Pediatric Hemostatic Disorders and Testing: A Case-Based Discussion, Protein C Deficiency

Georges E Rivard, MD

Centre Hospitalier Universitaire Sainte-Justine

Montréal, Canada

Mayo Clinic April 2009
Disclosure

I have no relevant financial relationship to disclose for this presentation
Overview of my presentation

• Protein C deficiency: clinical presentation
• Protein C physiology in a nut shell
• Protein C assessment for clinicians
Overview of my presentation

• Protein C deficiency: clinical presentation
• Protein C physiology in a nut shell
• Protein C assessment for clinicians
Case presentation # 1a

- Propositus is the second child of healthy parents with no known consanguinity. Normal pregnancy. Uneventful vaginal delivery at 40 w. June 26, 1991. BB boy, APGAR 8, 9, 9; 2345 g; looks N except for IUGR

- First child of the same parents. BB girl born 1989 with IUGR, microophtalmia and bilateral “cataracts”. Brain CT: “Many big cysts and foci of calcification”. Diagnosed as “Peter’s syndrome”. Died on day 3 in the context of “aseptic purpura fulminans”
Case presentation # 1b

On day 2, multiple “unusual ecchymoses”

BB transferred to CHU Sainte-Justine

Consultation in hematology
Case presentation # 1c

Hb 164; Leuko 13; Platelets 55

PT 18.5 s (11-15); APTT 50.8 s (26-36)

Fib 0.38 g/L; FII 0.22 IU/mL; FV 0.75 IU/mL; FVIII 0.5 IU/mL; FX 0.65 IU/mL

Blood smear: Moderate thrombocytopenia. Otherwise within normal limits for age
Case presentation # 1d

Chromogenic assay for Protein C
BB < 0.01 IU/mL
Mother 0.52 IU/mL
Father 0.55 IU/mL
Case presentation # 1e

• Immediately treated with FFP and heparin
• Protein C concentrate IV. Eventually Protein C ± 40 IU/kg IV or SC X 2/w, Coumadin with INR 3.5-4
• Over the years many episodes of purpura fulminans when suboptimal Coumadin or Protein C replacement. Very quick response to IV Protein C, with no skin scarring
• Currently on Coumadin with INR 4-4.5 and Protein C ± 40 IU/kg IV X 2/W
Case presentation # 1f

Brain CT, Bilateral retinal calcifications, many brain cysts and calcifications
Age 3 days
Age 15 years
Case presentation # 1g

• Homozygous for Protein C 3363 Ins C

• Both parents heterozygous for 3363 Ins C

• Both parents French Canadians but no demonstrable consanguinity
Case presentation # 1h

- Completely blind, mildly retarded
- Urinary incontinence
- More/less normal physical development
- 18 y old, attends special school
- Uses about $800,000/y Protein C concentrate
- Seems to be happy. Has a blind girl friend
Case presentation # 2a

- First child of healthy parents with no known consanguinity
- At 36 w, spontaneous rupture of membranes
- Fetal bradycardia, emergency CS, Sept. 14, 2006
- BB girl. APGAR 8,9,9. 1665 g. Normal except for evidence of IUGR
- Vit K given on left thigh and BB sent to mother’s room
Case presentation # 2b

- Day one, does not feed well, sleepy
- Big “ecchymoses” on left thigh and scalp which progressed within a few hours to become necrotic
- BB transferred to CHU Sainte-Justine
Case presentation # 2c

- Upon arrival, BB lethargic, no fever, no bleeding manifestation other than the two “ecchymoses”

- Consultation in hematology
Case presentation # 2d

- Cultures obtained from blood and “ecchymoses” (turned out to be negative)

- Initial lab results: Hb 141; Leuko 16.6; Platelet 41; APTT 46.7 s (23-34); PT 23 s (12-15); Fib 0.92 g/L (2-4); D-Di >2.0 μg/mL ( <0.25)

