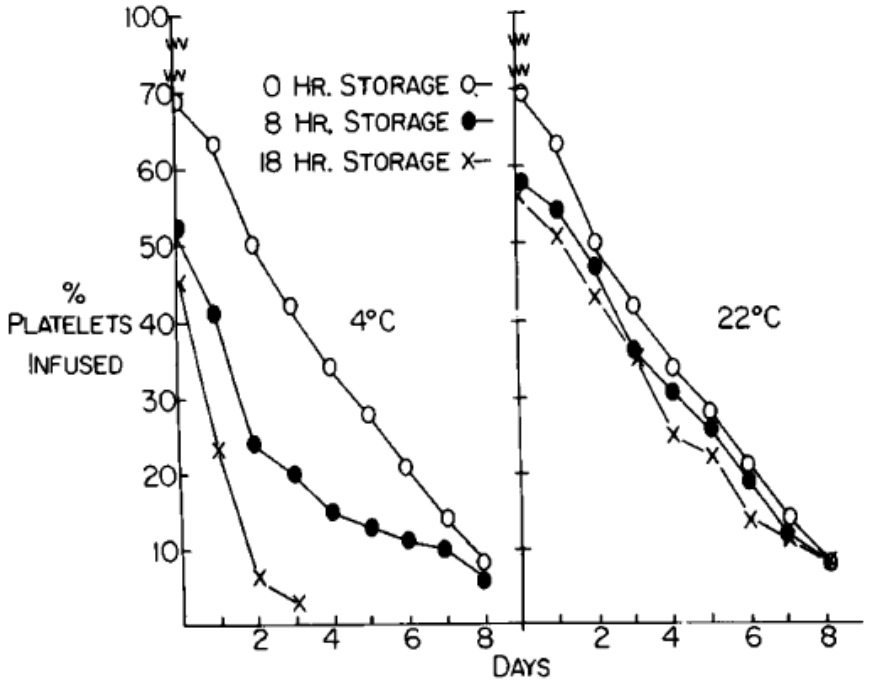

FIGURE 1. ^{51}Cr-*Labeled Platelet Life-spans after PRP Storage at 4°C and 22°C.*

Loss of cold-stored platelets after transfusion

Deadly blow for cold storage!

But

What about platelet function?

Are the superior *in vitro* hemostatic functions an advantage?

Hemostatic properties in bleeding patients?

Murphy and Gardner, *NEJM*, 1969; Prudent, *Hämostaseologie*, 2020

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CLINICAL IMPACT.

HAUKELAND UNIVERSITY HOSPITAL, BERGEN, NORWAY

Cardiothoracic surgery
Two-arm randomized pilot trial (**25 patients per arm**)
Room temp. vs 4°C A-PC in Intersol up to 7 days
+ Observational extension with 8 to 14 days cold PCs

