Phenotyping the blood of PV and ET patients – thrombotic risk

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Jan 2015
myeloproliferative neoplasms MPN

3 classic BCR/ABL-negative MPN:

- polycythemia vera (PV)
- essential thrombocythemia (ET)
- primary myelofibrosis (PMF)

They originate in a single pluripotent hematopoietic stem cell and share several clinical, hematologic and histological features.
MPN (PV-ET) & thrombosis

- magnitude of the problem & features of thrombosis in PV-ET patients
- thrombogenesis
- phenotyping coagulation: CAT
- 'microparticles' as biomarkers & players
- intermediate phenotypes:
  - conclusion with limits
- current recommendations & perspectives
Thrombosis in BCR-ABL neg MPN:
magnitude of the problem

20 - 40% patients with thrombosis at diagnosis

incidence during FU: 2 / 100 pt yr
incidence of recurrences: 9 / 100 pt yr

arterial events at least as frequent as venous events, and of greater clinical impact
Thrombosis in BCR-ABL neg MPN: some salient and puzzling features

- both arterial and venous sites
- unusual sites of venous thromboses
Thrombosis in BCR-ABL neg MPN:
some salient and puzzling features

- both arterial and venous sites
- unusual sites of venous thromboses
- inconsistent or no association with hypercytosis
<table>
<thead>
<tr>
<th>risk factor</th>
<th>A thrbs</th>
<th></th>
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<th>V thrbs</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PV</td>
<td>ET</td>
<td></td>
<td>PV</td>
<td>ET</td>
<td></td>
</tr>
<tr>
<td>Hb levels</td>
<td>no</td>
<td>no</td>
<td></td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>platelet count</td>
<td>no</td>
<td>no</td>
<td></td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>leukocytosis</td>
<td>yes</td>
<td>yes</td>
<td></td>
<td>no</td>
<td>no</td>
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</tr>
</tbody>
</table>

Thrombosis in BCR-ABL neg MPN:
some salient and puzzling features

- both arterial and venous sites
- unusual sites of venous thromboses
- inconsistent or no association with hypercytosis
- thrombosis despite normal CBC (latent MPN)
Thrombosis in BCR-ABL neg MPN: some salient and puzzling features

- both arterial and venous sites
- unusual sites of venous thromboses
- inconsistent or no association with hypercytosis
- thrombosis despite normal CBC (latent MPN)
- bleeding risk coexists

- heterogeneity of the thrombotic risk
Thrombosis in BCR-ABL neg MPN:
thrombogenesis
clonal expansion of myeloid cells

increased number of circulating cells

aberrant circulating cells

cell activation
Clinicaly relevant thrombosis in large vessels generally implies fibrin deposits.

Hence activation of coagulation must have occurred.
Coagulation activation

- Hypercoagulability
- TF (initiation)
coagulation activation

- hypercoagulability
- cell activation → initiation
in vivo / in vitro

coagulation activation

hypercoagulability

biomarkers

cell activation → initiation
in vitro

coagulation activation

hypercoagulability

phenotyping

cell activation → initiation
in vitro

thrombin (IIa) generation

phenotyping

challenge of a blood sample under appropriate conditions
in vitro IIa generation
in vitro IIa generation

thrombin (moles/L)

time (min)

TF (initiation)
In vitro IIa generation

Prothrombinase (IIa burst)
in vitro IIa generation

IIa inhibition by AT
in vitro IIa generation

clotting
in vitro IIa generation

fluorogenic substrate of IIa
Calibrated Automated Thrombography
**in vitro** IIa generation

can be studied in the presence of cells:
- platelets
- leucocytes
- endothelial cells (erythrocytes)
in vitro IIa generation

area under the curve = Endogenous Thrombin Potential

ETP
thrombogram parameters

- TTP (Thrombin Time)
- ETP (Extrinsic Thrombin Time)
- LT (Laboratory Thrombin Time)

The graph shows the thrombin concentration over time (in minutes). The peaks represent the time at which thrombin is measured.
PV and ET patients, JAK2 V617F (+)

Tissue Factor 1 pM; phospholipids 4 µM

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**Thrombin generation in platelet-depleted plasma.**

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag time (min)</td>
<td>6.16 ± 1.26</td>
<td>6.95 ± 2.10</td>
</tr>
<tr>
<td>ETP (nM.min)</td>
<td>1241 ± 287*</td>
<td>1457 ± 297</td>
</tr>
<tr>
<td>Peak (nM)</td>
<td>183 ± 61</td>
<td>211 ± 74</td>
</tr>
</tbody>
</table>

Results are presented as mean ± SD for lag time, ETP and peak. *p<0.05 patients vs. controls.

