Tissue Factor-positive Microparticles in Cancer-associated Thrombosis

Nigel Mackman, Ph.D., FAHA
John C. Parker Distinguished Professor of Medicine
Director of the UNC McAllister Heart Institute
Co-Director of the Thrombosis and Hemostasis Program
I have no disclosures
Goals

- Identify biomarkers that can be used to stratify the risk of VTE in cancer patients.
- To develop safer antithrombotic drugs that reduce the incidence of thrombosis in cancer patients.
Outline

- Background.
- Tumor-derived TF\(^+\) MPs in mouse cancer models.
- Tumor-derived TF\(^+\) MPs and venous thromboembolism (VTE) in cancer patients.
Tissue Factor (TF): The Primary Activator of Blood Coagulation

TF is the only clotting factor not present in blood. It can be encrypted (low activity) or de-encrypted (high activity).

Mackman Thromb Haemost 2007
Microparticles (MPs) were originally defined as small (0.1-1µm) membrane vesicles that are released from activated or apoptotic cells.

More recent studies have shown that MPs are typically 200-300 nm in diameter.

MPs are also referred to as microvesicles (MVs) or extracellular vesicles (EVs) (= any type of vesicle released from a cell).
Functions of Microparticles

- coagulation
- immune response
- inflammation
- cell activation
- angiogenesis
- transport

Diseases with Elevated Levels of Circulating TF+ MPs

- Cancer
- Endotoxemia
- Sickle cell disease
- Liver injury
- Cirrhosis
- Severe influenza A/H1N1 infection

MP TF = biomarker of thrombotic risk

Owens A and Mackman N Circ Res 2011
Risk Factor for Cancer-associated Thrombosis

- **Tumor characteristics**
  - Site: high risk = pancreatic, brain, lung, ovarian, lymphoma, myeloma, kidney, stomach, bone
  - low risk = breast, prostate
  - Stage: localized, metastatic

- **Blood cells**
  - Platelet count
  - Leukocyte count

- **Hemostatic System**
  - Prothrombotic variants
  - Anticoagulant deficiencies

- **Patient characteristics**
  - History of VTE
  - Age
  - Immobilization
  - Obesity

- **Treatment**
  - Chemotherapy
  - Radiotherapy
  - Surgery
  - CVC
  - Hormone therapy
  - Erythropoiesis stimulating agents
  - Anti-angiogenic agents

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Hisada Y, et al JTH 2015
Risk Stratification
# Predictive Model - Khorana Score

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site of Cancer</strong></td>
<td></td>
</tr>
<tr>
<td>Very high risk (stomach, pancreas)</td>
<td>2</td>
</tr>
<tr>
<td>High risk (lung, lymphoma, gynecologic, GU excluding prostate)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Platelet count &gt; 350,000/mm³</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Hgb &lt; 10g/dL or use of ESA</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Leukocyte count &gt; 11,000/mm³</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>BMI &gt; 35</strong></td>
<td>1</td>
</tr>
</tbody>
</table>

Khorana A et al, Blood 2008 (Brain not included)
Predictive Model Validation

Rate of VTE over 2.5 mos (%)

- Development cohort
- Validation cohort

D-dimer and soluble P-selectin have been added - Ay et al Blood 2010

Khorana et al Blood 2008
Prevention and treatment of VTE in cancer patients
Cancer-associated Thrombosis

Cancer patients are have a 4-7 fold higher risk of venous thromboembolism (VTE) than the general population.

~20% of patients with idiopathic VTE have an occult cancer.

Cancer patients treated with anticoagulants have an increased risk of bleeding.
Primary thromboprophylaxis is not routinely recommended in all cancer patients who are treated in the ambulatory setting (except for patients with multiple myeloma who receive thalidomide or lenalidomide in combination with chemo/dexamethasone).

However, some guidelines suggest thromboprophylaxis in some ambulatory cancer patients (with solid tumors) receiving chemotherapy at high VTE risk based on the tumor entity (e.g. pancreatic cancer) or on a high Khorana Score of ≥3.
Possible Mechanisms of VTE in Cancer Patients

Hisada Y et al. JTH 2015
Hypothesis: Tumor-derived TF$^+$ MPs Trigger Venous Thrombosis in Cancer Patients

Diagram:
- Tumor
- Platelet
- Leukocyte
- TF$^+$ MPs
- Valve pocket in a large vein
- Valve
- Fibrin
- Blood
- EC

Legend:
- Gray circle: Tumor
- Yellow circle: Platelet
- Gray and orange circle: Leukocyte
- Green line: EC
- Red lines: Fibrin
- Arrows: Flow of blood
Tumor-derived Microparticles (TMP)

Geddings and Mackman, Blood, 2013
Mouse Studies
Measurement of Thrombosis in the Infrarenal Vena Cava using Color Doppler Ultrasound

Can perform kinetic studies on thrombus formation

Geddings J et al. JTH 2014
Studies with the Human Pancreatic Cell Line BxPc-3

TF+ TMP Enhancement of thrombosis in tumor-bearing mice.

