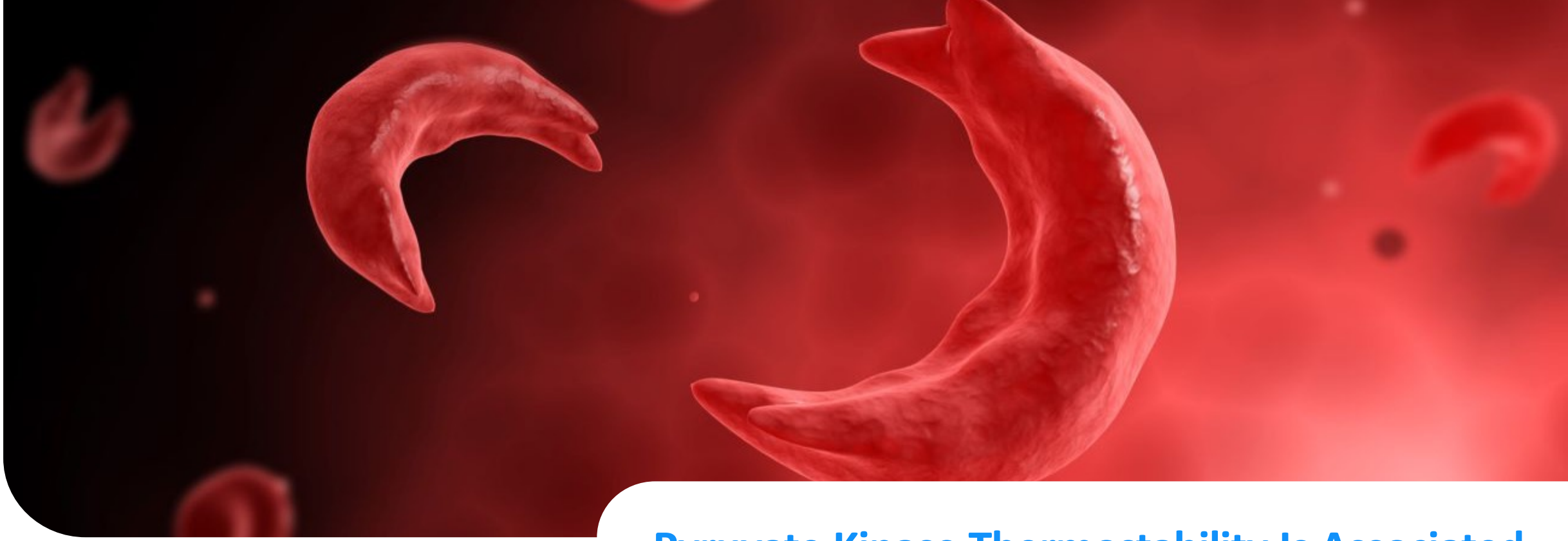

Pyruvate Kinase Thermostability Is Associated with Red Blood Cell Adhesion, Deformability and Oxygen Affinity in Patients with SCD

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UMC Utrecht

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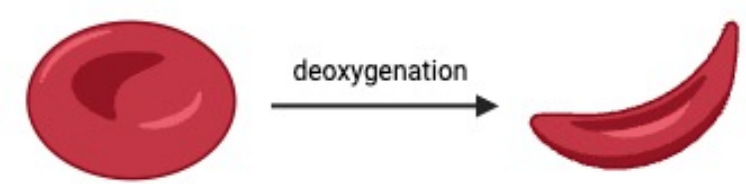
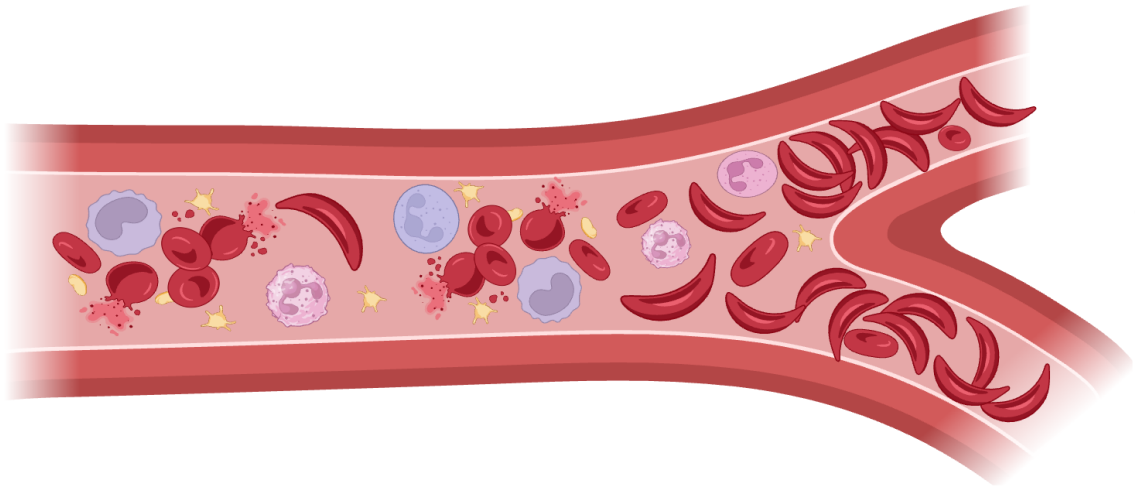
University Medical Center Utrecht – The Netherlands

