transcribed blood miRNA samples by RQ-PCR. Advanced *QBase Plus* software and SPSS were used for biostatistical analysis of the data.
Results: Whole blood samples were preferable to serum and plasma for greater detection and relative quantification of circulating miRNAs. Of 7 breast cancer specific miRNAs investigated, across 130 whole blood samples (106 preoperative samples from breast cancer patients and 24 from healthy age matched female controls), the levels of two miRNAs were found to be significantly higher in blood from breast cancer patients compared to controls ($p < 0.001$). Increased systemic miRNA levels in breast cancer patients are also reflected in breast tumours. We also report significant associations between circulating miRNAs and the clinicopathologic variables ER and HER2/*neu* receptor status, and nodal status.
Conclusion: This study is the first to demonstrate that miRNAs are detectable and in the circulation of breast cancer patients, compared to controls. The circulating miRNA expression profile correlates with the tumour miRNA expression profile and with various biopathologic parameters of breast cancer. In this, lies the expectation that circulating miRNAs will be clinically useful as a novel minimally invasive tool to aid in the early diagnosis and monitoring of breast cancer.

1307 POSTER

CXCL12/SDF1 expression by breast cancers is an independent prognostic marker of progression

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Purpose: The cytokine CXCL12 is synthesized by metastasis target tissues and has been shown to attract tumor cells that express the receptor, CXCR4. However, epigenetic silencing of CXCL12 has recently been reported to increase the metastatic potential of breast cancer cells and the reintroduction of the cytokine gene into MDA-MB-231 breast carcinoma cells decreases the number of metastases formed *in vivo*. We therefore wished to know whether CXCL12 expression correlates with relapse free and overall survival in human breast cancer patients.
Experimental Design: CXCL12 and CXCR4 expression was analyzed in one hundred archival breast cancer samples by immunohistochemistry and in two breast cancer microarray datasets of 408 cases. Data were analyzed by uni- and multivariate COX regression analysis.
Results: CXCL12 and CXCR4 are expressed by epithelial tumor cells and by stromal and endothelial cells. Microarray gene expression analysis and immunohistochemistry revealed that CXCL12 but not CXCR4 expression significantly correlates with disease free and overall survival in estrogen receptor positive and negative cancers. Expression of the estrogen receptor α and CXCL12 does not correlate.
Conclusions: CXCL12 is a strong, independent prognostic marker. We propose that saturation of the receptor through autocrine CXCL12 production reduces chemotaxis towards CXCL12 releasing metastasis target tissues.

1308 POSTER

Evaluating the prognostic role of serum extracellular domain (ECD) of HER-2/neu (s-HER2) in patients (PTS) with metastatic (M) breast cancer (BC): results of an observational study

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Background: BC is the leading cause of cancer-related death affecting women worldwide. Since the last decades, many efforts have been made to identify different subsets of BCs on the basis of molecular markers that might help in defining the prognosis. HER2/neu belongs to a family of 4 transmembrane tyrosine kinase (TK) cell membrane receptors mediating cell growth, differentiation and survival, its overexpression occurring in about 20%-30% of BCs and being associated with a more aggressive tumour behaviour and a poor prognosis. The ECD can be cleaved from the cellular surface and measured in the peripheral blood. A potential prognostic and predictive role of elevated s-HER-2 levels has been previously reported.
Material and Methods: we prospectively measured the s-HER2 levels of consecutive MBC pts [ELISA immunometric test (cut-off 13 ng/ml)], in order to investigate the possible correlation with the clinical situation. The assessment was done at the first visit (bs-HER2) and then every 3 months. All pts gave their written informed consent.
Results: 46 consecutive MBC pts have been enrolled (median age 63 ys (35−85) so far; ER+ 30 pts (65%); PgR+ 21 pts (46%); IHC evaluation of HER2 was available in 37 pts [16 pts (35%) positive (IHC3+ or IHC2+ FISH+), 21 pts (46%) negative, 9 pts (19%) unknown]. All pts were treated with standard hormone (8 pts) and/or chemotherapy (28 pts), including antracyclines and taxanes. Trastuzumab was given as needed (13 pts).
A total of 325 samples have been tested (mean = 7/pt). Elevated bs-HER2 were found in 27 (59%) pts, of which 12 HER2 positive and 12 HER2 negative; median ECD values were significantly higher in pts with HER2 positive ($P < 0.0001$).
A significant correlation was found between elevated bs-HER2 and number of M sites ($p < 0.05$), as well as between high ECD levels and visceral/nodal disease ($p < 0.05$). Noteworthy, a significant correlation has been found between the increase or the decrease of ECD values and PD or non-PD respectively ($p = 0.0001$). Moreover, with regard to OS, a statistically significant difference, regardless of HER2 status, has been observed between pts with elevated and lower ECD levels (log-rank test $p = 0.003$).
Conclusions: Our data seem to suggest that serum HER2 levels might be used as a "real-time" non invasive marker, allowing repeated evaluations of HER2 status during the whole disease history, with possible future therapeutic implications.

1309 POSTER

Invasive lobular carcinoma: Preliminary study of the efficacy of a blood based gene expression test for early detection of breast cancer

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Background: Mammography has limited efficacy for the detection of breast cancer of lobular origin. Invasive lobular carcinoma is particularly difficult to detect since this cancer type shows diffuse infiltration and often presents on mammography as a subtle distortion rather a solid mass. In addition, this cancer type is rarely associated with microcalcifications which are characteristic signs of malignancies. Due to the later detection of lobular cancers the likelihood of surviving lobular cancer is less than that seen for ductal cancer.
We have developed a blood based gene expression test for the detection of breast cancer using samples from women with ductal carcinoma due to the higher prevalence of this cancer type. We have previously reported detection of ductal carcinoma with accuracies ranging from 75−82% [1−3]. Here we report the findings from a preliminary study using lobular cancers and healthy controls (without mammographic findings) with a blood based gene expression signature developed from ductal breast cancer.
Materials and Methods: A 96-assay format gene expression signature developed from samples from 294 women was used to establish a PLS classifier. The resultant signature was used with blood samples from women with lobular cancer and healthy controls from a European/US cohort. Whole blood was collected in PAXgene™ tubes and shipped on dry ice to a central laboratory. RNA extraction was performed using PAXgene™ Blood RNA kit according to the manufacturer's instructions.
Results: Of the 14 lobular cases, 11 were correctly classified as cancer. Of the 14 healthy controls, 11 of 14 were correctly classified. These results are consistent with the accuracy of the test for ductal tumours.

	Mean age (y)	Lesion diameter (mm)	Correctly predicted
Stage 1 N = 10	65	8−20	8/10
Stage 2 N = 4	69	15−50	3/4
Healthy controls	51	NA	11/14

Conclusions: The present study provides an indication that lobular cancers are detected by the blood based gene expression test with implication that lobular and ductal cancers elicit a common response in whole blood which is recognised by the test.
The study suggests the test has a potential for detecting a form of cancer which is difficult to detect with normal mammographic procedures. Additional cases of lobular cancer are required to support these preliminary findings.

References

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