Thrombotic biomarkers for risk prediction of malignant disease recurrence in patients with early stage breast cancer

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**A full list of the Investigators of the HYPERCAN (HYPERcoagulation in CANcer) Study Group appears in the Appendix 1.

Supplementary material

Enrollment of resected breast cancer patients in the HYPERCAN study

Patients of both genders with a high-risk surgically treated breast cancer (stage I-II-III according to American Joint Committee on Cancer (AJCC) [1]) were enrolled. Inclusion criteria were having resected breast cancer (T2-T4 N0 M0, TX N+ M0), and being a candidate to adjuvant systemic chemotherapy treatment after tumor resection. Exclusion criteria were acute medical illnesses (including recent thrombosis), hospitalization or terminal conditions, use of anticoagulants (therapeutic dose), and life expectancy <3 months. The use of anti-platelet and anti-inflammatory agents was allowed. Patients have been recruited in 8 different Italian oncology centers, as listed in **Appendix 1.**

At enrollment, the following data were recorded: age, gender, tumor type and size, histological subtype, lymph node status, tumor grade, tumor biological characteristics, body mass index (BMI), current medications, performance status, relevant comorbidities, baseline prophylactic use of anticoagulants (any reason different to cancer), and time from surgery. After enrollment, patients were followed-up annually for a maximum of 5 years. At each follow-up visit, clinical information on any anti-tumor treatment and disease recurrence (DR) were recorded. The study protocol has been approved by the local Ethics Committee. Informed written consent is obtained from all subjects for participation in the study. Consent is also obtained for data recording, collection and storage of blood samples, to allow regulatory monitoring, statistical analysis and peer-review presentation and publication of the study results. The ethical conduct of the study is regulated by the last revision of the Helsinki Declaration.

1. Edge SB, Compton CC: The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 17:1471-4, 2010

Supplementary Table 1. Laboratory intra- and inter-assay coefficients of variability (range) for D-dimer, FVIIa-AT complex, F1+2 and Fibrinogen.

	Intra-assay CV	Inter-assay CV
D-dimer (ng/ml)	2 - 8.1%	6.5 - 10.5%
FVIIa-AT (pM)	2 - 6%	4.5 - 7%
F 1+2 (pmol/l)	3 - 4.2%	4 - 7%
Fibrinogen (mg/dl)	3.5 - 5.4%	4.7 - 6.8%

Supplementary figure 1. Remark diagram of patient included in the analysis. HER2: human epidermal growth factor receptor 2; ER: estrogen receptor; PGR: progesterone receptor.
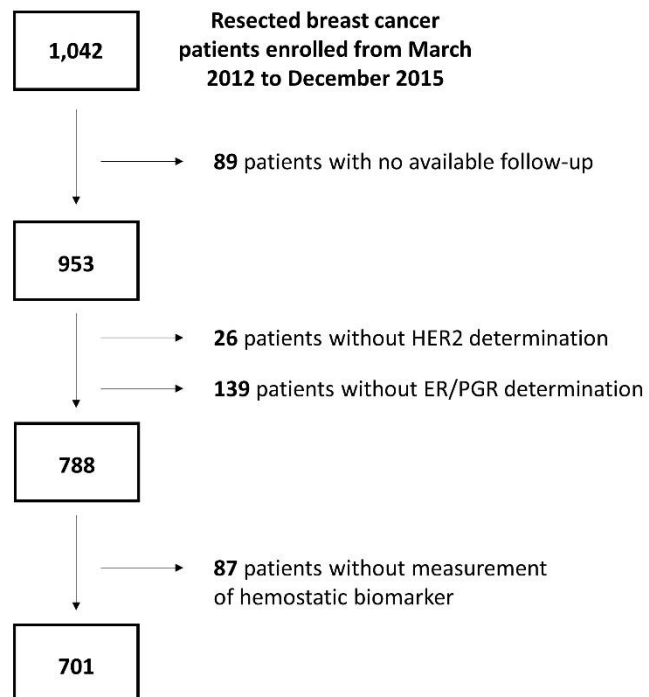
Supplementary figure 2. ROC curve of the score as a continuous variable for prediction of disease recurrence. AUC: area under the curve.