Background



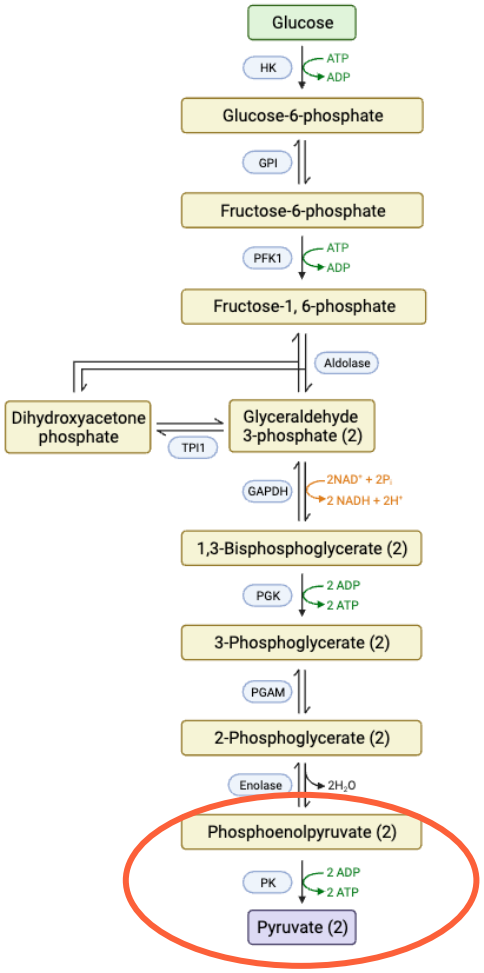


Vaso-occlusive episodes

Hemolysis

Figures were created with Biorender.com

Background



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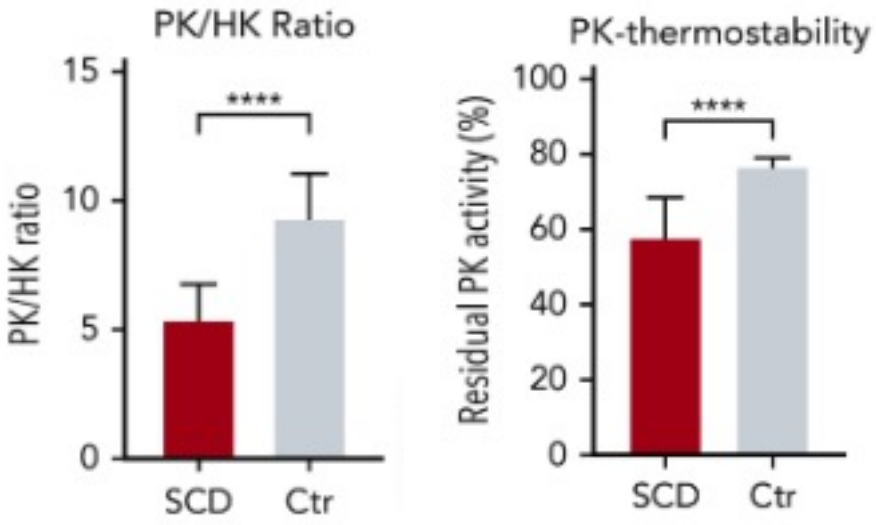

Reference: Rab et al. Blood. 2021.

Clinical trials with PK activators:

- Mitapivat
  - V Dijk et al. AJH. 2022
  - Thein et al. Blood. 2022
  - NCT05031780 (=recruiting)
- Etavopivat
  - Forsyth et al. CPDD. 2022
- AG-946
  - NCT04536792 (=recruiting)

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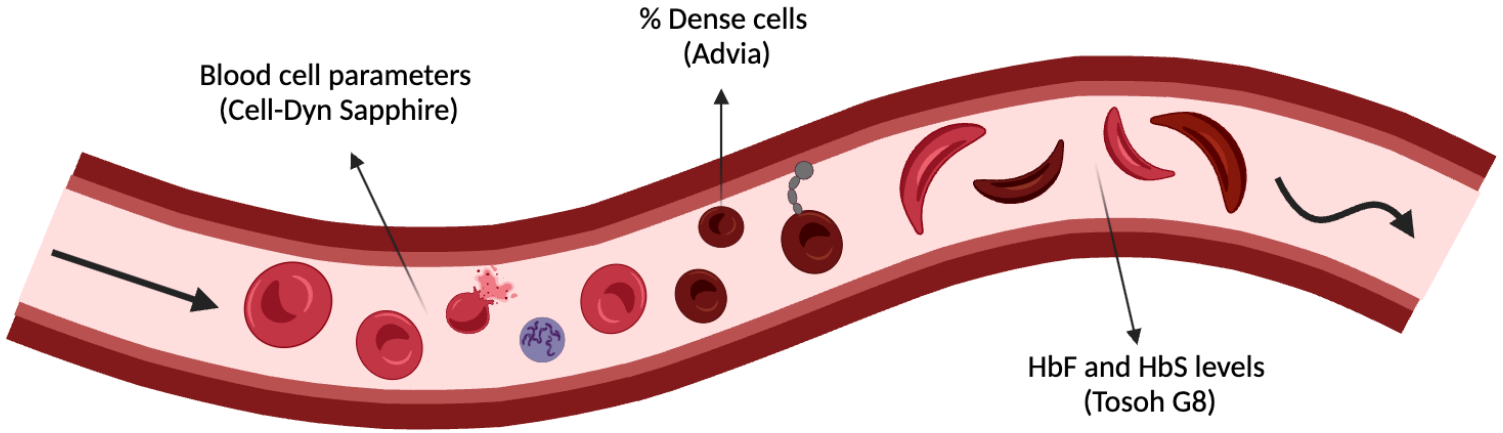
Research question

Are pyruvate kinase properties correlated to clinically important sickle red blood cell characteristics?