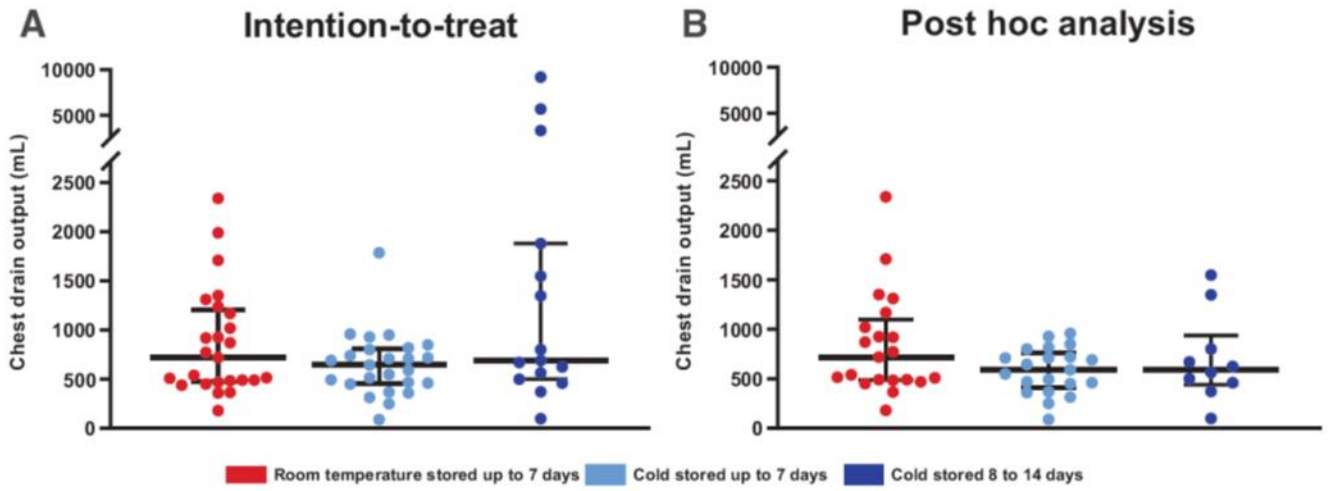
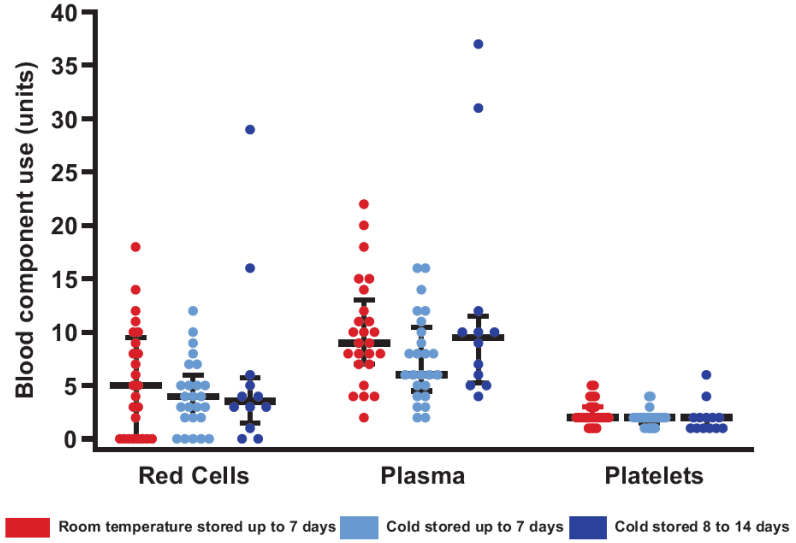
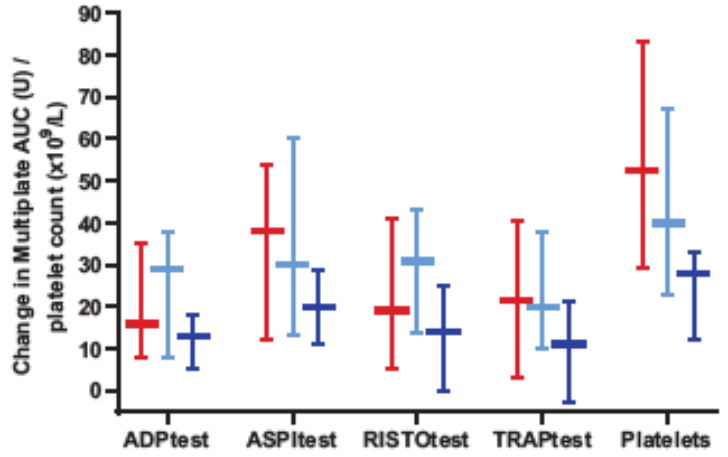


Fig. 2. Postoperative blood loss as measured by chest drain output for intention-to-treat patients (*A*) and *post hoc* analysis patients receiving room temperature–stored platelets, 7 days cold-stored platelets, and 8 to 14 days cold-stored platelets (*B*). The *dots* represent the individual study patients. The *lines* show median with interquartile range. The difference between the room temperature– and cold-stored for 7 days arms was not statistically significant ($P_{intention\text{-}to\text{-}treat}$ = 0.265, $P_{post\ hoc}$ = 0.115, two-tailed Mann–Whitney U test; SPSS Statistics for Windows, version 24.0, IBM, USA). The nonconcurrent stage II cold-stored for 8 to 14 days arm was not compared with the two stage I arms.





