Reviewer's report

Title: Analysis of and prognostic information from disseminated tumour cells in bone marrow in primary breast cancer. Report of a prospective observational study

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Reviewer: Vassilis Georgoulias

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Falck et al investigated the presence of DTCs in 401 patients with primary breast cancer and analyzed the prognostic implications of DTCs in bone marrow at the time of diagnosis. An additional aim was to stratify the patients according to lymph node status (positive, negative). They performed an immunofluorescence (IF) staining procedure in 327 patients and an immunocytochemistry (IC) technique in 74 patients. They used the breast cancer cell line MCF7 spiked into blood from healthy volunteers as a positive control for CK immunostaining and bone marrow aspirates from 76 healthy individuals. They detected cytokeratine (CK) positive cells in 152 (38%) out of 401 breast cancer patients and in 19 (25%) out of 76 healthy donors. They found that the detection of DTCs in bone marrow of patients with primary breast cancer was not related to either Distant Disease Free Survival (DDFS) or Breast Cancer Specific Survival (BCSS). Furthermore they showed that the presence of DTCs in the lymph node positive or negative group of patients had no statistically significant effect on DDFS.

Major Revisions

1) The authors describe an immunofluorescence assay for DTCs based on an antibody which can detect a wide range of cytokeratins. Moreover, they use another antibody for the ICH assay. There are several methodological problems which require clarifications before starting evaluate the clinical value of the detected cells in the cohort of patients: what is the the sensitivity and the specificity of the assay?? did the authors performed preliminary experiments using a breast cancer cell line spiked into normal bone marrow cells? did the authors performed experiments using different numbers of bone marrow mononuclear cells in order to define the optimum conditions for the preparation of cytospins? Did the authors compared the two different antibodies and the two different assays in both CK-positive and CK-negative samples as well as in MCF-7 cells spiked into normal bone marrow cells? This is extremely important if the data should be pooled for the final analysis. The high frequency of CK-positive cells in normal donors raises several questions concerning the specificity of the assay and limits the validity of the prognostication value of DTCs. Therefore, the authors have to demonstrate that the cells that they characterize as DTCs are really micrometastatic cells.

1) The data of the present study were mainly obtained an IF assay which detected a high frequency (25%) of CK positive events in healthy donor samples.
Moreover, in the background, the authors reported that comparisons between the different detection methods, IF and IC are not yet available. Krag et al. 2005 (Annals of Surgical Oncology, 12(9):753-760), attempted to validate the IF and IC methods used in several clinical studies. They observed a high rate of false-positive CK events in bone marrow samples from breast cancer patients and concluded that IF using markers for hematopoietic cells (HC) (e.g. CD45) allows the discrimination between CK positive cells of hematopoietic and non-hematopoietic origin. The related part of the background page 5 based on this publication should be appropriately modified. In addition, the high frequency of CK-positive cells in normal donors limits the validity of the prognostic value of DTCs.

2) Absence of CK positive DTCs in the Figure 2: according to Fehm et al. 2008, (Breast Cancer Research, 10:R76) a representative DTC of a patient with breast cancer has a high nuclear to cytoplasmic ratio, irregularities in the nucleus and CK stains the cytoplasm at the periphery of the cell causing a ring-like appearance. The authors have to present what they characterize as CK positive DTCs from patients.

3) In the material and methods, the authors reported that they used the breast cancer cell line MCF7 spiked into blood from healthy volunteers as a positive control for CK immunostaining. The authors have to present an image of MCF-7 spiked to normal PBMCs.

4) The 3 tables that the authors describe in the “Results” section are not included in the manuscript.

5) In the discussion, the authors reported that they also tested higher cut-offs for defining DTC positive events. These data should be presented in detail. In addition, the authors have to discuss in more details their findings which are in discordance with those of the literature (is this due to the used assay??)

Minor Revisions

1) Page 4, last paragraph, line 4: one of ‘node negative’ may be ‘node-positive’.

2) Page 5: The new AJCC classification have introduced the detection of micrometastatic cells either in the peripheral blood, the bone marrow or the lymph nodes as defining the M stage.

3) Page 12, last paragraph, line 1: ‘the bone marrow from adult healthy donors using both methods’ may be ‘the bone marrow from adult healthy donors was analyzed using both methods’.

4) Page 13, first paragraph, line 14 ‘and the data is mainly derived’ may be ‘and the data are mainly derived’.

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a
statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'