Methods

- Homozygous HbS (HbSS) and HbS/β^0 thalassemia patients were eligible

- Patients who received blood transfusion (<3 months) were excluded



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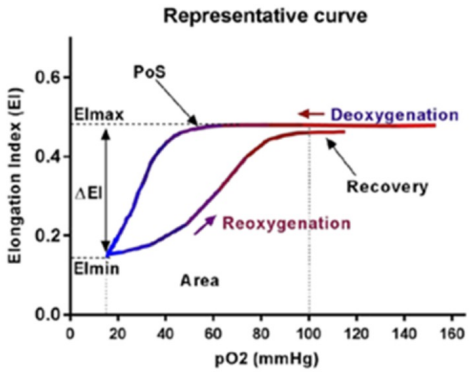
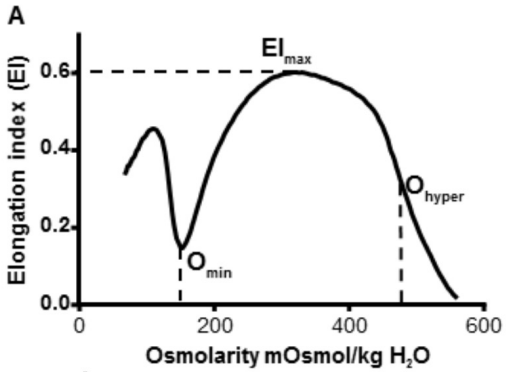
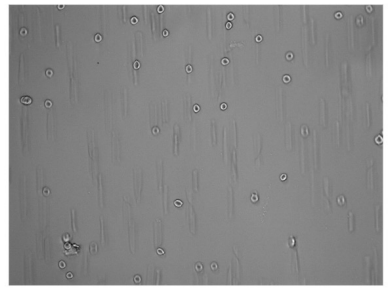
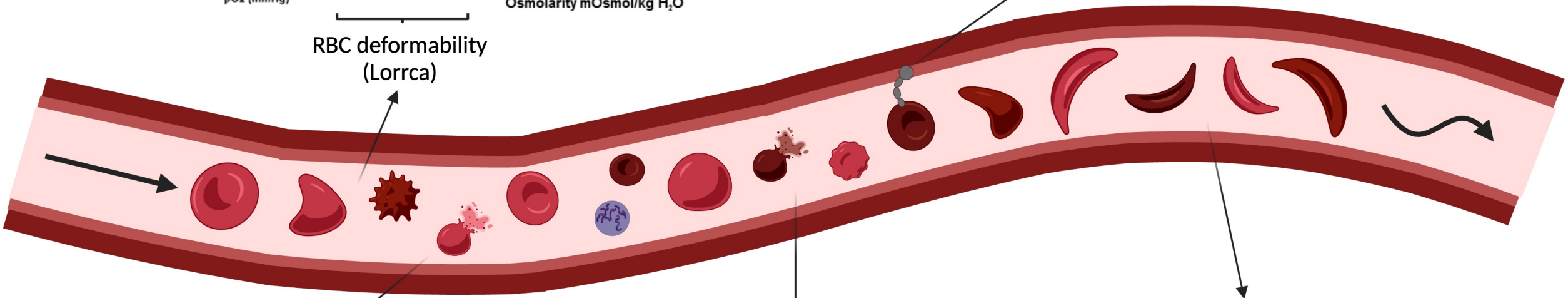




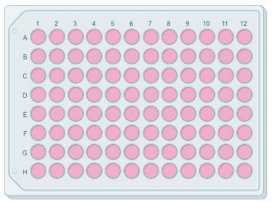
RBC deformability
(Lorrca)

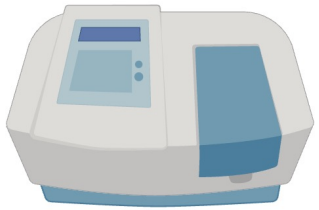


RBC adhesion to laminin
(IBIDI I slides)



PK and hexokinase (HK)
enzymatic activity

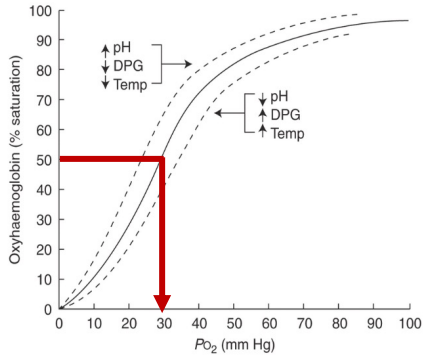




PK thermostability

600 rpm at 53°C

Oxygen affinity
(Hemox Analyzer)

