THANZ Scientific Workshop 2019
Oct 19th , 2019 in Perth

The impact of Clot Waveform Analysis for Diagnosis and Treatment of Hemophilia

Department of Pediatrics,, Nara Medical University
Midori Shima

- Assessment of clotting function of hemophilia A by APTT-CWA
- Monitoring of hemostatic therapy
- FVIIIa mimicking bispecific antibody and its monitoring
- New applications of APTT-CWA

Disclosures for: Midori Shima

CONFLICT	DISCLOSURE — IF CONFLICT OF INTEREST EXISTS
RESEARCH SUPPORT	Chugai, Bayer, Shire, Novo Nordisk, Pfizer, CSL-Behring, Kaketsuken, Sysmex
DIRECTOR, OFFICER, EMPLOYEE	
SHAREHOLDER	
HONORARIA	Chugai, Roche, Bayer, Shire, Novo Nordisk, Bioverativ, Pfeizer, Sysmex, Sekisui Medical
ADVISORY COMMITTEE	Chugai, Roche, Bayer, Shire, Bioverativ, CSL Behring, Novo Nordisk
CONSULTANT	Chugai

Laboratory methods for hemophilia

- APTT
- Factor VIII/IX activity assay:
  Clotting assay, Chromogenic assay
- Factor VIII/IX antigen assay:
  ELISA

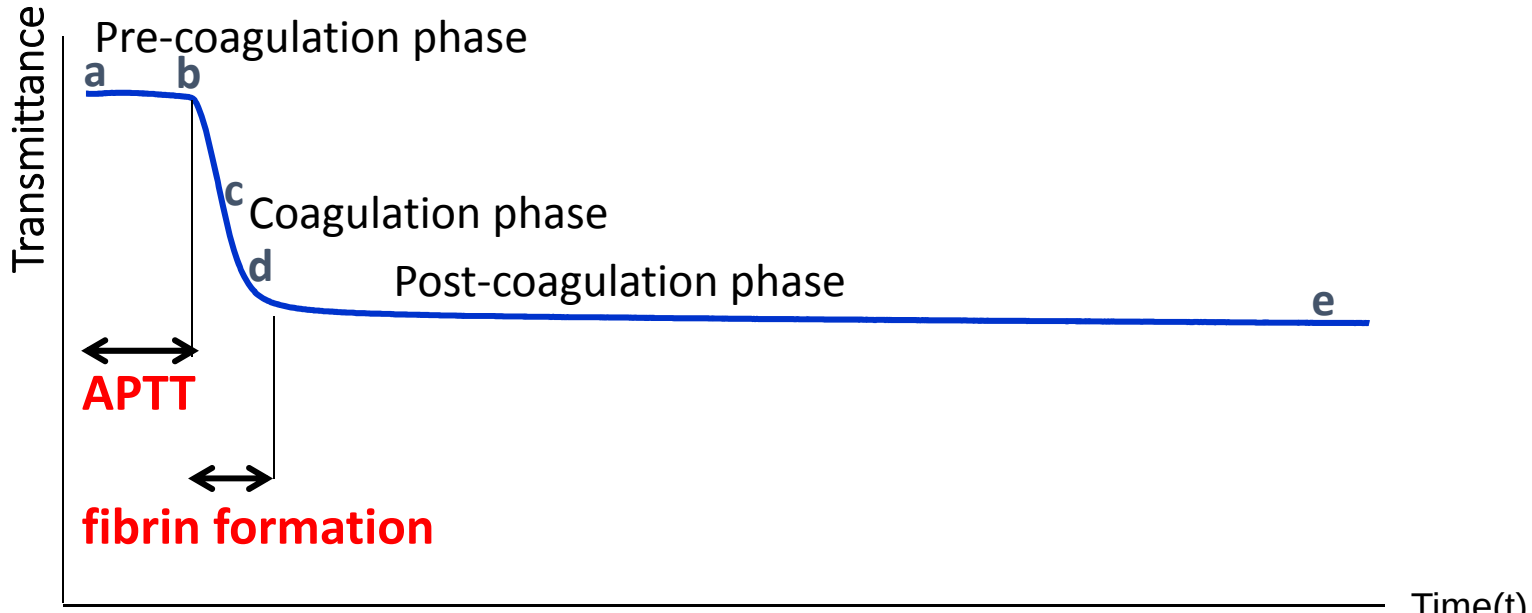
APTT or FVIII/FIX levels do not always reflect clotting function of hemophilia patients.

Global assays

- Thromboelastography (TEG), Rotating thromboelatometry (ROTEM)
- Thrombin generation assay
- Clot waveform analysis (CWA)

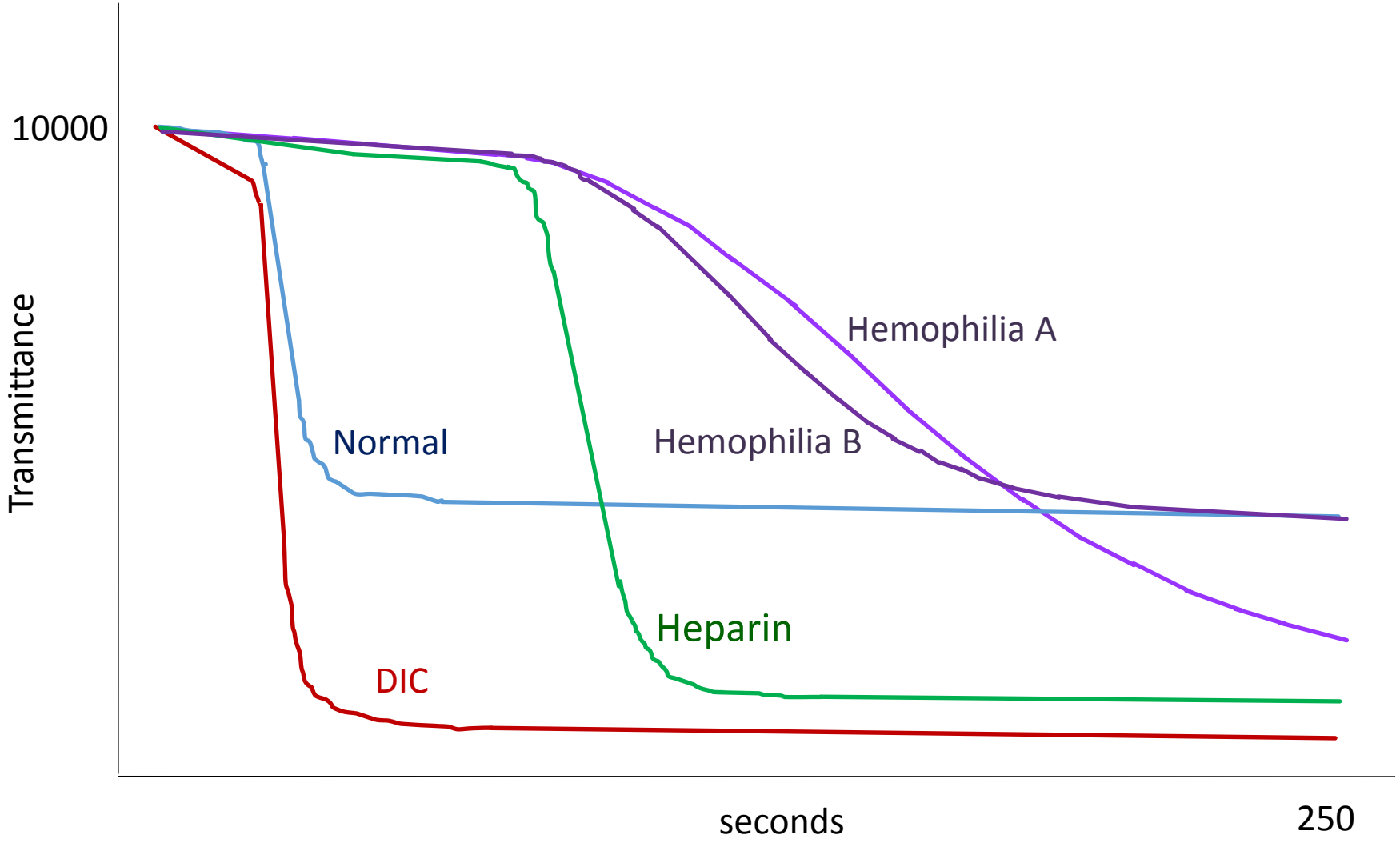
APTT has more information

If the transmittance during the aPTT is continuously monitored, it is possible to obtain waveform.



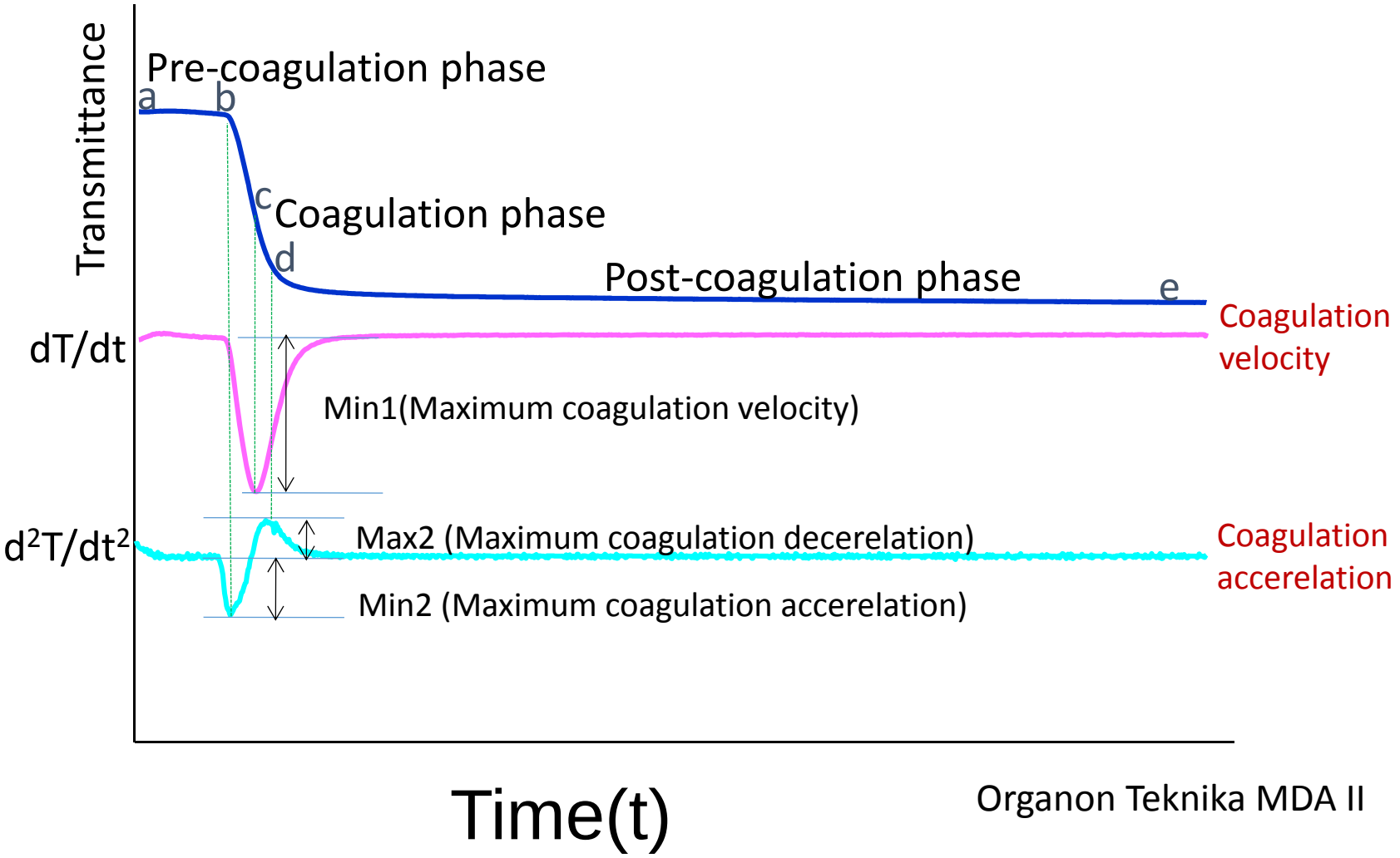
APTT clot waveform for normal plasma

- APTT reflects only pre-coagulation phase.
- CWA reflects whole clotting process.
- Qualitative evaluation is possible in various clinical settings: hemophilia, DIC, monitoring of anti-coagulant therapy.



Braun et al. Thromb Haemost 1997

Quantitative assessment can be performed by various parameters by CWA



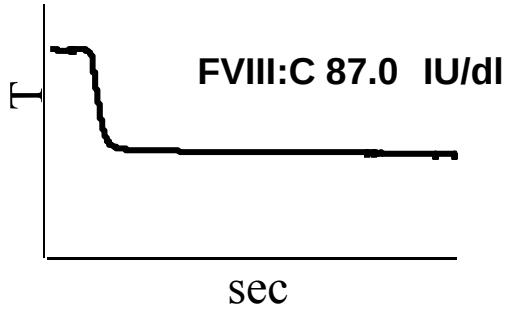
Organon Teknika MDA II

Braun et al. Thromb Haemost 1997

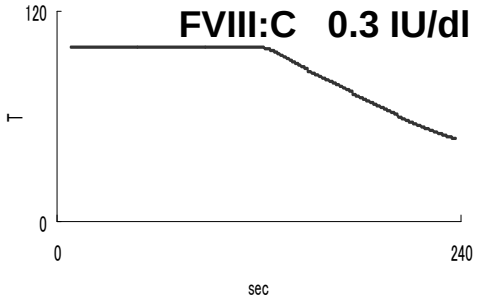
APTT clot waveforms of normal and severe Hemophilia A

Organon Teknika MDA II

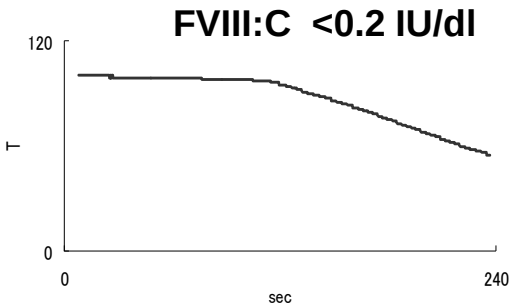
Normal plasma



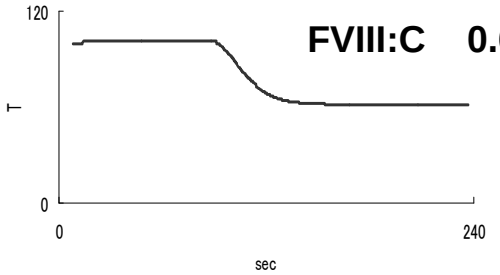
Case3



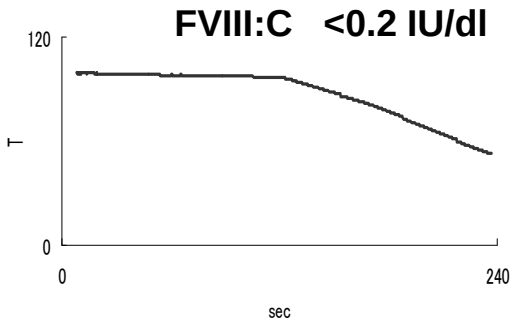
Case 1



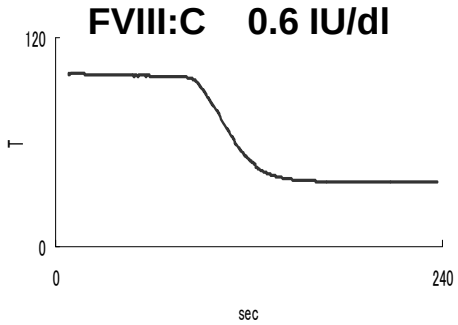
Case 4



Case 2



Case 5

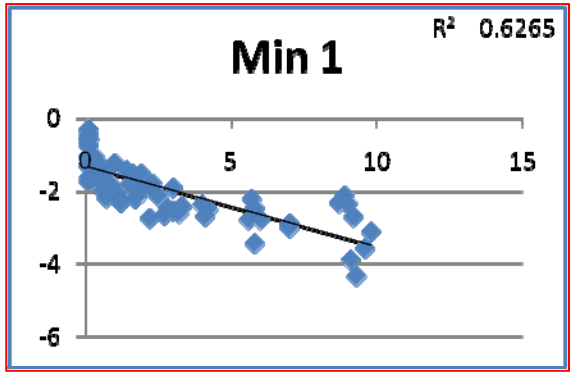


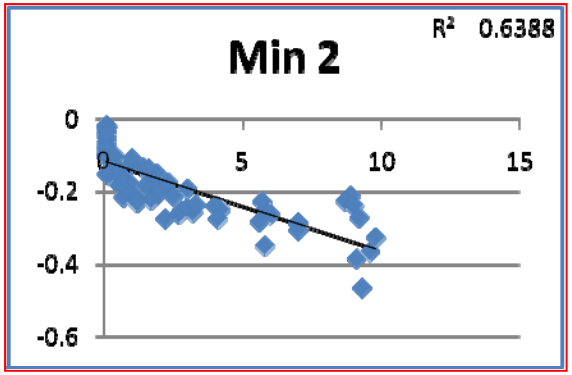
Shima M et al.
Thromb Haemost 2002

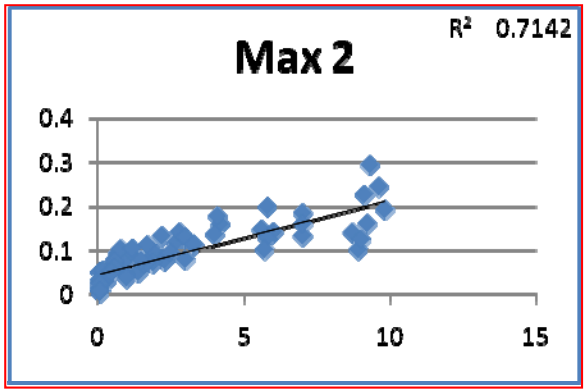
APTT CWA is sensitive to the low level of FVIII less than 1 IU/dl.

