The role of intrinsic pathway in sickle cell disease

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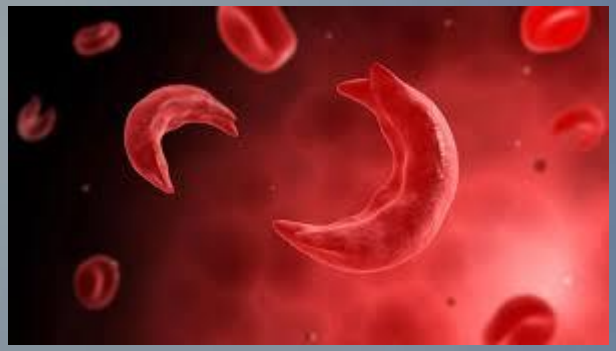
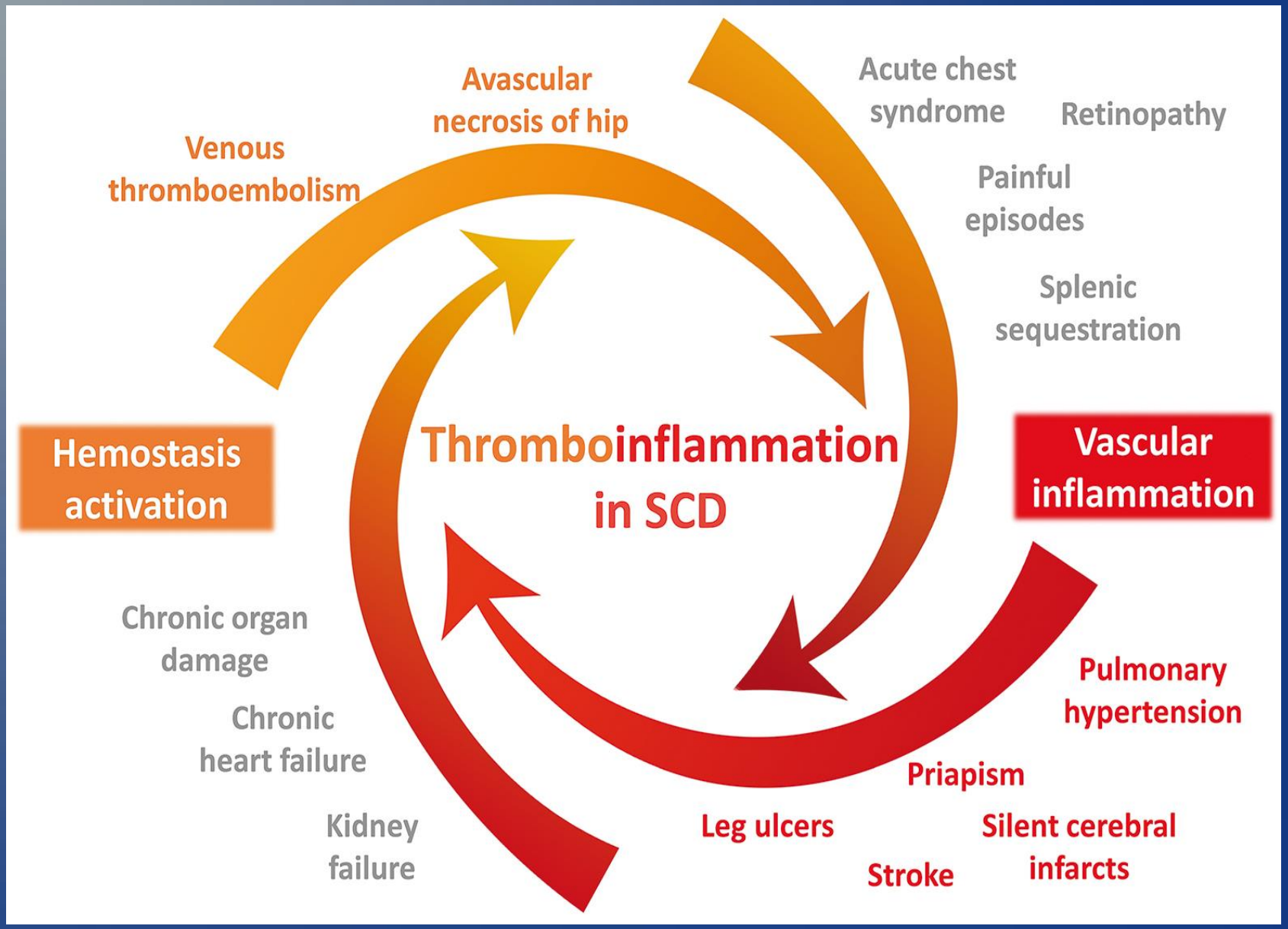


Sickle cell disease :