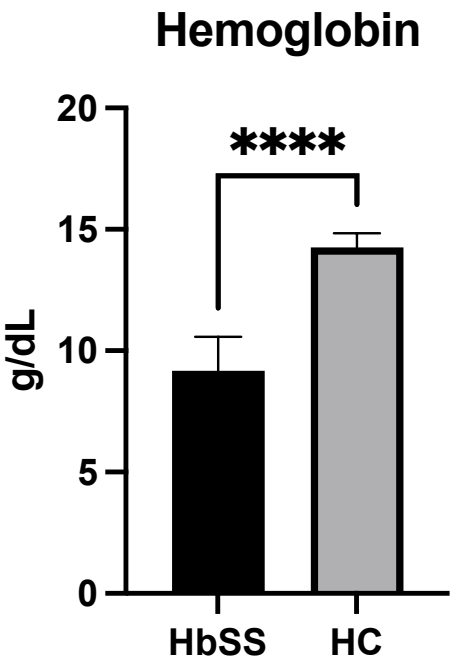


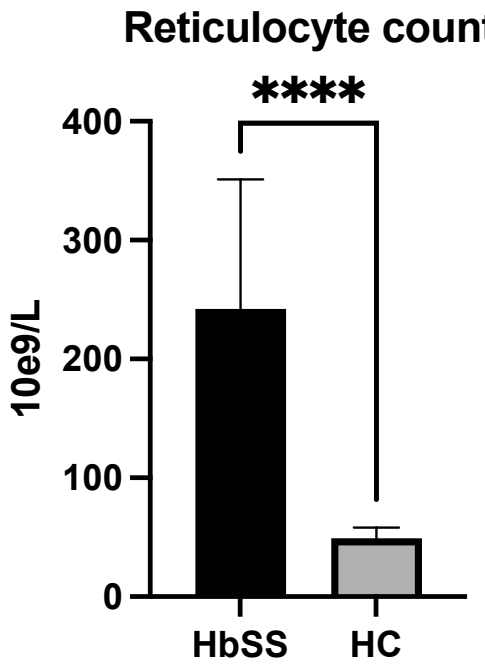
Results – Baseline characteristics

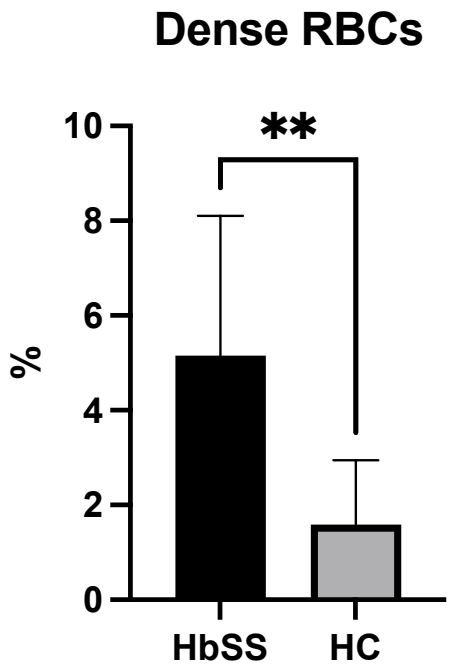
- 57 SCD patients were included (53 HbSS, 4 HbS/β^0 thalassemia)
  - 17 children (median age 14 years [range 6-17])
  - 40 adults (median age 36 years [range 18-58])

- Current treatment:
  - Hydroxyurea in 42/57 (74%) patients
  - Concomitant therapy:
    - Crizanlizumab (N=4)
    - Voxelotor (N=2)
    - N-acetylcysteine (N=1)
    - Hemopexin (N=1)

Results – RBC parameters







HC = Healthy control

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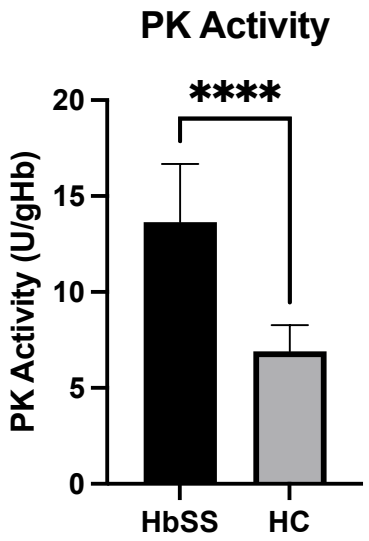
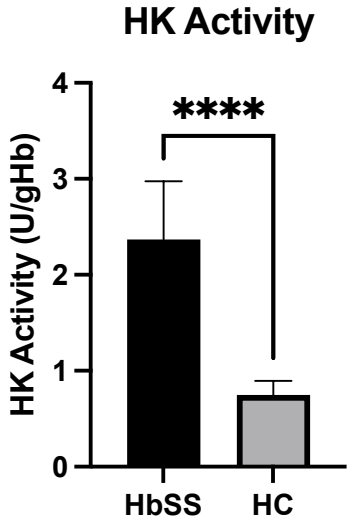
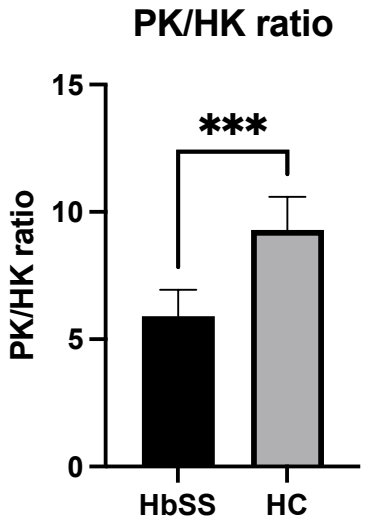
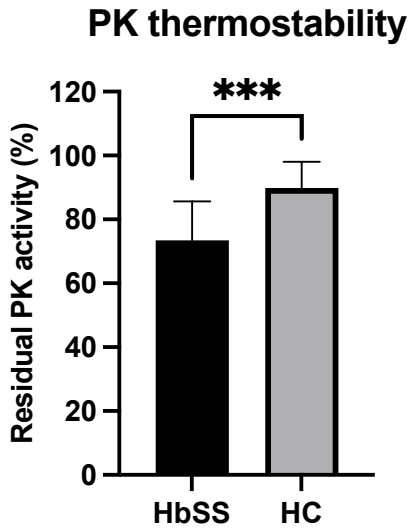
Mean ± SD, **** p<0.0001, ** p<0.01

Results – PK activity and thermostability









HbSS patients have higher enzymatic activities, but compromised PK activity (regarding the age of the cell) and thermostability

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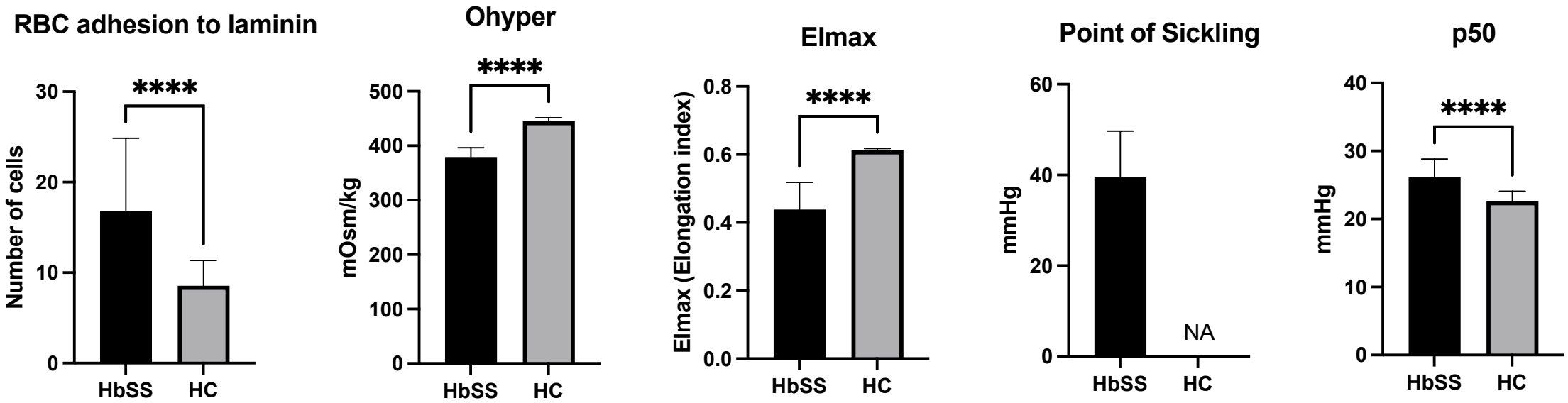
Mean ± SD, **** p<0.0001, *** p<0.001

