Plasma Kisspeptin: A Potential Biomarker of Tumor Metastasis in Patients with Ovarian Carcinoma

To the Editor:

More than 15,000 women in the US die from ovarian carcinoma annually (1). The most important determinant of survival in ovarian carcinoma cases is tumor stage. Most patients presenting with disease confined to the ovaries (stage 1) can be cured, with a 5-year survival rate of >90% (1). Spread of the tumor outside the ovaries (stages 2 to 4) confers a much poorer prognosis, however. Cure is uncommon in patients with tumor spread into the pelvis (stage 2), the abdomen (stage 3), or the liver/extra-abdominal region (stage 4), with a 5-year survival rate as low as 22% (1). It is therefore critically important to develop novel biochemical markers of ovarian carcinoma to stratify patients according to prognosis. Serum cancer antigen 125 (CA125) is a useful marker for screening and monitoring disease response but cannot be reliably used for staging, because 20% of tumors do not secrete CA125 (2). There is currently no diagnostic marker of tumor spread in patients with diagnosed ovarian carcinoma.

Kisspeptin, a peptide that activates the kisspeptin receptor, is encoded by the KISS1 (KiSS-1 metastasis-suppressor) gene. KISS1 expression negatively correlates with metastasis risk in lung, pancreatic, esophago-gastrointestinal, endometrial, renal cell, and bladder carcinomas (3). In ovarian carcinoma, low KISS1 expression is associated with aggressive disease and a poor prognosis, and KISS1 overexpression inhibits cell migration and reduces metastasis (4). Kisspeptin is therefore a putative metastasis suppressor in ovarian carcinoma. We compared plasma kisspeptin concentrations in patients with stage 1 ovarian carcinoma and patients with disease in stages 2 to 4 to determine its utility as a novel biomarker of tumor metastasis in ovarian carcinoma.

After obtaining ethics approval (reference: 04/Q0406/80) and informed consent, we studied 31 patients with ovarian carcinoma and 31 healthy volunteers without known cancer. Blood was collected into a tube coated with lithium heparin and containing 5000 kallikrein inhibitor units (0.2 mL) of aprotinin and centrifuged for 4 min at 1538 g. The separated plasma was stored at −20 °C. Plasma kisspeptin immunoreactivity was measured with a previously described RIA (5). The limit of detection was 2 pmol/L, and the intra- and inter-assay CVs at 20 pmol/L were 8.3% and 10%, respectively. CA125 was measured with a 2-step ARCHITECT immunoassay (Abbott Diagnostics), with a 1-kU/L analytical sensitivity and intra- and inter-assay CVs between 40–170 kU/L of <5%. Data are expressed as the mean (SD) unless stated otherwise. Multiple means were compared with one-way ANOVA and the Tukey multiple-comparison test.

Nine patients had stage 1 ovarian carcinoma, with a median plasma kisspeptin concentration of 17.4 pmol/L (range, 10.1–54.2 pmol/L; interquartile range, 12.9–36.1 pmol/L). Twenty-three patients had ovarian carcinoma of stages 2 to 4, with a median plasma kisspeptin concentration of 7.8 pmol/L (range, <2–41.2 pmol/L; interquartile range, 5.7–17.0 pmol/L). The median plasma kisspeptin concentration for the controls was 11.4 pmol/L (range, <2–29.9; interquartile range, 8.4–17.9 pmol/L).

The plasma kisspeptin concentration appeared dependent on ovarian carcinoma stage (Fig. 1). The mean kisspeptin concentration in the patients with stage 1 ovarian carcinoma was increased significantly, compared with the patients with ovarian carcinoma of stages 2 to 4 and with the controls [25.1 (15.2) pmol/L (stage 1), 11.8 (10.3) pmol/L (stages 2 to 4), and 13.1 (6.92) pmol/L (controls); P < 0.05, stage 1 vs stages 2 to 4; P < 0.01, stage 1 vs controls]. A plasma kisspeptin concentration <20 pmol/L had a 91.3% sensitivity (95% CI, 72%–99%) and a 44.4% specificity (95% CI, 14%–79%) with respect to patients with ovarian carcinoma in stages 2 to 4. A plasma kisspeptin concentration <10 pmol/L has a lower sensitivity (65.2%; 95% CI, 39%–80%) but a 100% specificity (95% CI, 66%–100%) with respect to patients with ovarian carcinoma in stages 2 to 4. By comparison, a serum CA125 concentration >1000 kU/mL had a 26.1% sensitivity (identified 6 of 23 patients with disease in stages 2 to 4) and a 100% specificity (excluded 9 of 9 patients with stage 1 disease) with respect to ovarian carcinoma in stages 2 to 4 (Fig. 1). A ROC curve analysis of plasma kisspeptin in patients with disease in stages 2 to 4 vs patients with stage 1 ovarian carcinoma yielded an area under the curve of 0.80 (95% CI, 0.65–0.96; P < 0.01).

Multiple lines of evidence implicate kisspeptin as a metastasis inhibitor in ovarian tumors, but plasma kisspeptin concentrations in patients with ovarian carcinoma have not been reported previously. This small study suggests that patients with stage 1 ovarian carcinoma have significantly increased concentrations of plasma kisspeptin compared with patients with ovarian carcinoma in stages 2 to 4 or compared with healthy controls. Furthermore, plasma kisspeptin may be more sensitive for detecting disease in stages 2 to 4.