Strandenes *et al*, *Anasthesiology*, 2020

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CLINICAL IMPACT.

HAUKELAND UNIVERSITY HOSPITAL, BERGEN, NORWAY

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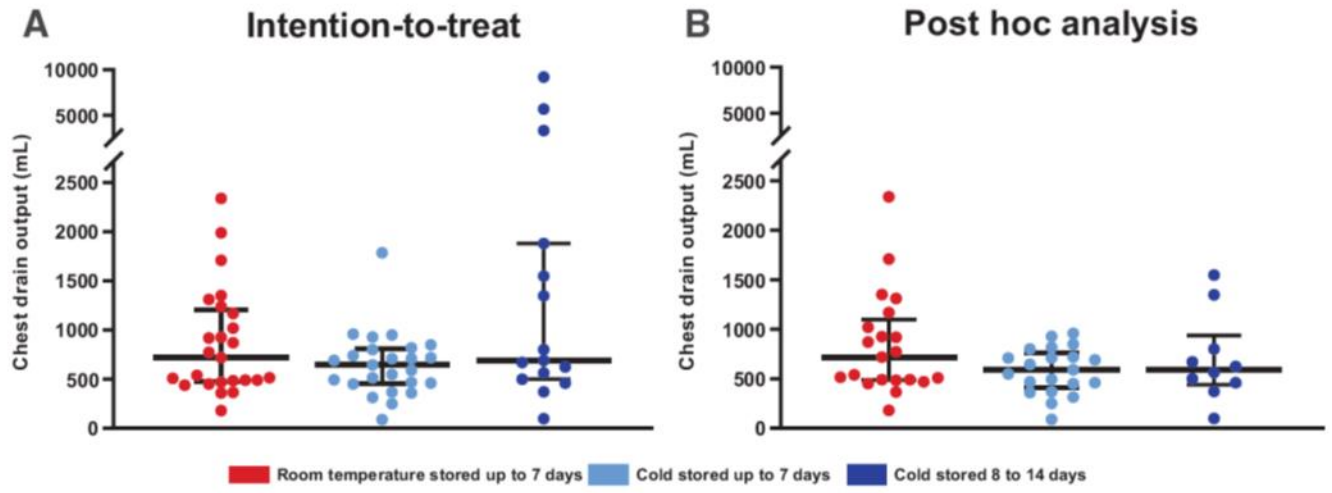

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Strandenes *et al*, *Anasthesiology*, 2020

Conclusions

- Blood loss: no significant differences
- Transfused blood products: no significant differences
- Increase of platelet count for all study arms
- Significant increase in aggregation response for all study arms: platelet functional up to 14 days at 4°C

- No transfusion reaction related to platelet transfusions, but number of patients too low

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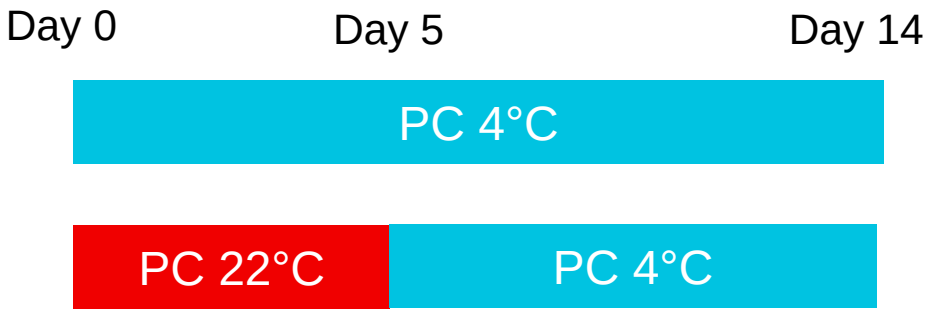
CLINICAL IMPACT.

