People's Democratic Republic of Algeria

Abdelhamid Ibn Badis Mostaganem University

Faculty of Natural and Life Sciences

Department of Biology

Dissertation

Presented for obtaining the

MASTER'S DEGREE IN BIOLOGICAL SCIENCES

Speciality: Applied Biochemistry

By

Kupe Francoise Taboka

&

Mwemba Rachael Mambori

THEME

Impact of mastectomy and chemotherapy on haematological parameters in breast cancer patients at Mostaganem University Hospital oncology center.

Defended on 11/06/2024

Members of the Jury:

President	NEBBACHE Salim	MCA	University of Mostaganem
Examiner	BOUABSSA Foufa	MAB	University of Mostaganem
Supervisor	DAHMOUNI Said	MAA	University of Mostaganem
Co-supervisor	BENGHARBI Zineb	MCB	University of Mostaganem

Academic Year: 2023/2024

Dedication

I dedicate this work to my family—my beloved father, mother, and sibling—who have supported me unwaveringly throughout my academic journey. I am deeply grateful for your help and unceasing prayers, which have made it possible for me to reach this point. A special thanks to my grandparents, who have been my biggest cheerleaders.

This dissertation is also dedicated to my relatives and friends, whose encouragement has lightened this journey.

Lastly, I dedicate this dissertation to my partner and friend Rachael. Your diligence, dedication, and support have been invaluable in accomplishing this work, and I will always appreciate it.

Kupe Francoise Taboka

Dedication

With sincere gratitude, I wholeheartedly dedicate this research work to:

My late grandfather;

Your unwavering insistence on my commitment in both my relationship with God and my education laid the foundation for achieving many goals in my life. You will always hold a special place in my heart, Babu.

My mother and my brother;

Your love, prayers, encouragement, and support have been instrumental in completing this work and achieving my dreams. I am forever grateful to God for having you in my life.

My extended family;

I recognize and deeply appreciate your contributions to my academics, whether in the form of resources, inspiration, or prayers. I continually thank God for all of your contributions.

My siblings in the Lord and my close friends;

You have continuously prayed with me, allowed God to use you to speak over my life, and made my academic journey a blissful experience. I greatly appreciate you.

My partner Francoise;

Words alone cannot express my gratitude for your support, patience, and cooperation. Completing this work was challenging, but you made it seem effortless. In short, I have learned and grown immensely by working with you. Thank you Sis.

Mwemba Rachael Mambori

☆*Abstract*☆

Breast cancer ranks among the most prevalent cancers affecting women. Mastectomy and chemotherapy are crucial elements of its treatment, markedly affecting patients' haematological parameters and often resulting in anaemia, leukopenia, and thrombocytopenia. This study aims to examine the alterations in haematological parameters before and after these treatment methods in breast cancer patients in Mostaganem, Algeria. The study included 72 patients from 2019 to 2024, with 70 receiving chemotherapy and 28 undergoing mastectomy.

Medical records from the Mostaganem University Hospital oncology centre were utilised, focusing on patients who had undergone six cycles of chemotherapy and those who had only undergone mastectomy. Data on erythrocytes and their indices, leukocytes, platelets, haemoglobin, and haematocrit levels before and after chemotherapy and mastectomy were analysed.

All patients exhibited anaemia following chemotherapy, evidenced by low erythrocyte counts (3.99 μL uniquely in the 40-49 age group), haematocrit (mean value 34.84%), and haemoglobin (mean value 11.52 g/dL). Neutrophil, leukocyte, and thrombocyte levels varied depending on age and chemotherapy cycles. Similarly, all patients were anaemic both before and after surgical resection, with consistently low erythrocyte counts (mean value 4 μL), haematocrit (mean value 35.40%), and haemoglobin levels. Neutrophil levels remained within the normal range, while thrombocyte and leukocyte levels showed age-related variations.

Chemotherapy and mastectomy for breast cancer can lead to decreased haematological parameters and anaemia in patients. However, subsequent clinical monitoring, the use of haematopoiesis boosters, and maintaining a well-balanced nutrition regimen can mitigate these adverse effects.

