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Singh, Heena

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Quantitative Expression of Gli-1 In Breast Cancer Using Histo-Rx Analysis

HEENA SINGH

Introduction

Early detection of breast cancer plays a vital role in increased overall survival and administration of less aggressive treatments used for therapy. Measuring receptor responsiveness for levels of estrogen, progesterone, and Her-2 allow for an accurate diagnosis and course of treatment administered. Another encouraging clinical biomarker that may aid in early detection is the Glioma associated oncogene Gli-1, found downstream of the Sonic Hedgehog (Shh) Pathway. Shh pathway is one of three Hedgehog signaling pathways that plays a crucial role during embryogenesis development. Deregulation of this pathway has also been correlated with the activation of tumors in lung, esophageal, gastric, and basal cell carcinoma. The Gli family, including Gli-1, Gli-2, and Gli-3, are promising transcription factors that may help in diagnosing aggressive breast cancer. The abnormal activation of Gli-1 and Gli-2 cause an uncontrolled self renewal of tumor cells that become resistant to treatment. The objective of this project is to analyze how the expression of Gli-1 correlates with overall survival using a newer method of gauging quantitative expression from a Histo-Rx machine.

Materials and Methods

Statistical Analysis

- 99 breast cancer patients administered Tamoxifen were taken from the 50/50 DNA micro-array database.
- Half of the database consists of patients that demonstrated overall good survival while the remainder demonstrated poor prognosis leading to death.
- Triplicate Gli-1 AQUA core series from the Cytoplasm, Nucleus, and Tumor Mask were used to correlate clinicopathological factors such as:
 - Hormonal proliferation
 - Common breast cancer prognostic markers (such as estrogen, progesterone, Her-2, and Src).
 - Overall patient outcome: Overall Survival (OS) (date of diagnosis to date of death or date of last visit by patient to Tom Baker Cancer Centre if patient is alive).
- AQUA scores were run through a Histo-Rx machine where continuous values were recorded. The use of a Histo-Rx machine allows for a non-observer biased result as previously viewed conclusions of DAB stain expressions varied diversely among individual pathologists.

Materials and Methods

Analysis Programs

- SPSS (version 16.0) software was used to correlate statistical significance of Overall Survival with the expression of Gli-1 AQUA core series and clinical variables.
- Survival curves and statistical significance were obtained by using Kaplan-Meier and Cox Regression tests and Pearson's- χ^2 test was performed to find clinical independence between two variables.
- X-tile software was used to specify cut points to convert continuous data into nominal data later used in SPSS.