- Rare red cell fragments on blood smear
Case presentation # 2e

Chromogenic assay for protein C
BB: < 0.01 IU/mL
Mother: 0.58 IU/mL
Father: 0.53 IU/mL
Meeting with 2 parents and 4 grand-parents: Active support discontinued: Died on day 3 of respiratory arrest. Autopsy refused
Further on family tree: French Canadians, consanguinity 6 generations back
Molecular diagnostic: 3363 Ins C BB homozygous, 2 parents heterozygous
Chorionic biopsy for prenatal diagnostic planned for future pregnancy
Overview of my presentation

- Protein C deficiency: clinical presentation
- Protein C physiology in a nut shell
- Protein C assessment for clinicians
Protein C Physiology in a Nut Shell

- Vit K dependant serine protease zymogen
- MW 62 000
- Activated by IIa in the presence of TM
- In the presence of free PS, anionic phospholipids, and Ca++, inactivates
  - FVα by cleavage at Arg 506, Arg 306, Arg 679
  - FVIIIα by cleavage at Arg 336, Arg 562, Arg 740
Protein C 3363 Ins C

Gene located on chromosome 2, at position 2q13-q21; comprises 9 exons, 1795 coding nucleotides for a 462 AA protein

The mutation 3363 is caused by the insertion of a C just after nucleotide 3363 and leads to a truncated protein with a proline instead of a histidine at AA 107, followed by 11 new AA and a STOP codon
The Mutation 3363 Ins C

Modified from Foster DC et al. Proc Natl Acad Sci 1985;82:4673-7
3363 Ins C protein C mutation

Tomczak JA, Ando RA, Sobel HG, Bovill EG, Long GL.

The new “Vermont” mutation identified was 3363 Inc C

One of the 55 mutations was 3363 Ins C present in 2 subjects of the same family

Study suggests a founder effect introduced in Québec by a couple of French settlers in the seventieth century.
Overview of my presentation

• Protein C deficiency: clinical presentation

• Protein C physiology in a nut shell

• Protein C assessment for clinicians
Protein C Assessment

- Immunological assays
  - EIA
  - ELISA
  - RIA

- Functional assays
  - Clotting
  - Amidolytic
Protein C Immunological Assays

- Measure total protein C
- Easy to perform
- Relatively inexpensive
- No discrimination of abnormal protein C
- Necessary for diagnosis of Type II deficiency, in association with a functional assay
Protein C Functional Assays: The Older Generations

• Using protein C separated from other plasma proteins with aluminum hydroxyde, barium citrate, monoclonal antibodies, others…
• Activation of protein C by human/bovine thrombin, with/without thrombomodulin
• Clotting end point: prolongation of APTT, Xa clotting time, others…
• Chromogenic and fluorogenic substrates end points
• Difficult to perform
• Reproducibility highly operator dependent
Protein C Functional Assays: The Newer Generations

FAST FUNCTIONAL PROTEIN C ASSAY USING PROTAC®,
A NOVEL PROTEIN C ACTIVATOR

J.L. Martinoli* and K. Stocker**
*Serbio Laboratories, Asnières, France
**Pentapharm Ltd., Basle, Switzerland

(Received 6.1.1986; Accepted in revised form 23.4.1986 by Editor E.A. Beck)
Agkistrodon contortrix contortrix
With me dosage of Protein C never fails
Protein C Functional Assays: The Newer Generations

- Using whole plasma
- Activation of protein C with Protac®, venom of *Agkistrodon contortrix contortrix*
- Clotting end point: prolongation of APTT or PT; potentially influenced by level of FV and FVIII, FV Leiden, LA, heparin
- Chromogenic or fluorogenic substrate end point
- Easy to perform
- Automation possible

FIG. 4
Correlation between Functional and Enzyme-Immunoassay for Protein C in Plasma from Patients under Oral Anticoagulation

CRYOcheck Clot C Assay (Precision BioLogic, Darmouth, Canada)