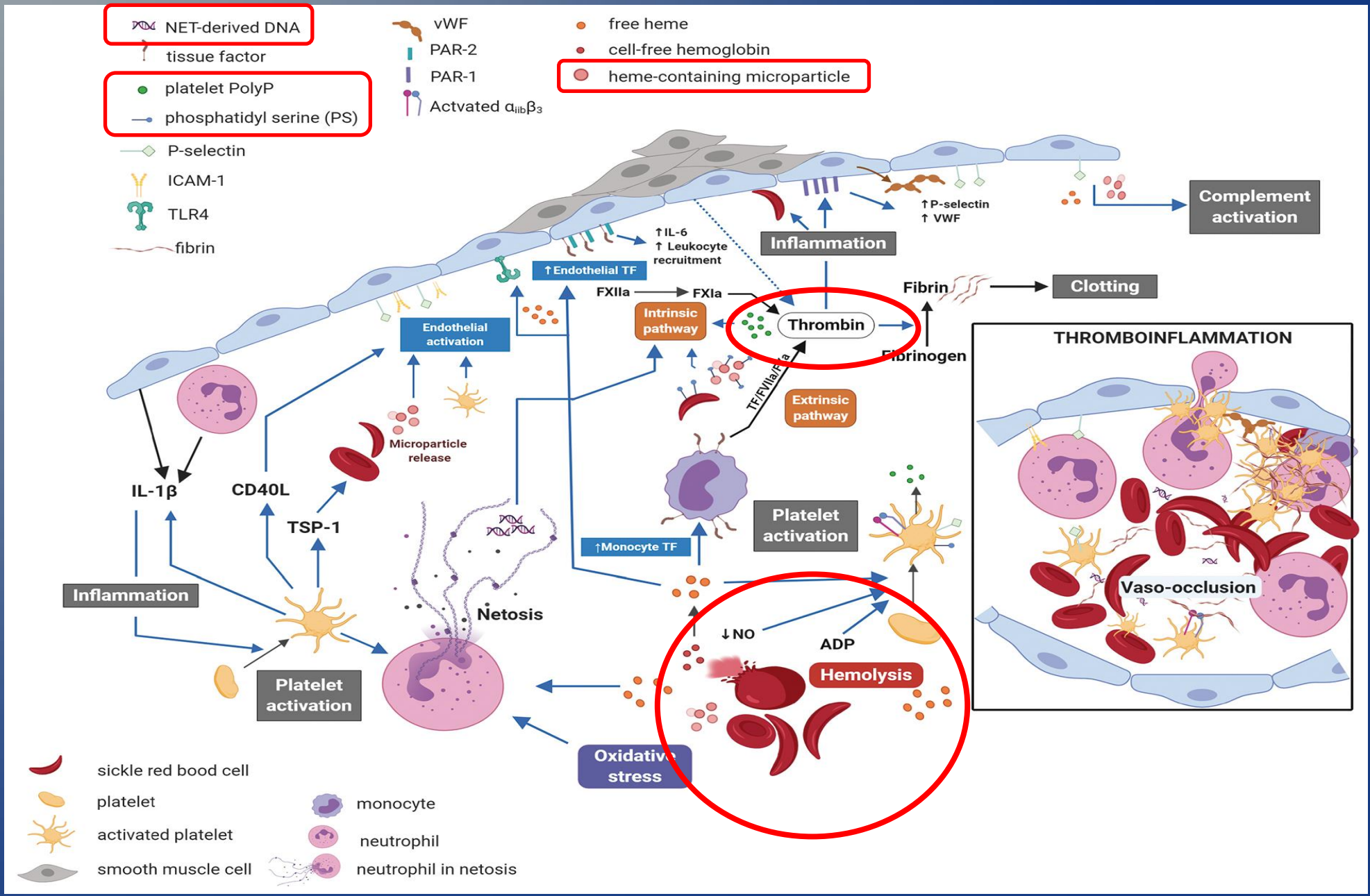
- Inherited hemoglobinopathy
- Point mutation in the β globin gene, (valine for glutamic acid) results in HbS production that polymerizes when deoxygenated
- Red blood cell deformation,
- Expression of adhesive molecules,
- Reactive oxygen species generation
- Complex interactions with endothelial cells, white blood cells and platelets
- Hemolysis, inflammation, microvascular obstruction, chronic organ ischemia and damage