MAYO CLINIC, ROCHESTER, MINNESOTA, USA

Transition from room temp. to cold-stored platelets
Retrospective study in context of shortage
5-day old PCs were converted to cold-stored PCs
61 PCs were transitioned
57% Cardiac surgery, 20% vascular surgery, 8% general surgery, 5% solid organ transplantation and 5% to hemorrhaging patients in emergency and ICU

Conclusions
- Hemostasis adequate in all cases
- No wastage
- Non documented or suspected transfusion reaction



Impact on platelets?

TABLE 1 Demographic and clinical characteristics of transfused patients (n = 40)

Variable	Median (IQR) or n (%)
Age (years)	68 (59-73)
Sex	
Female	17 (42)
Male	23 (58)
Comorbidities	
Coronary artery disease	19 (47.5)
Hypertension	27 (67.5)
Diabetes mellitus type 2	12 (30)
Stroke	5 (12.5)
Chronic kidney disease	8 (20)
Transfusion location	
Emergency department	1 (2.5)
Operating room	38 (95)
Intensive care unit	1 (2.5)
Surgery type (n = 38)	
Cardiac	23 (58)
General surgery	3 (7.5)
Vascular	8 (20)
Transplant	2 (5)
Other	4 (10)
Pretransfusion PLT count ($\times 10^9$/L)[a]	87 (67-106)
Posttransfusion PLT count ($\times 10^9$/L)[a]	115 (93-145)

[a]Available for 39 (97.5%) patients.

Warner *et al*, *Transfusion*, 2021

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DELAYED COLD STORAGE.