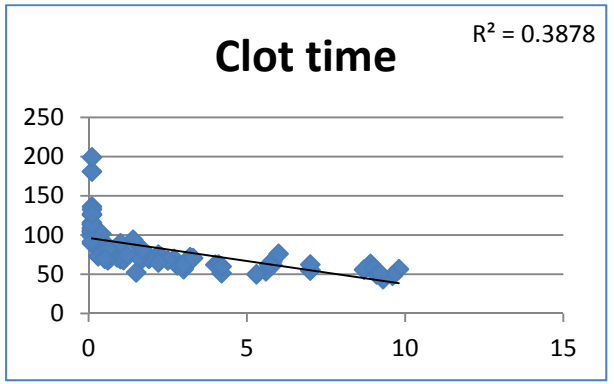
Correlation between Parameters and FVIII levels in 70 cases of Hemophilia A .

Hemophilia A patients with the FVIII level of 0-10 %.







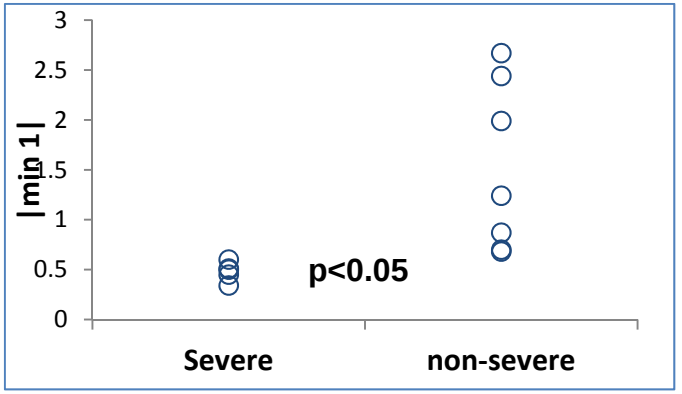


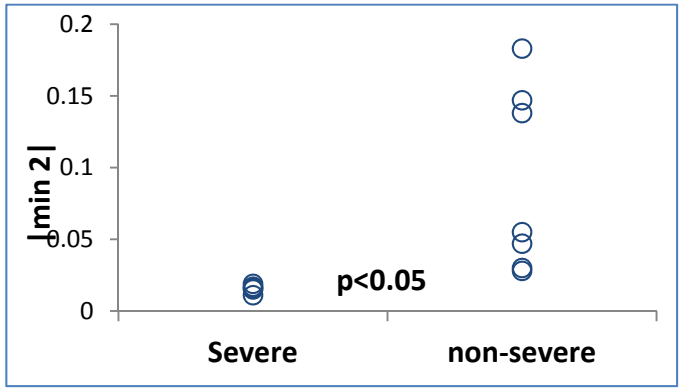
Sysmex 2000i

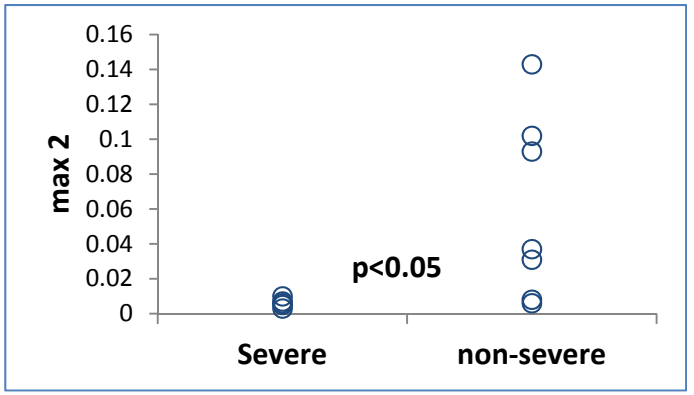
Min1, Min2 and Max2, reflecting coagulation phase, are useful for quantitative evaluation of clotting function of hemophilia A.

Clinical severity and CWA parameters of the severe hemophilia A patients (FVIII:C<1.0 IU/dL)

Clinically severe:
- Spontaneous bleeding <1 y.o.,
- Onset of joint/muscular bleeding < 3 y.o.
- Severe bleeding(ICH, refractory oral bleeding)







These results suggested that CWA reflect global clotting function.

Shima M. et al. J Thromb Haemost 2013; 11: 1417-20

Various clotting instruments have potential to perform CWA

Coagulation Analyzer		
CS-5100	Sysmex	WA
CS-2400/2500	Sysmex	WA
CS-1600	Sysmex	RAW
BCS XP	Siemens	RAW
ACL TOP 750/700 Series	IL	WA
	IL	WA
ACL TOP 550/500 CTS	IL	WA
ACL TOP 350/300 CTS	IL	RAW
ACL ELITE Series		RAW
CP2000/CP3000	Sekisui	RAW
STACIA	LSI Medience	Not
STA R Max/Compact Max	Stago	RAW
Cobas t 511/t 711	Roche	RAW
Coagtron-180	Kyowa Medix	

WA: CWA available, RAW: Raw data output for CWA available,
Not: CWA not available

Journal of Thrombosis and Haemostasis, **11**: 1417–1420 DOI: 10.1111/jth.12287

OFFICIAL COMMUNICATION OF THE SSC

Towards standardization of clot waveform analysis and recommendations for its clinical applications

M. SHIMA,* J. THACHIL,† S. C. NAIR‡ and A. SRIVASTAVA§
**Department of Pediatrics, Nara Medical University, Kashihara, Nara, Japan; †Department of Haematology, Manchester Royal Infirmary, Manchester, UK; ‡Department of Transfusion Medicine and Immunohaematology, Christian Medical College; and §Department of Haematology, Christian Medical College, Vellore, India*

Shima M et al. J Thromb Haemost 11:1417-1420, 2013

Case presentation

44 years old male, Mild hemophilia A (95kg).

The patient repeated the episode of severe bleeding such as massive intra-muscle bleeding in spite of enough baseline level (FVIII:C 20-30 IU/dL). In each bleeding episode, he required replacement therapy with FVIII concentrates. He was hospitalized for his massive intra-muscle bleeding.

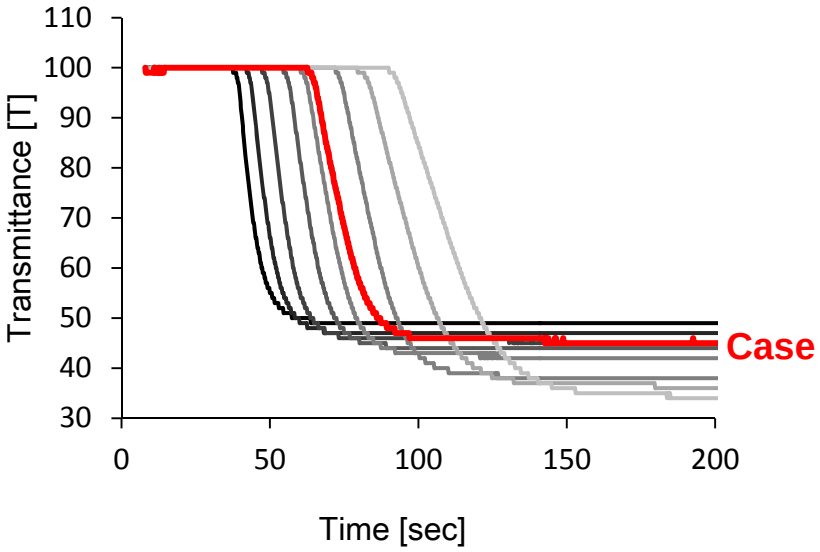
- FVIII:C 30 IU/dL (one-stage),
- FVIII:Ag 91.6 %
- VWF:Rcof 190 %, VWF:Ag 189.6 %

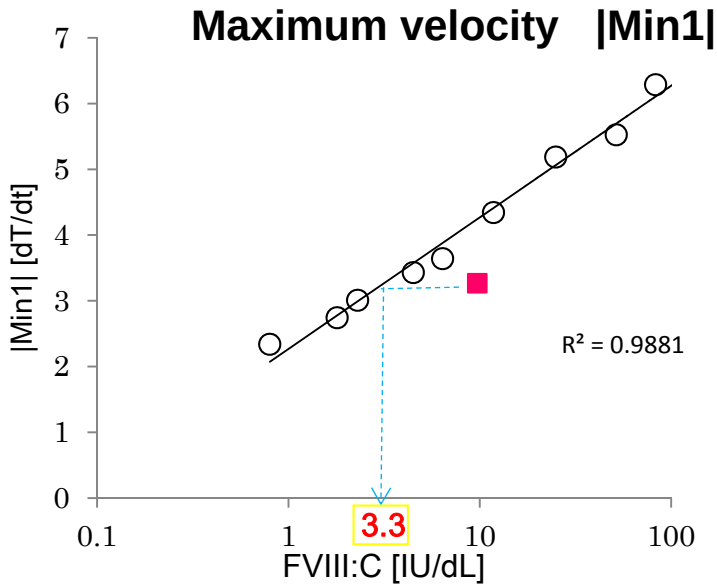
20-30 IU/dL should be enough for hemostasis.
Therefore, his FVIII level does not reflect clotting function.

→ Chromogenic assay 10.4 IU/dl.

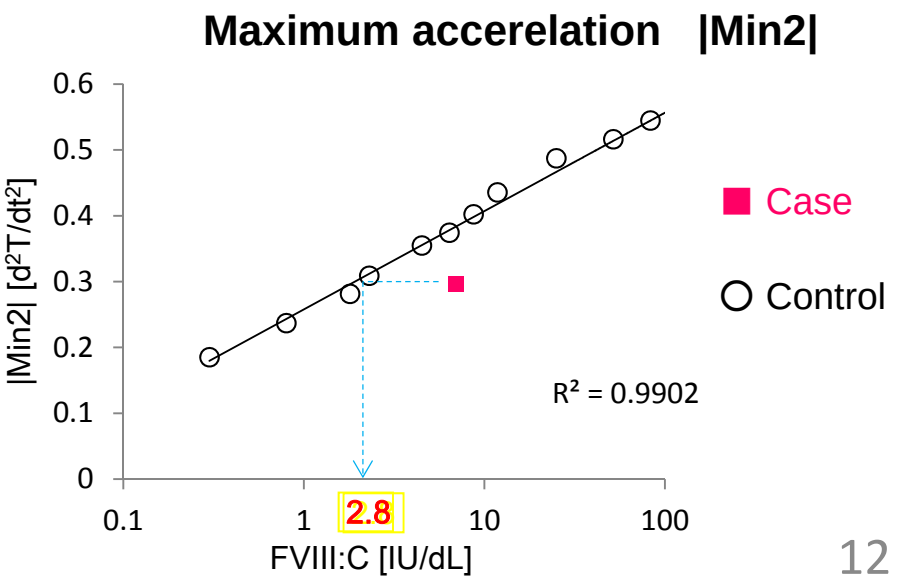
→ F8 gene analysis Thr 677 Ile (Exon 13)

FVIII:C measured by CWA parameters

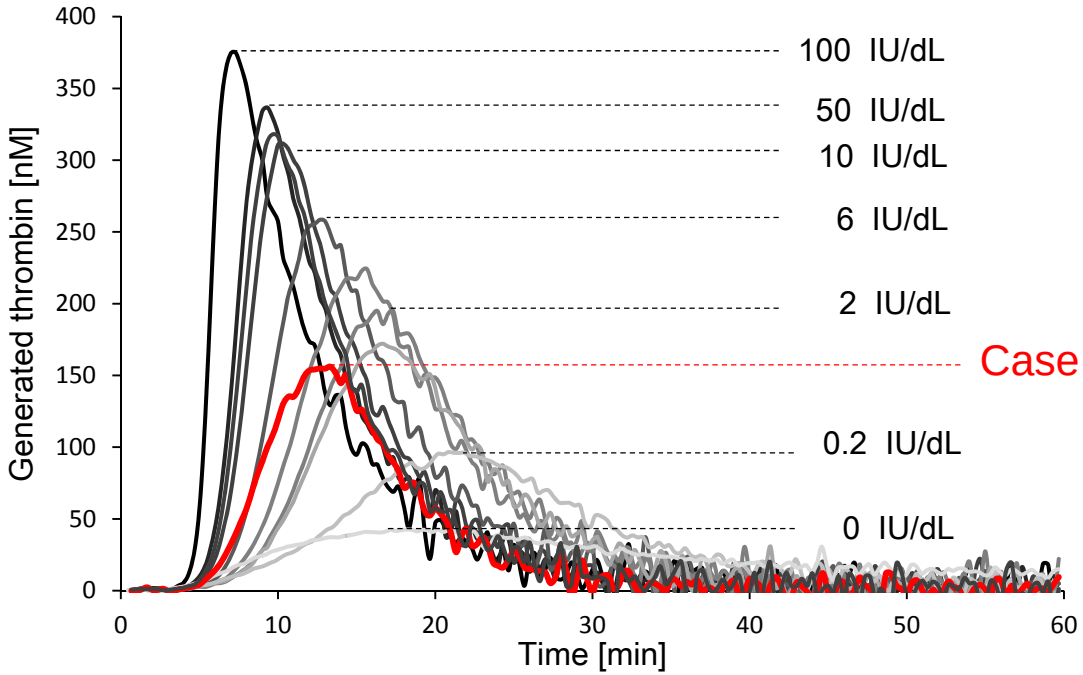




FVIII:C by |Min1|, |Min2| :FVIII:C ~3.0 IU/dL

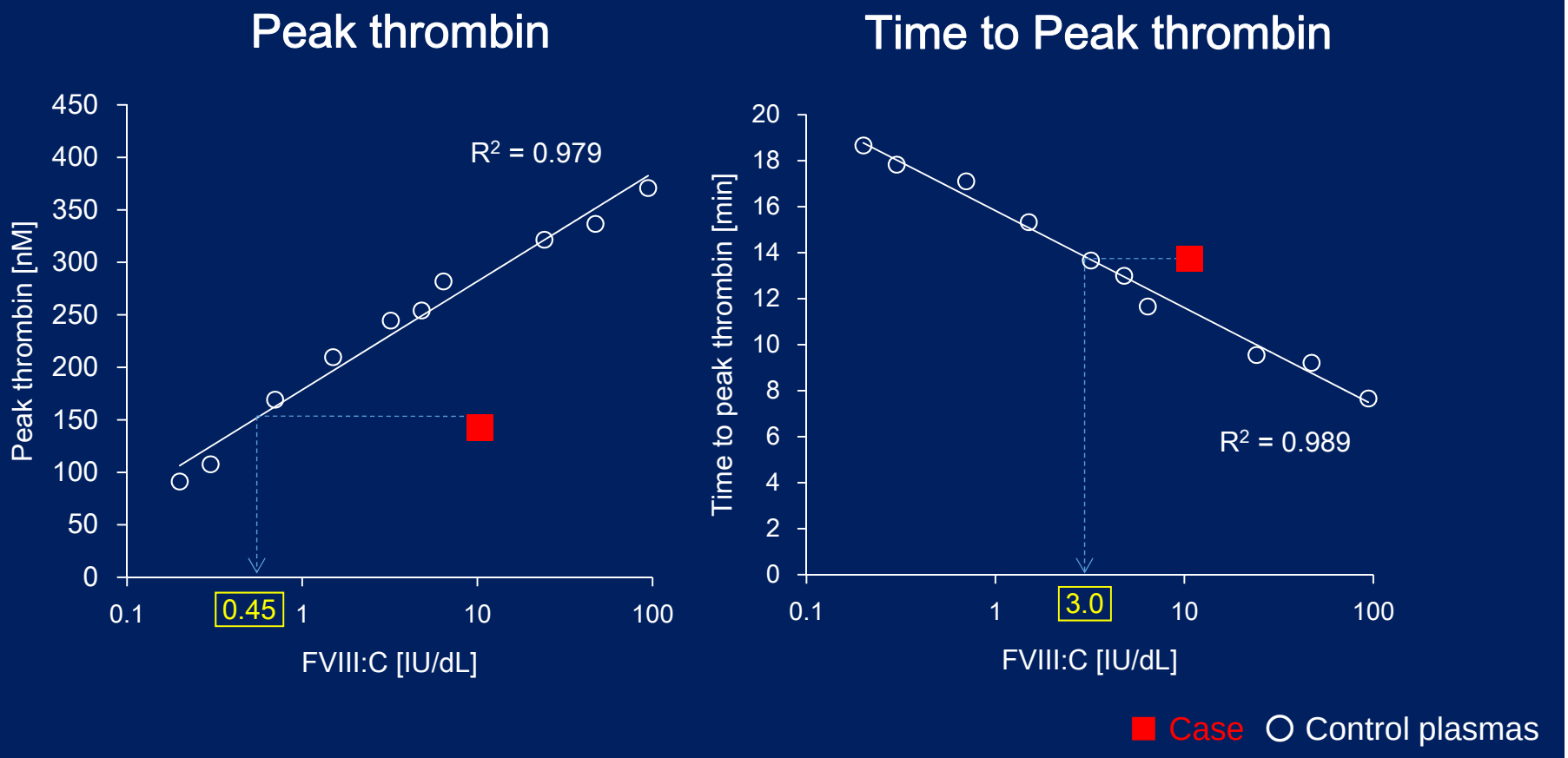


Thrombin generation test (TGT)

