

# Disease free survival and overall survival from Holliday Junction Recognition Protein (HJURP) expression in non-metastatic breast cancer patients

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**Abstract.** *Background and aim:* Holliday Junction Recognition Protein (HJURP) is a gene that integrates with Centromere Protein A (CENPA) which plays a role in cell proliferation. It is related to radiation response where high HJURP level give a good response to radiotherapy and a high rate of apoptosis. It is a prognostic factor for Disease Free Survival (DFS) and Overall Survival (OS) and a predictive biomarker on radiotherapy. This study aimed to analyze the correlation between HJURP expression with DFS and OS in breast cancer patients who received radiotherapy. *Methods:* This study used a quantitative method with a cross-sectional design. Data were collected from 57 non-metastatic breast cancer patients undergoing radiotherapy from 2019 to 2021. Patients were examined from paraffin blocks by IHC examination using HJURP antibodies which were then assessed for expression. Data were analyzed using survival analysis with Kaplan-Meier and Cox Regression test. *Results:* The majority of breast cancer patients who received radiotherapy had positive HJURP expression (68.4%). There was no statistically significant correlation between HJURP expression with DFS or OS in breast cancer patients who received radiotherapy. However, there was a statistically significant correlation between radiotherapy interruptions and OS where patients with interruptions >3 times had a risk of death 8.40 times faster than patients with interruptions ≤3 times (95% confidence interval [CI]). *Conclusions:* Although HJURP expression was not related with survival in breast cancer patients who received radiotherapy, it is a good candidate as a predictive biomarker of radiotherapy response. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** breast cancer, disease free survival, HJURP, overall survival, radiotherapy interruptions

## Introduction

Breast cancer is the most common malignancy in women with an estimated incidence of 2.1 million cases and a death rate of 626,679 cases in 2018. The incidence of breast cancer is estimated to have increased by more than 3.1% since the 1980s. The disease burden will continue to increase as the ratio of the female population reaches more than 49.5% of the world's population and the high proportion of cases in women aged over 60 years (1).

Various markers have been widely used in determining prognostic and predictive to estimate the response to a therapy. However, few breakthroughs have been made to identify prognostic or predictive biomarkers in breast cancer radiotherapy. One of the most studied markers is Holliday Junction Recognition Protein (HJURP), which is a gene that functions and integrates with Centromere Protein A (CENPA). This marker is still being studied as a prognostic and predictive marker in breast cancer radiotherapy. HJURP overexpression is associated with CENPA expression

and cancer aggressiveness (2). HJURP expression is related to radiation response where high HJURP values give a good response to radiotherapy and a high rate of apoptosis (3).

Hu et al. (2010) conducted an assessment of HJURP expression in determining prognostic and predictive factors in giving radiotherapy to breast cancer patients. The study showed that there was an increase in the value of HJURP expression in breast cancer and its relationship with the value of estrogen receptors, progesterone receptors, Ki-67, but not on HER2 expression. When compared HJURP expression with Ki-67, HJURP is predicted to have a better prognosis than Ki-67. It was concluded that HJURP expression is a prognostic factor for Disease Free Survival (DFS) and Overall Survival (OS) and a predictive biomarker on radiotherapy (3).

This study aimed to analyze the correlation between HJURP expression with DFS and OS in breast cancer patients who received radiotherapy in Padang City, West Sumatra, Indonesia.

## Methods

This study used a quantitative method with a cross-sectional design. It was conducted from May to June 2022 in the Radiotherapy section of Andalas University Hospital and Anatomical Pathology Laboratory of Dr. M. Djamil Central Hospital in Padang, West Sumatra. Samples were taken from non-metastatic breast cancer patients who received radiotherapy to the mass/chest wall and regional glands. The assessment of DFS and OS based on medical records. Patients were also contacted by telephone to find out whether the patient was still routinely receiving treatment control, disease progress, and metastases or had died.

