Author's response to reviews

Title: The effect of menopause and hysterectomy on Systemic Vascular Endothelial Growth Factor in women undergoing surgery for breast cancer.

Authors:

Aoife J Lowery (aoife.lowery@gmail.com)
Karl J Sweeney (karlj.sweeney@hse.ie)
Alan P Molloy (alanpmolloy@yahoo.com)
Emer Hennessy (emer.hennessy@nuigalway.ie)
Catherine Curran (Catherine.Curran@hse.ie)
Michael J Kerin (michael.kerin@nuigalway.ie)

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Author's response to reviews: see over
Dear Dr. Alam,

Many thanks for reviewing the above manuscript and for the suggestions of the reviewers. We have addressed the issues raised by the reviewers and have made the necessary changes to the above manuscript. The following is a point by point response to the concerns as requested.

**Reviewer: Ugur Coskun**

1. The tables have been amended to more clearly show the results and define the groups: **Table 1** (Tumor Characteristics) refers only to the breast cancer patients and they have been clearly divided into pre-menopausal and post-menopausal breast cancer patients with (n) numbers included.
Table 2 has been renamed “Serum Steroid Hormone and VEGF levels”. The groups are now clearly defined and presented as control-premenopausal, control-postmenopausal, breast cancer premenopausal, breast cancer postmenopausal with (n) numbers included.

The data on VEGF levels in patients with intact uterus compared to those who have had a previous hysterectomy all relates to postmenopausal patients. This data is represented in figure format for both controls (figure 1) and breast cancer patients (figure 2a & 2b). We feel that graphical representation suits this data better than tabular format. The (n) numbers have been included in the figure legends.

2. In this study we have found that postmenopausal women with an intact uterus actually have lower systemic VEGF levels than those who have had a previous hysterectomy. This finding is somewhat unexpected as the uterus is a major VEGF source in premenopausal women, however the role of the postmenopausal uterus in the regulation of VEGF levels has not been fully investigated to date; in view of the conflicting data on VEGF levels in postmenopausal patients and in relation to the uterus, we feel that this warrants further investigation and hope that this report will stimulate this. The papers investigating VEGF levels in breast cancer have been discussed in the discussion section as suggested by the reviewer and are referenced as numbers (19) Byrne et al Anticancer Res. 2007 Sep-Oct: 27 (5B):3481-7.
(27) Sancak et al Internal medicine journal. 2004;34:310-315
The effect of tamoxifen on vascular endothelial growth factor has also been referred to in the discussion using the reference (32) Coskun U et al. Effect of tamoxifen on serum IL-18, vascular endothelial growth factor and nitric oxide activities in breast carcinoma patients. Clinical and experimental immunology. 2004;137:546-551. This particular reference was chosen as this study included consideration of the endometrial thickness in relation to the effect of tamoxifen on VEGF levels.
Reviewer: N Bundred

1. The publications relating to serum and uterine VEGF levels mentioned by the reviewer have been added and referenced in the introduction and the discussion. Reference numbers 19 & 31. In the introduction, the previous statement “There is little data on serum or uterine VEGF levels in postmenopausal women” has been amended as per the reviewers comment that there are many publications in this area. The point is made that studies to date on VEGF levels in postmenopausal women have had conflicting results. Lines 20-24 page 4.

2. The commercial VEGF assay used (Quantikine; R&D System, Minneapolis, MN, USA). The minimal detectable dose of VEGF was 9.0pg/ml and maximum dose was 2000pg/ml. For serum samples the intra-assay variation of the assay kit used was 4.5-6.7% and the inter-assay variation was 6.2-8.8%. Paired samples were measured on the same plates. This information has been included in the Methods section lines 14-17 page 7.

3. In this series of patients there was no significant difference in VEGF levels between patients with in-situ carcinoma and those with invasive carcinoma. The sVEGFp level of the patient with low grade DCIS was 1.03 pg/10^6 and was not an outlier when compared to the other samples. In addition, non-parametric statistical tests were used to analyse the data, this should account for any outliers or heterogeneity within the groups.

4. All the patients in this series who had a hysterectomy were postmenopausal as established from their ovarian steroid hormone levels (table 2). The comparison of sVEGFp in patients and controls with an intact uterus versus previous hysterectomy was performed in postmenopausal cohort only.

5. The finding that there was no association between serum VEGF/ serum VEGFp and clinicopathological features of breast cancer is in concordance
with previous reports (Byrne et al, Anticancer Res 2007), and certainly implies that the use of serum VEGF as a tumour marker in breast cancer is limited due to the differing origins of circulation VEGF between pre-and post-menopausal women. However, the presence of high levels of circulating VEGF at the time of diagnosis and indeed at the time of surgery can increase vascular permeability and potentially contribute to tumour cell extravasation and metastasis formation. The emergence of anti-VEGF therapy as a potential adjuvant treatment for patients with breast cancer and other solid tumours is testament to the importance of this cytokine in the pathogenesis and progression of malignancy. We suggest in the discussion that large cohort study addressing the effect of previous hysterectomy on prognosis in breast cancer patients will identify the oncologic significance of our findings and that women who have had a previous hysterectomy may benefit from more rigorous surveillance programmes if they are found to be at increased risk of breast cancer progression.

We hope that these revisions are in keeping with the reviewers’ suggestions and are resubmitting this revised manuscript to be considered for publication in BMC Cancer. We feel that this study will be of interest to your readership and has a high value in future investigation of the significance of VEGF in breast cancer.

Sincerely,

Professor Michael Kerin
Department of Surgery
Clinical Science Institute
University College Hospital
National University of Ireland Galway
Ireland
e-mail: michael.kerin@nuigalway.ie
Tel: + 353 91 524390
Fax: + 353 91 494509