q 3 wks (FLN regimen). Seventy-six patients received 5-fluorouracil 200 mg/m2/die as intravenous continuous infusion, vinorelbine 20 mg i.v. as total dose on days1 and 3, plus cisplatin 60mg/m2 i.v. on day 1 q 3 wks (ViFuP regimen).

Results: The overall response rate (partial and complete remissions) according to radiological and clinical evaluation was 65% (95% C.I: 57%-72%.) In both the univariate and multivariate analyses the most important predictive factor was the baseline absence of expression of PgR ($p<0.01$), high baseline Ki-67 ($p<0.01$) and decrease of p53 positive cells ($p<0.01$). Conversely no significant effect according to other histological features was observed.

Conclusion: Our results indicated that baseline PgR and Ki-67 expression as well as changes during therapy of p53, should be considered in further studies on preoperative chemotherapy.

656 POSTER

Effect of elevated serum carboxyterminal telopeptide (ICTP) on survival in breast cancer patients with and without bone metastases

S.M. Ali[1,2], L. Demers[1], K. Leitzel[1], V. Chinchilli[1], L. Engle[1], L. Costa[3], J. Risteli[4], A. Lipton[1]. [1] *Penn State University, Hematology-Oncology, Hershey, PA, USA;* [2] *VA Medical Center, Hematology-Oncology, Lebanon, PA, USA;* [3] *Hospital Santa Maria, Hematology-Oncology, Lisbon, Portugal;* [4] *Oulu University Hospital, Clinical Chemistry, Oulu, Finland*

Purpose: Bone is made of Type I collagen fibrils that are crosslinked e.g. by pyridinium cross-links. Bone resorption releases the cross-links as free and peptide bound (telopeptide) fragments. There are 2 assays available for the carboxy-terminal telopeptide fragments, ICTP and Crosslaps. ICTP elevations have also been seen in non-bony disease and found to be prognostic in ovarian cancer patients. We evaluated the prognostic value of serum ICTP levels in patients without bone metastasis (BM-) and with bone metastasis (BM+).

Methods: ICTP levels were quantified in serum using an ICTP RIA produced by Orion Diagnostica (Espoo, Finland) and distributed in USA by Diasorin, Inc. (Stillwater, Minn). This was a retrospective study and used baseline serum that was obtained from 253 patients with metastatic breast cancer. These patients had participated in a double-blind randomized study of second-line hormonal therapy with a second-generation aromatase inhibitor (Fadrozole) vs. Megace.

Results: The mean followup for survivors was 868 days (range 264 - 1466). Normal serum ICTP level from a published report of 202 healthy postmenopausal women was 3.0 + 1.6 ug/L (mean + SD). The serum ICTP cutoff of 6.2 ug/L was established using the mean + 2 SD. Using this cutoff, 62/181 patients (34%) in the BM+ group had elevated serum ICTP levels as compared to 6/72 (8%) in the BM- group. Mean serum ICTP for the BM+ group [6 ug/L (1.7 - 24.6)] was significantly higher compared to the BM- group [4.2 ug/L (1.6 - 16.1)]($p= 0.0002$). Overall survival was significantly worse in patients who had elevated baseline serum ICTP levels in both the BM- ($p=0.02$) and BM+ groups ($p<0.0001$).

Conclusion: In summary, an elevated serum ICTP level predicts for decreased survival in breast cancer patients with and without clinically-detectable bone metastasis.

657 POSTER

KI67 and p53 are not predictive of tumor response or survival with trastuzumab-based therapy in metastatic breast cancer patients (pts) with her2/neu-overexpression

A. Schneeweiss[1], F. Lenz[1], F. Beldermann[1], M. Geberth[1], R. Goerner[1], P. Sinn[2], H.-J. Strittmatter[1], G. Bastert[1]. [1] *University of Heidelberg, Gynecology and Obstetrics, Heidelberg, Germany;* [2] *University of Heidelberg, Pathology, Heidelberg, Germany*