The target population in this study were 107 non-metastatic breast cancer patients who received radiotherapy recorded in the Andalas University Hospital medical record from 2019 to 2021. The research sample was paraffin block of non-metastatic breast cancer patients who received radiotherapy. The sample size was obtained from calculations using the Lemeshow formula on a finite/known population. Based on calculations using the formula, this study requires a minimum

of 56 samples. Of the 107 study population, there were only 78 paraffin blocks and only 57 patients out of 56 minimal samples met the inclusion and exclusion criteria. Inclusion criteria were non-metastatic breast cancer patients who received radiotherapy. Exclusion criteria were damaged paraffin blocks that could not be examined for IHC or incomplete, damaged or missing medical record data. Patients who were included in this study had met the inclusion and exclusion criteria, and had informed consent.

Patient characteristics, including age, stage, tumor size, lymph nodes status, histopathological type, differentiation degree, lymphovascular invasion, radiotherapy interruption, therapeutic procedures and HJURP expression were analyzed in this study. HJURP expression was gene expression taken from breast cancer tissue using immunohistochemistry (IHC) examination. The IHC staining intensity was defined as: negative (<25%), weak positive (25–50%), moderate positive (50–75%) and strong positive (75–100%). HJURP expression was categorized into positive (included weak, moderate and strong) and negative. The age variable used in this study was the age of the patient when diagnosed with breast cancer which is categorized into  $\leq 40$  years and  $> 40$  years. Stage was an assessment of the clinical stage of breast cancer based on the American Joint Committee on Cancer (AJCC) 2018, namely Local: I, II and Advanced Local: III. Tumor size was the diameter of the breast tumor measured at the start of treatment categorized into T1 (Tumor size  $\leq 20$  mm), T2 (Tumor size  $> 20$  mm but  $\leq 50$  mm), T3 (Tumor size  $> 50$  mm), T4 (Tumor with direct extension to the chest wall and/or the skin with macroscopic changes). Regional lymph node metastasis in breast cancer was categorized as positive and negative. Histopathology type was a microscopic description of histopathology by an expert anatomic pathologist. Histopathology was categorized into NST (No Special Type) and Non NST. It's called 'no special type' because the cancer cells have no features that class them as a special type of breast cancer when examined under the microscope. It is also called Invasive ductal carcinoma of the breast (IDC).

Differentiation degree was a grouping of cancer cells based on their appearance and properties on histopathological examination categorized into Grade I,

Grade II, Grade III. Lymphovascular Invasion (LVI) was a description of cancer cells infiltrating into blood vessels categorized into positive and negative. It had been a predictor of poorer survival. In breast cancer, it was defined as the presence of tumor cells within the endothelial (lymphatic or blood vessel) restricted space in the breast surrounding the invasive carcinoma. Interruption (radiotherapy fraction gap) was the distance/gap that occurs in the delivery of fractionation set in radiotherapy. Fractionation was one of the important characteristics of radiation therapy, and was carried out to exploit the differences between tumor and normal tissues, so as to achieve the desired therapeutic results with an acceptable toxicity rate. Because of this fractionated delivery, radiation therapy generally takes several weeks. However, various things happen during these weeks that can cause interruptions to the radiation therapy delivery process. Therapeutic procedures were breast cancer treatments that were given after a definitive diagnosis consisting of locoregional only, locoregional and systemic, and systemic only.

Data quality control was conducted at each stage of the study to ensure the accuracy and precision of the results. Each stage was carried out using an approved protocol with a filled-in procedure checklist. The object of supervision includes IHC examination by expert anatomic pathologists and medical records traced to the patient. All immunohistochemical assessments were performed blind by two examiners, and based on the results of these assessments, the present study compared the responses and characterized the results.

Survival analysis using Kaplan-Meier and Cox Regression tests with 95% confidence intervals was used to determine the correlation between HJURP expression, radiotherapy interruption and therapeutic procedures with DFS and OS in breast cancer patients who received radiotherapy. The proportional-hazard assumption was assessed with graphical approach using a log minus log survival plot. Meanwhile, the Chi-Square test with 95% confidence intervals was used to determine the association between patient characteristics and HJURP expression.