Keywords: Breast cancer, mastectomy, chemotherapy, haematological parameters

☆*Résumé*☆

Le cancer du sein est l'un des cancers les plus fréquents chez les femmes. La mastectomie et la chimiothérapie sont des éléments cruciaux de son traitement, impactant fortement les paramètres hématologiques des patientes et entraînant souvent une anémie, une leucopénie et une thrombocytopénie. Cette étude vise à examiner les modifications des paramètres hématologiques avant et après la mise en œuvre de ces méthodes de traitement chez les patientes atteintes de cancer du sein à Mostaganem, Algérie. L'étude a inclus 72 patientes de 2019 à 2024, dont 70 ont reçu une chimiothérapie et 28 ont subi une mastectomie.

Les dossiers médicaux des patientes du centre d'oncologie de l'hôpital universitaire de Mostaganem ont été utilisés, en se concentrant sur les patientes ayant subi six cycles de chimiothérapie et celles ayant uniquement subi une mastectomie. Les données sur les érythrocytes et leurs indices, les leucocytes, les plaquettes, l'hémoglobine et les niveaux d'hématocrite avant et après la chimiothérapie et la mastectomie ont été analysées.

Toutes les patientes ont présenté une anémie après la chimiothérapie, comme en témoignent les faibles niveaux d'érythrocytes (3,99 µL uniquement dans le groupe d'âge 40-49 ans), d'hématocrite (valeur moyenne de 34,84 %) et d'hémoglobine (valeur moyenne de 11,52 g/dL). Les niveaux de neutrophiles, leucocytes et thrombocytes variaient en fonction de l'âge et des cycles de chimiothérapie. De même, toutes les patientes étaient anémiques avant et après la résection chirurgicale, avec des niveaux d'érythrocytes constamment bas (valeur moyenne de 4 µL), d'hématocrite (valeur moyenne de 35,40 %) et d'hémoglobine. Les niveaux de neutrophiles restaient dans la plage normale, tandis que les niveaux de thrombocytes et de leucocytes montraient des variations liées à l'âge.

La chimiothérapie et la mastectomie pour le cancer du sein peuvent entraîner une diminution des paramètres hématologiques et une anémie chez les patientes. Cependant, une surveillance clinique ultérieure, l'utilisation de stimulants de l'hématopoïèse et le maintien d'un régime alimentaire équilibré peuvent atténuer ces effets indésirables.

Mots clés : Cancer du sein, mastectomie, chimiothérapie, paramètres hématologiques

★ الملخص ★

يعد سرطان الثدي من أكثر أنواع السرطانات شيوعًا التي تصيب النساء. تعتبر استئصال الثدي والعلاج الكيميائي عناصر حاسمة في علاجه، حيث تؤثر بشكل كبير على المعايير الدموية للمرضى وغالبًا ما تؤدي إلى فقر الدم، انخفاض عدد كريات الدم البيضاء ونقص الصفائح الدموية. تهدف هذه الدراسة إلى فحص التغيرات في المعايير الدموية قبل وبعد تنفيذ هذه الأساليب العلاجية لدى مرضى سرطان الثدي في مستغانم، الجزائر. شملت الدراسة 72 مريضًا من عام 2019 إلى عام 2024، منهم 70 تلقوا العلاج الكيميائي و28 خضعوا لاستئصال الثدي.

تم استخدام السجلات الطبية لمرضى سرطان الثدي الذين خضعوا لـ 6 دورات من العلاج الكيميائي وأولئك الذين خضعوا لاستئصال الثدي فقط في مركز الأورام بمستشفى جامعة مستغانم. تم تحليل بيانات كريات الدم الحمراء ومؤشراتها، كريات الدم البيضاء، الصفائح الدموية، مستويات الهيموجلوبين والهيماتوكريت قبل وبعد العلاج الكيميائي واستئصال الثدي.