Sickle cell disease : thrombosis and inflammation



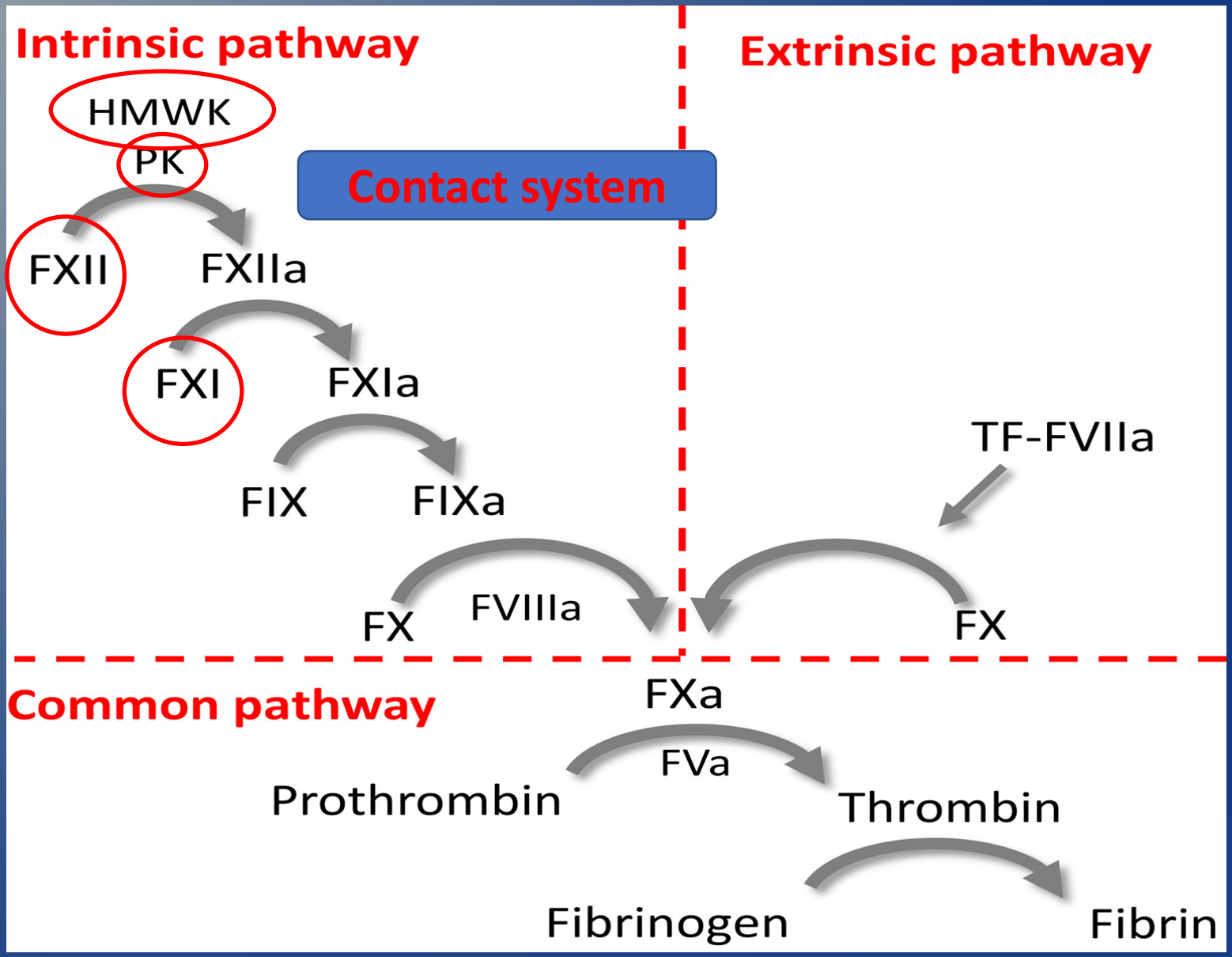
Conran N, Haematologica, 2020

Complex pathophysiology of sickle cell disease



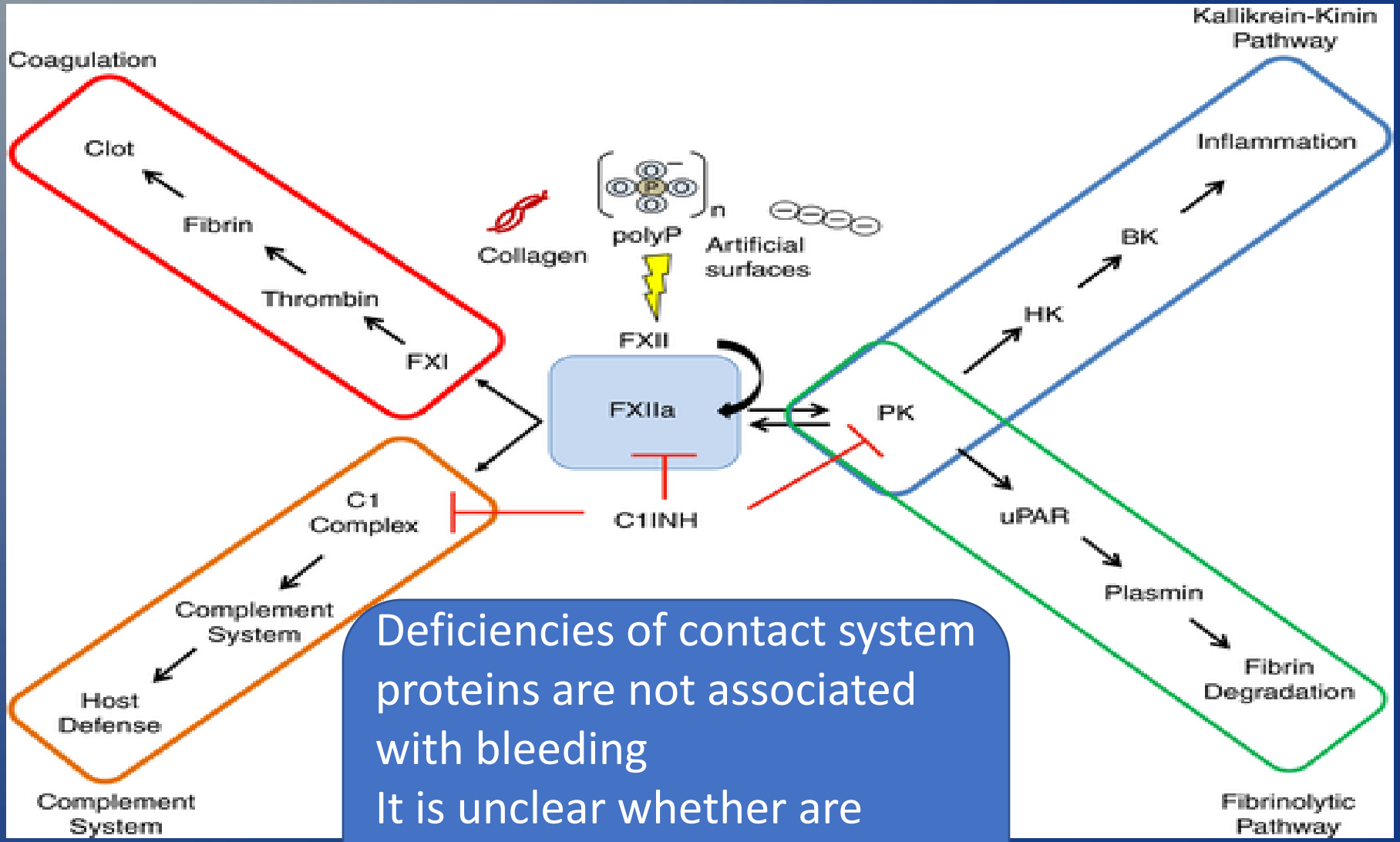
Conran N, Haematologica, 2020

The coagulation cascade



O'Donnell, Br J Haem 2019

Activation of contact system triggers inflammation and coagulation pathways



Deficiencies of contact system proteins are not associated with bleeding

It is unclear whether are associated with thrombosis in humans

Long et al. *J Thromb Haemost* 2015

Activation of contact system

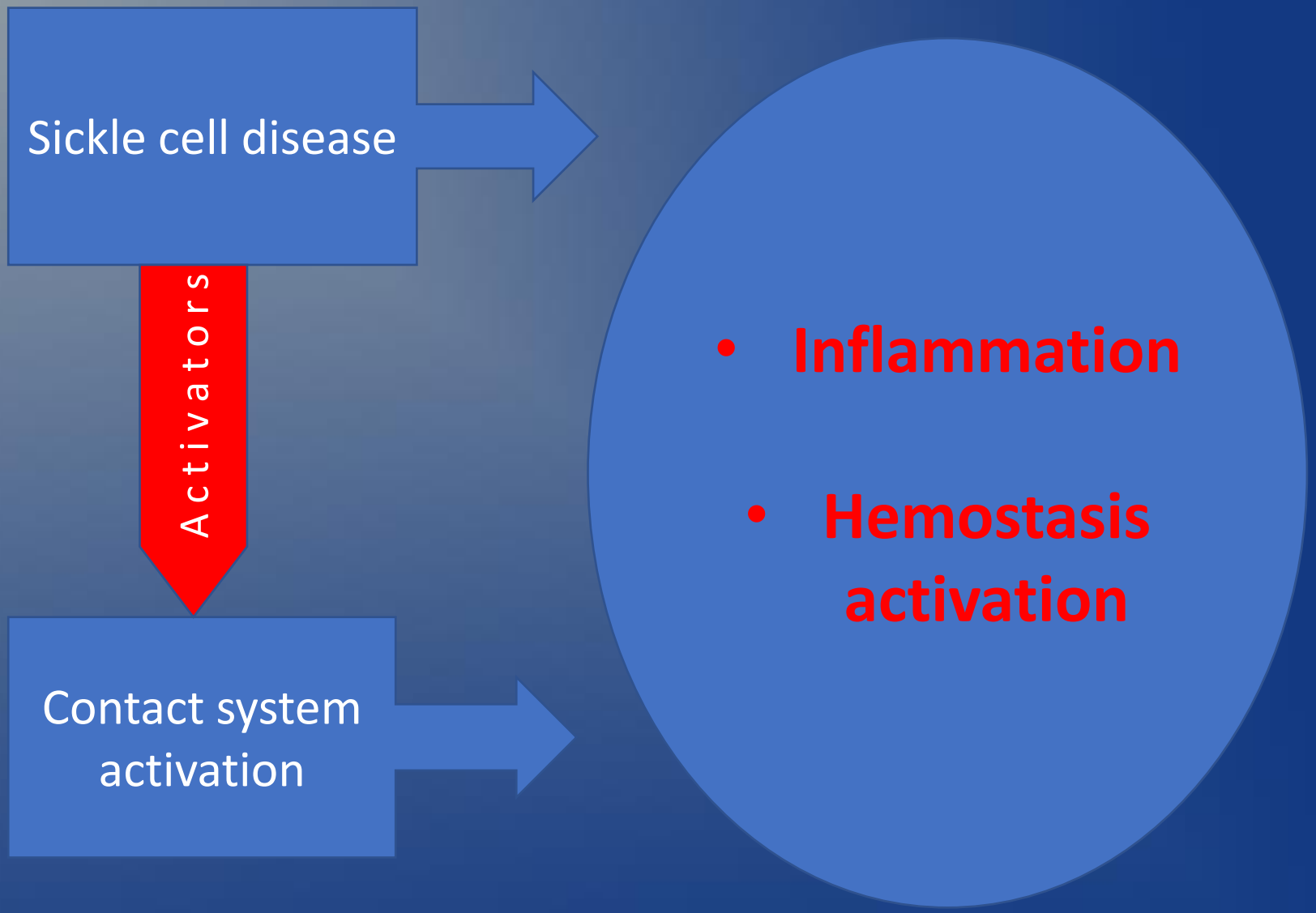
In vitro activators

- caolin
- elagic acid
- glass
- silica

➡ the base of aPTT test

In vivo activators

- RNA
- PolyPs
- NETs
- MPs
- PS
- Protein aggregates (i.e β-amyloid)
- Collagen
- Heparin


Sickle cell disease
Activators
Contact system activation
• Inflammation
• Hemostasis activation

Hypothesis:

Contact system might be involved in SCD pathogenesis

Objective

Measurement of FXII levels in patients with SCD and comparison with those of healthy blood donors

Investigation of any possible relationship of FXII with certain disease manifestations

Cross sectional study