From 2/99 to 3/01, 53 pts with metastatic breast cancer overexpressing HER2/neu were treated with trastuzumab alone (25 pts, group A) or in combination with chemotherapy (28 pts, group B). 3+ -positive HER2/neu overexpression was determined with immunohistochemical staining using antibodies (Ab) 3b5 or A0485. In group B, 19 pts received docetaxel, 5 pts paclitaxel, 2 pts vinorelbine, and 2 pts mitomycin/5-fluorouracil. Pts have been treated either as first-line therapy (17 pts), or as second-line (26 pts) or as third line (10 pts). Using immunohistochemistry staining on primary tumor sections, 23 of 40 pts (58%) showed equal or more than 35% KI67 positive cells (Ab MIB-1) and 17 pts showed equal or more than 50% p53 positive cells (43%, Ab DO7). KI67 and p53 expression in tumor cells were well balanced between groups A and B. To evaluate the predictive value of KI67 and p53 for response, chi-square tests were performed. The predictive value for survival was determined by univariate analysis (log-rank test). The overall response rate (ORR = CR + PR) and the clinical benefit rate (CBR = CR + PR + SD > 24 weeks) were 36% and 60% (group A: 48% and 76%; group B: 25% and 46%), respectively. After a median follow-up of 35 weeks (w) (range, 3 -93 w) the median progression-free survival (PFS) and overall survival (OS) were 25+ w (range, 2-93 w) and 35+ w (range, 3 -93 w) so far. Pts with high or low levels of KI67 or p53 positive tumor cells showed no difference in response rates (ORR: pKI67=0.84, pp53=0.21; CBR: pKI67=0.69, pp53=0.55) and survival rates (PFS: pKI67=0.72, pp53=0.79; OS: pKI67=0.58, pp53=0.56).

In conclusion the proportion of KI67 and p53 positive tumor cells on primary tumor sections might not predict response or survival for a trastuzumab-containing therapy in pts with HER2/neu overexpressing metastatic breast cancer.

658 POSTER

Upstaging of breast cancer patients with PT1 tumours by detection of micrometastasis in sentinel lymph node biopsy (SLND)

I. Langer[1], M. Zuber[2], W.R. Marti[1], D. Oertli[1], J. Torhorst[3], F. Harder[1]. [1] *University Hospital of Basel, Department of Surgery, Basel, Switzerland;* [2] *Kantonsspital Olten, Department of Surgery, Olten, Switzerland;* [3] *Institute for Pathology, University of Basel, Basel, Switzerland*

Purpose: Determination of axillary lymph node status is an integral part for patients with early breast cancer and the most important prognostic factor. 15-20% of patients with tumour-free axillary lymph nodes in conventionel H&E staining show recurrences and distant metastasis within 10 years. Serial sectioning and immunohistochemistry (IHC) of the sentinel lymph nodes (SLN) allow more accurate information about micrometastasis (≤2 mm)

Methods: Between September 1997 and April 2001 114 patients with pT1 breast carcinoma were prospectively enrolled and underwent SLND with tumour resection. SLN were detected using blue dye and/or 99mTc labeled colloidal tracer. Completion axillary lymphadenectomy of level I and II was performed in case of macrometastasis in the SLN. Sentinel nodes were examined by serial sectioning and IHC (Lu-5/CK22).

Results: SLN were identified in 108 (95%) of 114 patients. 37 (34%) patients with a pT1 tumour had a SLN metastasis, 71 (66%) being tumour-free. In 14 (38%) cases SLN contained micrometastasis, diagnosed by serial sectioning with H&E staining and by IHC in 7 patients each. 23 (62%) patients had macrometastasis. 12 of 14 micrometastasis and 22 of 23 macrometastasis in SLN were found in patients with pT1c tumours.

Conclusion: In our previous study we reported an incidence of axillary lymph node metastasis in pT1 tumours of 25% without SLN (n=185). Serial sectioning and IHC of SLN lead to an upstaging of one third in this group. Further studies are necessary to assess a potential benefit of ALND in this subgroup of breast cancer patients.

659 POSTER

Sentinel lymph node biopsy and axillary dissection in breast cancer: results and initial experience of a changed technique with subareolar intradermal blue dye injection only

M. Thalhammer, M. Hanschitz[1], L. Kronberger[1], P. Regitnig[2], V. Weigl[1], S. Lax[2], H.J. Mischinger[1], M.G. Smola[1]. [1] *Div. of General Surgery, Karl-Franzens University, Dept. of Surgery, Graz, Austira;* [2] *Pathology, Karl-Franzens University, Dept. of Pathology, Graz, Austria*

Introduction: Our study tries to assess the value of intraoperative efficacy to identify the sentinel lymph node in clinical management of breast cancer. We hypothesized that the axillary lymphatic drainage pattern in predicting the pattern of metastatic spread from primary breast carcinoma is independent from a particular breast quadrant and it might be useful to examine our hypothesis in using subareolar intradermal blue dye injection only.