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Perrin *et al* 2011
<table>
<thead>
<tr>
<th></th>
<th>Lag time (min)</th>
<th>ETP (nM*min)</th>
<th>Peak (nM)</th>
<th>Peak time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1.93±0.34</td>
<td>821±118</td>
<td>248±34</td>
<td>3.78±0.47</td>
</tr>
<tr>
<td>ET</td>
<td>1.94±0.33</td>
<td>760 ±177 *</td>
<td>242±54</td>
<td>3.63±0.43</td>
</tr>
<tr>
<td>PV</td>
<td>1.88 ±0.36</td>
<td>725±166 **</td>
<td>235±51</td>
<td>3.53±0.58 *</td>
</tr>
</tbody>
</table>

Marchetti et al 2008
in vitro
thrombin (IIa) generation

phenotyping

ETP is not increased but rather decreased in MPN!

???
in vitro
thrombin (IIa) generation

ETP is not increased but rather decreased in MPN!

=> in the presence of APC?
IIa generation in presence or absence of APC

'normalized'

\[ \text{nAPC}_{\text{sr}} = \Box \text{APC effect} \]
FII, FV, free PS levels: significantly lower in MPN patients / controls

levels of FV and of free PS: significant determinants of the nAPCsr

Marchetti et al 2008
Correlation between nAPCs\(\text{sr}\) and free PS / controls; ET and PV patients
\(R^2=0.32; P<0.001\)

Marchetti et al 2008
FII, FV, free PS levels: significantly lower in MPN patients / controls

levels of FV and of free PS: significant determinants of the nAPCsr plasma neutrophil elastase: inversely correlated to FV and free PS

Marchetti et al 2008
Effect of PMN on IIa generation

Typical curves
PPP: grey tracing; supplemented with PMN (10x10⁹/L): black tracing

MPN pt

added PMN

healthy subject

added PMN

PV and ET patients, JAK2 V617F (+)
Tissue Factor 1 pM; phospholipids 4 μM

Perrin et al 2011
**A**

Comparison of thrombin generation profiles between MPN patients and healthy subjects.

**B**

<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>Lag Time (min)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td>6.08 ± 1.26</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>6.96 ± 2.01</td>
</tr>
<tr>
<td><strong>PMN-RP 10^6/L</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td>5.85 ± 1.27*</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>6.53 ± 1.65*</td>
</tr>
</tbody>
</table>

PCA evaluated by difference PMN-RP – PPP: NS

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</thead>
<tbody>
<tr>
<td><strong>ETP (nM.min)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td>1329 ± 290</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>1486 ± 279</td>
</tr>
<tr>
<td><strong>PMN-RP 10^6/L</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td>1507 ± 313*</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>1668 ± 292*</td>
</tr>
</tbody>
</table>

NS

<table>
<thead>
<tr>
<th></th>
<th>PPP</th>
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<tbody>
<tr>
<td><strong>Peak (nM)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td>195 ± 60</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>221 ± 76</td>
</tr>
<tr>
<td><strong>PMN-RP 10^6/L</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td>234 ± 72*</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>273 ± 81*</td>
</tr>
</tbody>
</table>

NS

Perrin et al 2011
Effect of PMN on IIa generation

Typical curves
PPP: grey tracing; supplemented with PMN (10x10⁹/L): black tracing

MPN pt
healthy subject

The procoagulant effect of added PMN is not different when PMN are prepared from MNP patients or healthy subjects

J Perrin, D Ranta, F Empereur, C Vigneron, P Feugier, T Lecompte. Polymorphonuclear neutrophils from JAK2V617F positive MPD patients do not support hypercoagulability: a study with Calibrated Automated Thrombography (CAT).

