Clinical application of clot waveform analysis in hemophilia treatment

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I currently have, or I have had in the past two years, an affiliation or financial interest with business corporation(s):

(1) Consulting fees, patent royalties, licensing fees : No
(2) Research fundings : Yes, Shire, Novo Nordisk, Bayer, Chugai, Bioverativ
(3) Others: No
Since 1950 (start of univ.)
clinical and research on
thrombosis and hemostasis

Enrolled patients
Hemophilia ~600
VWD ~300

Chairman:
1st: Kunio Yoshida
2nd: Hiromu Fukui
3rd: Akira Yoshioka
4th: Midori Shima (present)
Hemophilia A and B (HA/HB) are congenital inherited bleeding disorders, occurred by genetic abnormalities of blood coagulation factor (F)VIII and FIX molecules, respectively, resulting in the frequently repeated intra-muscular and joints bleeding.

Alive patients in Japan (2018)
HA : 5,326 patients
HB : 1,129 patients
Routine laboratory test on diagnosis of hemophilia

Prolonged APTT
Normal PT
Normal platelet count
↓
Hemophilia ?
↓
Measurement of clotting factor activity
## Classical coagulation assays

<table>
<thead>
<tr>
<th></th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT / aPTT</td>
<td>Widely spread</td>
<td>Non-physiological condition</td>
</tr>
<tr>
<td></td>
<td>Measurable quickly</td>
<td>Reagent &amp; Institution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Influence of factors except for FVIII or FIX</td>
</tr>
<tr>
<td>One-stage</td>
<td>Measurable activity of clotting factor</td>
<td>Purity of deficient plasma</td>
</tr>
<tr>
<td>Two-stage</td>
<td></td>
<td>Low cons. of measurement</td>
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<tr>
<td></td>
<td></td>
<td>Technical complexity</td>
</tr>
<tr>
<td>Chromogenic assay</td>
<td>Not influence by other factors</td>
<td>Expensive</td>
</tr>
</tbody>
</table>
Diversity of clinical phenotype of severe hemophilia patients

Determinant factors for clinical phenotype

- Anticoagulation ($FV_{Leiden}$, PT20210G>A, PC deficiency)
- Fibrinolysis (TAFI, PAI-1)
- Genetic defects ($F7$ R353Q polymorphism)
- Pharmacokinetics (ABO group)
- Environmental factors (life style, activity)
- Inflammation (IL-10)

- Methodology

Nogami K. Semin Thromb Haemost 2015
What is Clot Waveform Analysis (CWA) ?

- Originally developed by Organon Technica (MDA-II®)
- Waveform by monitoring the transmittance changes during aPTT/PT
- Waveform analysis reflects whole blood clotting process.
- Coagulation velocity/acceleration can be evaluated by 1\textsuperscript{st}/2\textsuperscript{nd} derivative.
- Quantitative parameters, Min1, Min2 and Max2 can be measured.
- Fibrinolytic activity can be evaluated.

\begin{itemize}
\item [A] Transmittance (T)
\item [B] \(\frac{dT}{dt}\)
\item [C] \(|\text{Min1}|\)
\item [D] Max2
\item [E] Fibrinolytic activity
\item \(\frac{d^2T}{dt^2}\)
\end{itemize}

Braun et al. Thromb Haemost 1997
CWA parameters

- **|Min1|**: the absolute minimum value of 1st derivatives (dT/dt) that reflect the **maximum velocity** of the change in light transmission.
- **|Min2|**: the absolute minimum value of 2nd derivative (d²T/d²t) that reflects the **maximum acceleration**.
- **|Max2|**: the maximum value of 2nd derivative (d²T/d²t) which reflects the **maximum deceleration** in the clotting process.
Coagulation analyzer for clot waveform analysis