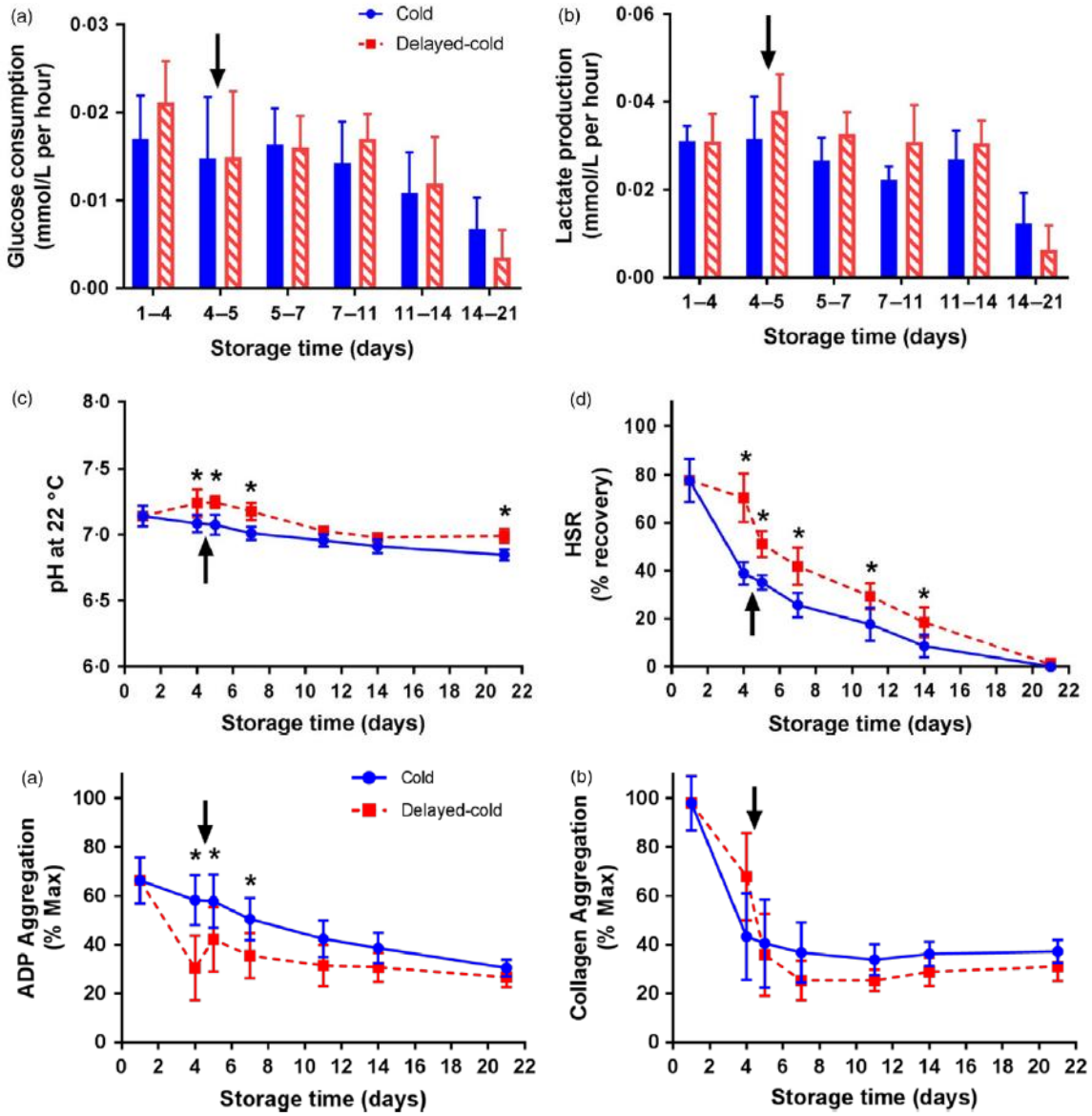
Day 0 Day 4 Day 21

PC 4°C

PC 22°C PC 4°C

Transition from room temp to cold-stored platelets
RT to 4°C at day 4:

- Small differences around the 4 days of storage between delayed-cold and cold-stored PCs or along the storage (pH, HSR and PAC-1)
- Metabolic and activation profiles equivalent



Wood *et al*, *Vox Sanguinis*, 2018

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ONGOING CLINICAL TRIALS.

CHIPS: Chilled platelet study – Recruiting – March 2024
Phase 3 randomized study
Cold vs RT-stored PCs
Acute blood loss (complex cardiac surgery)
Primary outcome: hemostatic efficacy
1000 patients
Sponsors: Philip Spinella

CriSP-HS: Cold stored platelets in Hemorrhagic Shock – Active, not recruiting – Dec 2023
Phase 2 randomized study
Urgent release of cold-stored PCs vs standard care
Trauma, Hemorrhage (hemorrhagic shock)
Primary outcome: feasibility of recruitment
200 patients
Sponsors: Jason Sperry

Extended cold stored Apheresis Platelets in cardiac surgery patients (CHASE) – Recruiting – Oct 2024
Phase 1-2 randomized study
Cold vs RT-stored PCs
Hemorrhage, Defect bleeding, Surgical blood loss (cardiac surgery)
Primary outcome: feasibility of recruitment
30 patients
Sponsors: Moritz Stolla

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CONCLUSIONS.