Results – Functional RBC properties



RBCs from patients with HbSS ->

- Increased adhesion to laminin
- More dehydrated
- Decreased deformability
- Decreased oxygen affinity

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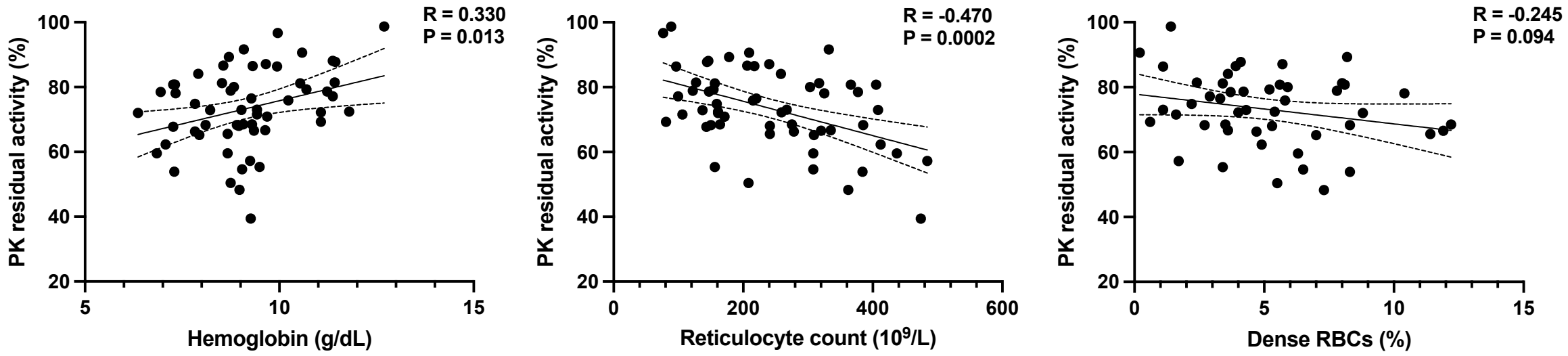
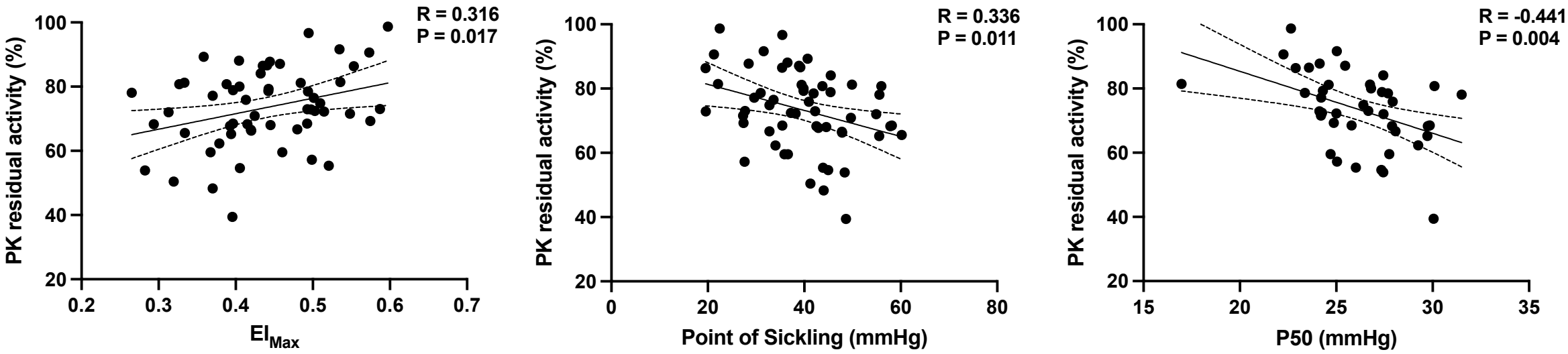
Mean ± SD, **** p<0.0001

Results – Correlation of PK thermostability with RBC parameters



Decreased PK thermostability is signifantly correlated with hemoglobin and reticulocytes, however not with % dense cells

Results – Correlation of PK thermostability with functional RBC properties



Patients with decreased PK thermostability had less deformable RBCs which sickled at a higher oxygen tension