This research has passed the ethical review of the health research ethics committee of Dr. M. Djamil Central Hospital with ethics number: LB.02.02/5.7/182/2022 on May, 20th 2022.

## Results

The majority of breast cancer patients who received radiotherapy had positive HJURP expression (68.4%) (Table 1). The results of the HJURP expression examination can be seen in Figure 1. The majority of breast cancer patients with positive HJURP expression were over 40 years old (70.0%), in advanced local stage (73.5%), had T1 tumor size (100.0%), had positive lymph nodes status (69.2%), were Non NST type (75.0%), in grade III (85.7%), and had positive LVI (71.4%) (Table 1). Based on Chi-Square tests, it was found that age, stage, tumor size, lymph nodes status, histopathological type, differentiation degree and LVI were not statistically significantly associated with HJURP expression in breast cancer patients who received radiotherapy ( $p > 0.05$ ).

The majority of breast cancer patients who received radiotherapy did not relapse (77.2%) (Table 2). From the Kaplan-Meier graph in Figure 2, it can be seen that the median value in patients with radiotherapy interruptions  $\leq 3$  times was 46 months, meaning that 50% of breast cancer patients who received radiotherapy had a recurrence by the 46th months. Meanwhile, in patients with radiotherapy interruptions  $> 3$  times, the median survival value was not obtained, because there was no intersection of survival lines, meaning that the probability of recurrence did not reach 50%. The median value in patients who only underwent systemic procedure was 46 months, meaning that 50% of breast cancer patients who received radiotherapy had a recurrence by the 46th months. Meanwhile, in patients who underwent both locoregional and systemic procedures, there was no median survival, because there was no intersection of survival lines, meaning that the probability of recurrence did not reach 50%. None of the patients who only underwent locoregional procedure had recurrence. The median value in patients with negative HJURP expression was 46 months, meaning that 50% of breast cancer patients who received radiotherapy had a recurrence by the 46th months. Meanwhile, in patients with positive HJURP expression, there was no median survival, because there was no intersection of survival lines, meaning that the probability of recurrence did not reach 50%. Based on Cox Regression test in Table 2,

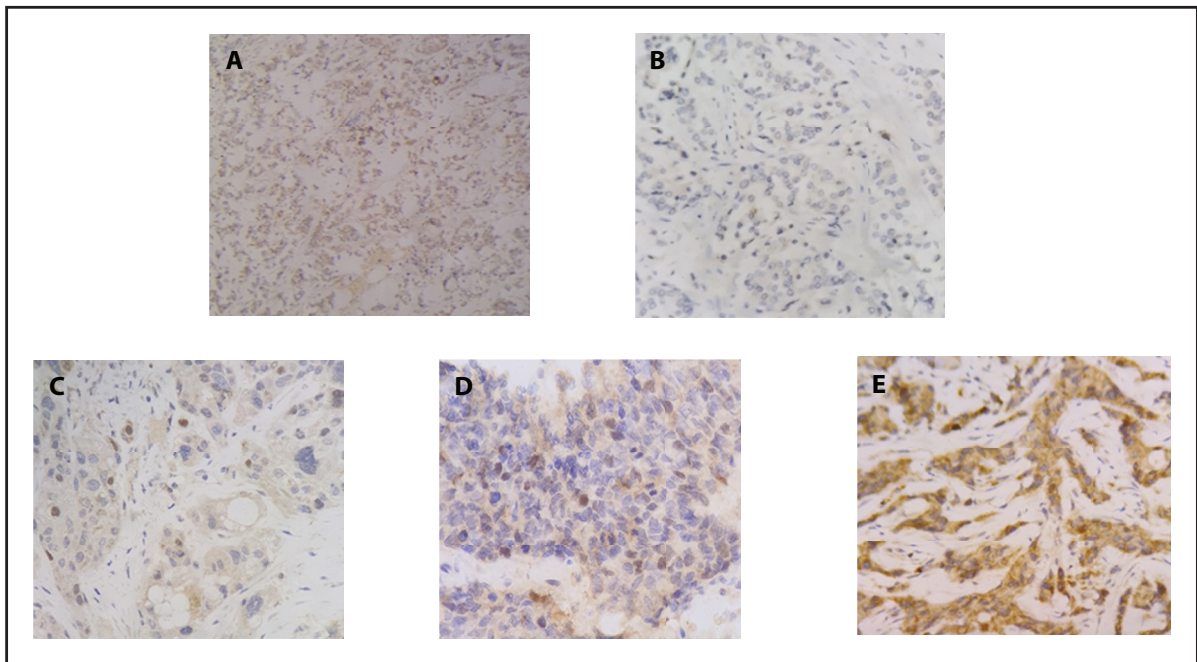
**Table 1.** Association between patient characteristics and HJURP expression.