Storage at 4°C (up to 14 or 21 days)

In vitro data

- Clear impact of prolonged cold storage
- Better maintenance of key metabolic parameters / Modifications of proteins
- Phenotype and functional data: superior hemostatic functions but assay-dependent / Further experiments are required

Clinical data

- Focus on bleeding patients
- Published data showed the feasibility of using cold platelets but not the superiority / Clinical trials ongoing
- Advantage for blood supply

Further experiments

- Advanced analyses upon stimulation to fully characterize the platelets (ongoing)
- Function beyond the recirculation using labeled platelets for instance (Muret *et al*, *Transfusion*, 2022 and BioCAP project ongoing in our lab)

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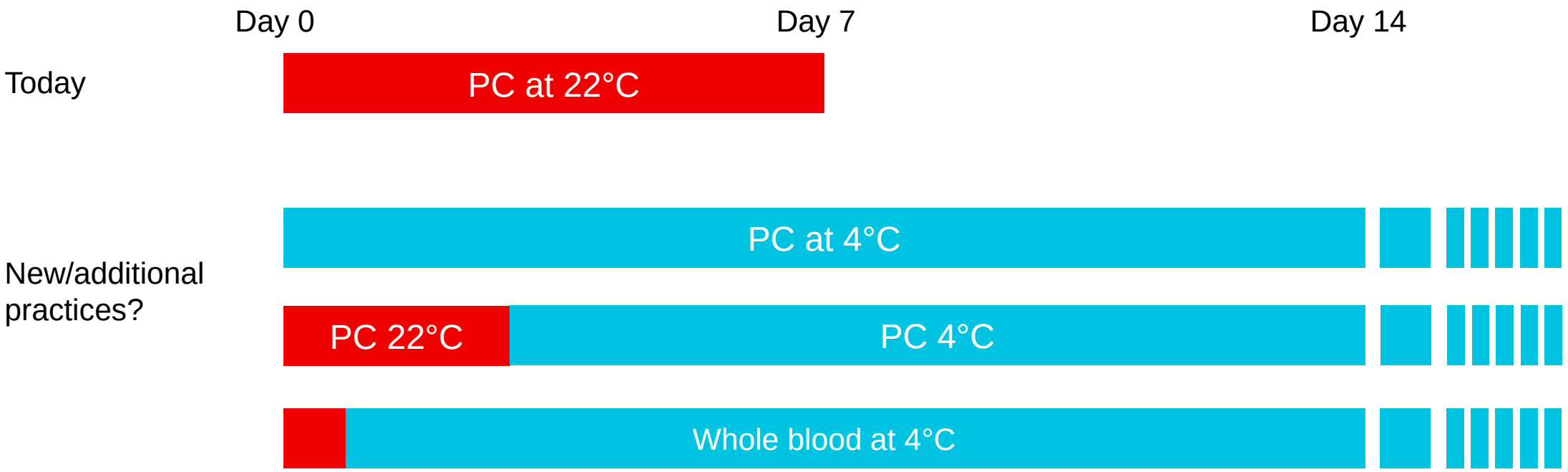
INTERREGIONALE BLUTSPENDE SRK

FUTURE.



Cold platelets:

- Improve platelet supply
- Get out of one-size-fits-all

TRANSFUSION INTERREGIONALE CRS
INTERREGIONALE BLUTSPENDE SRK

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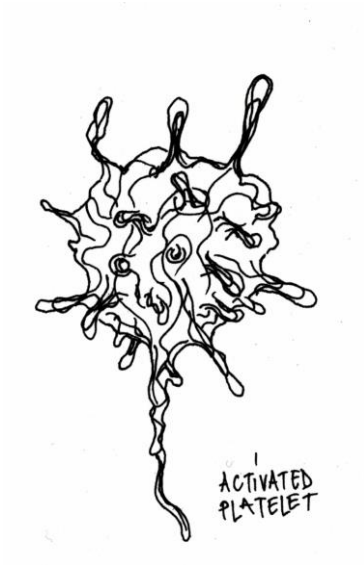
HUMANITÄRE STIFTUNG SRK
FONDATION HUMANITAIRE CRS
FONDAZIONE UMANITARIA CRS

MERCI POUR VOTRE ATTENTION



Courtesy G Sonego