Methods: Between 5/99 and 12/2000 we investigated 86 consecutive patients (85 women, 1 men) using 2 ml of Lymphazurin 1%, which was injected intra- and subdermal into the subareolar area, immediately before definitive surgical treatment of primary breast carcinoma without massage of the breast. The average age of the patients was 59 years (range37-83).In these 86 patients we performed peritumoral blue dye injection in 13 cases, intradermal subareolar injection in the remaining 73 cases. Out of these we found verification of breast cancer in 60 cases, in which complete axillary lymphadenectomy was done and 13 cases with DCIS and/or no completed axillary dissection.

Results: Sentinel nodes were detected in this first period in 51 of the 60 patients (85%). A mean number of 1.47 and a median number of 1 with a range from 1 to 5 sentinel nodes per patient was found. Within the first two levels for routine histology 14 out of 60 cases (23.3%) were positive. In addition 7 cases (11.7%) got positive through serial sections and another 4 cases (6.7%) through immunohistochemistry. Finally in 25 pts. (41.7%) negative SLND predict negative axillary status, in 7 pts (11.7%) positive SLND predict positive axillary status, in 18 pts (30%) the only positive node was the sentinel node. Only in one case of identified negative sentinel nodes, the remaining axillary nodes were positive (1.17%).

Conclusion: In the large majority of patients with breast cancer, areolar intradermal blue-dye injection technique alone can be succesfully used to locate the sentinel node, and thereby provide important and correct information about the axillary status. Our results are comparable with combined blue dye and radioguided peritumoral guided surgery, whereas the method is more simple, less expensive and practicable for all trained surgeons and not related to major centers.

660 POSTER

Clinicopathological study of p73 gene expression in breast carcinomas: an immunohistochemical study with special reference to apoptosis and cell proliferation

K. Oda[1], T. Kubota[1], K. Miyazaki[1], U. Nimura[1], M. Haguchi[2], S. Matsuda[2]. *[1] Nagoya University Graduate School of Medicine, Division of Surgical Oncology, Department of Surgery, Nagoya, Japan; [2] Nagoya University Graduate School of Medicine, Molecular Pathogenesis, Nagoya, Japan*

Purpose: To analyze the functional role of the p73 gene in breast carcinomas, it is necessary to examine the fine expression of the p73 gene in carcinoma cells in order to prevent contamination by other cells expressing p73 gene. In this study, we analyzed the immunohistochemical expression of p73 protein in breast carcinomas and its relationship with cell proliferation, apoptosis and clinical features of the carcinoma.

Materials and Methods: Development of the anti-p73 polyclonal antibody; A rabbit was immunized with GST-p73 (amino acids from TQVVKRCPNH to YEPPQVGTEF) for the production of the anti-p73 antibody. Tissue specimens were obtained from 76 patients with breast carcinoma undergoing mastectomy. For the immunohistochemistry of p73, the TSA indirect system (NEN Life Science Products, Inc.) was used. Immunohistochemical expression of the p73 protein was categorized as p73 positive [p73 (+)] (p73 positive carcinoma cell>0.5%) or p73 negative [p73 (-)].

The number of cells displaying mitotic morphology was expressed as the percentage of the total number counted (Mitotic Index, MI). The apoptotic index (AI) was defined as the ratio of TUNEL-positive tumor cells to all counted tumor cells. For statistic analysis, the Mann-Whitney U-test was used. P-values less than 0.05 were considered to be statistically significant.

Results: Sixteen out of 76 (21.1%) cases were p73 (+). The AI (mean ±SD) of p73 (+) cases (1.89 ± 0.90%) was significantly higher than those of p73 (-) cases (0.75 ± 0.73%) ($p < 0.01$). The MI of p73 (+) cases (2.25 ± 1.65%) was also significantly higher than those of p73 (-) cases (0.82 ± 0.96%) ($p < 0.01$).

In 30 cases with high MI (MI > 1%), AI of p73 (+) cases (n: 13, 2.16 ± 0.76) was significantly higher than those of p73 (-) cases (n: 17, 1.52 ± 0.60)($p < 0.05$). In low MI cases (MI<1%), however, there was no significant difference in AI between p73 (+) and p73 (-) cases.

In 8 cases (p73 (+) 2, p73 (-) 6*) of 30 high MI cases, AI was less than 1.2%, and in 22 cases (p73 (+) 11, p73 (-) 11) was higher than 1.2%. All 4 cases with distant metastases in this series were included in this P73 (-) and low-AI (<1.2%) group*.

