Intérêt du dosage des microvésicules dans la thrombose associée au cancer

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Microvesicles

- Extracellular vesicles generated by membrane budding
- Size: 100 – 1000 nm
- Vector of procoagulant activities

Vallier et al. Semin Thromb Haemost 2017
Endogenous MVs accumulate at the site of vessel injury and promote thrombus formation.

Before injury

After injury

Panc02-GFP

Tumeur - GFP

After injury + Mab blocking P-selectin


Role of MVs in thrombus associated with cancer
**Role of cancer MV-TF in VTE**

*Inferior Vena Cava stenosis model*

**Thomas et al, JTH 2015:**
Injection of Panco2 MVs

![Graph showing thrombus formation in control and experimental groups](image)

* TF from tumoral MVs is essential to their VTE-promoting activity

**Geddings. et al, JTH 2016**
Injection of BxPC3 MVs

![Graph showing platelet activation](image)

* TF-from tumoral MVs enhance VTE also activating platelets

**Isada Y, et al, JTH 2017**

*Human pancreatic BxPC3 in nude mice tumors:* TF MVs from tumor increase VTE
<table>
<thead>
<tr>
<th>Patients</th>
<th>Wo VTE / W VTE</th>
<th>Follow-up</th>
<th>MV phenotype</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locally advanced or metastatic pancreatic cancer</td>
<td>11/02</td>
<td>Every 4 weeks for 20 weeks</td>
<td>TF + MV</td>
<td>Functional assay</td>
<td>Khorana, JTH, 2008</td>
</tr>
<tr>
<td>Cancer Wo VTE</td>
<td>60/5</td>
<td>1 year</td>
<td>TF + MV</td>
<td>Impedance FMC</td>
<td>Zwicker, Clin. Cancer Res., 2009</td>
</tr>
<tr>
<td>Cancer Wo VTE</td>
<td>299 (48 pancreatic) / 49 (12 pancreatic)</td>
<td>2 years</td>
<td>TF + MV</td>
<td>Functional assay (chromogenic end point and kinetic)</td>
<td>Thaler, JTH, 2012</td>
</tr>
<tr>
<td>Cancer Wo VTE at study entry</td>
<td>43/5</td>
<td>6 months</td>
<td>Annexin V + MV</td>
<td>FCM</td>
<td>Van Doormaal, Thromb. Haemost., 2012</td>
</tr>
<tr>
<td>Pancreatic Cancer</td>
<td>252/40</td>
<td>10 months</td>
<td>TF + MV</td>
<td>FCM</td>
<td>Hernandez, Thromb. Haemostat., 2013</td>
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<tr>
<td>Cancer Wo VTE at study entry</td>
<td>43/5</td>
<td>6 months</td>
<td>TF + MV</td>
<td>Functional assay</td>
<td>Van Doormaal, Thromb. Haemost., 2012</td>
</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>61/11</td>
<td>7 months</td>
<td>TF + MV</td>
<td>FCM</td>
<td>Sartori, Thromb. Haemost., 2013</td>
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<tr>
<td>Cancer Wo VTE</td>
<td>608 (90 pancreatic) / 40 (10 pancreatic)</td>
<td>180 days</td>
<td>TF + MV</td>
<td>Functional assay (Fibrin generation and FXa generation)</td>
<td>Van Es, Thromb. Res., 2018</td>
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<tr>
<td>Pancreatic cancer</td>
<td>41/12</td>
<td>1 year</td>
<td>TF + MV</td>
<td>Functional assay</td>
<td>Faille, Oncotarget, 2018</td>
</tr>
</tbody>
</table>
Tissue Factor-bearing MPs and the risk of venous thrombosis in cancer patients: A meta-analysis

Chan-juan Cui¹, Guo-jing Wang¹, Shuo Yang¹, Sheng-kai Huang¹, Rui Qiao² & Wei Cui³

OR = 1.76 (1.21-2.56)

Bharthuar 2013
Sartori 2013
Thaler 2012
Bucciarelli 2012
Campello 2011
Zwicker 2009
Overall
Clinical studies synthesis

✓ MVs: potential to predict cancer-associated thrombosis

✓ Study limitations:
  - results driven by pancreatobiliary cancers
  - methodological heterogeneity and lack of standardisation
Limited association between MVs and CAT

Hypothesis

1) Diversity of mechanisms

2) More complex role of MVs in hemostasis

3) Methodological issues
Microvesicles, polyP and thrombosis


FXIIa MV activity

Intrinsic pathway inhibition protects against cancer MV-dependent mortality

Tumor EVs exhibit PolyP

Nickel et al. Blood 2015

Tumor-dependant thrombosis mechanisms

Stark et al ATVB 2018
Nickel et al Blood 2015
Rield et al Blood 2017

Lacroix et al STH in press
Adapted from Mackman et Hisada Blood 2017
Limited association between MVs and CAT