Population: patients with SCD followed in a SCD unit

control group: healthy blood donors

Study design

In a typical visit of the patient:

- Physical examination
- FBC (+DEC, nucleated RBC's)
- Biochemical profile + LDH

Coagulation tests

- PT, aPTT, INR, fibrinogen
- **FXII**, VWF
- CRP, C3, C4

Patient records

- Demographic information
- Type of disease, HbS/F
- Treatment
- Splenectomy
- Clinical manifestation

- Acute chest syndrome
- Stroke
- Acute painful episodes
- Osteonecrosis
- Liver/kidney disease
- DVT/PE

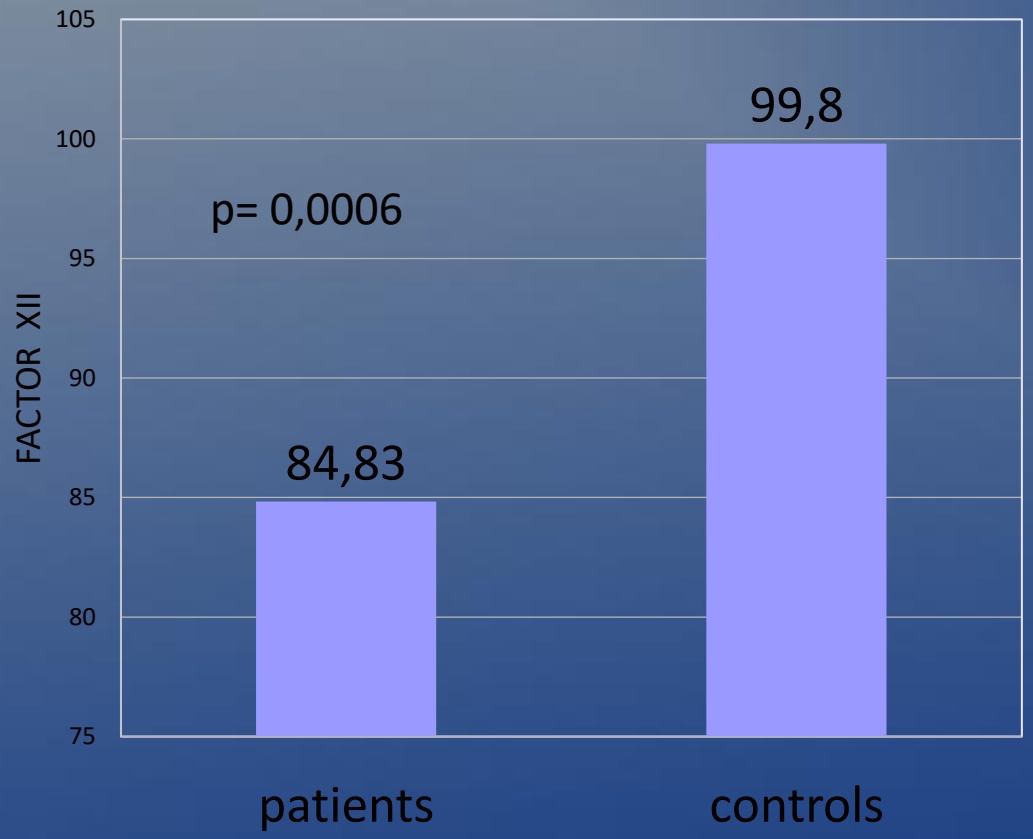
If acute vaso occlusive episode: repeat of blood tests

Control group: FXII levels

Results

Demographic and clinical patient characteristics		
SEX (men/women)	29	12 (41,4%) / 17 (58,6%)
AGE (years)		48,3 ± 11,6
GENOTYPE	Homozygotes (βs/βs)	4 (13,8%)
	Compound heterozygotes	25 (86,2%)
TREATMENT	Transfusion /exchange transfusion	10 (34,5%)
	Hydroxyurea	16 (55,2%)
	Not on special treatment	8 (27,5%)
CLINICAL MANIFESTATIONS/ COMPLICATIONS	Acute painful episodes	10 (34,5%)
	Avascular necrosis	18 (62%)
	DVT/PE	7 (24,1%)
	Acute chest syndrome	6 (20,7%)
	Splenic sequestration	6 (20,7%)
	Stroke	4 (13,8%)
	Liver disease	3 (10,3%)
	Pulmonary hypertension	3 (10,3%)
	Kidney disease	3 (10,3%)