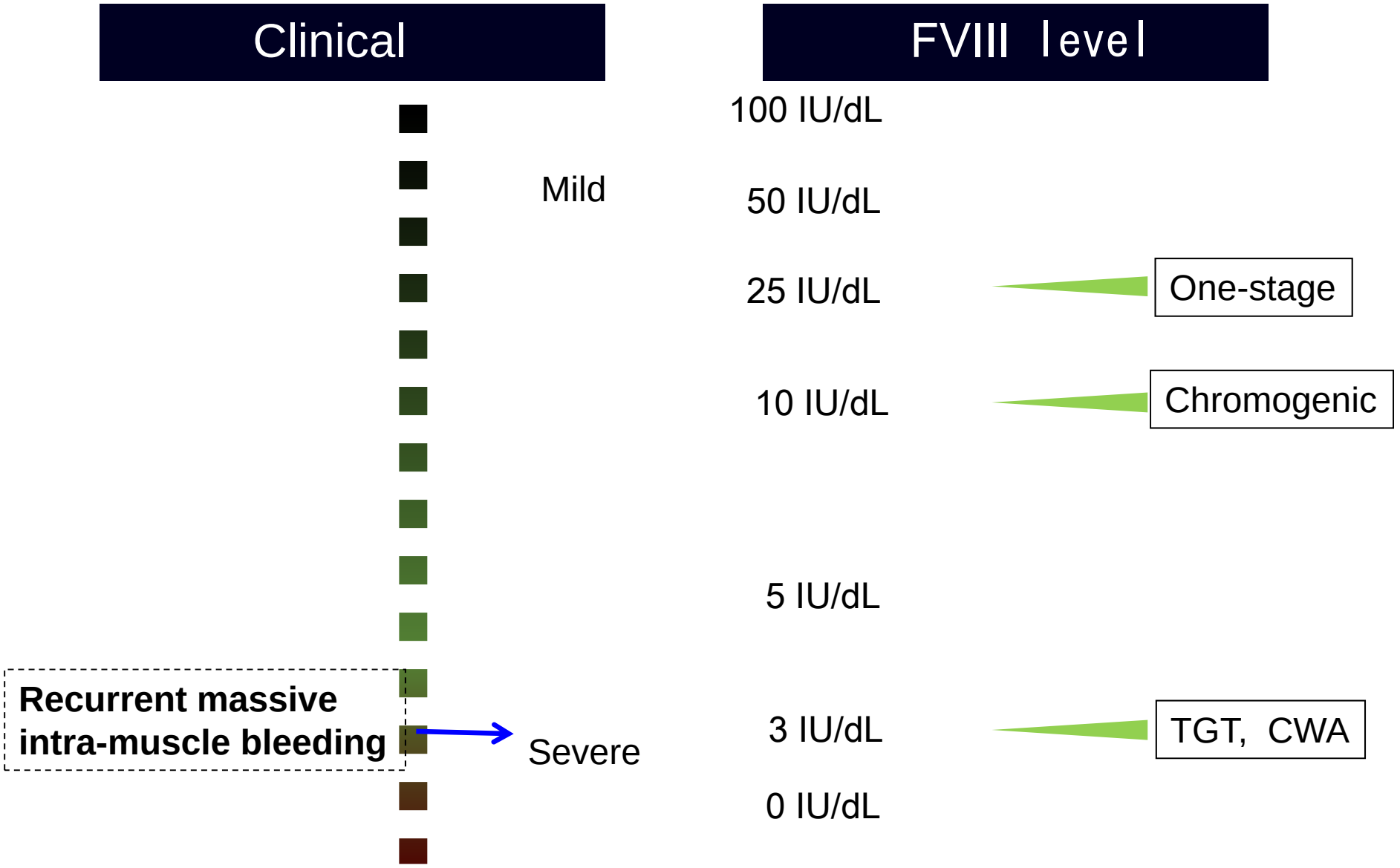


Dose-dependent thrombogram of FVIII deficient plasma with various level of FVIII

Measurement of FVIII:C by TGT parameters



FVIII:C by Peak thrombin: 0.45IU/dL
FVIII:C by Time to Peak thrombin: 3.0 IU/dL



Global test is useful in the assessment of clotting function of some hemophilia A patients whose factor level is not corresponding to the clinical severity.

Application of CWA to bypassing therapies

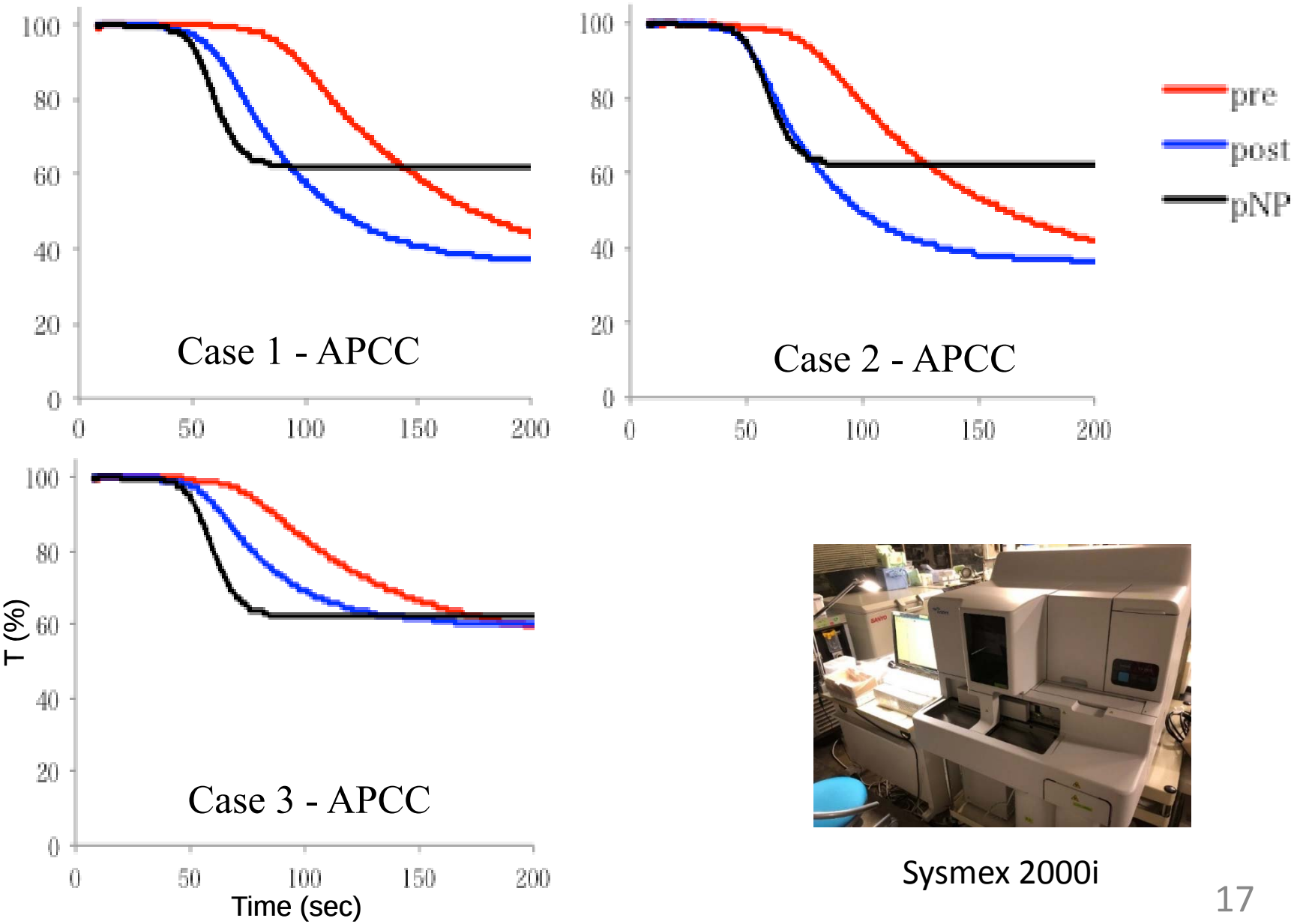
Bypassing therapies for hemophilia A with inhibitor

- APCC (Feiba)
- rFVIIa (Novoseven)
- pd FVIIa/FX (Biclot:only in Japan)

How to monitor?

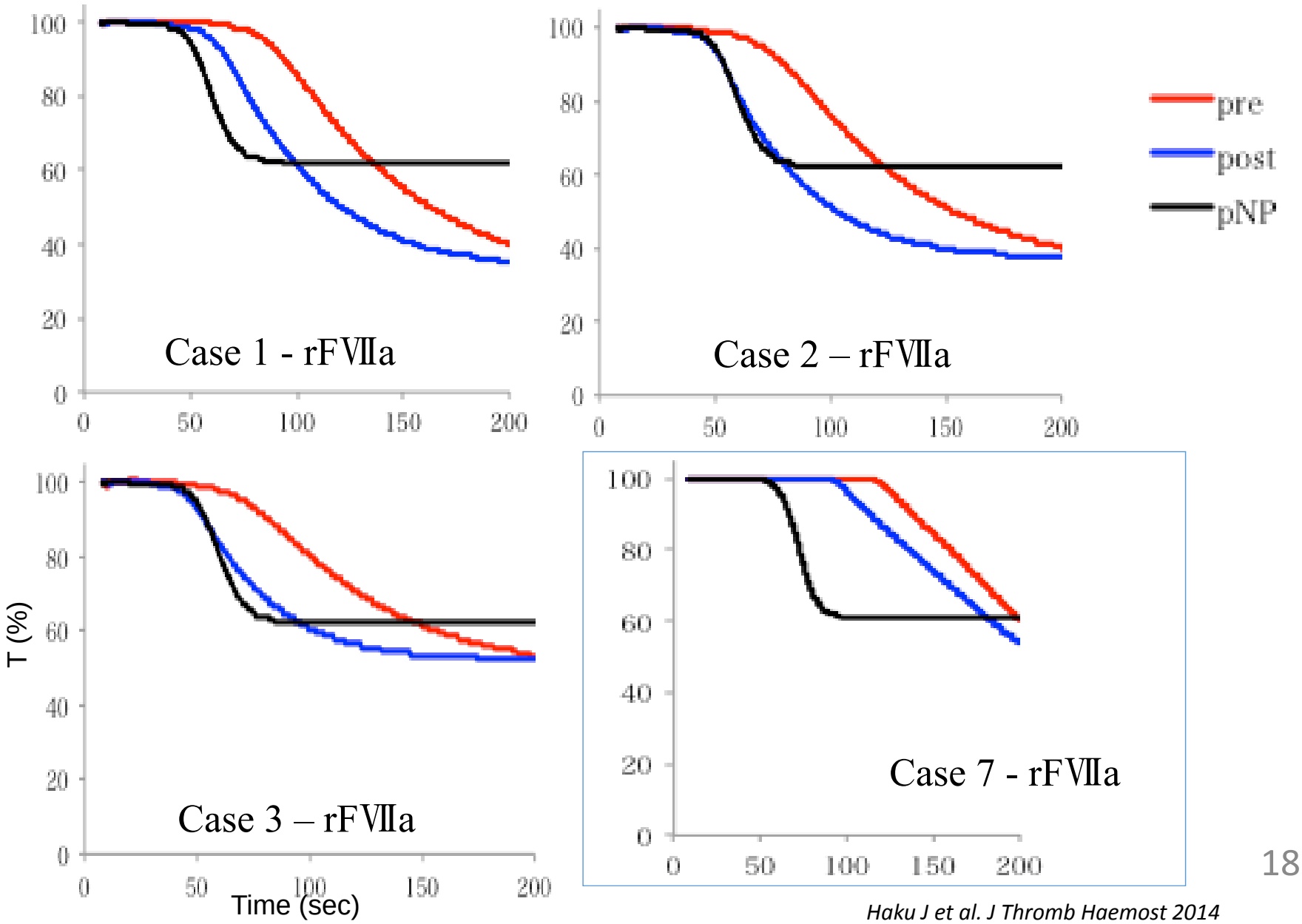
TEG/ROTEM
TGT
CWA

Waveform in HA patients with inhibitor receiving APCC as a bolus (in Elg-TF)



Sysmex 2000i

Waveform in HA patients with inhibitor receiving rFVIIa (in Elg-TF)

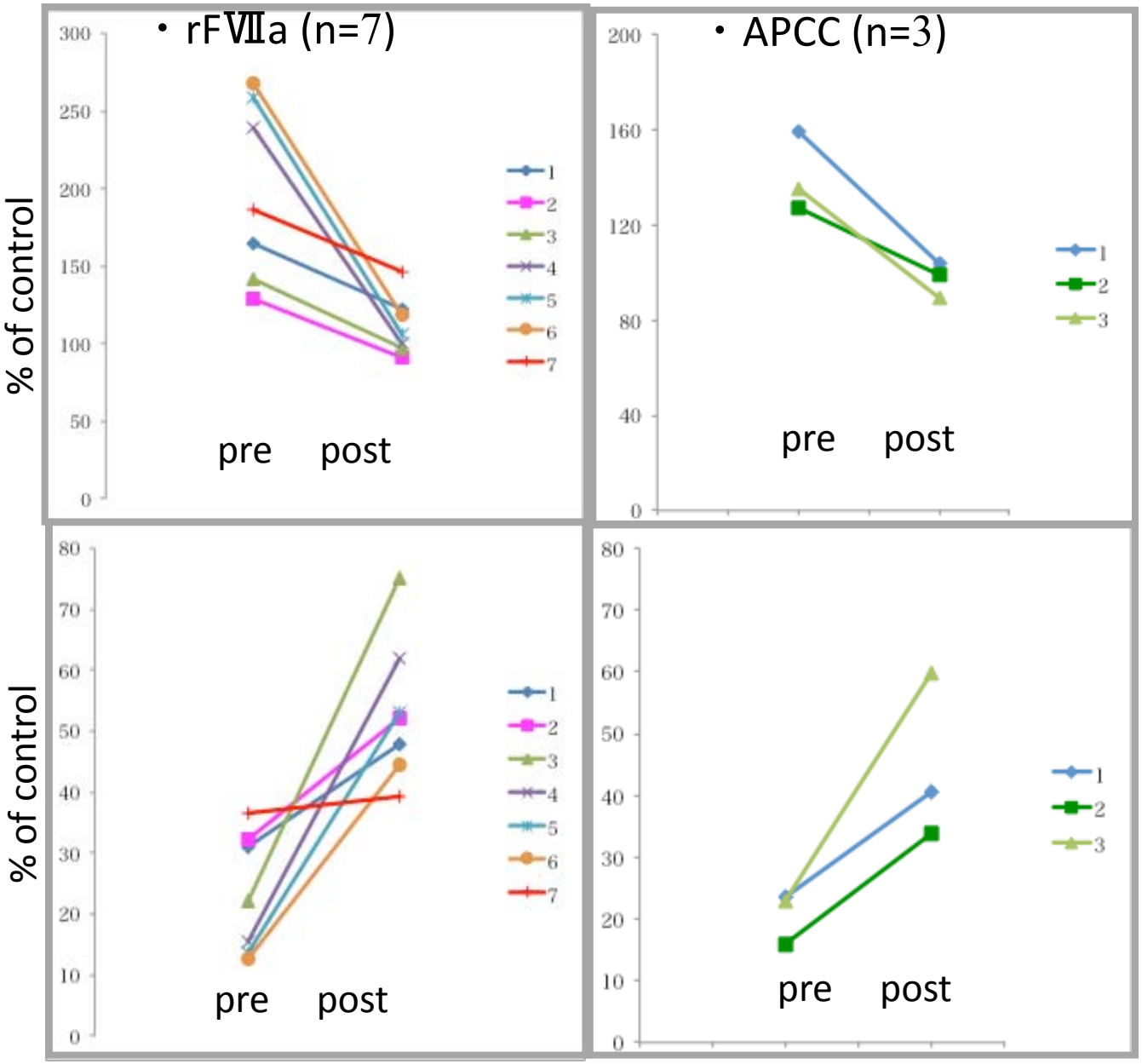


Haku J et al. J Thromb Haemost 2014

Parameters in HA patients with inhibitor receiving rFVIIa or APCC (in Elg-TF)