Results

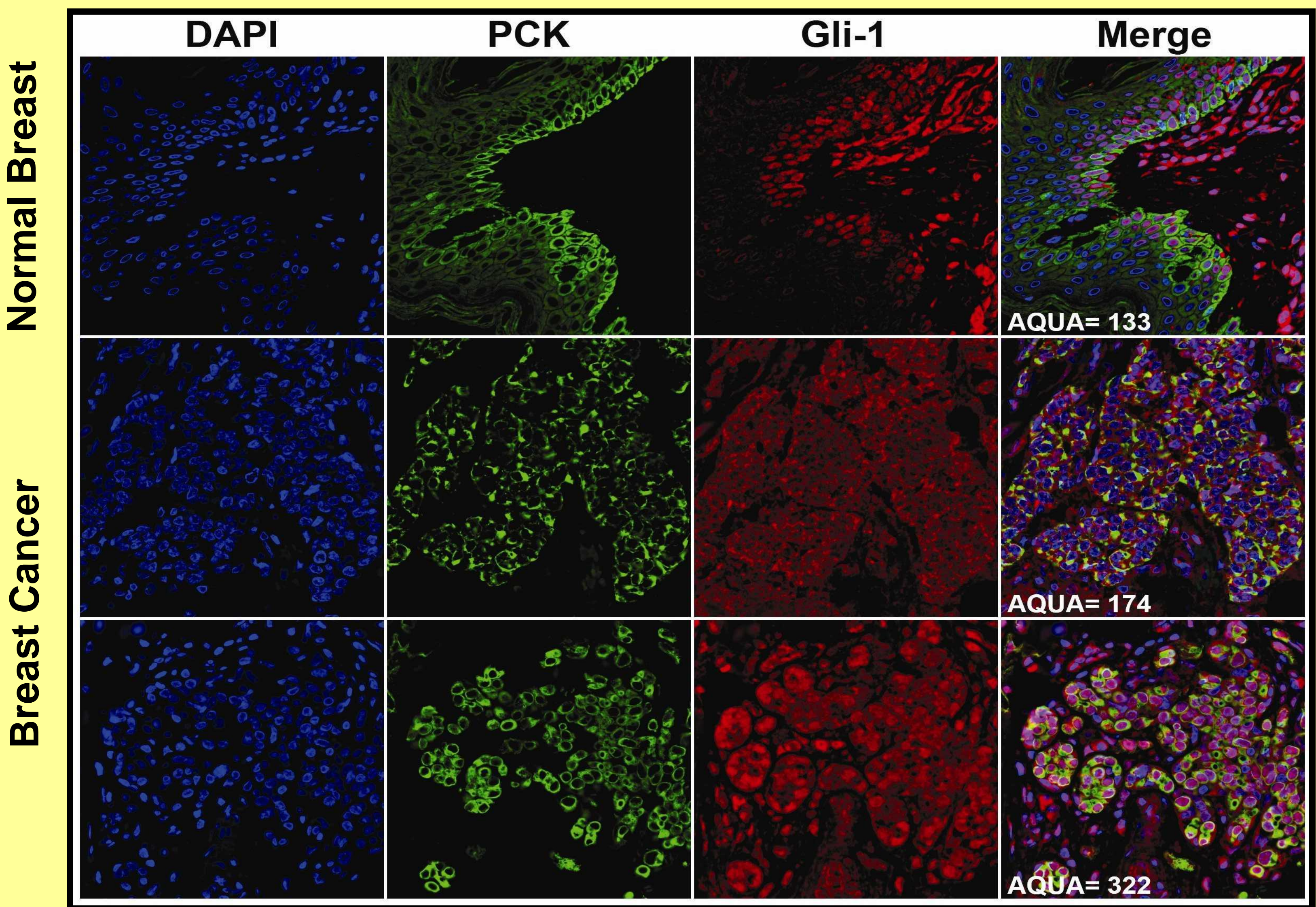


Figure 1: A DAPI (4',6-Diamidino-2-Phenylindole) fluorescent DNA staining was used as a nuclear marker while a Pan-cytokeratin antibody stain identified the tumor and cytosolic markers in normal and breast epithelial cells. In normal breast tissue, expression of Gli-1 diminishes as they become terminally differentiated into keratinocytes. However, in breast cancer cells expressing lower quantities of Gli-1 (AQUA score 174), tissues are largely concentrated in the cytoplasm whereas higher expression of Gli-1 expression demonstrates a nuclear tissue make-up. (Brightness enhancement was applied for greater clarity of Histo-Rx images.)