Levels of FXII in patients and controls



FXII levels were significantly lower in patients than in healthy blood donors

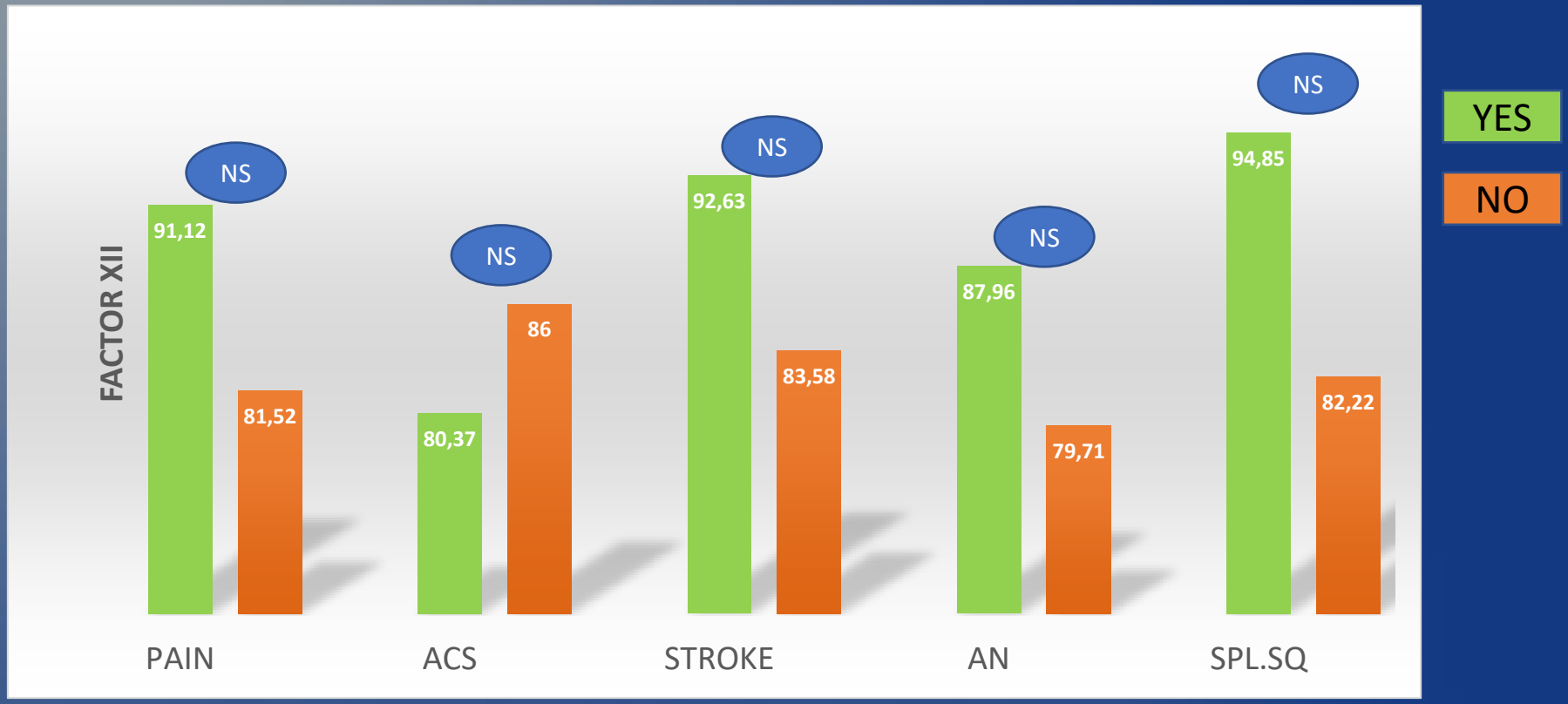
Implication of contact system activation

FXII levels and treatment

No difference in FXII levels between patients

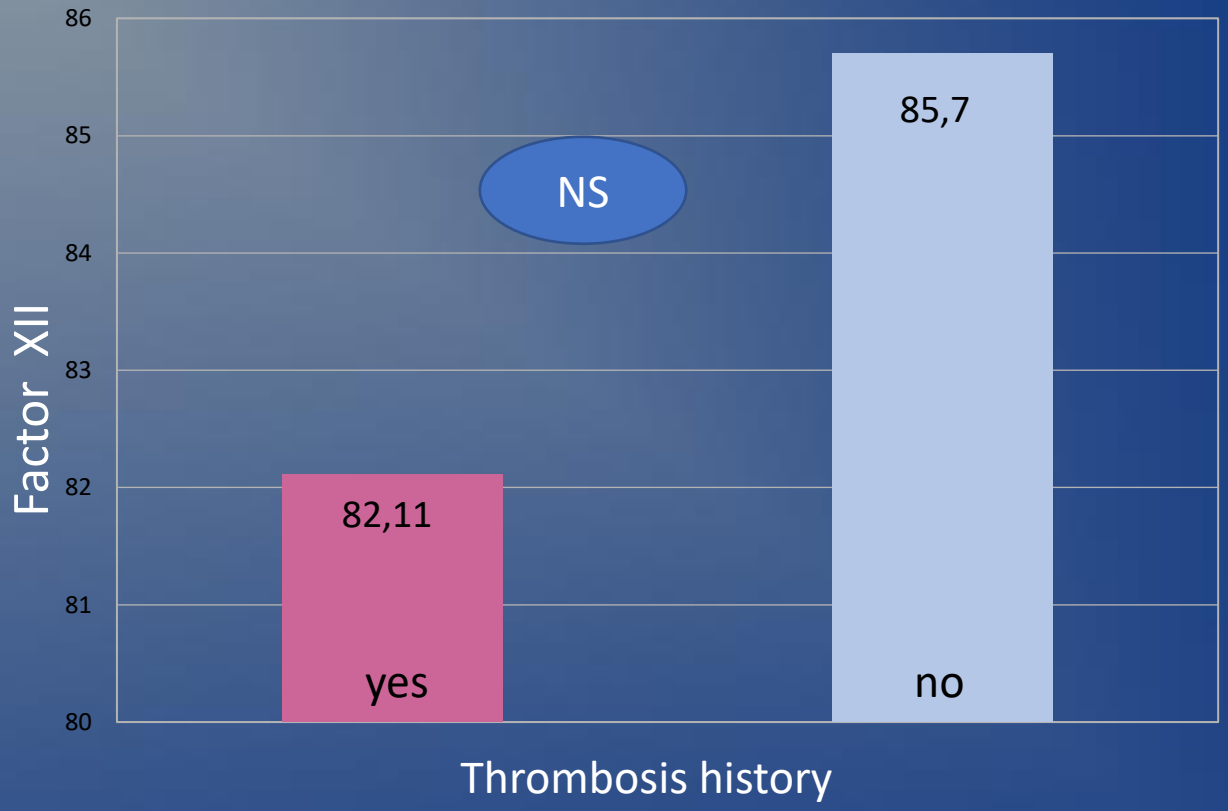
- transfused or not transfused
- on hydroxyurea or not