Figure 1. CRYOcheck Clot C assay calibration curve, evaluation of linearity. Standard diluted one in 33 in Precision Biologic C&S Diluent. Protein C range 10 to 215 U/dl $R^2 = 0.9755$ (x–x) and protein C range 10–130 U/dl $R^2 = 1.000$ (○–○).
Table 1. Interquartile ranges and median levels of protein C for PC clot, PC antigen and PC chromogenic in normal subjects (n = 20), subjects heterozygous for FVL (n = 20) and subjects with LA (n = 25)

<table>
<thead>
<tr>
<th>Inter quartile range</th>
<th>PC clot</th>
<th>PC antigen</th>
<th>PC chromogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>70–95</td>
<td>75–100</td>
<td>67–98</td>
</tr>
<tr>
<td>Second</td>
<td>95–108</td>
<td>100–109</td>
<td>98–108</td>
</tr>
<tr>
<td>Third</td>
<td>108–129</td>
<td>109–120</td>
<td>108–126</td>
</tr>
<tr>
<td>Fourth</td>
<td>129–176</td>
<td>120–160</td>
<td>126–196</td>
</tr>
<tr>
<td>Median</td>
<td>108</td>
<td>109</td>
<td>108</td>
</tr>
<tr>
<td>Factor V Leiden (Heterozygous)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>77–94</td>
<td>80–95</td>
<td>81–98</td>
</tr>
<tr>
<td>Second</td>
<td>94–102</td>
<td>95–100</td>
<td>98–104</td>
</tr>
<tr>
<td>Third</td>
<td>102–108</td>
<td>100–118</td>
<td>104–123</td>
</tr>
<tr>
<td>Median</td>
<td>102</td>
<td>100</td>
<td>104</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>54–88</td>
<td>66–93</td>
<td>68–95</td>
</tr>
<tr>
<td>Second</td>
<td>88–94</td>
<td>93–103</td>
<td>95–106</td>
</tr>
<tr>
<td>Third</td>
<td>94–128</td>
<td>103–121</td>
<td>106–133</td>
</tr>
<tr>
<td>Fourth</td>
<td>128–194</td>
<td>121–205</td>
<td>133–213</td>
</tr>
<tr>
<td>Median</td>
<td>94</td>
<td>103</td>
<td>106</td>
</tr>
</tbody>
</table>
Conclusion/Opinion

• Severe Protein C deficiency carries a major risk for pre-/neonatal thrombosis

• Proper testing, genetic counselling, prenatal diagnosis, and option for timely therapeutic abortion should be offered to couples at risk

• There are many good techniques for immunological and functional assay of Protein C

• Except for convenience and local availability, there is no evidence for an outstanding immunological technique

• Functional assays activating Protein C with Protac® are the easiest to use

• The first line assay for Protein C deficiency screening should be a functional assay

• For unusual situations functional clotting assay should be done in association with an immunological assay.
Protein C Genetics

Which of the following statement is true?
The gene for protein C is located on chromosome:

1- X
2- 2
3- 7
4- 14
5- Y
The gene for protein C is located on chromosome 2-2
Protein C Physiology

Which of the following statements is true?

1- Kalikrein is a major activator of protein C
2- Production of protein C requires the presence of vitamin K
3- The serine esterase function of protein C requires the presence of thrombomodulin
4- Factor Va is a good substrate for activated protein C
5- Factor VIIIa is not a good substrate for activated protein C
The following statements is true

4- Factor Va is a good substrate for activated protein C
Protein C Testing

Which of the following statements is false?

1- With vitamin K deficiency, protein C antigen is higher than protein C function measured with a clotting assay
2- In liver diseases, protein C measured with a clotting assay is comparable to protein C measured with a chromogenic assay
3- Protac® requires thrombomodulin for complete activation of protein C
4- Activation with Protac® makes testing of protein C easier than with activation by thrombin
The following statements is false

3- Protac® requires thrombomodulin for complete activation of protein C