Clot time

Min2



Application of CWA to FVIIIa mimicking bispecific antibody

Unmet needs for current hemophilia A treatment

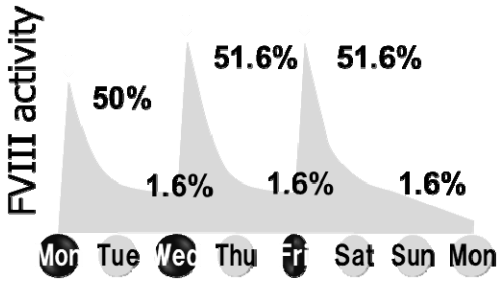
Gene therapy

Non-clotting factor

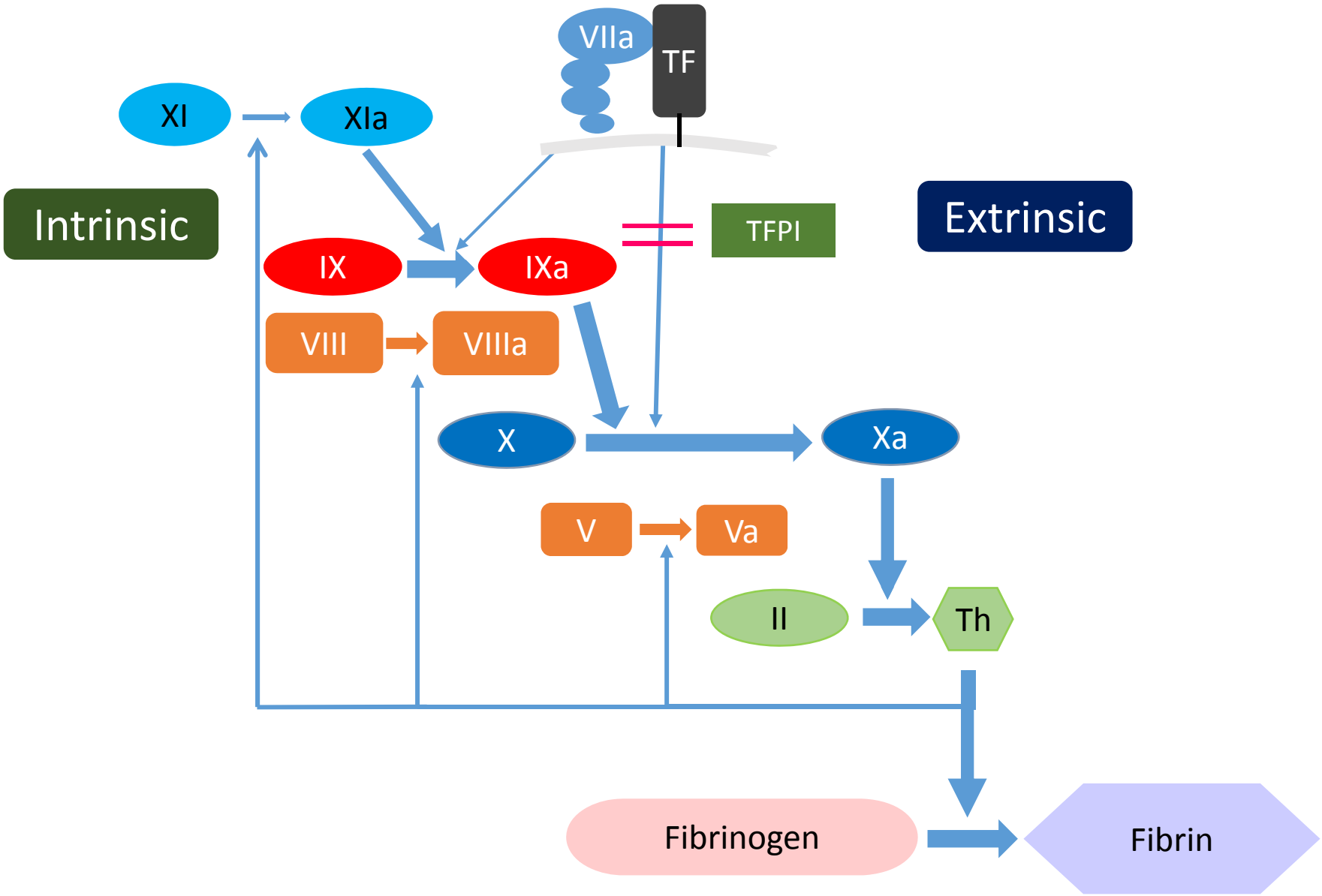
Multiple intravenous infusions
FVIII inhibitor
Low trough level
Progress in hemophilic arthropathy

Regular prophylaxis

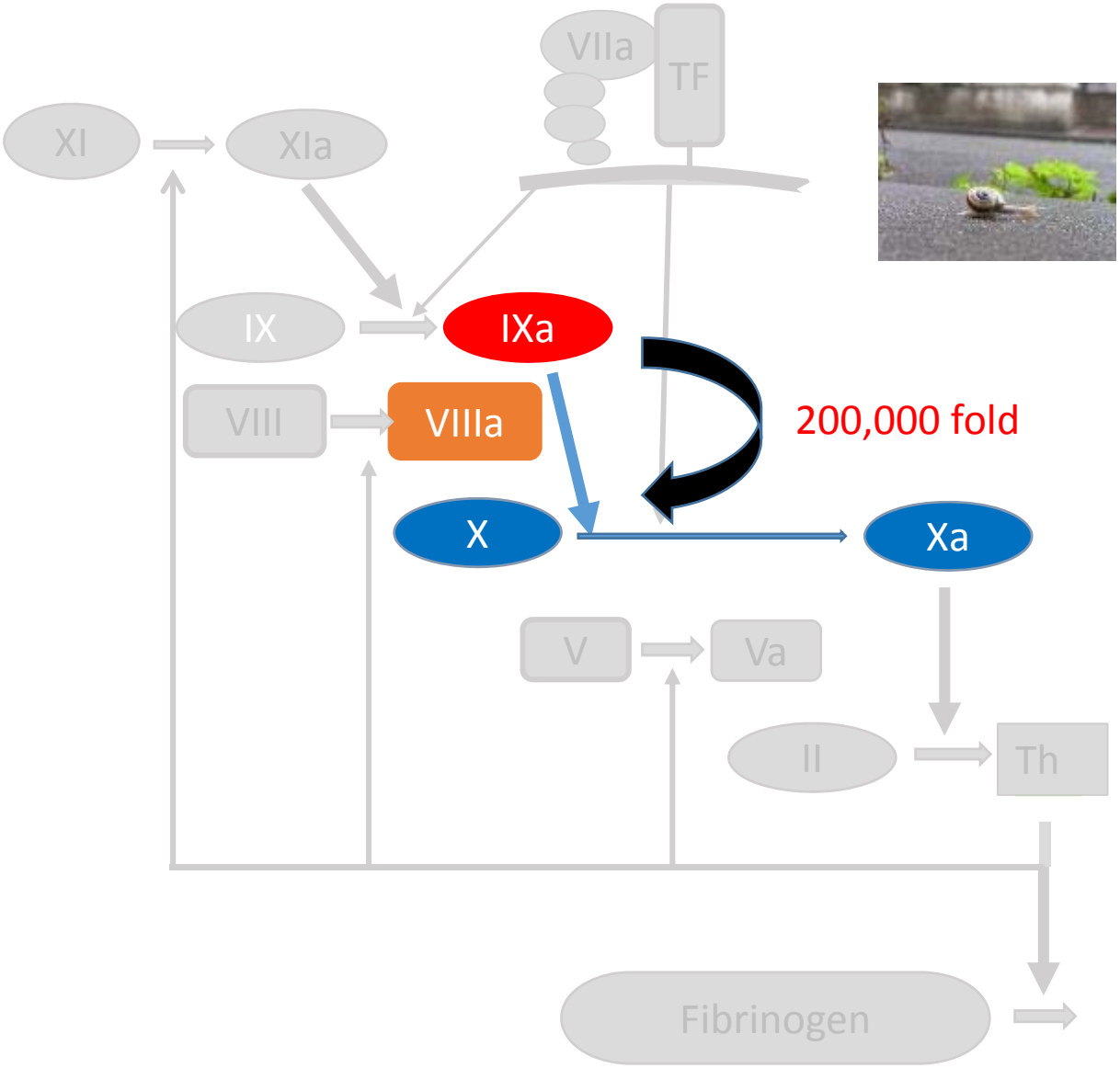
Episodic treatment



The role of FVIII

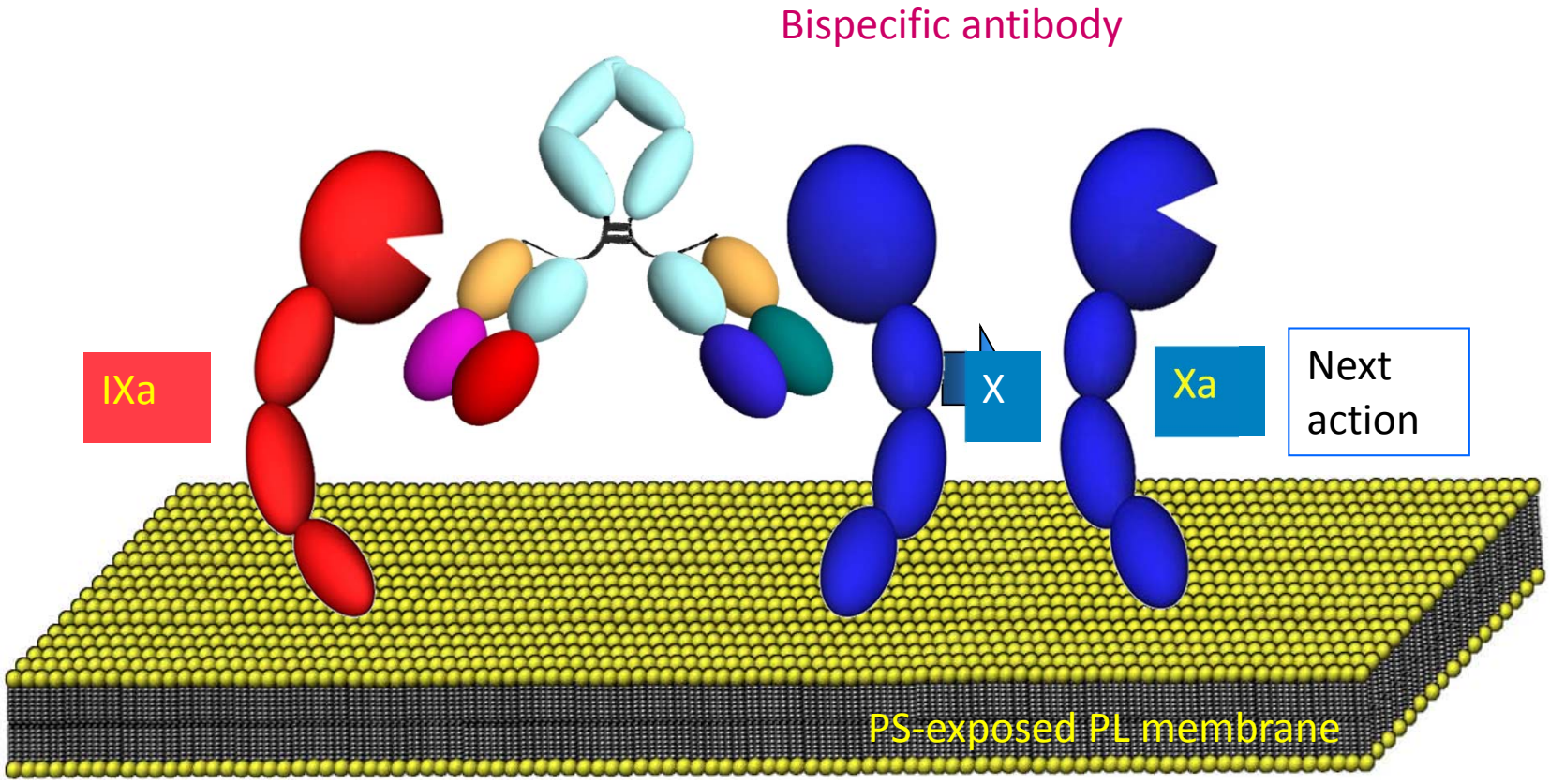


FVIIIa promotes FIXa catalyzed FXa generation



X-43

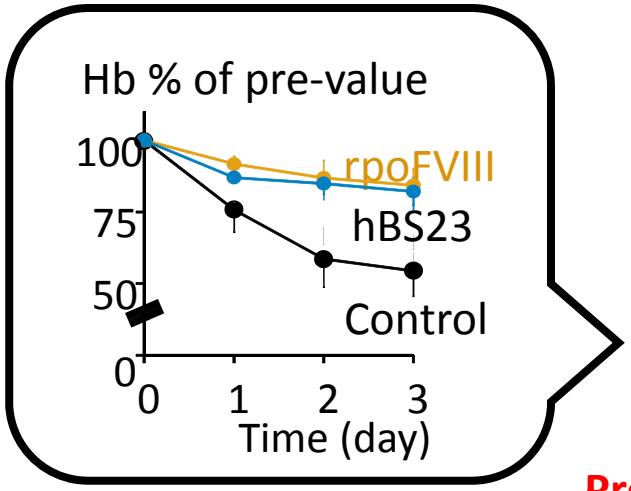
FVIIIa mimetic bispecific antibody



Kitazawa, Shima et al. Nature Med 2012

Anti-FIXa/FX bispecific antibody could exert FVIII cofactor function by promoting the interaction b/w FIXa and FX.

Pre-clinical development of emicizumab in Japan



In vivo effects on on-going bleeding & spontaneous bleeding by emicizumab (JTH&Blood)

In vitro, in vivo effect of hBS23 (Nature Med), nearly identical to emicizumab

2014

2013 Antibody engineering of emicizumab (Plos One)

2012 Multi-dimentional optimization by 2400 pattern

~2008 Lead antibody from 40,000 bispecific antibodies

Project re-started

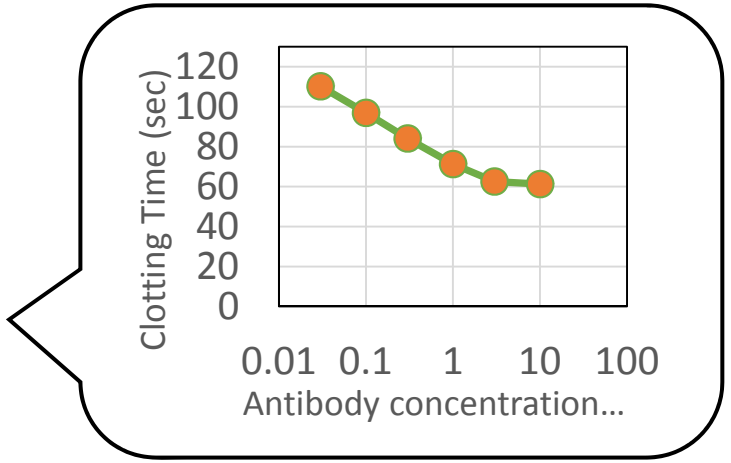
2006 WFH (Vancouver)

2005 ISTH (Sydney)

2003 Nara Medical University

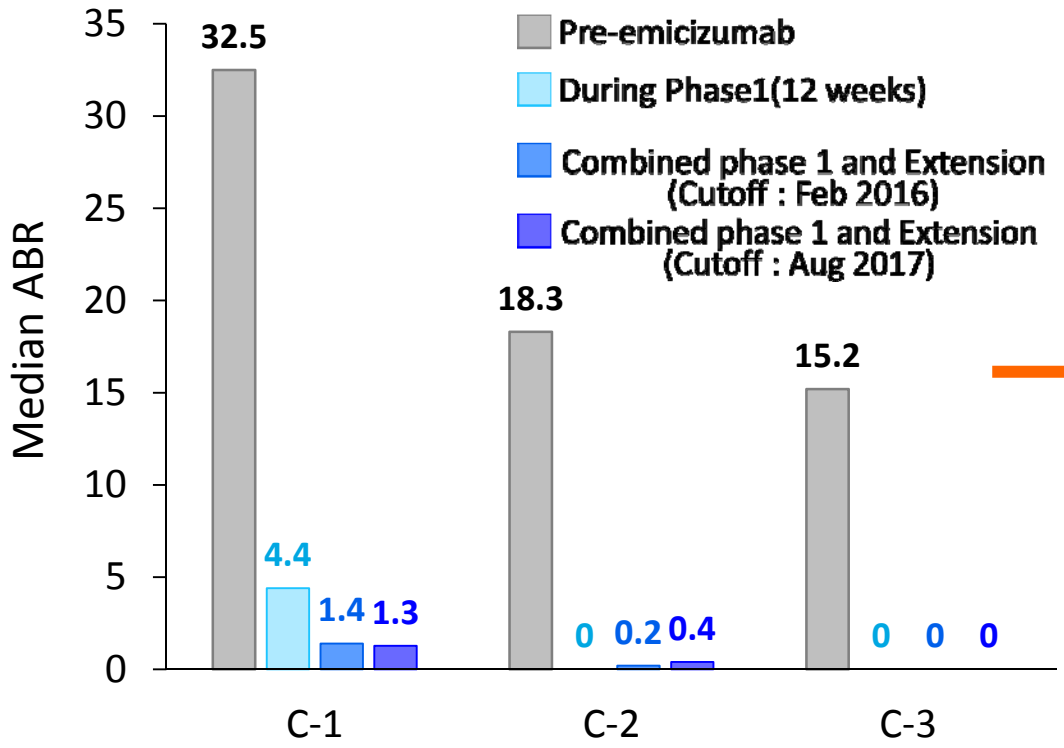
~2000 XB12/SB04 from 460 bispecific antibodies

Project started



Changes in ABR in the phase ½ studies for emicizumab prophylaxis (2013-).