| Patient Characteristics | HJURP Expression – n (%) |                      | p-value |
|-------------------------|--------------------------|----------------------|---------|
|                         | Positive – 39 (68.4)     | Negative – 18 (31.6) |         |
| Age                     |                          |                      |         |
| ≤40 years old           | 7 (70.0)                 | 3 (30.0)             | 1.00    |
| >40 years old           | 32 (68.1)                | 15 (31.9)            |         |
| Stage                   |                          |                      |         |
| Local: I, II            | 3 (37.5)                 | 5 (62.5)             | 0.09    |
| Advanced Local: III     | 36 (73.5)                | 13 (26.5)            |         |
| Tumor Size              |                          |                      |         |
| T1                      | 1 (100)                  | 0                    | 0.19    |
| T2                      | 3 (42.9)                 | 4 (57.1)             |         |
| T3                      | 6 (54.5)                 | 5 (45.5)             |         |
| T4                      | 29 (76.3)                | 9 (23.7)             |         |
| Lymph Nodes Status      |                          |                      |         |
| Positive                | 36 (69.2)                | 16 (30.8)            | 0.65    |
| Negative                | 3 (60.0)                 | 2 (40.0)             |         |
| Histopathological Type  |                          |                      |         |
| NST                     | 27 (65.9)                | 14 (34.1)            | 0.73    |
| Non NST                 | 12 (75.0)                | 4 (25.0)             |         |
| Differentiation Degree  |                          |                      |         |
| Grade I                 | 1 (50.0)                 | 1 (50.0)             | 0.22    |
| Grade II                | 26 (63.4)                | 15 (36.6)            |         |
| Grade III               | 12 (85.7)                | 2 (14.3)             |         |
| LVI                     |                          |                      |         |
| Positive                | 25 (71.4)                | 10 (28.6)            | 0.75    |
| Negative                | 14 (63.6)                | 8 (36.4)             |         |

it can be seen that there was no statistically significant correlation between radiotherapy interruption, therapeutic procedure and HJURP expression with DFS in breast cancer patients who received radiotherapy ( $p>0.05$ ).

Furthermore, it was found that the majority of breast cancer patients who received radiotherapy were alive (87.7%) (Table 2). From the Kaplan-Meier graph in Figure 3, it can be seen that the median value in patients with radiotherapy interruptions >3 times was 25 months, meaning that 50% of breast cancer patients who received radiotherapy had died by the 25th month. Meanwhile, in patients with radiotherapy interruptions ≤3 times, the median survival value

was not obtained, because there was no intersection of survival lines, meaning that the probability of recurrence did not reach 50%. The median survival for either patients who underwent both locoregional and systemic procedures or only systemic procedure was not known, because there was no intersection of survival lines, meaning that the probability of death in these patients does not reach 50%. However, it can also be seen that patients who only underwent systemic procedure have a higher probability of death than patients who underwent both locoregional and systemic procedures. Meanwhile, none of the patients who only underwent locoregional procedure died. The median survival for both patients with positive and