<table>
<thead>
<tr>
<th>Coagulation analyzer</th>
<th>Company</th>
<th>Application of CWA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-7000</td>
<td>Sysmex</td>
<td>Raw data output</td>
</tr>
<tr>
<td>CS-5100</td>
<td>Sysmex</td>
<td>Available</td>
</tr>
<tr>
<td>CS-2000, CS-2100i</td>
<td>Sysmex</td>
<td>Available</td>
</tr>
<tr>
<td>CA-1500</td>
<td>Sysmex</td>
<td>Raw data output</td>
</tr>
<tr>
<td>BCS</td>
<td>Siemens</td>
<td>Raw data output</td>
</tr>
<tr>
<td>BCT</td>
<td>Siemens</td>
<td>Raw data output</td>
</tr>
<tr>
<td>ACL-Top 700</td>
<td>IL</td>
<td>Available</td>
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<tr>
<td>ACL-Top 500 CTS</td>
<td>IL</td>
<td>Available</td>
</tr>
<tr>
<td>ACL-Top 300 CTS</td>
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<tr>
<td>ACL-Advance</td>
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<tr>
<td>ACL-FURURA</td>
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<tr>
<td>ACL-ELITE pro</td>
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<tr>
<td>ACL-7000</td>
<td>IL</td>
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</tr>
<tr>
<td>CD-X</td>
<td>Dia Med</td>
<td>Raw data output</td>
</tr>
<tr>
<td>MDA-II</td>
<td>Trinity</td>
<td>Available</td>
</tr>
<tr>
<td>Coapresta2000</td>
<td>Shimazu</td>
<td>Raw data output</td>
</tr>
</tbody>
</table>

Shima M et al. JTH 2013
APTT-based clot waveform patterns of various conditioned plasmas

Braun et al. Thromb Haemost 1997

Shima M et al. JTH 2013
FVIII-dependent change of APTT-based clot waveform

FVIII-def plasma with various FVIII conc.

HA plasmas with various FVIII levels

Shima M et al. JTH 2013
Evaluation of CWA of FVIII:C using various APTT reagents (CS-2000i)

Clot time

|Min1|

|Min2|

|Max2|

Matsumoto, Nogami, Int J Hematol. 2017
Correlation between very low levels of FVIII:C and aPTT clot time or $|\text{min2}|$ in severe HA

**aPTT clot time**

Clot time (sec) vs. FVIII:C (IU/dl)

$r = 0.363$

**$|\text{Min2}|$**

$|\text{min2}|$ (%T/sec^2) vs. FVIII:C (IU/dl)

$r = 0.720$

*Shima M. Thromb. Haemost 2002*
aPTT-based clot waveform on plasmas with severe HA (FVIII:C <1 IU/dl)

Shima M et al. Haemophilia 2006
CWA parameters and clinical severity on patients with severe HA

Clot Time

|Min1|

|Min2|

|Max2|

Shima M et al. JTH 2013
Case presentation 1

44 y.o. M, Mild hemophilia A

He repeated the severe bleeding (massive intra-muscle bleeding), despite baseline level of FVIII:C 20-30 IU/dL. In bleeding episode, he required hemostatic therapy with FVIII products. He was hospitalized for his massive intra-muscle bleeding.

FVIII:C 30.2 IU/dL (OS)
FVIII:Ag 91.6 IU/dL
VWF:RCo/Ag 190/189 IU/dL

FVIII:C 20-30 IU/dL level should be enough for hemostasis. His FVIII level does not reflect the clotting function.

Chromogenic assay 10.4 IU/dL

Yada K, Nogami K. Annual Meeting of JSH 2013
Equivalent FVIII:C levels by CWA parameters

Clot waveform

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

Yada K, Nogami K. Annual Meeting of JSH 2013
CWA is useful to assess the clotting function of HA patients whose levels do not correspond to clinical severity.
Case presentation 2

27 y.o. Severe hemophilia A

2 y.o. He was diagnosed as severe HA when difficult of bleeding of traumatic bleeding. After then, he rarely had bleeds, and received the FVIII infusion, resulting in the no arthropathy.

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII:C</td>
<td>0.9 IU/dl (OS)</td>
</tr>
<tr>
<td>FVIII:Ag</td>
<td>1.8 IU/dl</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>160 IU/dl</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>140 IU/dl</td>
</tr>
<tr>
<td>Chromogenic assay</td>
<td>4.5 IU/dl</td>
</tr>
<tr>
<td>Arg1781His mutation</td>
<td></td>
</tr>
</tbody>
</table>

Yada, Nogami,. Thromb Haemost 2013
Equivalent FVIII:C levels by CWA parameters

Equivalent FVIII:C  13~15 IU/dl

Higher binding affinity of R1781H-FVIII and FX

Yada, Nogami,. Thromb Haemost 2013
Hemostatic monitoring in bypassing therapy for HA patients with inhibitor

- Selection and dosing of bypassing agents
- Monitoring of efficacy and safety
- Determination of changing to other agent

- PT and APTT is not useful.
- TEG may be useful.

Ideal method has not been established or standardized.