TF+ TMP Enhancement of thrombosis by injection of exogenous TF+ MPs.
Tumor-bearing Mice have Enhanced Thrombosis

Geddings J et al JTH 2015
Exogenous Tumor-derived TF\(^+\) MPs Enhance Thrombosis in the IVC Stenosis Model

Geddings and Mackman unpublished data

57% 57% 57% 71% 100% 100% 100% 100%

14% 57% 57% 42%

Geddings J et al JTH 2015
Do tumor-derived TF+ MPs enhance thrombosis by activating platelets?
Human Pancreatic Tumor-derived TF⁺ MPs Induce Platelet Aggregation in a Thrombin-dependent Manner

BxPc-3 = high TF expressing human pancreatic cancer cell line
L3.6pl = low TF expressing human pancreatic cancer cell line

Geddings J et al JTH 2015
What is the effect of the antiplatelet drug clopidogrel on TF⁺ MP enhancement of thrombosis?
Clopidogrel Reduces TF$^+$ MP Enhancement of Thrombosis in Mice

Geddings J et al JTH 2015
These results suggest that platelet inhibitors may be useful in prevention thrombosis in cancer patients.
Human Studies
Hypothesis

MP TF activity may be a useful biomarker to identify cancer patients at risk for venous thrombosis.

Perform prospective studies.
Levels of MP TF Activity Increased in Pancreatic Cancer Patients before VTE

Khorana A et al JTH 2008
Geddings J and Mackman N Blood 2014
Conclusion

Increased MP TF activity in plasma was associated with VTE and decreased survival in pancreatic cancer patients.

However, there was no association between MP TF activity and VTE or survival in brain, stomach or colorectal cancers.
A Prospective Randomized Multicenter Study of Dalteparin Prophylaxis in High-Risk Ambulatory Cancer Patients

Prophylaxis High-Risk Ambulatory Cancer Study (PHACS) – Drs. C. Francis and A. Khorana.
Study Design

- Prospective, randomized, open-label parallel group study
- Inclusion: starting OP chemotherapy, high risk
- Randomize
  - Standard treatment (no prophylaxis)
  - Dalteparin 5000U/day
- Duration: 12 weeks with 6 month follow-up (1-4 samples/patient)
- Efficacy endpoint: Symptomatic VTE and asymptomatic VTE found by screening US and CT
- Safety endpoint: Bleeding
- Safety and efficacy endpoints adjudicated by blinded committees
- TF and other hemostasis assays
## Patient Characteristics

<table>
<thead>
<tr>
<th>cancer type</th>
<th>number of sample</th>
<th>Incidence of VTE</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>gastric</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pancreatic</td>
<td>39</td>
<td>7</td>
<td>17.95</td>
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<td>GE junction</td>
<td>12</td>
<td>2</td>
<td>16.67</td>
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<tr>
<td>lung</td>
<td>13</td>
<td>3</td>
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<td>breast</td>
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<td>100</td>
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<td>0</td>
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<td>ovarian</td>
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<td>unknown</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td><strong>98</strong></td>
<td><strong>16</strong></td>
<td></td>
</tr>
</tbody>
</table>
Low Risk and High Risk Groups

Khorana score

- >3 UC
- 2 or less

Low risk group - 101 Patients

Positive

- High risk group
  - LMWH
    - 50 patients
  - Placebo
    - 48 patients

Negative
Clinical Results

- Placebo group VTEs were observed in 10/48 (21%) patients and 1/48 (2%) had a major bleed.
- LMWH group VTEs were observed in 6/50 (12%) patients and 7/50 (14%) had a major bleed.
- VTE HR = 0.69, 95% CI 0.23-1.89.
- Bleeding = HR 7.0, 95% CI 1.2-131.6.
- Conclusion - thromboprophylaxis is associated with a non-significant reduction in the risk of VTE and a significant increase of clinically relevant bleeding in this underpowered study.

Khorana A et al submitted
Measurement of Biomarkers of Activation of Coagulation

- MP TF activity (UNC)
- FVIIa-AT (UNC)
- F1+2 (Rochester)
- TAT (UNC)
- D-dimer (Rochester)
MP TF Activity

A

Low risk

High risk

P = 0.0006

Mean 0.18 0.97

B

All high risk patients

P = 0.1395

Mean 0.96 0.97

Khorana A et al in prep
A
Non-pancreatic cancer

$P = 0.2156$

B
Pancreatic cancer

$P < 0.0001$

Khorana A et al in prep.
Conclusion

MP TF activity may be a useful biomarker to identify pancreatic cancer patients at risk for venous thrombosis
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