**Figure 1.** a) Positive hjurp expression in normal brain tissue as control; b) Negative HJURP expression in breast cancer patient tissue; c) Weak positive HJURP expression; d) Moderate positive HJURP expression; and e) Strong positive HJURP expression in breast cancer patient tissue.

negative HJURP expression was not known, because there was no intersection of survival lines, meaning that the probability of death in these patients does not reach 50%. However, it can also be seen that patients with positive HJURP expression have a higher probability of death than patients with negative HJURP expression. Based on Cox Regression test in Table 2, it can be seen that there was a statistically significant correlation between radiotherapy interruption and OS in breast cancer patients who received radiotherapy ( $p < 0.05$ ). Patients with interruptions  $> 3$  times had a risk of death 8.40 times faster than patients with interruptions  $\leq 3$  times (95% confidence interval [CI]). The log minus log survival plot showed that the independent variables of radiotherapy interruption form parallel lines in each category and none of them intersect or cross each other so that the proportional hazard assumption is met (Figure S1). However, there was no statistically significant correlation between therapeutic procedure and HJURP expression with OS in breast cancer patients who received radiotherapy ( $p > 0.05$ ).

## Discussion

There was a statistically significant correlation between radiotherapy interruption and OS in breast cancer patients who received radiotherapy. Patients with interruptions  $> 3$  times had a risk of death 8.40 times faster than patients with interruptions  $\leq 3$  times (95% confidence interval [CI]). The time factor is very influential in therapy. If the treatment time is too short, there will be incomplete improvement. However, if the treatment is too long, it will give the opportunity for cells to proliferate or repair slowly. Among the cells that survive after radiation, there will be an increase in the cell cycling and a decrease in the cell cycle time (4). In Yao et al. (2018), compared to patients with RTI  $> 5$  days, patients with RTI  $\leq 5$  days had a significantly lower rate of LRFS (97% vs. 83%;  $P < 0.001$ ). In multivariate analysis, RTI was a risk factor independently associated with LRFS (HR = 9.64, 95% CI, 4.10–22.65), but not for OS (HR = 1.09, 95% CI, 0.84–1.64) (5). In Fesinmeyer et al. (2010), patients with laryngeal tumors who experienced an interruption

**Table 2.** Disease free survival and overall survival from radiotherapy interruption, therapeutic procedures and HJURP expression in non-metastatic breast cancer patients.

| Variable                  | Event/Total | HR (95% CI)       | p-value | p-log rank |
|---------------------------|-------------|-------------------|---------|------------|
| Disease Free Survival     |             |                   |         |            |
| Radiotherapy Interruption |             |                   |         |            |
| ≤3 times                  | 8/40        | 2.95 (0.88-9.87)  | 0.08    | 0.07       |
| >3 times                  | 5/17        |                   |         |            |
| Therapeutic Procedure     |             |                   |         |            |
| Locoregional              | 0/3         | 1.89 (0.63-5.68)  | 0.26    | 0.51       |
| Locoregional + Systemic   | 8/44        |                   |         |            |
| Systemic                  | 5/10        |                   |         |            |
| HJURP Expression          |             |                   |         |            |
| Positive                  | 9/39        | 0.65 (0.19-2.18)  | 0.49    | 0.48       |
| Negative                  | 4/18        |                   |         |            |
| Overall Survival          |             |                   |         |            |
| Radiotherapy Interruption |             |                   |         |            |
| ≤3 times                  | 2/40        | 8.40 (1.56-45.15) | 0.01    | 0.003      |
| >3 times                  | 5/17        |                   |         |            |
| Therapeutic Procedure     |             |                   |         |            |
| Locoregional              | 0/3         | 3.01 (0.71-12.66) | 0.13    | 0.29       |
| Locoregional + Systemic   | 4/44        |                   |         |            |
| Systemic                  | 3/10        |                   |         |            |
| HJURP Expression          |             |                   |         |            |
| Positive                  | 6/39        | 0.30 (0.04-2.54)  | 0.27    | 0.24       |
| Negative                  | 1/18        |                   |         |            |

in radiotherapy had a 68% (95% confidence interval, 41-200%) increased risk of death, compared with patients with no interruptions. Treatment interruptions seem to influence survival time among patients with laryngeal tumors completing a full course of radiotherapy (6).