Clinical Chemistry 58:6
1061–1066 (2012)

Letters to the Editor
It is interesting to speculate whether the observed relationship between plasma kisspeptin and tumor stage is causative. In accordance with previous studies suggesting that KISS1 expression inhibits ovarian tumor spread (4), an increased plasma kisspeptin concentration may signify a low intrinsic metastatic capacity; however, an increased plasma kisspeptin concentration during stage 1 ovarian carcinoma may merely be a consequence of early tumorigenesis.

Our pilot study suggests that plasma kisspeptin could provide a novel method to aid ovarian cancer staging. We observed that most cases of ovarian carcinoma metastasis had plasma kisspeptin concentrations <10 pmol/L (91.3% sensitivity), and no cases of curative disease had a plasma kisspeptin concentration >10 pmol/L. Larger studies are required to further examine the relationship between plasma kisspeptin and other biomarkers, such as CA125 and HE4, with regard to tumor spread in patients with ovarian carcinoma.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

**Authors’ Disclosures or Potential Conflicts of Interest:** Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflict of interest:

**Employment or Leadership:** None declared.

**Consultant or Advisory Role:** None declared.

**Stock Ownership:** None declared.

**Honoraria:** None declared.

**Research Funding:** The Section of Investigative Medicine is funded by grants from the Medical Research Council, the Biotechnology and Biological Sciences Research Council (BBSRC), and the National Institute of Health Research (NIHR), by an Integrative Mammalian Biology (IMB) Capacity Building Award, by an FP7-HEALTH-2009–241592 EuroCHIP grant, and is supported by the NIHR Imperial Biomedical Research Centre Funding Scheme. C.N. Jayasena, NIHR Clinical Lectureship, Academy of Medical Sciences/Wellcome Starter Grant for Clinical Lecturers, and Society for Endocrinology/Early Career Grant; A.N. Comninos, Imperial College Healthcare NHS Charity Fellowship and Wellcome/GlaxoSmithKline Translational Research Fellowship; W.S. Dhillon, NIHR Career Development Fellowship.

**Expert Testimony:** None declared.
Survival of Patients after ST-Elevation Myocardial Infarction: External Validation of a Predictive Biomarker Model

To the Editor:

Early risk stratification has the potential to play an important role in ST-elevation myocardial infarction (STEMI) patients who are to be treated with primary percutaneous coronary intervention (PCI). Several risk scores have been developed for STEMI patients; however, most risk scores require many variables, making them more difficult to use in clinical practice. The long-term prognostic value of biomarker measurements for glucose, N-terminal pro–brain type natriuretic peptide (NT-proBNP), and estimated glomerular filtration rate (eGFR) taken early after admission has recently been demonstrated for STEMI patients (1). Damman and coworkers have shown that a multimarker model including these biomarkers improved the prediction of mortality over that provided by established risk factors derived from the Thrombolysis In Myocardial Infarction (TIMI) score, which include age, body mass index, diabetes, hypertension, systolic blood pressure, heart rate, anterior myocardial infarction, and time to treatment (1, 2). Moreover, a simplified risk score developed with the 3 biomarkers identified low-, intermediate- and high-risk subgroups with respect to mortality. The best way to evaluate such a model is to perform an external validation study of the predictors in a new and independent STEMI cohort (3).

To assess the general validity of this multimarker model and the simplified risk score, we evaluated both in an external STEMI cohort treated with primary PCI in a large single center in Groningen, the Netherlands. We analyzed the data of all 1645 consecutive STEMI patients treated with primary PCI from 2006 to 2010. A STEMI cohort of 1321 patients was defined according to the criteria of the original cohort (1). The baseline characteristics of the validation cohort and the original cohort were generally comparable. Blood samples for the biomarker measurements were routinely obtained at admission before the primary PCI. The diagnostic methods used were all identical to the systems used for the original cohort (1). The primary end point of the study was all-cause mortality. These data were collected from the municipal civil registry, which has complete information regarding the vital status of all residents registered in the Netherlands. Statistical analysis was performed with IBM SPSS Statistics (version 18.0) and R software.

In the multimarker study, the discriminative value was estimated for the multimarker model with only established risk factors and for the multimarker model with the glucose, NT-proBNP, and eGFR biomarkers in addition to established risk factors. Discriminative value was measured with the Harrell C index, the categoryless reclassification improvement (NRI), and integrated discrimination improvement (IDI). These indices were calculated for patients with a complete follow-up at 2 years. These analyses have previously been described in detail (1). In the validation cohort, the ability to predict mortality was high for the model with only established risk factors (Harrell C index, 0.855). The discriminative power increased for the model with all 3 biomarkers added [Harrell C index, 0.872; NRI, 0.25 (P < 0.05); IDI, 0.04 (P < 0.01)].

For the simplified risk score, points were assigned on the basis of the hazard ratio coefficients of the original cohort: 2 points for a glucose value of 144–162 mg/dL (8–9 mmol/L), an NT-proBNP value of 150–599 ng/L, or an eGFR value of 60–89 mL/min; 3 points for a glucose value ≥180 mg/dL (≥10 mmol/L) or an NT-proBNP value ≥600 ng/L; and 4 points for an eGFR value <60 mL/min. The total point score classifies patients into low-risk (≤4 points), intermediate-risk (5 or 6 points), and high-risk (≥7 points) subgroups. All-cause mortality for the risk subgroups was estimated with the Kaplan–Meier method, and the groups were compared with the log-rank test. In the validation study, 103 patients died at a median follow-up of 2.1 years. Using the simplified risk score, we classified 830 patients (63%) within the