Which parameters must be checked in order to avoid thrombotic complications in CDG patients?

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Spain

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Which parameters must be checked in order to avoid *thrombotic complications* in CDG patients?

Unfortunately we are not able to firmly answer this question yet.

Further studies are required, increasing the number of patients, parameters and clinical features.

*We are going to show*

- A general view of the hemostatic system
- The most recent results of this system in CDG patients
- A protocol to manage vascular complication in CDG patients

_from San Joan de Deu Hospital (Barcelona, Spain)_
HAEMOSTATIC SYSTEM
System developed during $430 \times 10^6$ years

Vascular Damage

Bleeding

Death

Simple: few elements
Strong and fast: No protein synthesis

Coagulation cascade

Platelets
1. Vascular damage exposes connective tissue. Platelet adhesion and activation.

2. Platelet plug

3. Fibrin clot traps other blood cells

Procoagulant surface
- Platelets
- Endothelial cells
- Monocytes
- Calcium and other plasma factors

Prothrombin → Thrombin

Fibrinogen → Fibrin

Avoid bleeding
**Procoagulant surface**
- Platelets
- Endothelial cells
- Monocytes
- Calcium and other plasma factors

- **Prothrombin**
- **Thrombin**
- **Fibrinogen**
- **Fibrin**

**SPACE AND TIME REGULATION**

**ANTICOAGULANTS & PROFIBRINOLYTICS**

- **Antithrombin**
  - $\text{IX} + \text{VIII}^*$
  - $X + V^*$
  - **Protein C**
  - **Protein S**

- **TF pathway inhibitor**
- $\text{VIIa} + TF^*$
- **Fibrinolysis**
  - **Fibrin clot**
  - Cross-linked clot
HAEMOSTATIC SYSTEM

- Procoagulant activity
- Antifibrinolytic activity
- Fibrinolytic activity
- Anticoagulant activity

Normal Haemostasis
Thrombosis

Procoagulants

Procoagulants

Balanced equilibrium

Procoagulants

Procoagulants

Anticoagulants

Anticoagulants

Haemostatic disorders

Thrombosis

FV Leiden (APCr)

Prothrombin

Antithrombin

Protein C

Protein S

Variants

Deficiencies

Antithrombin

Protein C

Protein S

Haemostasis disorders

Bleeding

FII, V, VII, VIII, FIX, FX, FXI

Thrombopenia

Procoagulants

Procoagulants

Anticoagulants

Anticoagulants
How is the hemostatic system in CDG patients?

All plasma coagulation factors are N-glycoproteins. Platelets contain surface proteins highly glycosylated.
FOLDING AND SECRETION

IMMUNOGENICITY

SPECIFICITY TO INTERACTION

SENSITIVITY TO PROTEASES

HALF-LIFE AND STABILITY

FUNCTION

Glycoprotein

- GlcNAc
- Mannose
- GalNAc
- Galactose
- Fucose
- Sialic Acid

O-glycans

N-glycans

lumen/extracellular

cytosol
Role of N-glycosylation on the secretion of antithrombin and TFPI

Site directed mutagenesis of N-glycosylation signals, recombinant expression and analysis of protein secreted to the conditioned medium of mammalian cells

<table>
<thead>
<tr>
<th>Number of N-glycans</th>
<th>Antithrombin (4 N-glycans)</th>
<th>TFPI (2 N-glycans)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
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<tr>
<td>4</td>
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<td>4</td>
</tr>
</tbody>
</table>
Levels of hemostatic proteins in CDG patients

✓ A systematic review on PMM2-CDG patients (1990 to 2013 in literature*)
✓ Our cohort N=20 (PMM2-CDG and one ALG12-CDG)

N= 100 patients in total


-Literature review N= 79 patients-

Anticoagulants: Deficiency
Antithrombin      39/54 (72%)
Protein C         34/47 (72%)
Protein S         17/32 (53%)

Procoagulants: Deficiency
Factor XI         22/38 (58%)
-Our cohort of 20 patients-

TFPI deficiency (Severe)

Anticoagulant side of the hemostatic balance is severely affected

High risk of thrombosis
Mice, with a great resistance to thrombosis

Our cohort of 20 patients-
Absolute values in certain patients

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Male (1 year)</th>
<th>Male (1 year)</th>
<th>Female (9 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>17%</td>
<td>17%</td>
<td>9%</td>
</tr>
<tr>
<td>PC</td>
<td>50%</td>
<td>17%</td>
<td>16%</td>
</tr>
<tr>
<td>TFPI</td>
<td>20%</td>
<td>10%</td>
<td>8%</td>
</tr>
</tbody>
</table>

No thrombosis
Why?
FXI deficiency: the other side of the coin

• FXI deficiency was first described as hemophilia C by Rosenthal et al. in 1953
• Even in individuals with FXI levels <20 IU/dL, serious spontaneous haemorrhage is not common.