Step	Emicizumab dosing group	
	Multiple SC	N
C-1	1.0→0.3** mg/kg	6
C-2	3.0→1.0** mg/kg	6
C-3	3.0 mg/kg	6



Phase1

12w

Extension

Median follow up period

C1: 2.7 years
C2: 2.3 years
C3: 1.8 years

(Clinical data cutoff : Feb 2016)

Median dosing duration

C1: 4.3 years
C2: 3.8 years
C3: 3.3 years

Up to 4.3 years

(Clinical data cutoff : Aug 2017)

Shima et al. N Engl J Med. 2016;374(21):2044.

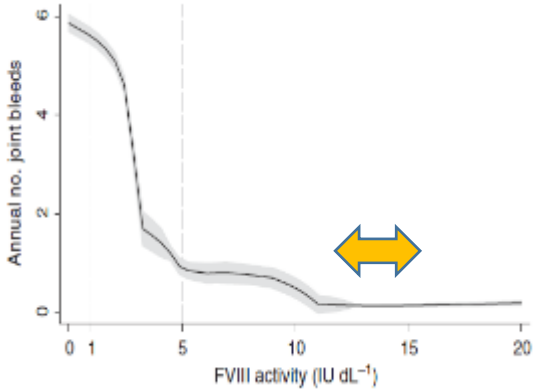
Shima et al. Blood Adv. 2017;1(22):1891

Shima et al. ISTH 2019

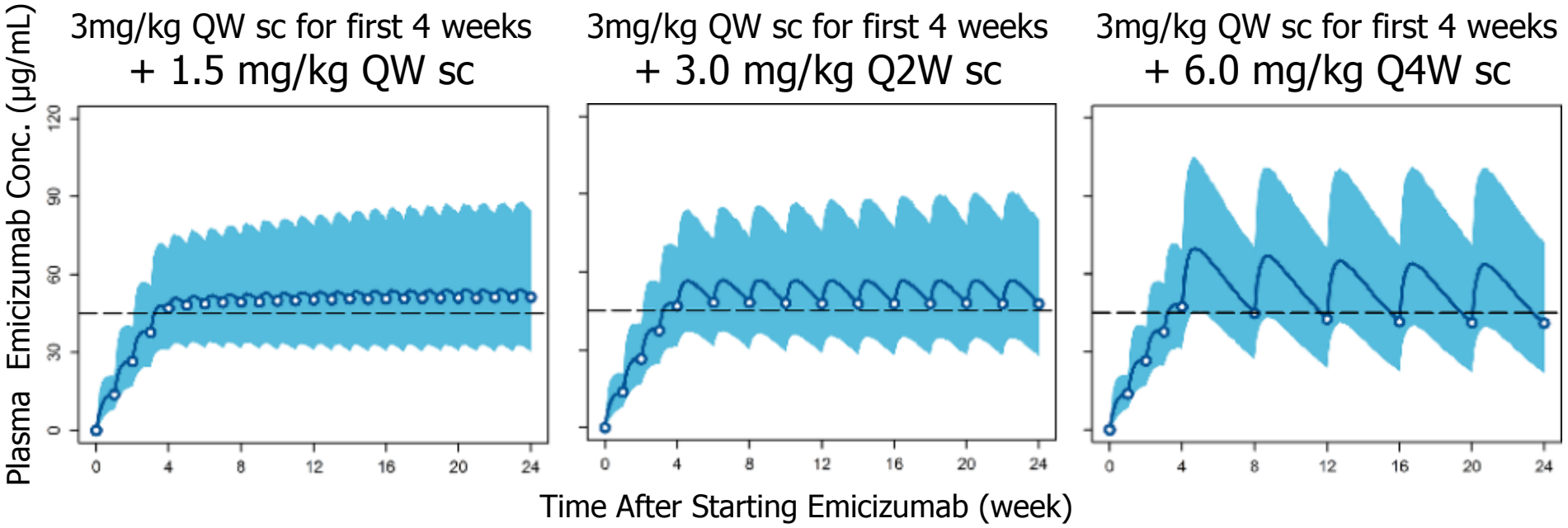
Goal of emicizumab and dose determination for phase 3 studies

FVIII>12-15%

45～50μg/mL



Uijl et al. Haemophilia 2011



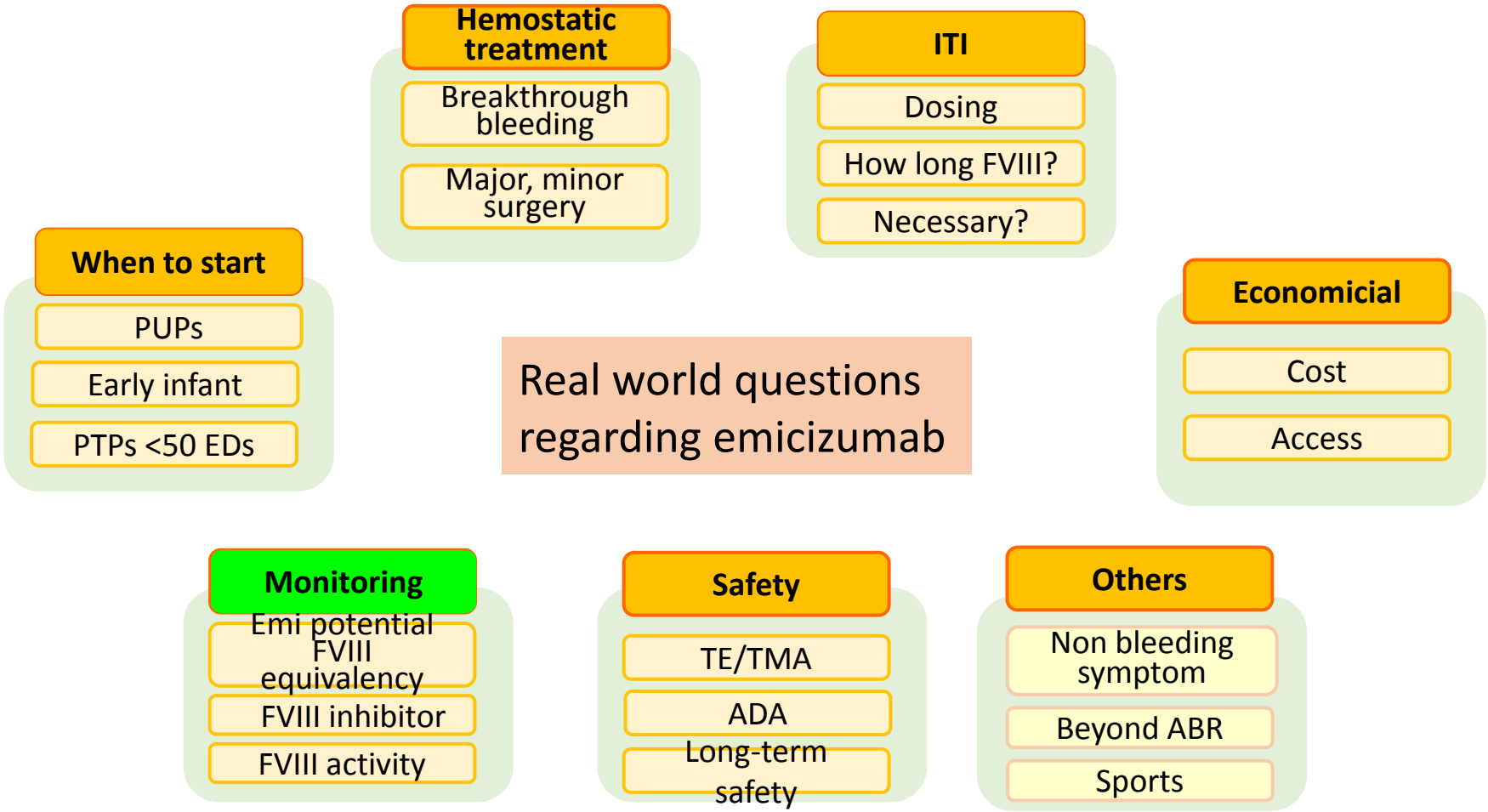
1. Yoneyama, Shima, et al. *Clin Pharmacokinet.* 2017. 2. Shima et al. *N Engl J Med.* 2016;374(21):2044.

Summary of the international phase 3 studies for Emicizumab

Emicizumab clinical trials	Eligible Patients	Number of patients	Dosing*	ABR (% reduction vs ref arm)	Patients with 0 treated bleeds (%)	safety
HAVEN 1 (NCT02622321)	Inhibitor patients (age ≥12)	113	QW	2.9 (87% reduction)	62.9	2 TEs/3 TMAs 1ADA
HAVEN 2 (NCT02795767)	Paediatric inhibitor patients (age 0–11)	88	QW Q2W Q4W	0.3 0.2 2.2	76.9 90.0 60.0	No TE/TMA 2ADA
HAVEN 3 (NCT02847637)	Non-inhibitor patients (age ≥12)	152	QW Q2W	1.5 (96% reduction) 1.3 (97% reduction)	55.6 60.0	No TE/TMA
HAVEN 4 (NCT03020160)	Inhibitor and Non-inhibitor patients (age ≥12)	48	Q4W	2.4 (expansion cohort)	56.1	No TE/TMA

*: QW: once-weekly(1.5mg/kg/week)
Q2W: every 2 weeks(3.0mg/kg/2weeks)
Q4W: every 4 weeks(6.0mg/kg/4weeks)

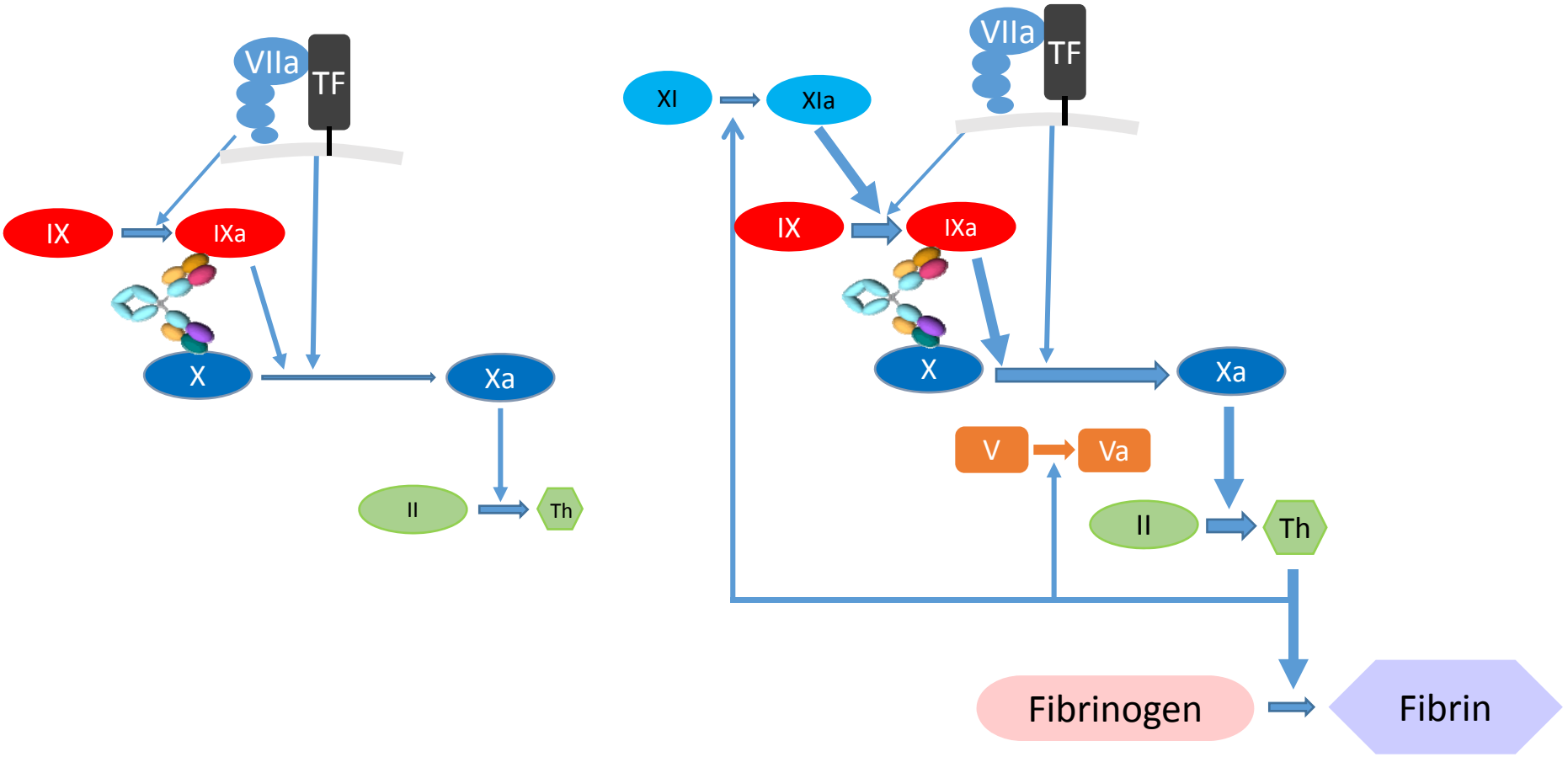
Oldenburg J, et al. N Engl J Med 2017;377:809–18; Young G, et al. ASH 2018; Mahlangu J, et al. NEJM 2018;379:811–22; Pipe S, et al. Lancet Haem 2019; e295-e305. Shima M, et al. ASH 2018 ; Shima M, et al. JSTH 2019


Hemostatic treatment
Breakthrough bleeding
Major, minor surgery
ITI
Dosing
How long FVIII?
Necessary?
When to start
PUPs
Early infant
PTPs <50 EDs
Real world questions regarding emicizumab
Economicial
Cost
Access
Monitoring
Emi potential
FVIII equivalency
FVIII inhibitor
FVIII activity
Safety
TE/TMA
ADA
Long-term safety
Others
Non bleeding symptom
Beyond ABR
Sports

Mode of action of emicizumab

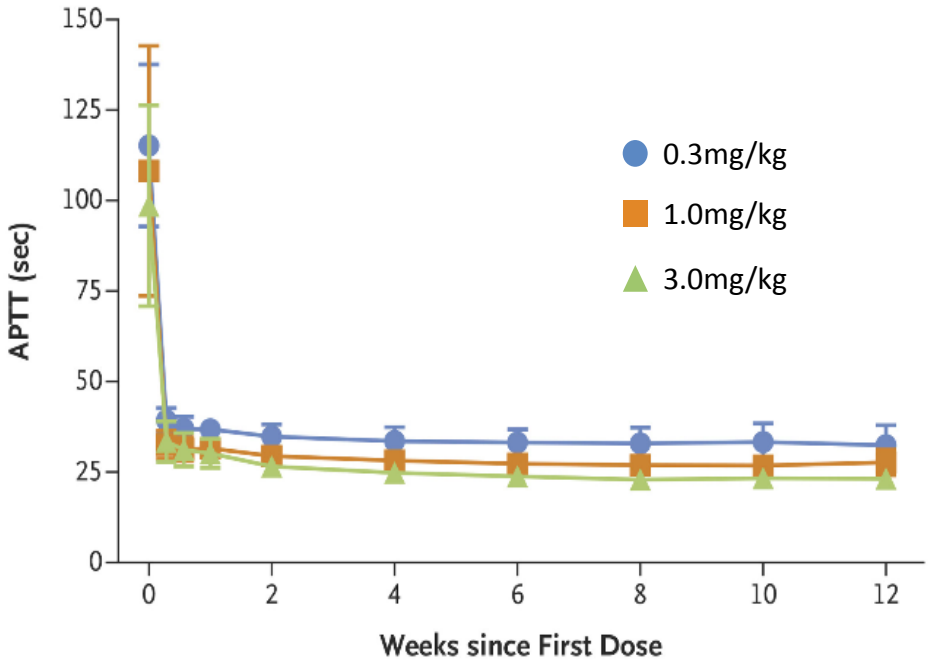
A Emicizumab-driven initial reaction

B Emicizumab-driven propagation reaction



Eicizumab can react from beginning FVIIIa reaction continues

How to monitor the potential of emicizumab?



