

Breast Cancer Research and Treatment

Marc E. Lippman, MD, editor-in-chief

Special Issue
27th Annual
San Antonio Breast Cancer Symposium



0167-6806(2004)88:S1;1-A

Kluwer Academic Publishers

Ind
ed.
nic,
reut
ing
nitc
rin
e d
) co
se i
2).
or r
osit
the
nd
rin
de

compared to archived standard masses. This device will triage between a palpable mass vs. palpable fibrocystic change. Of those identified palpable masses, it may distinguish between benign and suspicious palpable masses.

METHODS: Seventy-two patients who were referred to our breast clinic with a complaint of a breast mass were examined. In addition to clinical physical examination, ultrasound, and mammograms, each patient underwent a mechanical imaging scan (MIS). This table top device uses a broad-based transducer that measures variable degrees of hardness (elasticity) within the breast. As the transducer scans the area of interest, a real-time display of the palpable area is digitally recorded. Scan measurements include size of the lesion, distribution of firmness within the lesion and maximum hardness. Final histologic and/or follow-up data (minimum 6 months) was correlated with preoperative hardness/elasticity data. **RESULTS:** MIS evaluation separated patients into two groups; those with a truly palpable mass and those with glandular nodularity. Of the entire group, 40/44 patients were triaged as a dominant firm mass according to MIS data, while 25/28 were considered glandular nodularity without dominant mass. Using the MIS data, further triage was performed of the truly palpable masses into suspicious masses (cancers) and probably benign masses (firm cysts and fibroadenomas).

CONCLUSION: We describe the use of a unique table-top mechanical imaging scan that documents palpable breast masses with high sensitivity and specificity. Patients with truly palpable masses (cancers, benign tumors and firm cysts) were found to have increased firmness (decreased elasticity) while those with nodular breast tissue (fibrocystic change) had less firmness. The use of MIS allowed initial separation between benign and suspicious truly palpable masses. A reproducible record of the breast physical exam is created that allows objective review by multiple examiners at varied times. Further work is necessary to optimize examination methods, improve real-time software interpretation and define the array of diagnostic capabilities.

6013

Isolation of highly purified DNA from Papanicolaou (Pap) preparations of ductal lavage (DL) samples with small cell numbers: how low can we go?

Bhandare D, Motchoulskaia N, Melnikov A, Khan SA, Levenson V. Northwestern University Feinberg School of Medicine, Chicago, IL; Northwestern University, Chicago, IL

Background: Biomarker analysis on DL samples is limited by low epithelial cell yield. DNA isolated and purified from Pap stained slides of DL, and amplified using Polymerase chain reaction (PCR), provides a potential source of material for genomic studies. However, DNA purification involves losses which can be important when the starting amount of material is low. To find the best procedure for DNA isolation from a limited number of cells and apply this protocol to isolate genomic DNA (gDNA) from Pap stained slides, we compared DNA yield, quality, and ease of use of four commercially available kits.

Methods: Freshly cultured MCF-7 cells were serially aliquoted to obtain single cell suspensions with 10, 100, 1000, 10^4 and 10^5 cells. Four commercially available kits were used to isolate DNA: DNazol[®] Genomic DNA kit (Invitrogen Corp.), GenomicPrep[™] cells and tissue DNA kit (Amersham Biosciences Corp.), Wako DNA extractor WB kit (Wako Chemicals USA, Inc.) and DNeasy tissue kit (Qiagen Inc.) following manufacturers' protocols. DNA was quantified using the Hoefer[™] DyNA Quant 200 fluorometer. To verify the presence of genomic DNA (gDNA) in low range samples (isolated from <1000 cells), we used a nested PCR amplification of a 484 base pair fragment of the progesterone receptor gene. To assess the isolated gDNA purity, we treated it with methylation specific restriction enzyme (MSRE), and evaluated digestion efficiency in unmethylated region of hMLH1 gene. To determine whether the same technique can be used for clinical sample analysis (e.g. ductal lavage and ductoscopy washings) we isolated gDNA from Papanicolaou stained slides with varying

numbers of MCF-7 cells and from ductoscopy washings.

Results: Variability of gDNA yields increased with reducing cell numbers. The best and most consistent yields were obtained with GenomicPrep[™] kit, while DNazol[®] was a close second and much easier to use. All tested kits produced gDNA of sufficient quality to perform restriction digests with selected MSRE. In model experiments we obtained sufficient gDNA from Papanicolaou stained slides with 19 cells (detected by nested PCR). The quality of this gDNA was sufficient to perform analysis of DNA methylation using newly developed MSRE-PCR technique.

Conclusions: The most consistent yield of gDNA was obtained with GenomicPrep[™] kit. However, DNazol[®] protocol is better suited for clinical lab utility purposes, producing gDNA that can be used for downstream procedures, including DNA methylation analysis.

6014

Influence of primary tumor chemotherapy in breast cancer on circulating tumor cells. Indications for massive cell release into circulation concurrent with tumor size reduction.

Pachmann K, Camara O, Pachmann UA. Jena, Germany; Friedrich Schiller-University Jena, Jena, Germany; Transfusionsmedizinisches Zentrum, Bayreuth, Germany

Having developed a method for easy and rapid quantification of peripherally circulating tumor cells (fig. 1) we now examined the possibility to use their response to cytostatic agents for therapy monitoring in solid tumors. For this purpose the reactivity of the circulating cells was compared to that of the primary tumor in the neoadjuvant setting in breast cancer patients. During the applied combination therapy three different phases could be observed, a first decline in the number of circulating cells during the E (epirubicin) containing part of the regimen followed by a steep increase during taxol treatment and a subsequent decrease if a third segment with CMF (cyclophosphamid /methotrexate/fluorouracil) was applied before surgery (Fig.2). Whereas we could show that the initial decrease in circulating tumor cells correctly predicted final tumor reduction in patients with her2/neu negative tumors this correlation was less pronounced in her2/neu positive patients who additionally received antibody treatment. Although accompanied by tumor size reduction the increase in the number of circulating cells during taxol treatment did not show a correlation with the extend of final tumor reduction. The reason for this phenomenon is discussed. These results indicate that monitoring of circulating tumor cells can contribute to our understanding of tumor/blood interaction and may provide a valuable tool for therapy monitoring in solid tumors.

