RESEARCH PROTOCOL

STUDY TITLE

Neutrophil/lymphocyte ratio and overall survival in patients with breast cancer: a cohort study in a Latin-American hospital

STUDY INVESTIGATORS

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PURPOSE OF STUDY

The primary objective of this study will be to determine the association between NLR and OS in patients with breast cancer in the gynecology department of Hospital Nacional Hipólito Unanue (HNHU), Lima, Peru between 2012 and 2014.

BACKGROUND

Breast cancer is a disease of high mortality globally. In Latin America, this pathology is the most important cause of death by neoplasms among women (1). Therefore, it is fundamental to determine the factors associated with overall survival (OS). There is research that associates an elevated Neutrophil/lymphocyte ratio (NLR) with lower OS in patients with breast cancer; however, these were developed in populations with early-stage diagnosis, not in advanced stages. Furthermore, differences exist in breast cancer phenotype, prevalence of oncological infections (2), and the presence of social risk factors (3,4). This highlights the

need for studying the OS associated with breast cancer and its prognostic indices in this context.

STUDY DESIGN

An observational, longitudinal, analytical survival study will be carried out.

STUDY LOCATION

The study will be conducted in Hospital Nacional Hipólito Unanue (HNHU), III-I establishment in East Lima, Peru. It will be a single-center study.

STUDY POPULATION

This study will include all the female patients diagnosed with breast cancer seen in Hospital Nacional Hipólito Unanue (HNHU) between 1st January 2012 and 31st December 2014. A total of 241 female patients will be included.

STUDY PERIOD

This study will be carried out between July 2020 and November 2020.

ELIGIBILITY CRITERIA

Inclusion criteria

This study will include patients with:

1. Histopathological and immunohistochemical diagnosis of breast cancer.

2. Legal age (Over 18 years old)

3. Medical record with complete clinical information and follow-up from the moment of diagnosis until the last consultation or death.

4. Start of treatment in the hospital

Exclusion criteria

This study will exclude patients with:

1. Immune-suppressing diseases or a HIV/AIDS infection

STUDY OUTCOME

Primary outcome

The outcome variable of this study will be the overall survival (OS) of the patients diagnosed with breast cancer, time will be measured in months, from the time of diagnosis until death or date of last follow-up.

Variables

Exposure variable:

- Neutrophil/lymphocyte ratio NLR. It will be measured from the first blood count obtained at the time of diagnosis that appears in the medical record. The rate will be categorized as low if NLR<3 and high if NLR≥3, according to Enríquez *et al*. (5).

Confounders variables:

- Age. This will be categorized in two groups (>55 years, <55 years)
- Clinical stage of breast cancer from the imaging analysis registered in the medical record at the time of diagnosis. This will be classified in four stages.

Classification of clinical stage of breast cancer (6)

Stage I	Small and invasive tumor, with a capacity to spread to lymph nodes
Stage II	Cancer spread to lymph nodes without evidence of tumor in breasts
Stage III	Cancer spread to 4-9 axillary or internal mammary lymph nodes, a tumor greater than 50 mm may be found
Stage IV	Presence of metastasis

● Molecular subtype: This will be classified as Luminal A, Luminal B, HER 2, and triple-negative (7).

Study variables and operationalization

Variables	Definition	Type	Indicator	Scale	Category	Values	Source
Neutrophil/lymphocyte ratio (NLR)	Division of absolute neutrophil count over absolute lymphocyte count	Qualitative	Neutrophil /lymphocyte ratio	Ordinal	High	≥ 3	Medical record
					Low	<3	
Overall survival (OS)	Time from the time of diagnosis until death or date of last follow-up	Quantitative	Years	Ratio			Medical record
Age	Time of existence of a person	Qualitative	Years	Ordinal		<55	Medical record
						>55	

Stage	Stage or period in the evolution of breast cancer	Qualitative	Stage	Ordinal	I II III IV	1 2 3 4	Medical record
Molecular subtype	Classification of type of cancer	Qualitative	Subtype	Ordinal	Luminal A Luminal B HER 2 Triple-negative	1 2 3 4	Medical record

STUDY PROCEDURES

Data collection

Data collection will be made from medical records during the months of January and February 2020.

Follow-up

The follow-up will be carried out retrospectively from the medical evaluations recorded in the clinical history.

Procedure

Technique: Documentation. Medical records of each patient will be reviewed and filled out in a data collection sheet.

Research instrument: This will be designed specifically for this study. This will be filled out with the patient's information, such as age, date of breast cancer diagnosis, molecular subtype, clinical stage, absolute neutrophil count, absolute lymphocyte count, NLR, date of last follow-up, and, if applicable, the date of death and cause of death.

Data monitoring

a) The participants will be selected from the same hospital, which contributes to the fact that the groups have fairly similar clinical and social characteristics.

b) The medical records will be thoroughly reviewed to avoid information bias.

c) An analysis adjusted for confounders will be considered to avoid erroneous conclusions.

Study programs

Microsoft Excel (RRID: SCR_016137)

Stata (RRID:SCR_012763) 15.0

DATA ANALYSIS

Sample size

We will not have a sample, because we will work with the entire population.

Statistical power

The statistical power of this number of participants to find an expected OS difference, as reported by a similar study (8), of 86.2% for the exposed group and 97.9% for the unexposed group, will be 98%; this will give us a low probability of making a type 2 error when testing the hypothesis of our main objective.

Statistical methods

We will use frequencies and percentages for the qualitative variables, and central tendency and dispersion measures for quantitative variables, prior evaluation of its normality using the Shapiro-Wilk test.

The Kaplan–Meier method will be applied to generate survival curves, which will be compared using the log-rank test. Likewise, Cox regression will be used to find crude and adjusted Hazard Ratios, with its respective 95% confidence intervals. A p-value<0.05 will be considered statistically significant.

ETHICAL CONSIDERATIONS

Ethical approval

This study will be evaluated by the research ethical committee of Hospital Nacional Hipólito Unanue in order to get the approval.

Confidentiality

All the patients' data will remain in absolute confidentiality by encrypting their personal identification.

Consent

Patients, or their relatives, will sign a written informed consent at the time of admission to the hospital, in favor of performing medical procedures and handling their data for teaching and research purposes.

Timetable

STEPS	2019		2020			
	November	**December**	**January**	**February**	**March**	**April**
Drafting of the project	X					
Project approval		X				
Data collection			X	X		
Data processing and analysis				X		
Preparation of the article					X	

						X
Review and approval of the article						X
Article review by specialists in the area						X
Submission of article for publication						X

Budget

Item	Estimated amount (soles)
Stationery	250.00
Specialized support	500.00
Logistics	300.00
Internet	120.00
Refreshment	300.00

Mobility	100.00
Database access	500.00
Review by specialists in the area	3600.00
Total	**2270.00**

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