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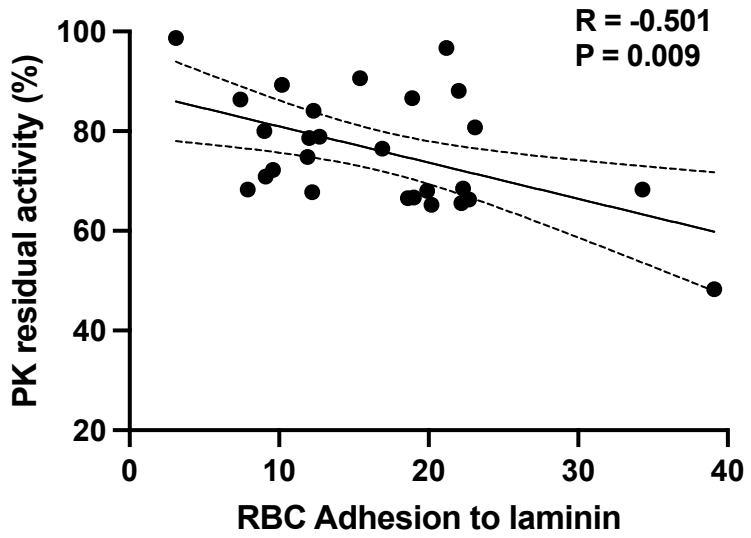
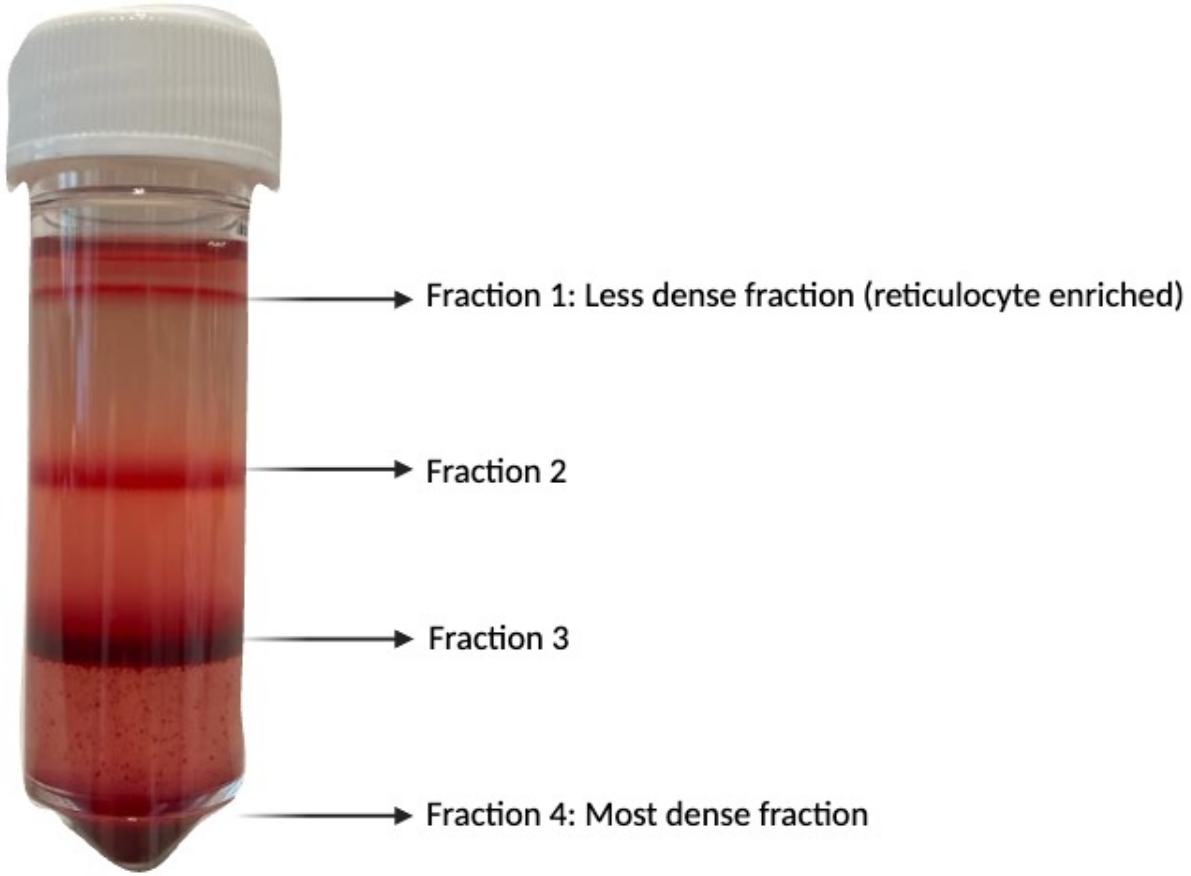
Results – Correlation of PK thermostability with adhesion to laminin

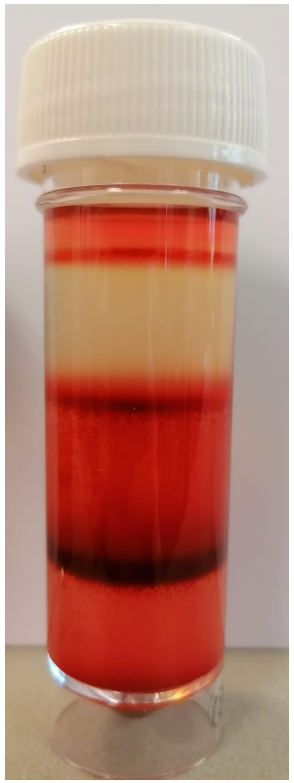


Decreased PK thermostability is associated with more RBC adhesion to laminin

Results – Density separation



Results – Density separation: 5 HbSS patients



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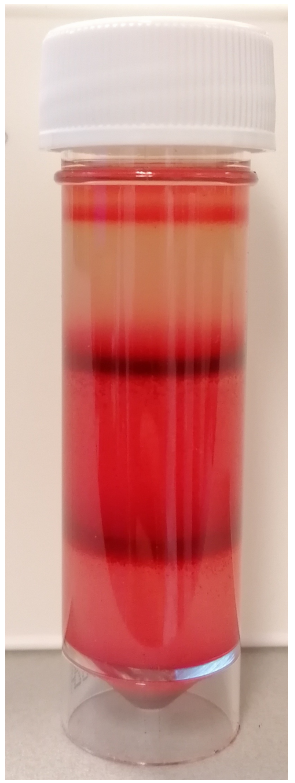