Conclusion: In breast carcinomas with high proliferative activity; 1. The p73 gene may play a role in the induction of apoptosis. 2. Loss of p73 gene expression and apoptotic regulation of carcinoma may be associated with advanced carcinogenesis and metastatic potential in breast carcinomas.

661 POSTER

Comparison of clinical, radiological and pathological assessment of response to neoadjuvant chemotherapy for primary breast cancer

R.J. Burcombe[1], G.D. Wilson[1], P.I. Richman[1], S. Allen[2], D. Wright[2], M. Pittam[2], A. Makris[1]. *[1] Mount Vernon Hospital, Marie Curie Research Wing, Northwood, UK; [2] Luton & Dunstable Hospital, Department of Radiology, Luton, UK*

Clinical and radiological (mammography and/or ultrasound) assessment based on UICC criteria is commonly used to evaluate response to neoadjuvant chemotherapy in primary breast cancer. More recently, pathological assessment of the post-treatment resected specimen has been advocated. This study compares clinical, radiological and pathological assessments of response to neoadjuvant chemotherapy.

72 patients received 6 cycles of neoadjuvant treatment using FEC (54), AC (10), MMM (5) or CMF (3) chemotherapy. All patients were reassessed both clinically and radiologically (by ultrasound (35), mammography (14) or both modalities (23)) and categorised into complete (CR), partial (PR) or non-responders (NR). Pathological response in the surgical specimen was defined as follows: CR - no residual tumour; PR - histological tumour response; NR - no tumour response.

Response (percent) categorised by various methods of assessment:

	Clinical	Mammography	Ultrasound	Pathological
CR	23 (32)	5 (14)	3 (5)	6 (8)
PR	35 (49)	20 (54)	41 (71)	25 (35)
NR	14 (19)	12 (32)	14 (24)	41 (57)
n	72	37	58	72

Mammographic and ultrasound categorisation of response was identical in 22 of the 23 patients assessed by both modalities. All 23 clinical complete responders achieved a radiological response (CR or PR) with the exception of one patient with stable disease both mammographically and pathologically. However, pathological response varied widely amongst clinical complete responders: 5 CR, 10 PR and 8 NR. Of the 41 patients (57%) with pathological NR, the majority were judged to have responded by other methods: clinical examination 10 NR, 23 PR, 8 CR; radiological assessment 16 NR, 22 PR, 3 CR.

Conclusion: This study highlights the inadequacies of clinical assessment of response to neoadjuvant chemotherapy. A substantial proportion of patients with complete response, either clinically or radiologically, had stable disease by pathological examination. The clinical significance of these results may be revealed by correlation with long-term survival data.

662 POSTER

Preoperative hemoglobin levels do not influence the dissemination of isolated tumor cells in the bone marrow of breast cancer patients

C. Kentenich, W. Janni, M. Zerzer, D. Rjosk, F. Hepp, C. Schindlbeck. *I. Frauenklinik of the Ludwig-Maximilians-Universitaet, Munich, Germany, Dept. of Gynecology and Obstetrics, Munich, Germany*

Background: Perioperative anemia is thought to increase the risk for relapse in patients with breast cancer. It has been speculated that anemia might enhance the hematogenous dissemination of isolated tumor cells (ITC) by inadequate cytokine production. This study evaluates the influence of hemoglobin (Hb) levels on the immunocytochemical detection of ITC in the bone marrow (BM) of breast cancer patients at the time of presentation

Methods: We analyzed bone marrow aspirates from 396 patients at the time of primary diagnosis of primary breast cancer, in which preoperative Hb-level was available. Carcinoma cells were detected using monoclonal antibody A45-B/B3 directed against a common epitope of the cytokeratin (CK) polypeptides, including the heterodimers CK8/18 and CK8/19 and the APAAP technique.

Results: At the time of primary diagnosis, 108 of 396 patients (27%) presented with ITC in the BM. The median Hb-level was 13.8 g/dl. Among those patients with Hb-levels £ 13.8 g/dl, 50 patients (25%) had a positive bone marrow finding, while 58 patients (29%) with Hb-levels > 13.8 g/dl presented with ITC in the bone marrow ($P = 0.63$). In CART analysis, no predictive Hb cutoff levels in terms of tumor cells dissemination could be found.

Conclusion: In a considerable number of patients with primary breast cancer, minimal residual disease can be detected. There is no direct proof that low Hb-levels at the time of presentation increases the risk for hematogenous tumor cells dissemination.