• FXI deficiency induced in animals by specific antibodies or gene targeting exerts an antithrombotic effect in arterial or venous models of thrombosis without compromising hemostasis.
• Congenital FXI deficiency is protective against ischemic stroke and venous thrombosis
• Reducing factor XI levels in patients undergoing elective primary unilateral total knee arthroplasty was an effective antithrombotic method and appeared to be safe with respect to the risk of bleeding (Büller et al. Factor XI antisense oligonucleotide for prevention of venous thrombosis. N Engl J Med. 2015 Jan 15;372(3):232-40)
9 (53%) have FXI deficiency (levels below 50% of a reference plasma)
Mean FXI levels: 52.9%

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<td>TFPI</td>
<td>20%</td>
<td>10%</td>
<td>8%</td>
</tr>
<tr>
<td>FXI</td>
<td>27%</td>
<td>22%</td>
<td>24%</td>
</tr>
</tbody>
</table>

COMPENSATORY MECHANISM?
Excellent correlations between AT and FXI or TFPI and FXI

**AT vs FXI**

- $R^2 = 0.378$
- Psperman=0.769
- $p=0.000$

**TFPI vs AT and asialoTf**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT(%)</td>
<td>100</td>
<td>17</td>
<td>17</td>
<td>48</td>
<td>46</td>
<td>35</td>
<td>22</td>
<td>28</td>
<td>43</td>
<td>100</td>
</tr>
<tr>
<td>asialoTf</td>
<td>0.0</td>
<td>21.5</td>
<td>27.6</td>
<td>7.9</td>
<td>11.0</td>
<td>10.0</td>
<td>23.6</td>
<td>11.9</td>
<td>5.9</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Inverse correlation of hypoglycosylation (asialoTf) and AT, FXI or TFPI**

**AT vs asialoTf**

- $R^2 = 0.445$
- Psperman=-0.625
- $p=0.003$

**FXI vs asialoTf**

- $R^2 = 0.363$
- Psperman=0.604
- $p=0.010$
POTENTIAL USEFULNESS OF THE RATIO AT/FXI FOR ESTIMATING THE RISK OF THROMBOSIS SHOULD BE EVALUATED IN FURTHER STUDIES

Procoagulant
Procoagulant
Procoagulant
Procoagulant
Procoagulant

Balanced equilibrium

Healthy subject

FXI
AT
PC
TFPI

Relatively balanced equilibrium

CDG

FXI
Procoagulant
Procoagulant
Procoagulant
Procoagulant

AT
PC
TFPI

Reduced flexibility, increased sensibility to even minor changes
Usefulness of global hemostatic tests in CDG

Many people question the usefulness of these tests to prevent the risk of thrombosis.

They probably reflect the capacity of the hemostatic system to respond to a procoagulant stimulus, but not the risk of thrombosis.

**aPTT Best example**

Prolonged 19/25 CDG (76%). All our patients had prolonged aPTT.

Bleeding

Thromboelastography

Hyperfibrinolysis

Cloting Time prolonged

Bleeding

Not association of aPTT with the risk of bleeding in CDG

Thrombin generation

Lag time prolonged

Less trombin generation

Bleeding
## Vascular events

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>PMM2-CDG patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding</td>
<td>16%</td>
</tr>
<tr>
<td>Seizures</td>
<td>14%</td>
</tr>
<tr>
<td>Venous thrombosis</td>
<td>10%</td>
</tr>
<tr>
<td>Stroke-like</td>
<td>13%</td>
</tr>
<tr>
<td>Thrombosis+Stroke</td>
<td>7%</td>
</tr>
<tr>
<td>Thrombosis+Bleeding</td>
<td>3%</td>
</tr>
<tr>
<td>Disseminated Intravascular Coagulation</td>
<td>5%</td>
</tr>
</tbody>
</table>


14% CDG had arterial thrombosis or venous thrombosis

14% CDG also presented bleeding episodes

Normal prevalence rate of thrombosis in children: 0.07–0.14 per 10,000 for venous thrombosis and 0.3–0.8 per 10,000 for arterial thrombosis
Platelets in CDG

- Normal platelet count \((287 \pm 64 \times 10^9/L)\) and normal size \((8.4 \pm 1.3 \mu m^3)\) (N=20)
- Normal platelet reactivity against all agonists but spontaneous hyperreactivity (Van Geet C, et al., 2001)
- Platelet N-glycoproteins are neither quantitatively nor qualitatively significantly affected (de la Morena-Barrio ME et al., 2014).
Other thrombophilic factors in CDG

Only common but mild prothrombotic polymorphisms might be evaluated in CDG patients

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Frequency</th>
<th>Risk of thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden</td>
<td>4-8%</td>
<td>4-fold</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>2-4%</td>
<td>2-fold</td>
</tr>
<tr>
<td>Non-O blood group</td>
<td>50%</td>
<td>1.4-fold</td>
</tr>
</tbody>
</table>

No study evaluating these prothrombotic polymorphisms in the severely affected hemostatic system of CDG

In our cohort ONE patient carried a prothrombotic polymorphism (Factor V Leiden) but with NO thrombotic event so far
1. **Stroke-like episodes:**
   - Antiepileptic treatment
   - No antiaggregant therapy except previous arterial thrombosis.

2. **Hemorrhagic cerebrovascular accidents:**
   - Fresh-frozen plasma (after administration measure: PT and aPTT)
   - No procoagulant concentrates (prothrombin complex concentrates or recombinant factor VIIa supplementation)

3. **Surgical interventions**
   - Before: PT (when >1.2s levels of factors II, V, VII and X) and apTT (always levels of IX and XI).
   - Fresh-frozen plasma according to factor levels
   - No procoagulant concentrates (prothrombin complex concentrates or recombinant factor VIIa supplementation)

4. **Thrombotic risk prevention**
   - Thrombophilia study: prothrombin G20210A and FV Leiden
   - Ratios: AT/FIX and AT/FXI
   - In situations with thrombotic risk (surgery, immobilization...):
     - Heparin (LMWH), prophylactic doses with anti-FXa controls
     - Or Antithrombin concentrates
     - Avoid immobilization after surgery

5. **Disseminated intravascular coagulation**
   - Fresh-frozen plasma
   - Or Antithrombin concentrates
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To all patients and their families