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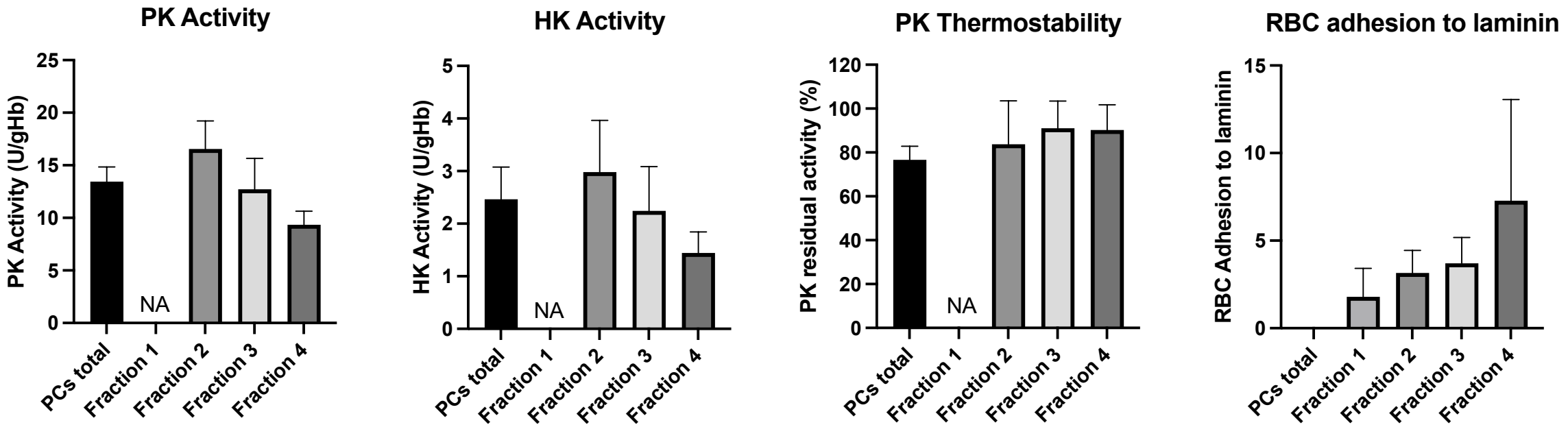
Results – PK properties and RBC adhesion to laminin



RBC age-dependent effect on enzymatic and adhesion properties

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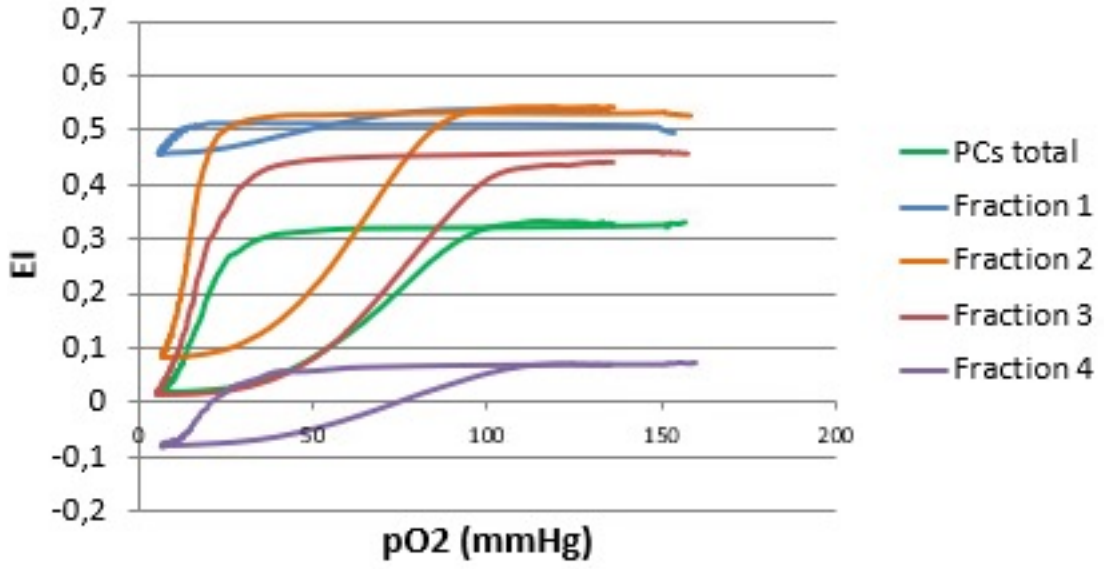
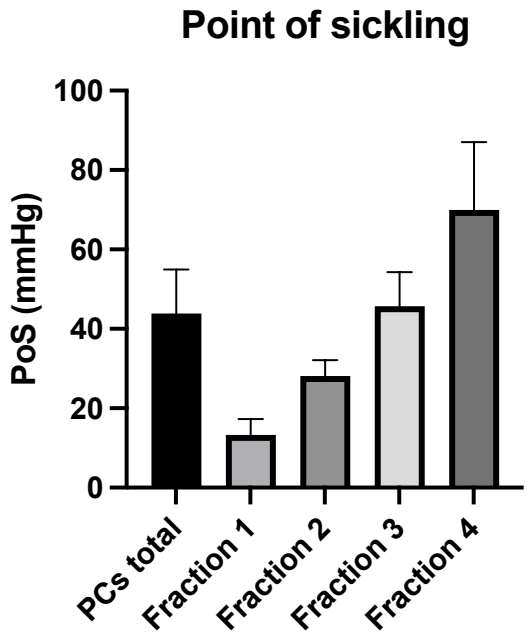
PC = packed cells; NA = not applicable

Results – Deformability and point of sickling





RBC age-dependent effect on RBC sickling properties

Conclusion

- Decreased PK thermostability is associated with impaired clinically important RBC (functional) properties
  - Hemoglobin levels ↓
  - Reticulocyte count ↑
  - RBC adhesion to laminin ↑
  - Deformability ↓
  - Point of sickling ↑
  - Oxygen affinity ↓

- Enhancing activity and stability of PK with PK activator therapy might improve other pathophysiological features outside of RBC metabolism

Acknowledgements

All patients who participated in this study.