FXII and disease complications



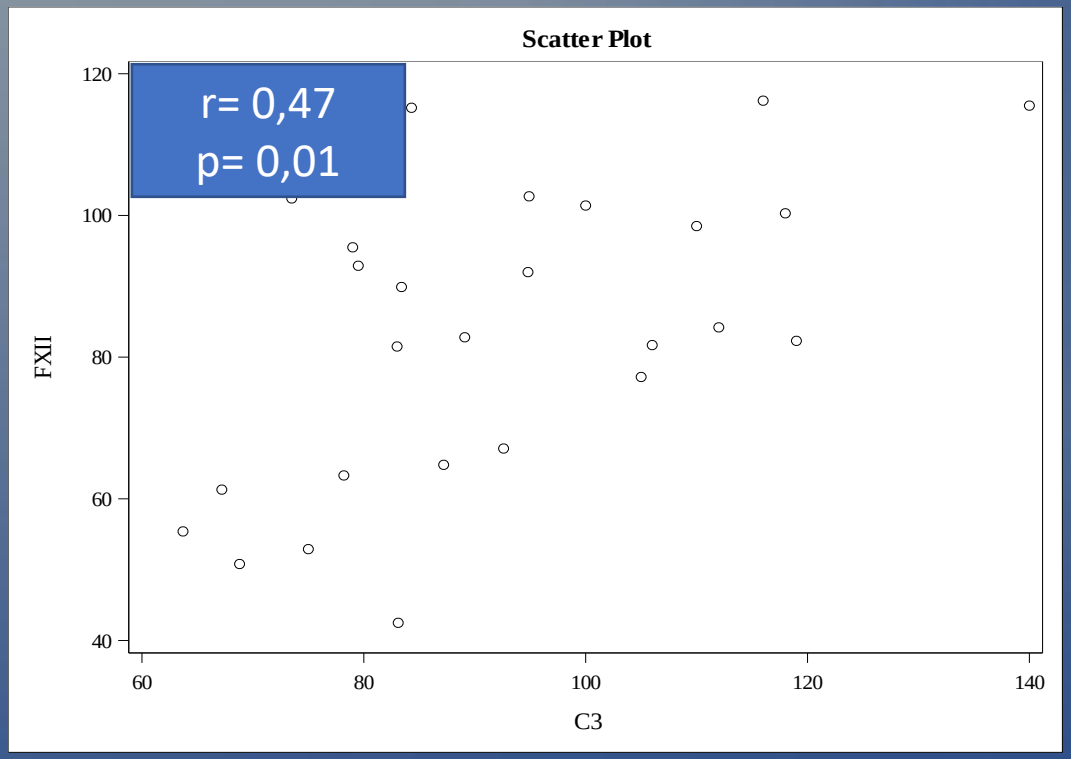
No difference in FXII levels between patients with and without specific complication history

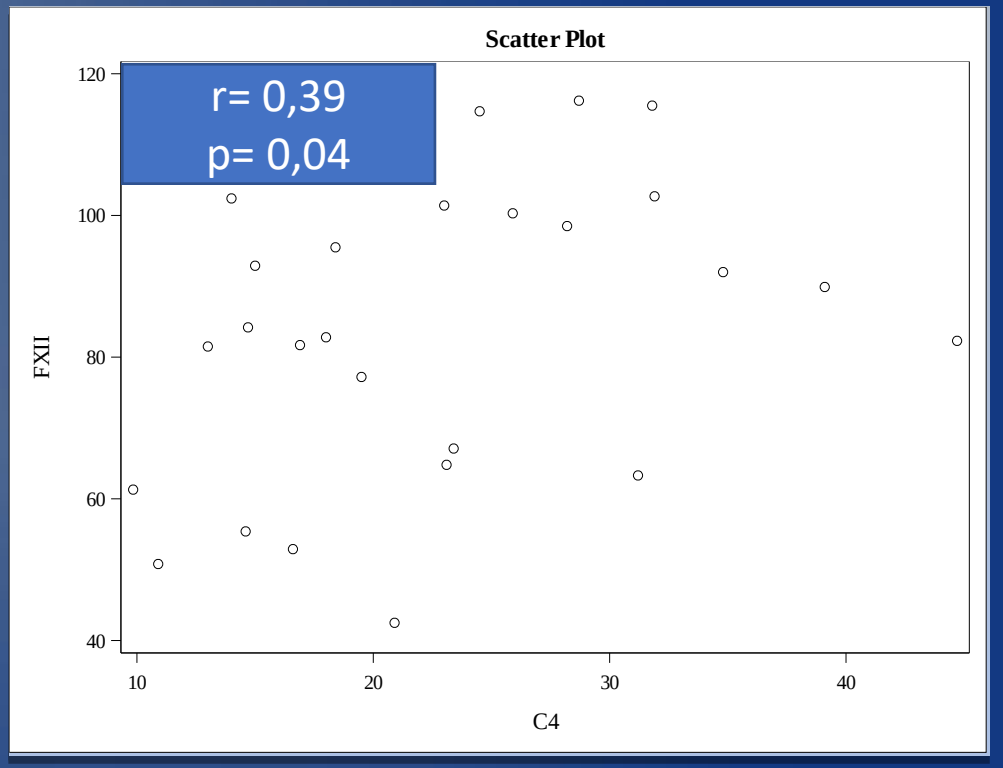
FXII levels and thrombosis history



No difference in FXII levels between patients with or without thrombosis history

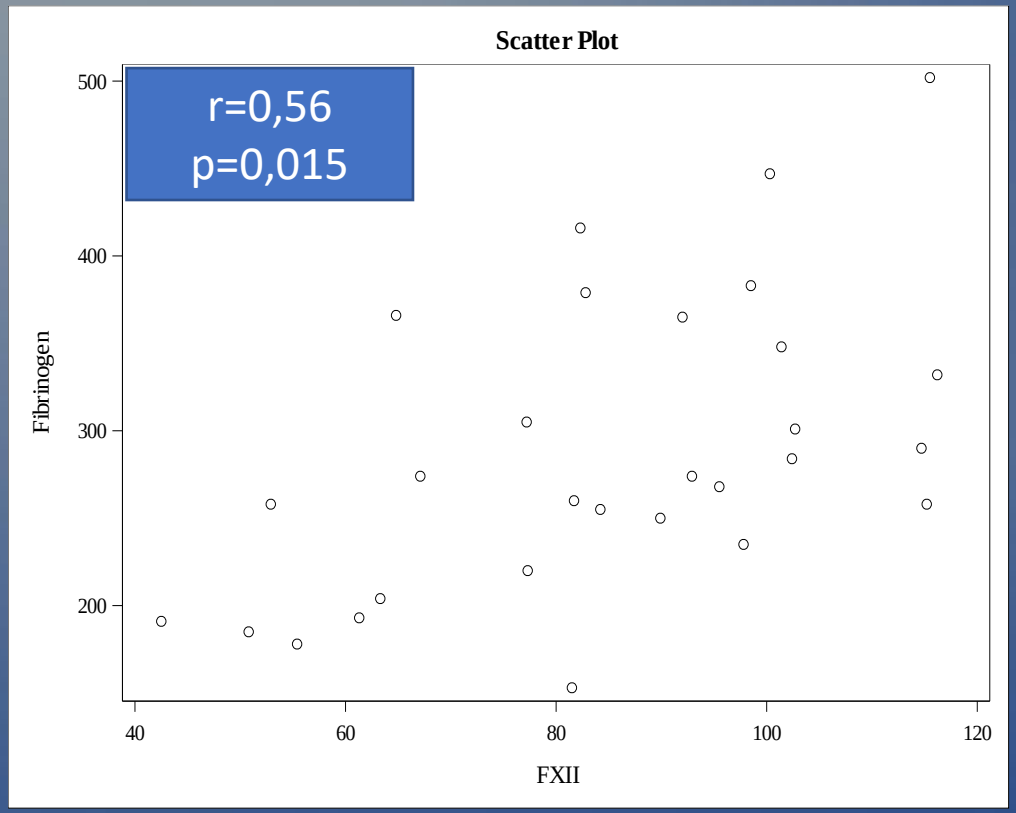
Correlation of FXII with biological markers