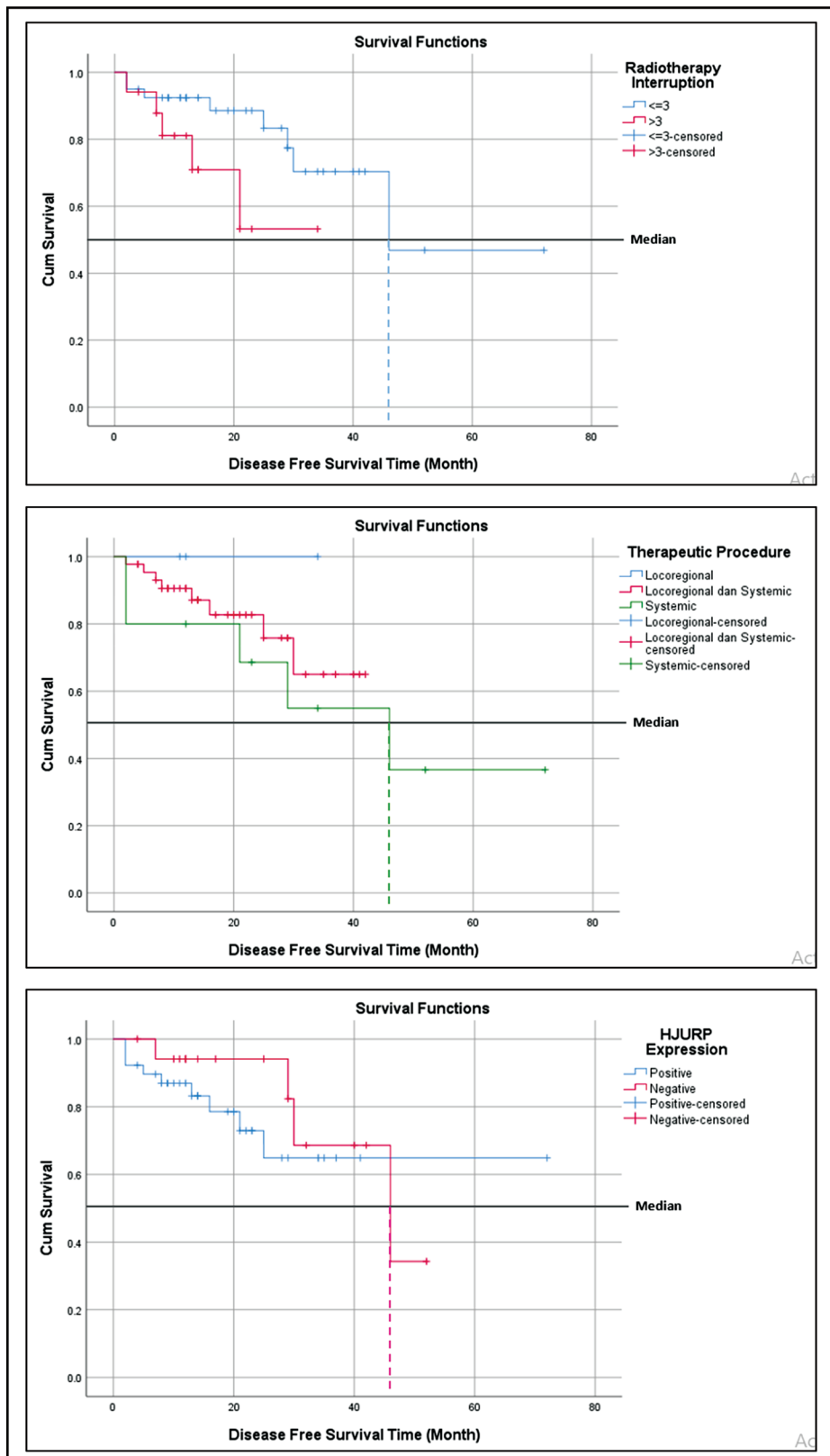
There was no statistically significant correlation between therapeutic procedure with DFS and OS in breast cancer patients who received radiotherapy. Meanwhile, a multivariate proportional hazards model found that women treated with surgical resection with free margins, when compared with those not surgically treated, had superior prognosis, with a hazard ratio of 0.61 (95% confidence interval 0.58,0.65) (7).