Hypothesis

1) Diversity of mechanisms

2) More complex role of MVs in hemostasis

3) Methodological issues
Procoagulant and profibrinolytic MV activities

MV-plasmin generation capacity


- Endothelial, leukocyte and tumoral MVs are catalytic surfaces supporting plasmin generation
- MV-PGC is dependent on u-PA and t-PA depending on the MV origin
- MVs activate Pg on their own surface but also bound to other surfaces
- MV-PGC is detectable in circulating MVs
LPS-MVs induce clot dissolution \((\text{in vitro})\)

LPS-MVs: whole blood stimulated with LPS (70,000 g pellet, washed x2 HEPES)

Fluorescent thrombus: platelet rich plasma + Alexa 488 plasminogen

\[ \rightarrow \text{LPS-MV induce a thrombus lysis} \]

\( S. \) Cointe unpublished data
A new assay to evaluate microvesicle plasmin generation capacity

Cointe et al., J. Extra. Ves. 2018

• A new immunomagnetic functional assay to measure the plasmin generation capacity of MVs in a robust manner.

• Increased procoagulant and fibrinolytic activities in lung cancer MVs
Limited association between MVs and CAT

Hypothesis

1) Diversity of mechanisms

2) More complex role of MVs in hemostasis

3) Methodological issues
<table>
<thead>
<tr>
<th>Assays</th>
<th>Strategy</th>
<th>Target</th>
<th>Method</th>
<th>Preanalytics</th>
<th>EV commercial kit</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Flow cytometry</td>
<td>Antigenic</td>
<td>PS, TF, TF/TFPI</td>
<td>Immunofluorimetry</td>
<td>PFP</td>
<td>No</td>
<td>Poncelet et al Trasci 2015, Nolan et al Platelets 2017</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Antigenic</td>
<td>Fibrin</td>
<td>Immunofluorimetry</td>
<td>PFP</td>
<td>No</td>
<td>Mege et al Oncotarget 2017</td>
</tr>
<tr>
<td>Scanning confocal microscopy</td>
<td>Antigenic</td>
<td>TF</td>
<td>Immunofluorimetry</td>
<td>PFP / Centrifugation</td>
<td>No</td>
<td>Hisada et al Thromb Res 2017</td>
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<tr>
<td>Calibrated Automated</td>
<td>Functional</td>
<td>PS or TF</td>
<td>Thrombin generation</td>
<td>PFP</td>
<td>Yes</td>
<td>Hemker et al, Pathophysiol. Haemost. Thromb. 2002</td>
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<tr>
<td>Thrombogram</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>STA-Procoag PPL</td>
<td>Functional</td>
<td>PS</td>
<td>Coagulation time</td>
<td>PFP</td>
<td>Yes</td>
<td>Exner et al, Blodd Coagul. Fribinolysis, 2003</td>
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<tr>
<td>Zymuphen-MP ELISA Kit</td>
<td>Combined</td>
<td>PS</td>
<td>Thrombin generation</td>
<td>Immunocapture (PS) on PFP</td>
<td>Yes</td>
<td>Laroche et al Platelets 2017</td>
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<tr>
<td>Zymuphen-MP TF</td>
<td>Combined</td>
<td>TF</td>
<td>FXa generation</td>
<td>Immunocapture (PS) on PFP</td>
<td>Yes</td>
<td>Laroche et al Platelets 2017</td>
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<tr>
<td>Fibrin generation assay</td>
<td>Functional</td>
<td>PS + TF</td>
<td>Fibrin generation</td>
<td>Centrifugation</td>
<td>No</td>
<td>Berckmans et al, Blood, 2011)</td>
</tr>
</tbody>
</table>
Detection of TF-MVs by flow cytometry

Can we really detect TF-MVs by FCM?

BxPC3-derived MVs

TF-decorated beads

TF copies/Mp

Poncelet al. CYTO 2017

Quantification of TF-MVs on clinical samples remains a challenge
Fibrin-bearing microparticles: marker of thrombo-embolic events in pancreatic and colorectal cancers

Diane Mege¹,², Lydie Crescence¹, Mehdi Ouaissi², Igor Sielezneff¹,², Regis Guieu³, Françoise Dignat-George⁴, Christophe Dubois¹ and Laurence Panicot-Dubois¹
Highly sensitive MV-TF assay

Vallier et al. Thromb Res, in revision

- Modified Factor Xa generation assay

Methodological development for new generation of functional tests with better sensitivity/specificity

High specificity for TF

Lower detection limit

Higher sensitivity

WT vs. KO-TF
Objective: Comparison of the sensitivity and the specificity of assays to measure TF-EVs in plasma samples

2019-2021

- Lab registration and detailed description of methods to measure TF-MVs
- Sample preparation and characterization
- Sample shipment
- Sample analysis, data collection and analysis according a scoring strategy integrating the analytical performances (Tripodi et al J Thromb Haemost 2004, 2008)

More information: romaric.lacroix@univ-amu.fr
Conclusion

Microvesicles and Cancer associated thrombosis

Limited association between MVs and CAT

1) Diversity of mechanisms: PopyP-MVs, PDPN-MVs

2) More complex role of MVs in hemostasis: Coagulolytic-balance of MVs

3) Methodological issues: Fibrin-MVs, more sensitive and robust TF-MVs assays
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