Shima et al. NEJM 2016

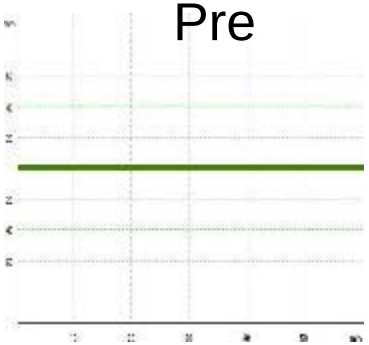
- Chromogenic assay with human components
- ROTEM/TEG: *Yada et al. Int J Hematol. 2019*
- Clot waveform analysis: *Nogami et al. JTH 2018*

Monitoring of emicizumab by ROTEM（NATEM)

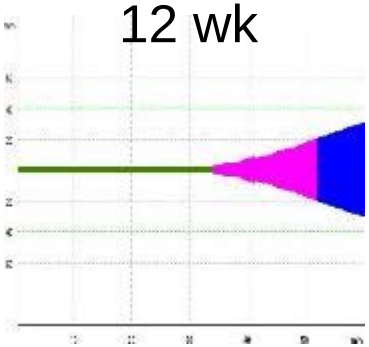
(Phase 1/2 study)

Patient 2 : Inhibitor, in C1 arm (1 mg/kg loading followed by weekly SC 0.3 mg/kg)

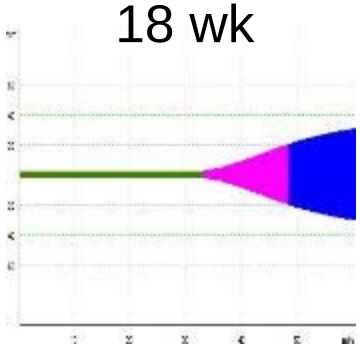
Pre



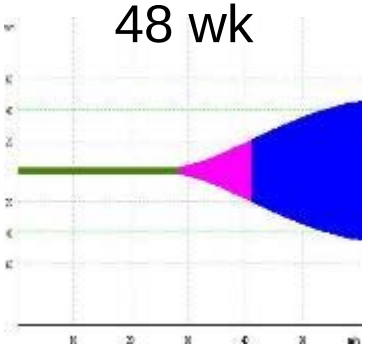
12 wk



18 wk

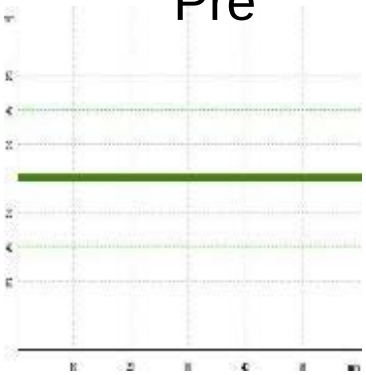


48 wk

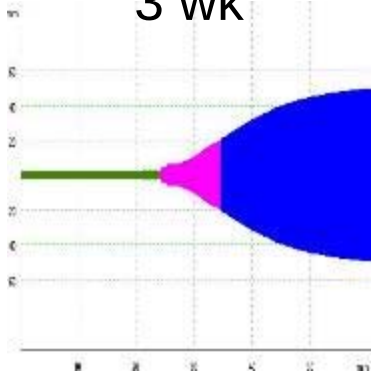


Patient 4: Non-inhibitor, in C3 arm (3 mg/kg weekly SC)

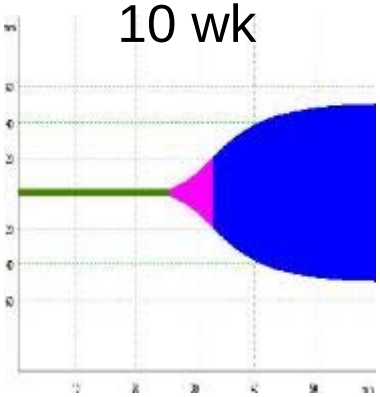
Pre



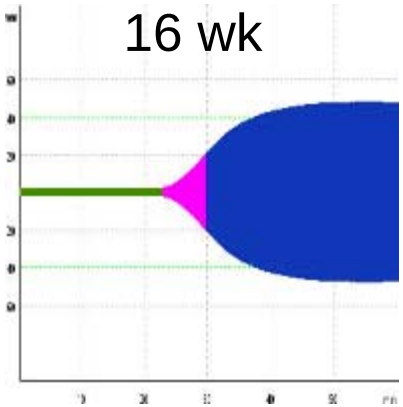
3 wk



10 wk



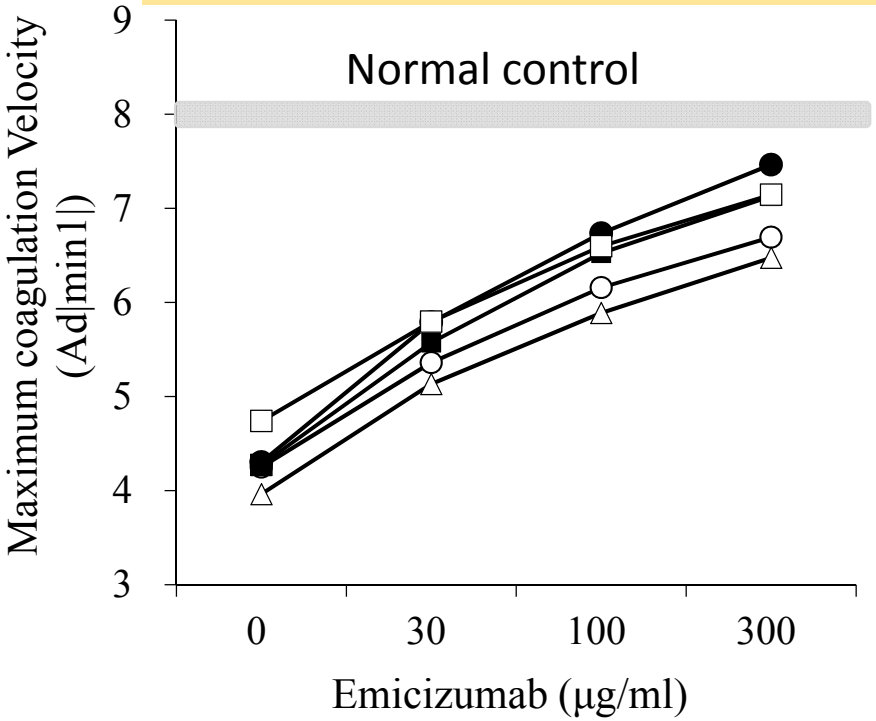
16 wk



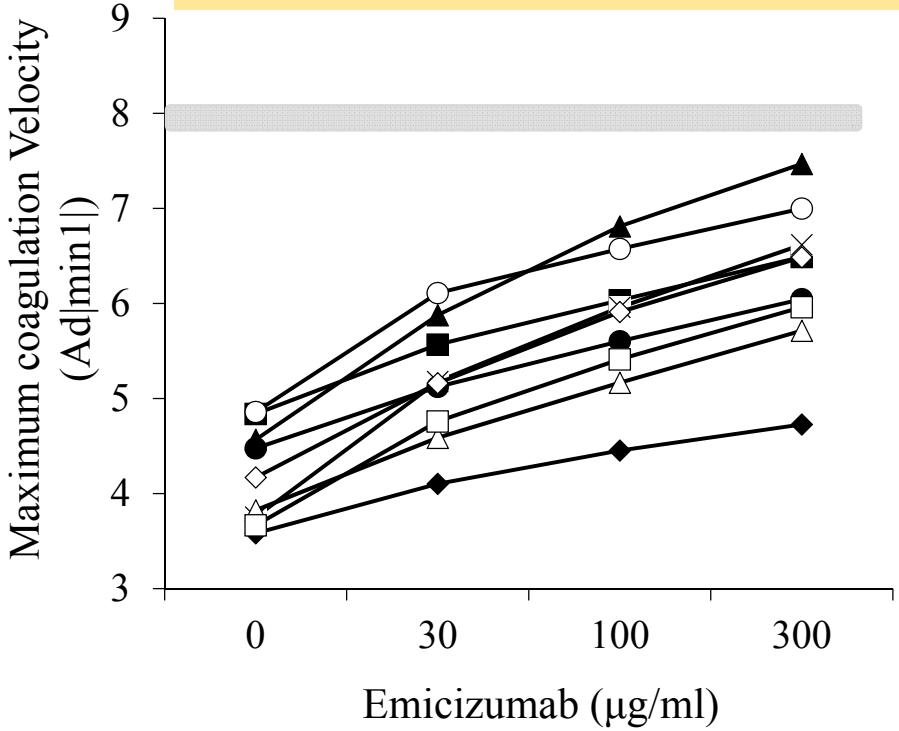
Yada K et al. Int J Hematol 2019

Mdified CWA in HA patients' plasmas without or with inhibitor after addition of emicizumab

Plasma samples from severe HA patients without inhibitors(n=5)



Plasma samples from severe HA patients with inhibitors(n=9)



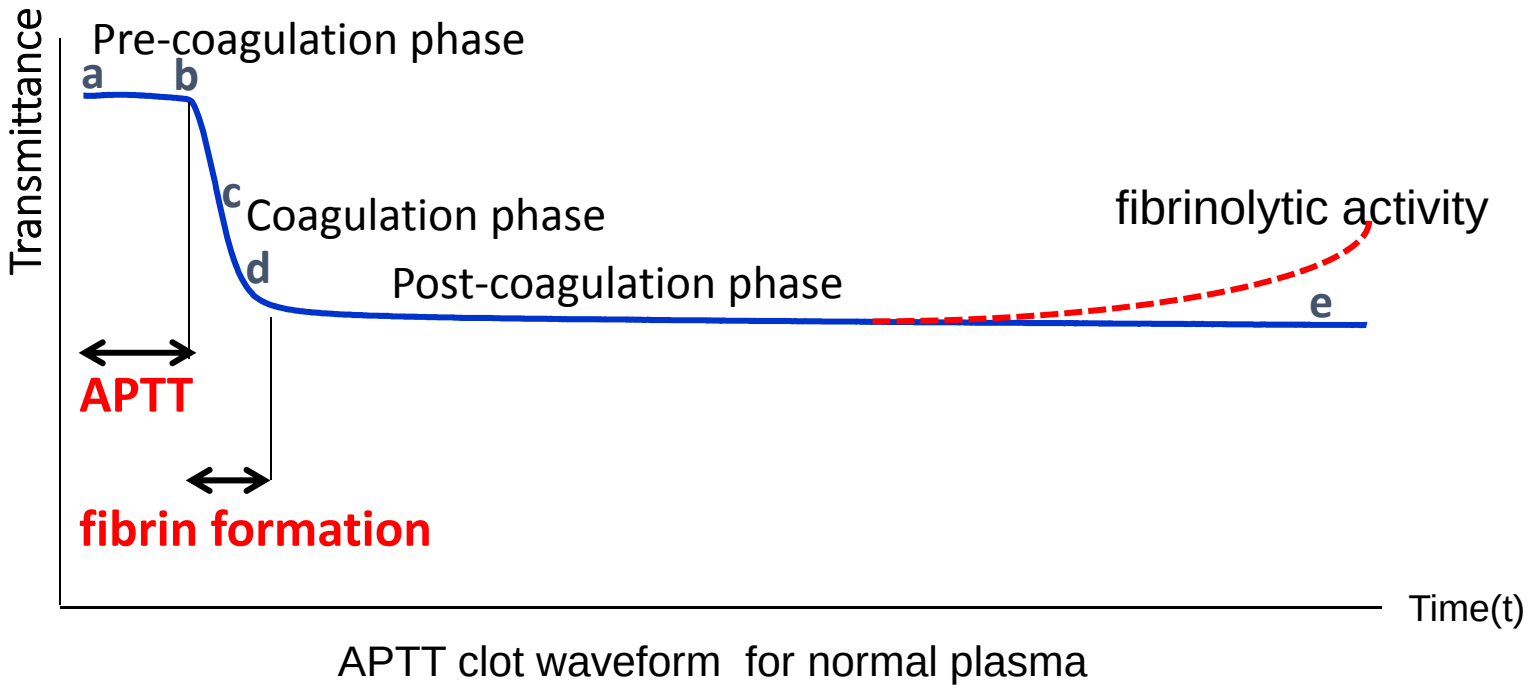
Mixed reagents :PT/APTT/buffer, 1 : 15 : 135

- The PT/APTT reagent-triggered adjusted CWA could provide a useful means of assessing global coagulation potential in emicizumab-treated HA patients

Nogami et al. J Thromb Haemost. 2018 Apr 12 [Epub ahead of print]

New application of APTT CWA for fibrinolytic activity

A global assay for assessing both coagulation fibrinolysis would be useful.

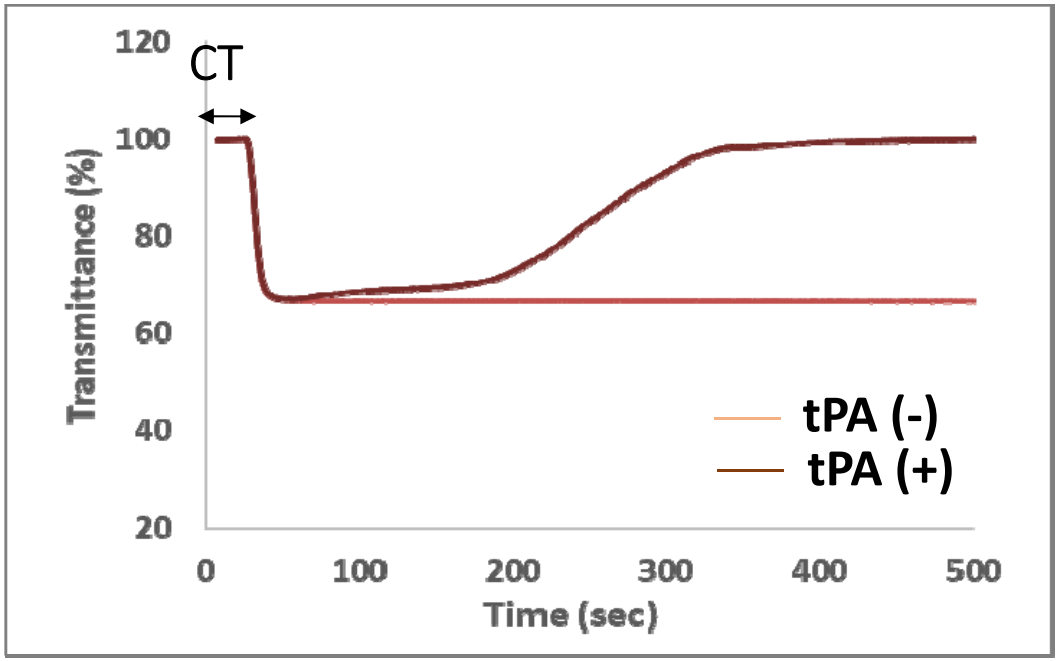


APTT clot waveform for normal plasma

It takes very long time for assessment of fibrinolysis

To assess fibrinolysis more rapidly, we modified APTT CWA by addition of tPA.

APTT-based clot-fibrinolysis waveform analysis (CFWA)



Normal plasma+ APTT reagents

$CaCl_2$

r-tPA
(0.63μg/ml)

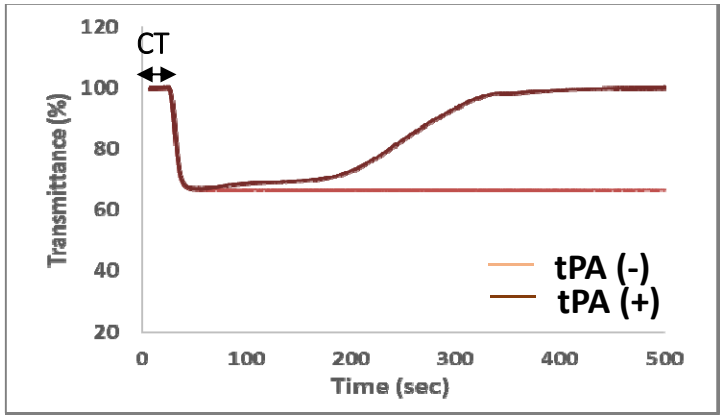
CWA

Sysmex CS 2000i

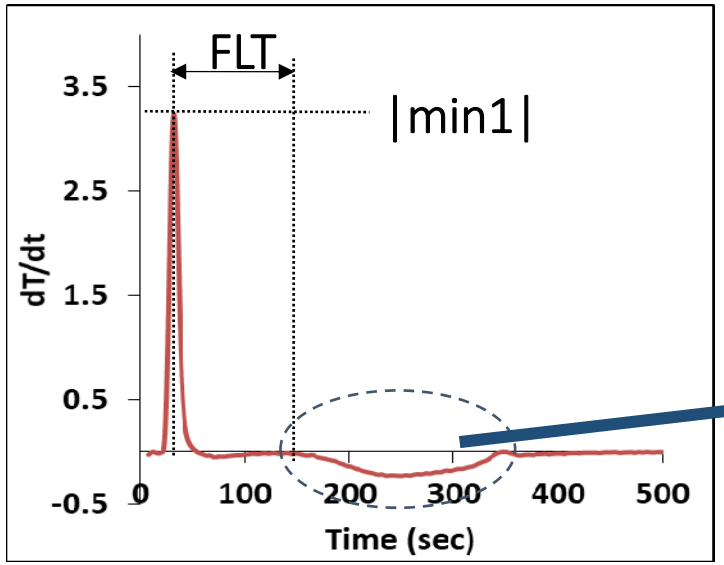
The series of clot-fibrinolysis measurements can be performed within 500 seconds at this concentration of r-tPA.