There was no statistically significant correlation between HJURP expression with DFS and OS in breast cancer patients who received radiotherapy. Meanwhile, in Hu et al. (2010), an assessment of HJURP expression was carried out in determining prognostic

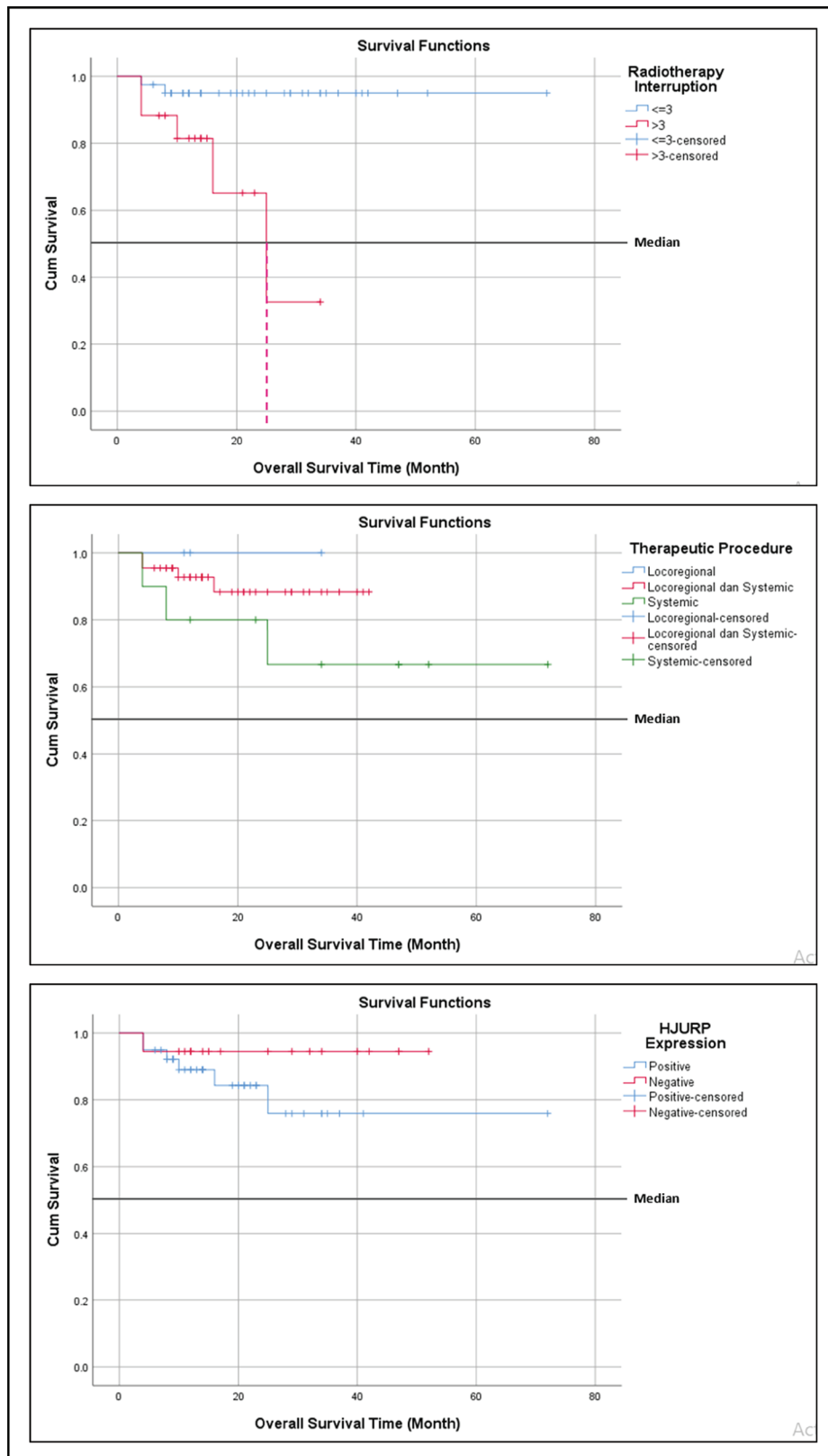
and predictive factors in giving radiotherapy to breast cancer patients. There was an increase in the value of HJURP expression in breast cancer patients. HJURP expression is a prognostic factor for Disease Free Survival (DFS) and Overall Survival (OS) and a predictive biomarker on radiotherapy (3). Although in this study HJURP expression was not related with survival in breast cancer patients who received radiotherapy, it is a good candidate as a predictive biomarker of radiotherapy response.