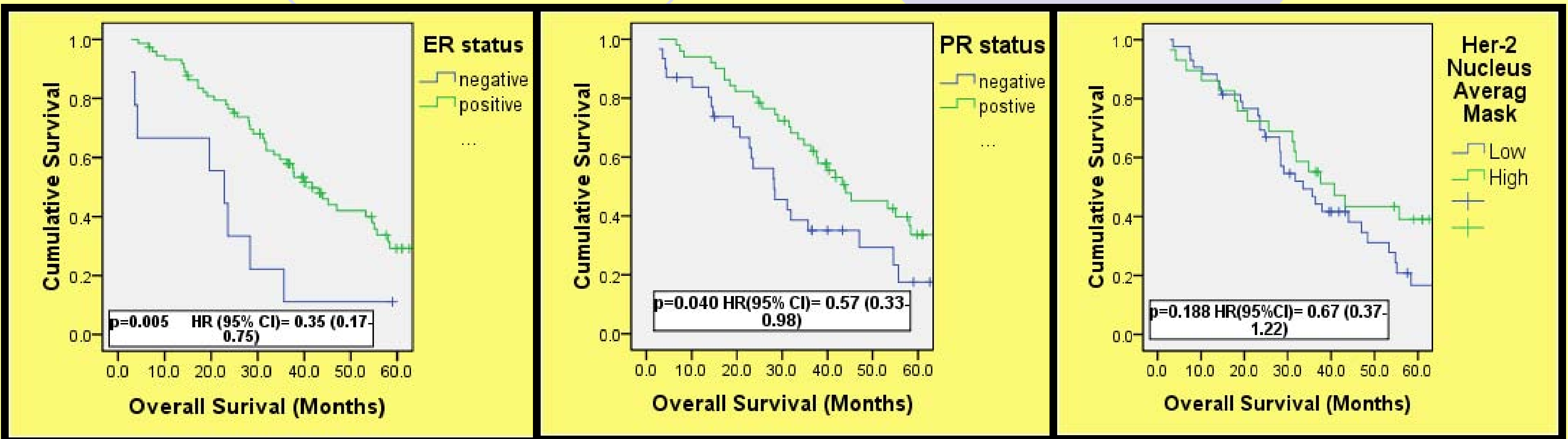
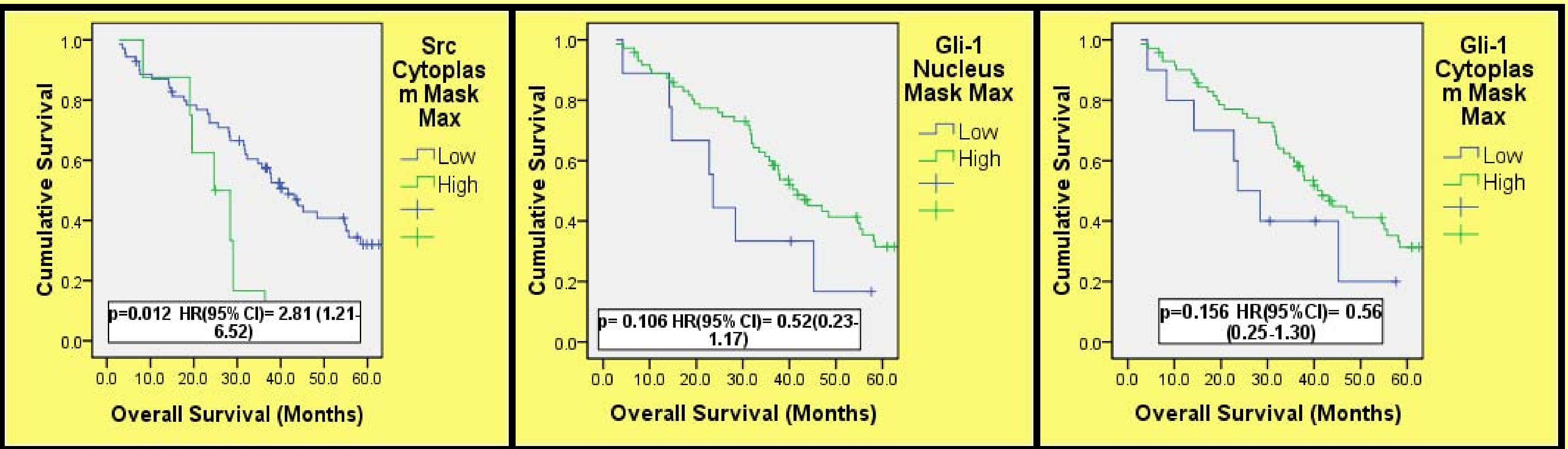


Figure 2: Clinical marker such as ER/PR positive and low cytoplasmic Src (maximum scores) expression have a better prognosis after five years. In contrast, the average nucleus Her-2 mask, maximum nucleus Gli-1 mask and maximum cytoplasmic Gli-1 masks have a tendency to demonstrate good prognosis, however are statistically insignificant with overall patient survival.

Conclusion

- Patients expressing a higher ER tumor and nucleus maximum and average scores and lower maximum scores in the Src cytoplasm demonstrated a better overall survival for a period of 5 years.
- Clinical indicators that associated with good OS include patients diagnosed with Stage I breast cancer, LN negative status, tumor sized 0.40-1.90 cm, patients aged 38-60 years and ER/PR positive patients.
- Overall Survival did not correlate with Gli-1 maximum and nuclear expression. However individual AQUA score 3 in the tumor, nucleus, and cytoplasm masks proved to be significantly linked with OS.

Acknowledgments

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