Blood Cells Mol. Dis. 2011
Hypercoagulability = IIa generation association with

- JAK2 V617F status and allele burden
Figure 1. ETP-based nAPC$sr$ in controls (C) and ET and PV patients.
ETP-based nAPC$sr$ are shown according to disease type (left panel) and to JAK2$^V617F$ mutational status (right panel). C = controls; Neg = negative; Het = Heterozygous; Hom = Homozygous.
* $=$ $p<0.01$ vs Controls

Marchetti et al 2008
Hypercoagulability = IIa generation

association with

- JAK2 V617F status and allele burden
- other genetic abnormalities
- no studies in CALR mutated patients
microparticles' & MPN
23 PV patients compared with
10 controls (CTR)
and 21 secondary polycythaemia (SP) pts

Tan et al 2013
• prospective study in northern France (GHICL and CHRU Lille): 2011-2013

• consecutive ET patients
• at diagnosis (WHO 2008)
• prior to any cytoreductive therapy

- 45 JAK2-mutated
- 15 CALR-mutated

Charpentier et al ASH 2014
ET patients haematological data

<table>
<thead>
<tr>
<th></th>
<th>CALR+</th>
<th>JAK2+</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet count</strong></td>
<td>861 [677-916]</td>
<td>664 [572-852]</td>
<td>0.04</td>
</tr>
<tr>
<td>(x10^9/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hemoglobin</strong></td>
<td>13.8 [13.2-14.5]</td>
<td>14.3 [14-15.6]</td>
<td>0.04</td>
</tr>
<tr>
<td>(g/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td>41 [40-43]</td>
<td>44 [42-47]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Leukocyte count</strong></td>
<td>8.0 [7.4-9.1]</td>
<td>8.7 [8.1-10.8]</td>
<td>0.06</td>
</tr>
<tr>
<td>(x10^9/L)</td>
<td></td>
<td></td>
<td></td>
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</table>

Charpentier et al. ASH 2014
Plasma MPs phenotype / flow cytometry

- Preanalytical / testing procedures:
  
  ISTH standardization committee

- Platelet-Free Plasma (2 centrifugations 2500g, 15 min)

- FC500 Flow cytometer, Beckman-Coulter™

- all annexin V (+)

- CD41: Platelet-MP, PMP
- CD62P + CD41: P-Selectin
- CD142 + CD41: Tissue Factor
- CD235a: Red cell-MP, RMP
- CD14: Monocyte-MP, MoMP
- CD11b: Granulocyte-MP, GMP
- CD144: Endothelial-MP, EMP

Charpentier et al ASH 2014
<table>
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<th>CALR+</th>
<th>JAK2+</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>total</strong></td>
<td>3300 [1662-4240]</td>
<td>6040 [3830-10650]</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Platelet (&amp;MGKC)</strong></td>
<td>3100 [2068-3887]</td>
<td>5702 [3423-10257]</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>RBC</strong></td>
<td>120 [60-178]</td>
<td>215 [123-262]</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Monocytes</strong></td>
<td>43 [27-52]</td>
<td>75 [22-135]</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Endothelial</strong></td>
<td>99 [32-110]</td>
<td>96 [51-140]</td>
<td>0.5</td>
</tr>
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</table>

Charpentier et al ASH 2014
'microparticles' & coagulation

Correlation of

IIa generation as assessed with Zymuphen MP activity - Hyphen & CAT

with the number of 'microparticles' in blood

Charpentier et al 2014

II generation experiments with CAT performed in Geneva (Aurélien Lebreton & Thomas Lecompte)

Financial support: Dubois Ferrière – Dinu Lipatti foundation
Intermediate laboratory phenotypes:

- Calibrated Automated Thrombography
- circulating microparticles

LIMITS:
- preanalytical & analytical issues
- no clinical validation in well-designed prospective studies
Thrombosis BCR-ABL neg MPN

CONCLUSIONS
Intermediate laboratory phenotypes:

- Calibrated Automated Thrombography
- circulating microparticles

- further investigations warranted
- potential clinical use of simplified tests
Thrombosis BCR-ABL neg MPN / CONCLUSIONS

Rely on current guidelines: See table, clinical parameters
<table>
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<tr>
<th>Risk Factor</th>
<th>A thrbs</th>
<th>V thrbs</th>
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<tbody>
<tr>
<td></td>
<td>PV</td>
<td>ET</td>
</tr>
<tr>
<td>Cardiovasc</td>
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<td>yes</td>
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<tr>
<td>Age &gt; 60y</td>
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<tr>
<td>Prior thrbs</td>
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<td>yes</td>
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<tr>
<td>JAK2 V617F</td>
<td>/</td>
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<tr>
<td>Hb levels</td>
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<td>no</td>
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<tr>
<td>Leukocytosis</td>
<td>yes</td>
<td>yes</td>
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Thrombosis BCR-ABL neg MPN / CONCLUSIONS

Rely on current guidelines:
See table, clinical parameters

Debate on the use of the mutational status:

PV: JAK2 V617F allele burden
ET: JAK2 V617F vs CALR vs others
Thank you for your attention!