أظهرت جميع المريضات فقر دم بعد العلاج الكيميائي، كما يتضح من انخفاض مستويات كريات الدم الحمراء (3.99 ميكرولتر في الفئة العمرية 40-49 سنة)، الهيماتوكريت (متوسط القيمة 34.84%) والهيموجلوبين (متوسط القيمة 11.52 جم/ديسيلتر). تنوعت مستويات العدلات، كريات الدم البيضاء والصفائح الدموية اعتمادًا على العمر ودورات العلاج الكيميائي. وبالمثل، كانت جميع المريضات يعانين من فقر دم قبل وبعد الاستئصال الجراحي، مع مستويات منخفضة باستمرار من كريات الدم الحمراء (متوسط القيمة 4 ميكرولتر)، الهيماتوكريت (متوسط القيمة 35.40%) والهيموجلوبين. بينما بقيت مستويات العدلات ضمن النطاق الطبيعي، أظهرت مستويات الصفائح الدموية وكريات الدم البيضاء تباينات مرتبطة بالعمر.

يمكن أن يؤدي العلاج الكيميائي واستئصال الثدي لعلاج سرطان الثدي إلى انخفاض المعايير الدموية وفقر الدم لدى المرضى. ومع ذلك، يمكن أن يخفف المراقبة السريرية اللاحقة، واستخدام محفزات تكون الدم والحفاظ على نظام غذائي متوازن من هذه التأثيرات السلبية.

الكلمات المفتاحية: سرطان الثدي، استئصال الثدي، العلاج الكيميائي، المعايير الدموية

LIST OF TABLES

List of appendices

LIST OF FIGURES

x

LIST OF ACRONYMS

A

Adenomatous polyposis coli
  APC
Adriamycin and cyclophosphamide
  AC

B

Body mass Index
  BMI
Breast cancer
  BC
Breast Cancer gene 2
  BRAC2
Breast Cancer gene 1
  BRAC1

C

Cadherin 1
  CDH1
Cancer stem-like cells
  CSCs
Complete blood count
  CBC
C-X-C chemokine receptor type 4
  CXCR4
C-X-C motif chemokine ligand 12
  CXCL12
Cyclin dependant kinase inhibitor
  p27Kip1
Cyclin-dependent kinases
  CDKs
Cyclin D1
  CCND1

D

Danger-associated molecular patterns
  DAMPs
Deoxyribonucleic acid
  DNA

Dichlorodiphenyltrichloroethane
  DDT
Direct intravenous injection
  DIV
DNA-binding domain
  DBD
Ductal carcinoma in situ
  DCIS

E

Estrogen receptor
  ER
Ethylene diamine tetra acetic
  EDTA
Extracellular matrix
  ECM

F

Fluorouracil
  5-FU
Fluorouracil, epirubicin, and
  cyclophosphamide
  FEC

G

Glycogen synthase kinase 3
  GSK3
Granulocyte colony-stimulating factor
  G-CSF
Growth factors
  GF
Growth factor receptor-bound protein 2
  GRB2