## Conclusions

From this study it can be concluded that there was no statistically significant correlation between HJURP expression with DFS and OS in breast cancer patients who received radiotherapy. However, there was a



**Figure 2.** Disease free survival from radiotherapy interruption, therapeutic procedure and HJURP expression.



**Figure 3.** Overall Survival from radiotherapy interruption, therapeutic procedure and HJURP expression.



statistically significant correlation between radiotherapy interruptions and OS where patients with interruptions >3 times had a risk of death 8.40 times faster than patients with interruptions ≤3 times (95% confidence interval [CI]). In addition, it can also be concluded that age, stage, tumor size, lymph nodes status, histopathological type, differentiation degree and LVI were not statistically significantly associated with HJURP expression in breast cancer patients who received radiotherapy.

HJURP expression plays a role in the process of cell proliferation which may not affect the survival of breast cancer patients who receive radiotherapy, but the possibility of response to radiotherapy will provide promising opportunities as predictive biomarkers of radiotherapy results. Other researchers can continue this study to analyze the correlation of HJURP expression with radiotherapy outcomes in inoperable advanced local stage of breast cancer patients.

**Conflict of Interest:** Each author declares that he has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

**Ethical Approval:** This research has passed the ethical review of the health research ethics committee of Dr. M. Djamil Central Hospital with ethics number: LB.02.02/5.7/182/2022 on May, 20th 2022.

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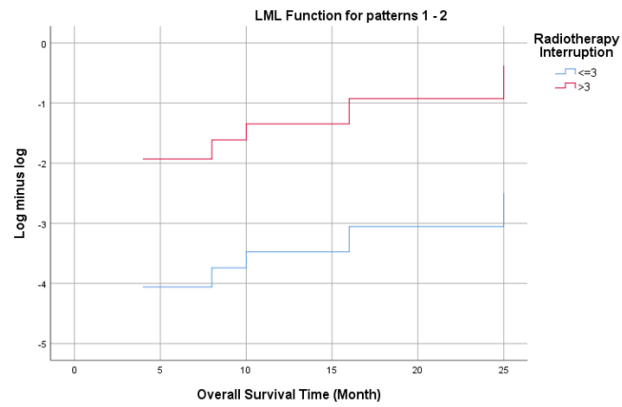
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## Appendix – Supplementary files



**Figure S1.** Log Minus Log Survival Plot from Radiotherapy Interruption.