University Medical Center Utrecht – Red Blood Cell Research Laboratory:

- Minke A.E. Rab
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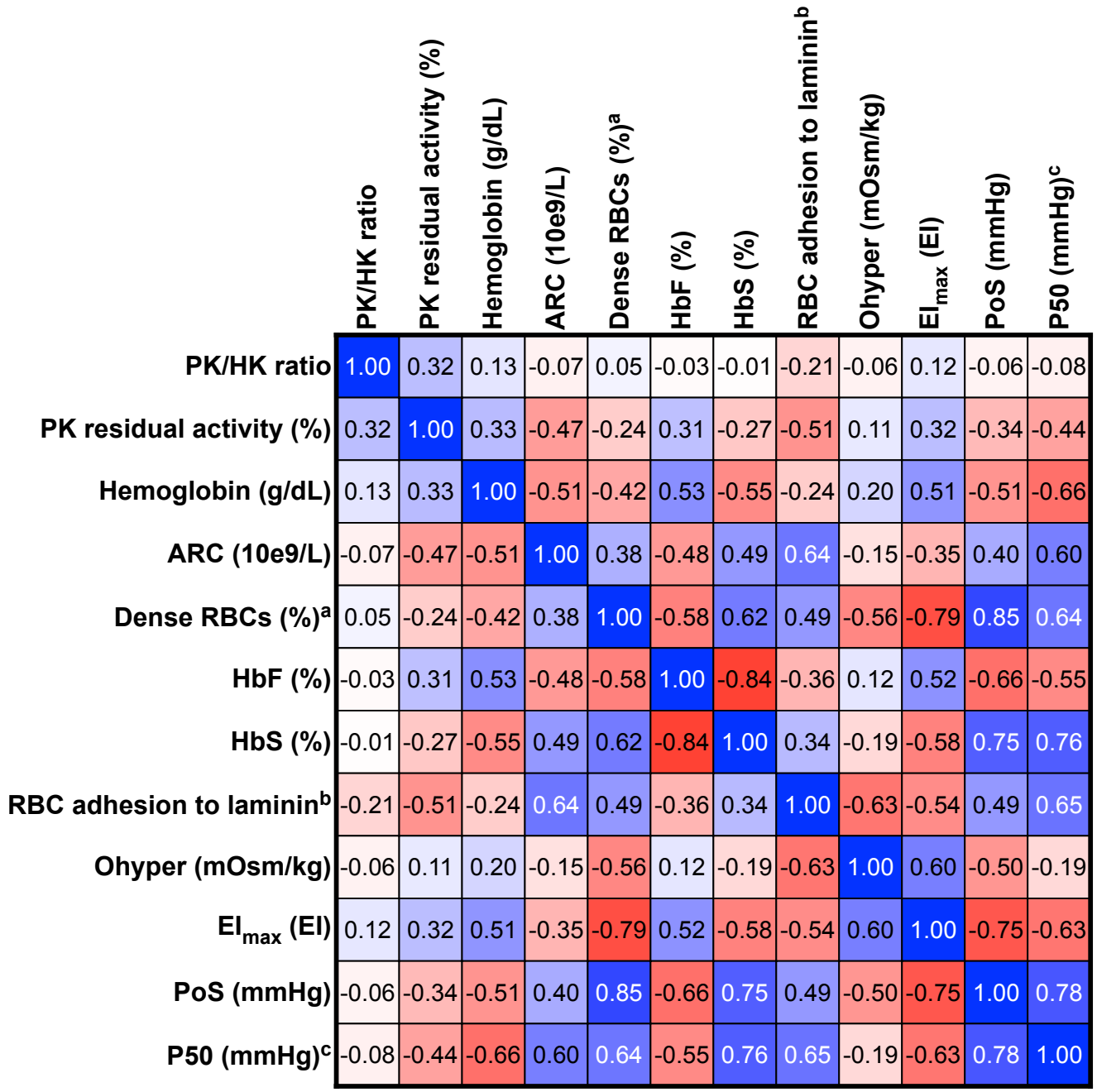
Radboud University Medical Center: Saskia E.M. Schols

Time for discussion


PK/HK ratio
PK residual activity (%)
Hemoglobin (g/dL)
ARC (10e9/L)
Dense RBCs (%)a
HbF (%)
HbS (%)
RBC adhesion to lamininb
Ohyper (mOsm/kg)
Elmax (EI)
PoS (mmHg)
P50 (mmHg)c
PK/HK ratio 1.00 0.32 0.13 -0.07 0.05 -0.03 -0.01 -0.21 -0.06 0.12 -0.06 -0.08
PK residual activity (%) 0.32 1.00 0.33 -0.47 -0.24 0.31 -0.27 -0.51 0.11 0.32 -0.34 -0.44
Hemoglobin (g/dL) 0.13 0.33 1.00 -0.51 -0.42 0.53 -0.55 -0.24 0.20 0.51 -0.51 -0.66
ARC (10e9/L) -0.07 -0.47 -0.51 1.00 0.38 -0.48 0.49 0.64 -0.15 -0.35 0.40 0.60
Dense RBCs (%)a 0.05 -0.24 -0.42 0.38 1.00 -0.58 0.62 0.49 -0.56 -0.79 0.85 0.64
HbF (%) -0.03 0.31 0.53 -0.48 -0.58 1.00 -0.84 -0.36 0.12 0.52 -0.66 -0.55
HbS (%) -0.01 -0.27 -0.55 0.49 0.62 -0.84 1.00 0.34 -0.19 -0.58 0.75 0.76
RBC adhesion to lamininb -0.21 -0.51 -0.24 0.64 0.49 -0.36 0.34 1.00 -0.63 -0.54 0.49 0.65
Ohyper (mOsm/kg) -0.06 0.11 0.20 -0.15 -0.56 0.12 -0.19 -0.63 1.00 0.60 -0.50 -0.19
Elmax (EI) 0.12 0.32 0.51 -0.35 -0.79 0.52 -0.58 -0.54 0.60 1.00 -0.75 -0.63
PoS (mmHg) -0.06 -0.34 -0.51 0.40 0.85 -0.66 0.75 0.49 -0.50 -0.75 1.00 0.78
P50 (mmHg)c -0.08 -0.44 -0.66 0.60 0.64 -0.55 0.76 0.65 -0.19 -0.63 0.78 1.00

PK Thermostability