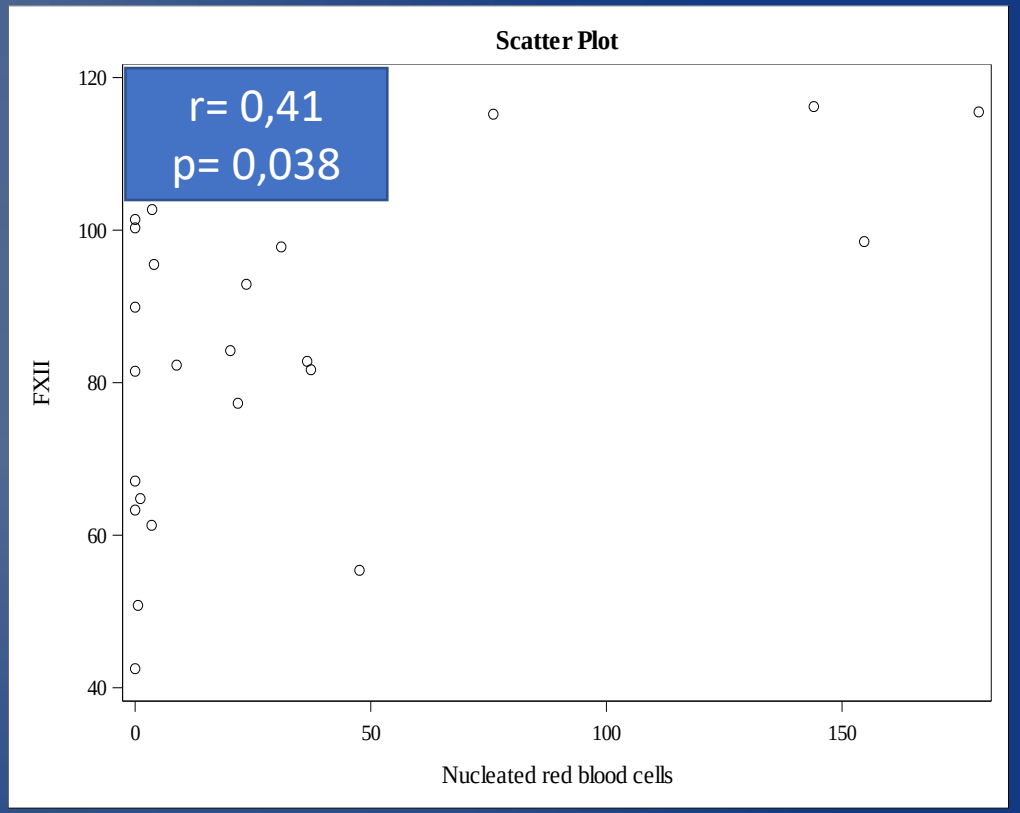




Positive correlation with C3 and C4

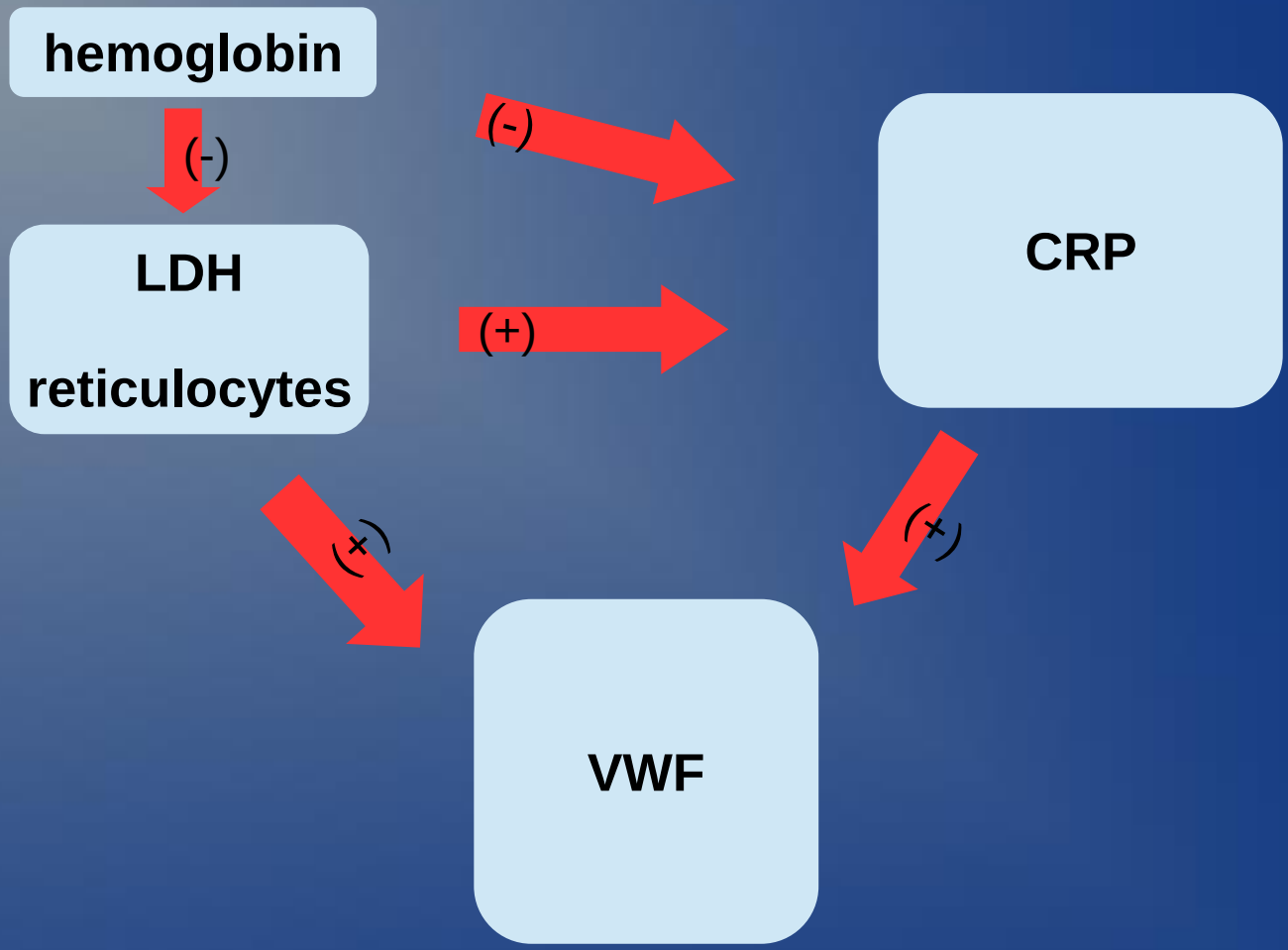
Correlation of FXII with biological markers