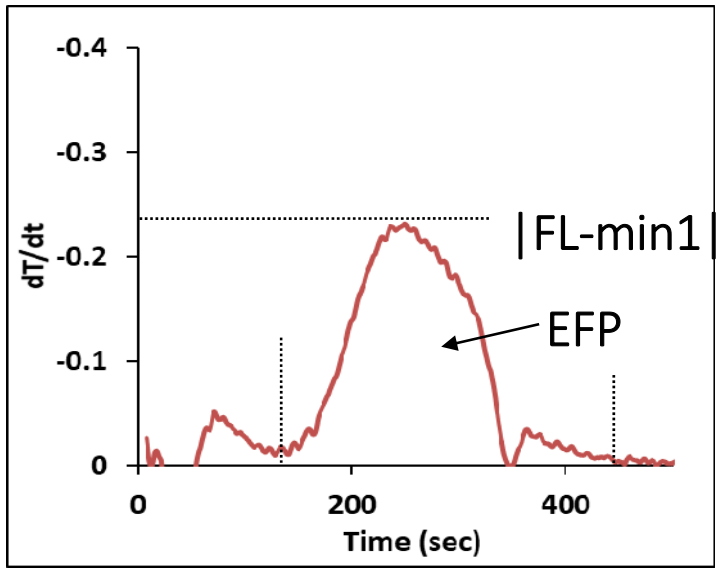
This CFWA could be useful for clinical setting.

Clot fibrinolysis waveform assay (CFWA)



CT: Clot time,
|min1|: Maximum coagulation velocity
FLT :Fibrinolysis time;
|FL-min1|: Maximal fibrinolysis velocity,
EFP :Endogenous fibrinolysis of potentials.



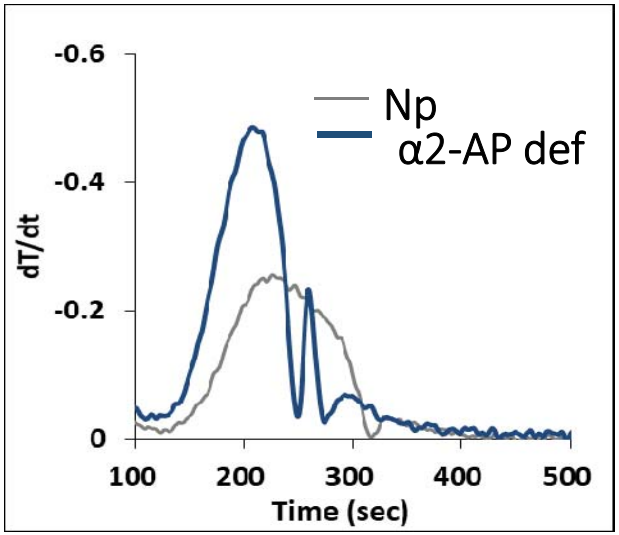


Nogami et l . Br J Haemotl 2019

Sysmex CS 2000i

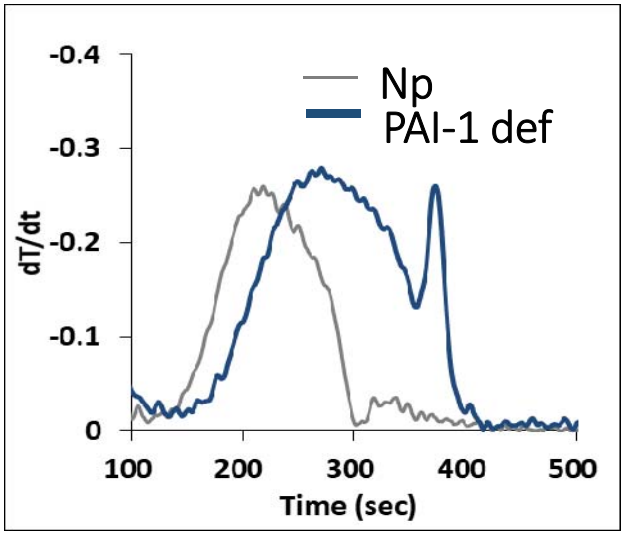
Specificity on the fibrinolysis-related factor-def. plasmas by CFWA

α2-AP def. plasma



■Higher and sharper fibrinolysis velocity peak (FL-min1)
■Higher endogenous fibrinolysis reaction

PAI-I def. plasma



Higher endogenous fibrinolysis reaction (EFP)

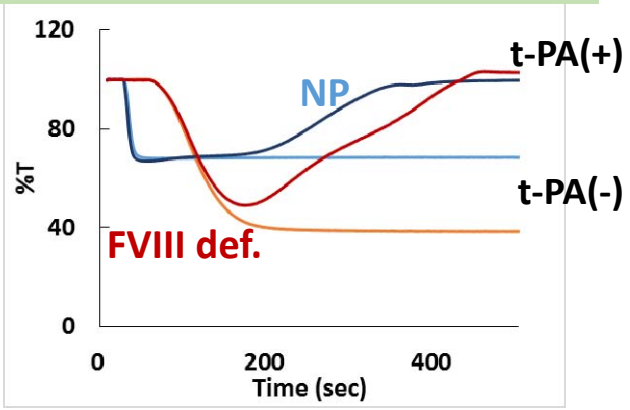
Hyper-fibrinolytic plasmas showed different fibrinolysis patterns.

Nogami et l. Br J Haemotl 2019

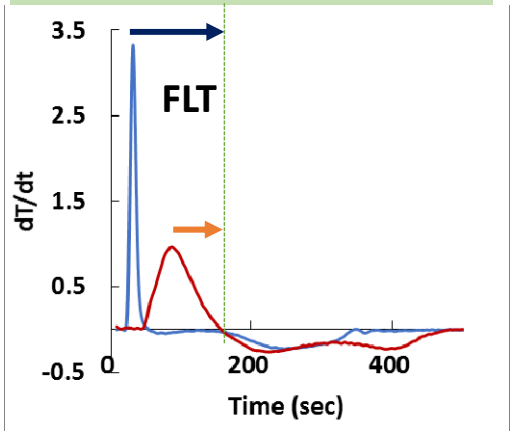
Sysmex CS 2000i

Hemophilia A plasmas on CFWA

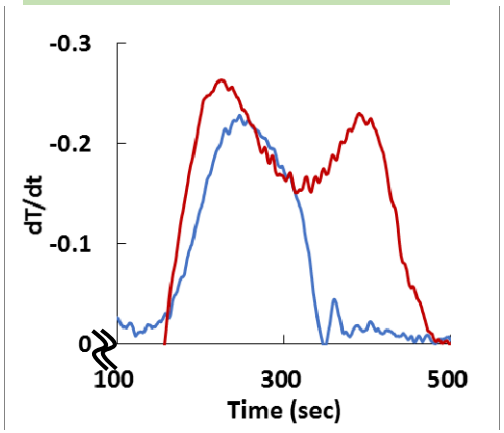
Changes in light transmittance



Coagulation phase



Fibrinolysis phase



Sysmex CS 2000i

Prolonged pre-coagulation phase
Gradually declined coagulation phase
Fibrinolysis initiated prior to the completion of fibrin formation

Low coagulation velocity peak.

Fibrinolytic reaction was bi-phasic and marked increase of EFP

Fragile formation of fibrin clots in FVIII deficient plasma

Nogami et l . Br J Haemotl 2019

New applications of APTT CWA

Measurement of FVIII by template matching method using weighted center on APTT clot waveform.

Facial recognition

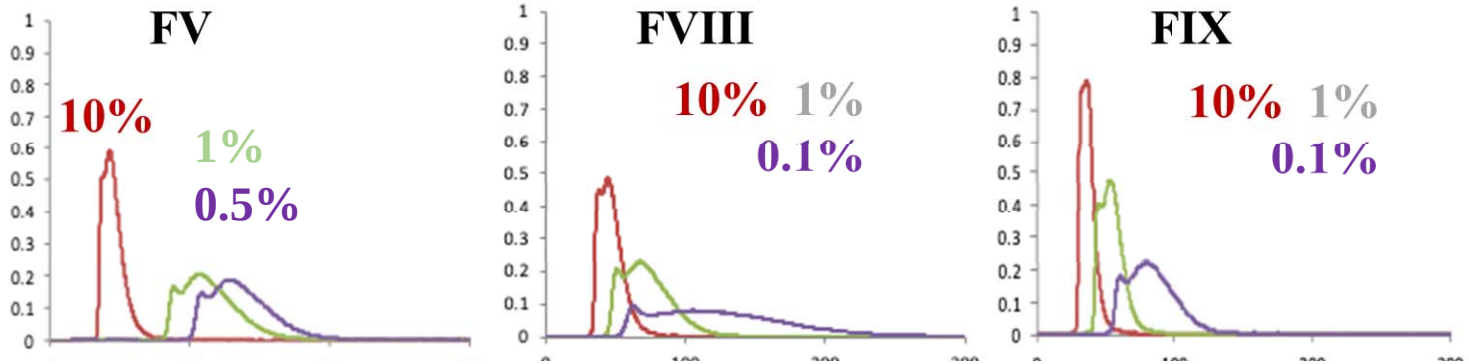
Parameters of characteristic of each person

- Longitudinal and vertical ratio
- Distance between eyes
- Position of mouth
- Position of eyes

Application of APTT CWA to diagnosis of hemophilia by template matching ?

APTT Clot waveform (Coagulation velocity) before and after smoothing

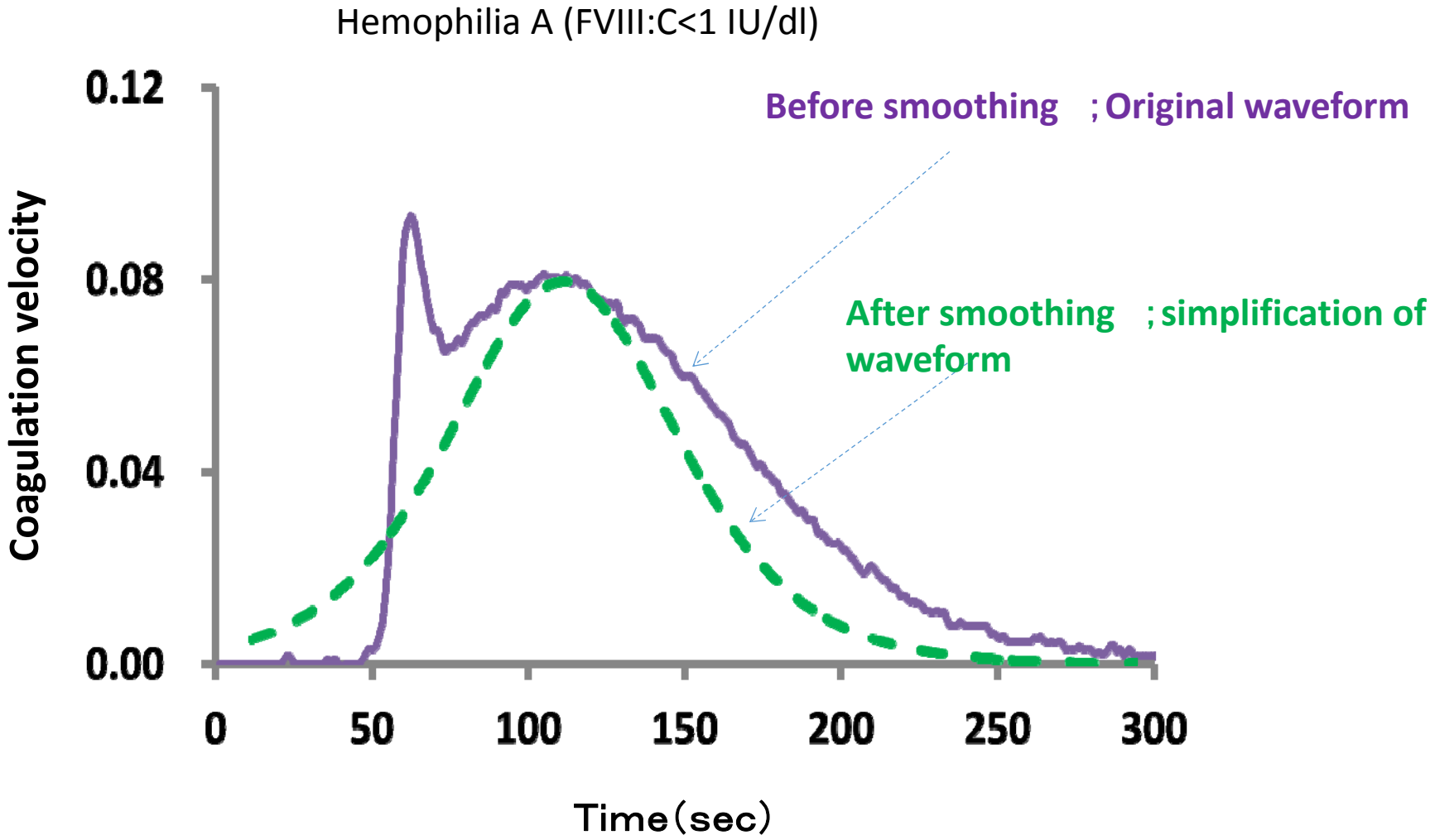
Before smoothing



CP3000™

Non-smoothed waveforms may include more important information of clotting function

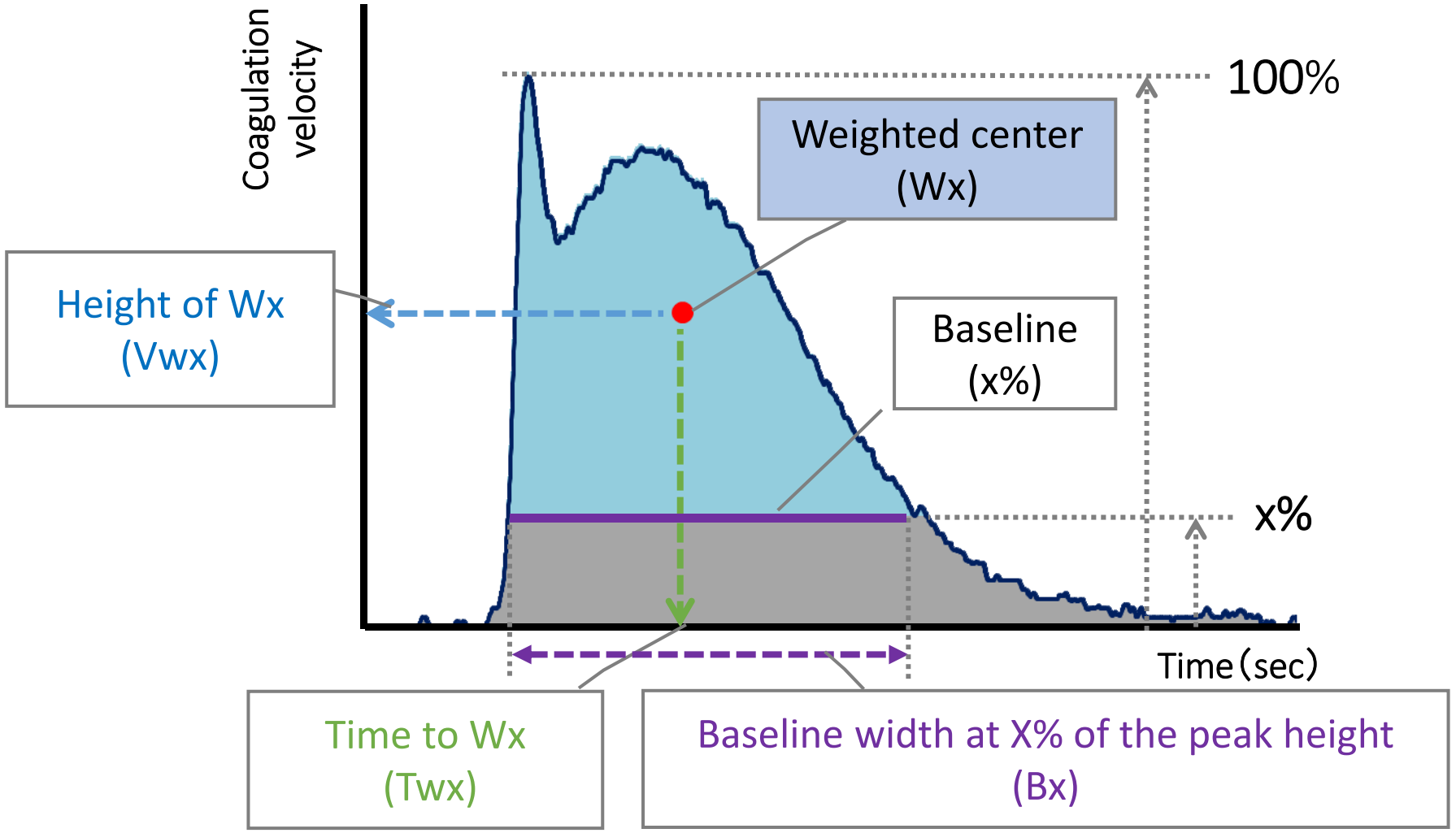
Comparison of clot waveform before and after smoothing