Usefulness of CWA for hemostatic monitoring during bypassing therapy in HA with inhibitor?
Clot waveforms of FVIII-def. plasma with rFVIIa

Ellagic acid

Ellagic acid/TF

TF

Haku, Nogami et al JTH 2014
Clot waveforms of FVIII-def. plasma with aPCC

Ellagic acid

TF

Ellagic acid/TF

%Transmittance vs. time (sec)

Haku, Nogami et al JTH 2014
Clot waveforms in HA inhibitor with rFVIIa infusion (Elg/TF-trigger)

Case 1

Case 2

Case 3

Case 4

%Transmittance

Pre

Post

NP

Poor improvement

Haku, Nogami et al JTH 2014
Clot waveforms in HA inhibitor with aPCC infusion (Elg/TF-trigger)

Haku, Nogami et al JTH 2014
Acquired hemophilia A (AHA)

AHA is a severe acquired bleeding disorder due to anti-FVIII autoantibody developed in non-hemophiliac individual who have had no bleeding tendency nor bleeding symptoms.

Typical severe subcutaneous hemorrhage

Huth-Kuhne et al. haematologica 2009
Why is massive bleeding pattern in AHA, despite the presence of mild or moderate FVIII:C levels in plasmas??

Coagulant hemostatic potentials in AHA is different from those in congenital HA??

Establishment of assays that evaluate global assay are required.
Parameters of CWA in moderate HA and acquired HA with similar levels of FVIII:C

Moderate HA; 2.1±0.9 IU/dl
Acquired HA; 2.0±1.9 IU/dl

Matsumoto T, Nogami K et al.
Thromb Haemost 2012
Novel non-clotting factor products

- Bispecific antibody mimicking FVIII (Emicizumab)
- siRNAs targeting antithrombin (Fitusiran)
- Anti-TFPI antibody (Concizumab)

- Subcutaneous administration
- Longer acting
- Greater efficacy, irrespective of inhibitor
Concept of a FVIIIa-mimetic bispecific antibody

- The actions of FVIIIa is to promote FIXa-catalyzed FX activation.
- The cofactor function of FVIIIa is enhancing the FIXa-FX interaction.
- Bispecific antibody recognizing FIXa with one arm and FX with the other arm may exert FVIIIa cofactor activity.
- FVIIIa-mimicking antibody preparation is difficult, since the antibody has to place FIXa and FX in spatially appropriate positions and angles.

APTT is unsuitable in the presence of emicizumab.

Emicizumab has a FVIIIa-mimicking function and markedly shortens the aPTT in FVIII-deficient plasma irrespective of presence of inhibitor.

FVIII:C and Bethesda unit based on the aPTT-based one-stage clotting assay should be influenced by emicizumab.

Kitazawa et al Nat Med 2012
CWA (CS-2000i) and 1st derivative

Adjust-CWA and 1st derivative

aPTT/PT mixture trigger

CWA on HAs’ plasmas with addition of emicizumab
(PT/aPTT mixed-trigger)

Inhibitor (−)

Inhibitor (+)

Emicizumab activity appears unlikely to affect FVIII activity

- Emicizumab has a much lower binding affinity for FIXa than FVIIla does.

- In the presence of emicizumab, FVIII has an additive effect*

![Diagram showing binding affinities and effects](image)

CWA on HA patients’ plasma with emicizumab in the co-presence of rFVIIa or aPCC

Summary

Clot waveform analysis (CWA) that has broad utility as a simple global test is possible to …

- evaluate the various bleeding disorders
- evaluate the very low levels of FVIII:C
- judge the diagnosis and severity of hemophilia A
- differentiate between mild/moderate HA and acquired HA
- monitor hemostatic management of bypassing therapy
- monitor hemostatic management of emicizumab treatment
Towards standardization of clot waveform analysis and recommendations for its clinical applications

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The future of CWA monitoring

- Recently, the number of appropriate analyzers for CWA has been increasing.

- To use the monitoring of CWA widely-spread, the assessment and standardization of parameters obtained by combination of different platforms and trigger reagent will be required.

- It is hoped that CWA become the clinical testing that has higher clinical utility with more research in future.
Acknowledgements

Dept. Pediatrics, Nara Medical University, Nara, Japan

Midori Shima, MD, PhD (Professor)
Tomoko Matsumoto, PhD
Koji Yada, MD, PhD
Kenichi Ogiwara, MD, PhD
Junka Haku, MD

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