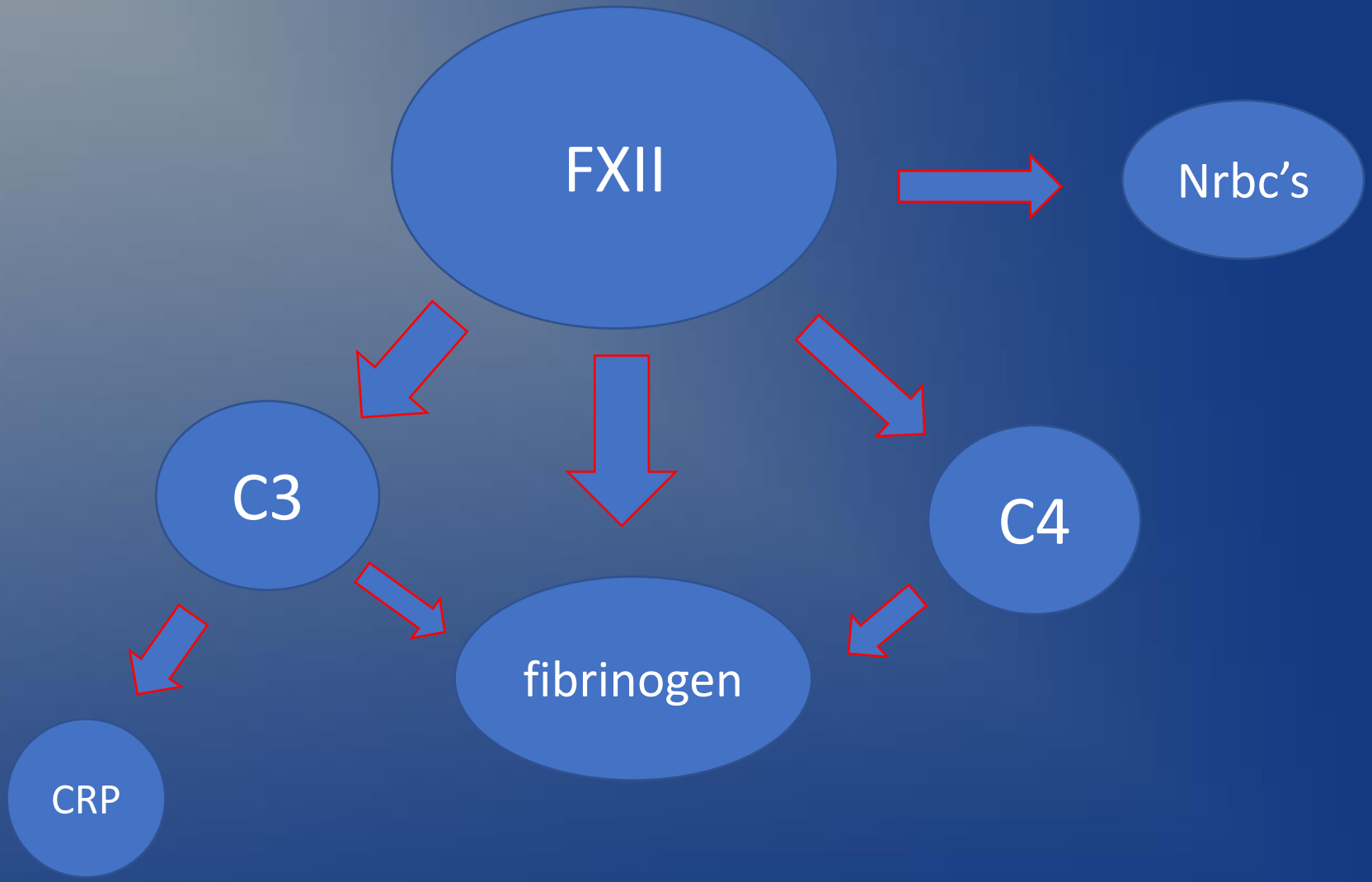
Positive correlation with fibrinogen and nucleated RBC's

Significant correlations between biological markers



Interrelation of hemolysis-coagulation-inflammation

Overall…



Conclusion

- FXII levels are lower in SCD patients in steady state compared to normal subjects
- Hemolysis is an important contributor in inflammation and hemostasis activation seen in SCD
- FXII participates in hemolysis and inflammation pathways

- **Possible involvement of FXII (and contact system) in SCD pathophysiology**

Evidence of contact system involvement in SCD

- Two studies in early 1980's showed decreased levels of FXII, PK, HK in sickle cell disease steady state with further reduction during vaso-occlusive episodes *(Miller et al 1983, Gordon et al 1985)*
- Markers of in vivo thrombin generation show only moderate or no correlation with whole blood TF-procoagulant activity ⟶ may reflect the contribution of intrinsic pathway in thrombin generation *(Noubouossie 2016)*
- RMPs contribute to FXI-dependent thrombin generation that was reduced by 50% with anti-human FXI *(van Beers, 2009)*
- FXII and HK deficiency significantly attenuated thrombin generation in sickle cell mice *(Sparkenbaugh 2016)*
- FXII plays an important role in apoptotic cells-mediated procoagulant activity which is attenuated with anti-FXII antibody *(Yang 2018)*

Thank you very much
for your attention