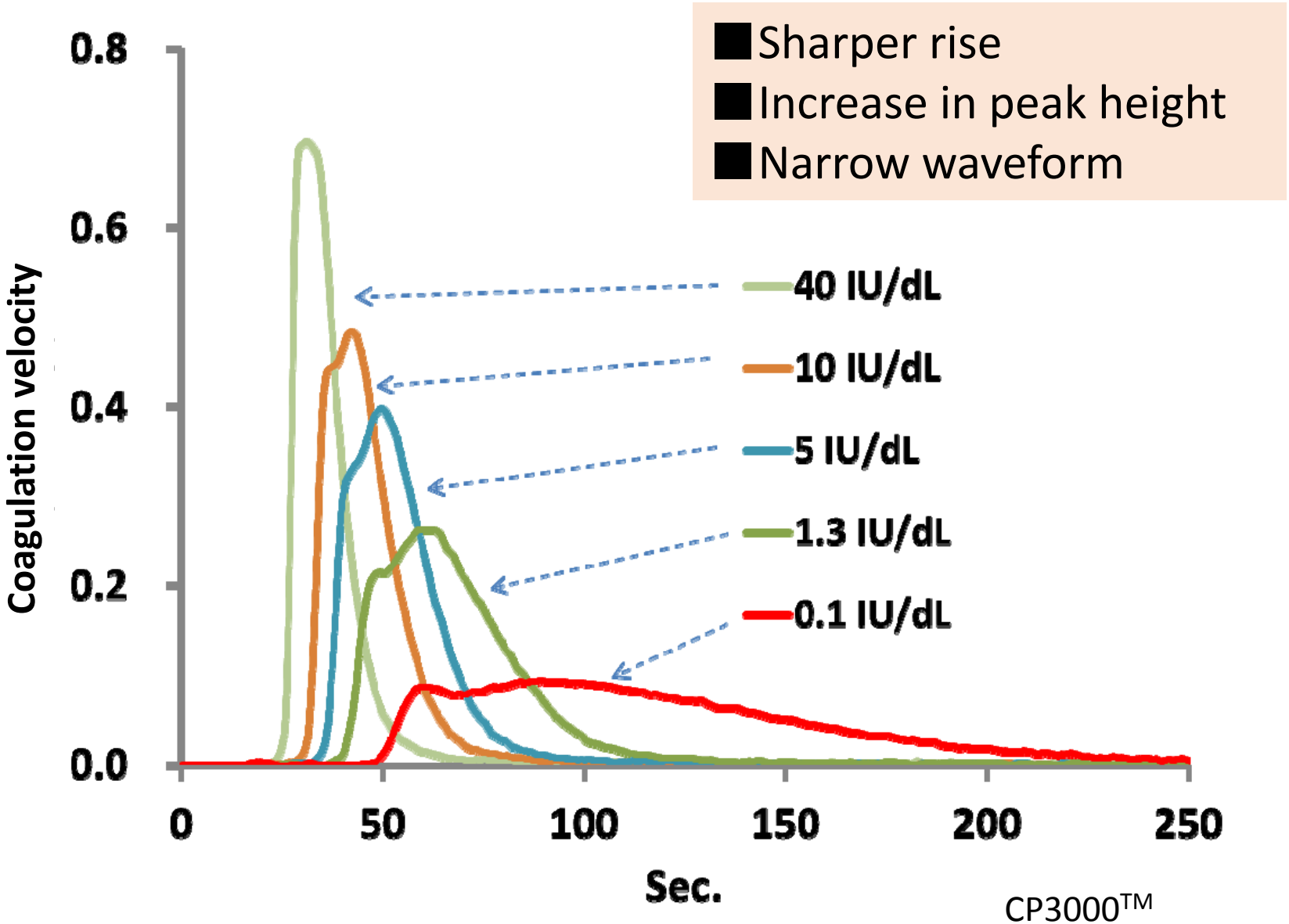
How to obtain suitable parameter reflecting the original waveform?

Parameters based on weighted center method

Weighted center in an area surrounded by the curve
and baseline at X% of **the** peak height (100%)



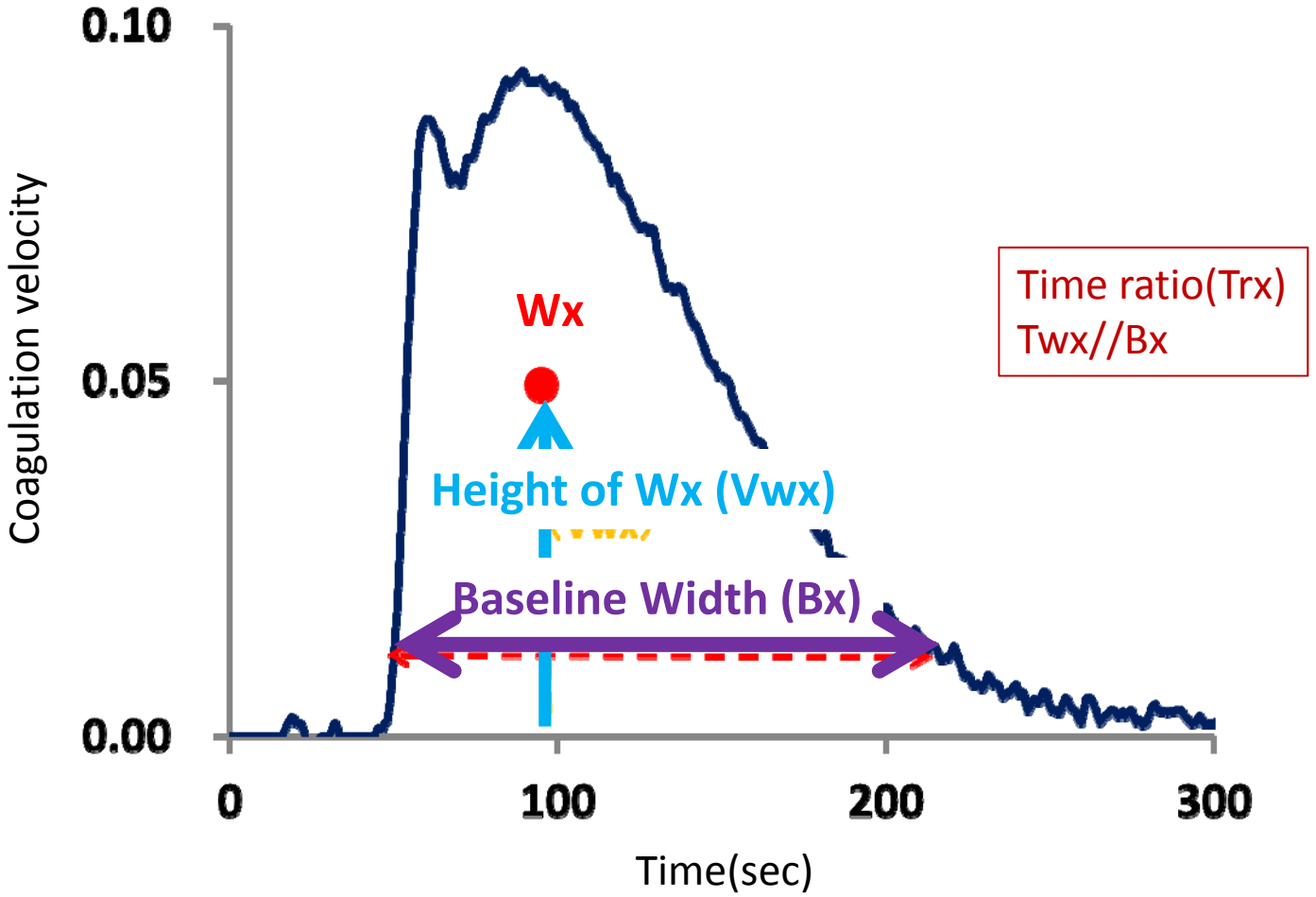
Peak sharpening with FVIII:C



CP3000™

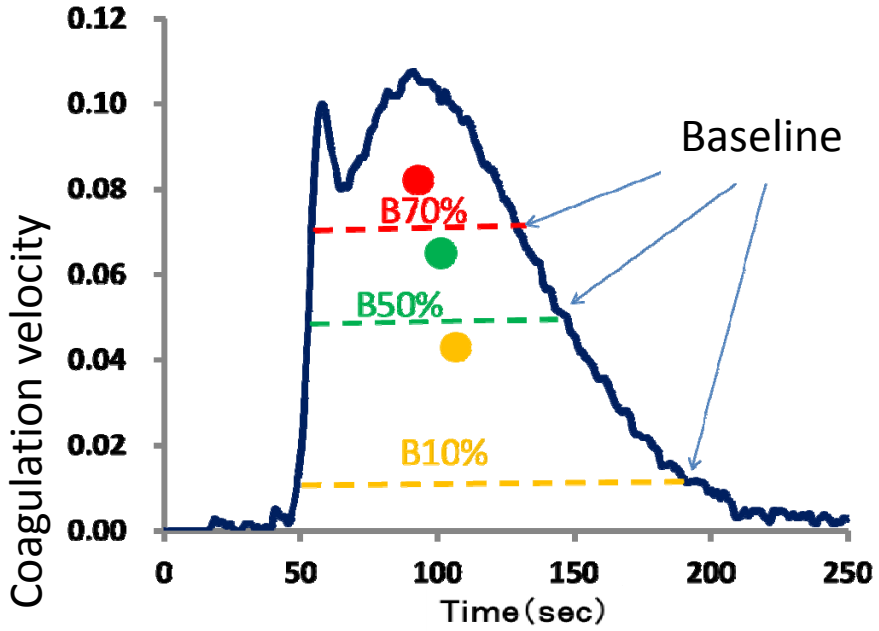
Peak sharpening Indicator

Aspect ratio(Ar) = Height of Wx / Baseline width at X% = Vwx / Bx



Sample : F VIII:C 0.1IU/dL

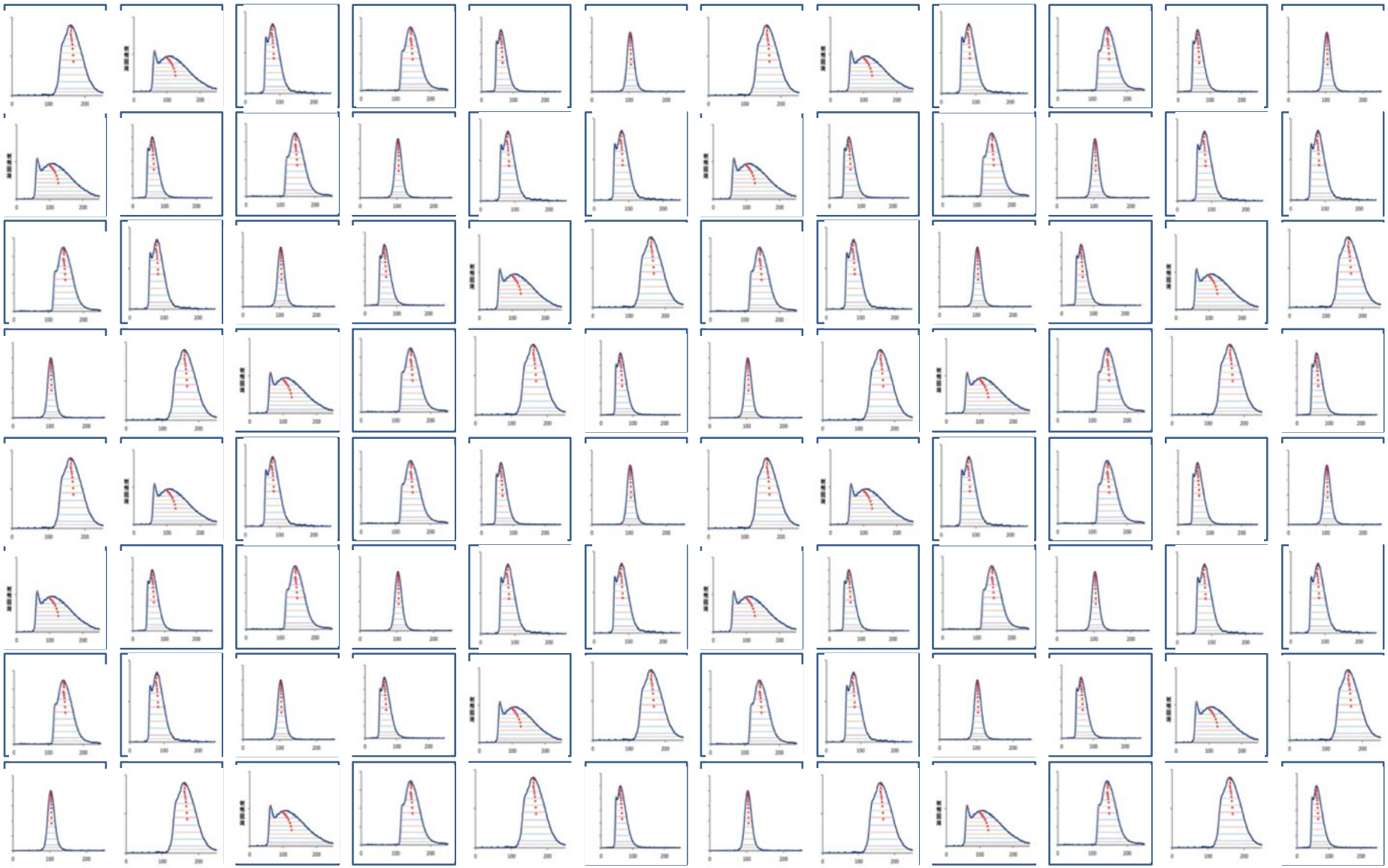
Totally 50 parameters based on weighted center without smoothing



X	Bx	Vwx	Twx	Arx	Trx
5	B5	Vw5	Tw5	Ar5	Tr5
10	B10	Vw10	Tw10	Ar10	Tr10
20	B20	Vw20	Tw20	Ar20	Tr20
30	B30	Vw30	Tw30	Ar30	Tr30
40	B40	Vw40	Tw40	Ar40	Tr40
50	B50	Vw50	Tw50	Ar50	Tr50
60	B60	Vw60	Tw60	Ar60	Tr60
70	B70	Vw70	Tw70	Ar70	Tr70
80	B80	Vw80	Tw80	Ar80	Tr80
90	B90	Vw90	Tw90	Ar90	Tr90

Weighted center varies with the movement of baseline and 50 (10 × 5) parameters were created for each sample by raising X from 5 to 90 in 10 steps.

Template matching for diagnosis of hemophilia by APTT CWA



Summary

■APTT CWA is simple assay for assessment of global clotting function by continuous monitoring of APTT.

■Quantitative assessment is possible by various parameters such as, clotting time (CT), maximum coagulation velocity (Min1)and maximum coagulation acceleration (Min2) .

■APTT CWA is useful for evaluation for clotting function of hemophilia in various clinical setting.

■Emicizumab, a bispecific antibody against FIXa/FX, is now being new option for hemophilia A therapy irrespective of the inhibitor. Modified APTT CWA (as well as ROTEM) is useful for monitoring.

■In the presence of tPA, clotting and fibrinolytic reaction can be assessed simultaneously within 10 minutes (CFWA).

■Template matching by data set of new parameters based on the weighted center by APTT CWA would be applied to diagnosis of hemophilia A and assess severity.

Acknowledgement

- **Department of Pediatrics Nara Medical University**
  Thrombosis and Hemostasis group

  Keiji Nogami
  Masahiro Takeyama
  Yasuaki Shida
  Kenichi Ogiwara
  Hiroshi Yada
  Tomoko Matsumoto
  Shoko Furukawa
  Hiroaki Minami
  Hiroaki Yaoi
  Satoshi Ochi

- **Sysmex investigators**

  Nobuo Arai et al.

- **Sekisui Medcial investigators**

  Yukio Oda
  Toshiki Kawabe

Thank you for attention