H

Haematocrit
  Ht
Haemoglobin
  Hb
Hematopoietic stem cells
  HSCs

Human epidermal growth factor receptor
   HER 2

Hydrocortisone
   HHC

Hypothalamic–pituitary–adrenal
   HPA

Hypoxia inducible factor alpa 1
   HIFIA

I

Infiltrating ductal carcinoma
   IDC

Infiltrating lobular carcinoma
   ILC

Interleukin
   IL

L

Lobular carcinoma in situ
   LCIS

M

Major histocompatibility complex class 1
   MHC1

Mammalian target of rapamycin
   mTOR

Mean corpuscular hemoglobin
   MCH

Mean corpuscular hemoglobin
   concentration
   MCHC

Mean corpuscular volume
   MCV

Metastases
   M

Mitogen-activated protein kinase
   MAPK

Mitogen-activated protein kinase kinase
   MEK

Mouse double minute 2 homolog
   MDM2

N

Natural killer
   NK

Neurofibromatosis type 1
   NF1

Nipple areola complex
   NAC

No special type
   NST

Nodal involvement
   N

Nuclear factor kappa beta
   NF-kB

O

Oestrogen response elements
   EREs

P

Pathogens-associated molecular patterns
   PAMPs

Phosphatase and Tensin genes
   PTEN

Phosphatidylinositol 4,5-bisphosphate 3-
   kinase
   PI3K

Polychlorinated biphenyl
   PCB

Polyunsaturated fatty acids
   PUFA

Potential of hydrogen
   pH

Progesterone receptor
   PR

Prostaglandins
   PG

Protein kinase B

   AKT

R

Rat sarcoma virus

RAS
Rapidly accelerated fibrosarcoma
   RAF
Reactive oxygen species
   ROS
Receptor activator of NF-Kb ligands
   RANKL
Receptor activator of NK-Kb
   RANK

S

Serine /threonine kinase
   STK11
Son of sevenless
   SOS

Sympathetic nervous system
   SNS

T

Taxotere plus cyclophosphamide
   TC
Toll-like receptors
   TLRs
Triple-negative breast cancer
   TNBC
Tumour microenvironment
   TME
Tumour protein
   p53
Tumour size
   T

W

Wingless-related integration site 4
   Wnt4

World Health Organization
   WHO

TABLE OF CONTENTS

Chapter 3: Immune system

Chapter 4: Breast cancer therapies

Chapter 5: Materials and methods

Chapter 6: Results and discussion

GENERAL INTRODUCTION

Introduction

Cancer encompasses a group of malignant diseases characterized by abnormal cell growth with the potential to invade other parts of the body. It is the second leading cause of death globally, following heart disease. The rising incidence of cancer worldwide results in millions of casualties annually, underscoring the urgent need to develop effective pharmaceuticals for various cancers.

Breast cancer (BC) arises in the tissues of the breast, typically the ducts and lobules. It is a prevalent malignancy affecting millions globally, with mastectomy and chemotherapy as fundamental components of its treatment. While these interventions are crucial for managing the disease and improving survival rates, they can profoundly affect hematological parameters, including erythrocytes, leukocytes, and thrombocytes. Understanding the relationship between breast cancer treatments and hematological parameters is essential for optimizing patient care and minimizing treatment-related complications.

Chemotherapy, a cornerstone of systemic breast cancer treatment, uses cytotoxic agents to target rapidly dividing cancer cells. However, these agents also impact hematopoietic progenitor cells in the bone marrow, leading to myelosuppression and alterations in haematological parameters. Common chemotherapeutic agents, such as anthracyclines and taxanes, can induce dose-dependent cytopenias, including anaemia, leukopenia, and thrombocytopenia, compromising patients' overall well-being and quality of life. The timing, duration, and intensity of chemotherapy regimens significantly influence the severity and duration of haematological toxicity. Cumulative doses of cytotoxic agents and the interval between treatment cycles play crucial roles in determining the extent of bone marrow suppression and subsequent haematological recovery. Therefore, meticulous monitoring of haematological parameters during chemotherapy cycles is imperative to guide supportive interventions, such as blood transfusions, hematopoietic growth factors, and dose modifications, to mitigate treatment-related complications and maintain optimal haematological function.

Mastectomy, a surgical procedure involving the removal of breast tissue, can elicit systemic physiological responses and alter haematological indices. Surgical trauma and associated inflammatory processes may lead to transient decreases in haemoglobin levels and erythrocyte count, often necessitating perioperative interventions to manage potential

blood loss and maintain hemostasis. Additionally, the extent of surgical resection and the surgical approach (e.g., lumpectomy vs. mastectomy) can influence the magnitude of haematological changes. Individual patient characteristics, including age, comorbidities, genetic polymorphisms, and baseline haematological status, can modulate susceptibility to treatment-induced haematological toxicity. Personalized risk assessment and tailored interventions are essential to optimize treatment outcomes while minimizing adverse effects on haematological parameters.

A complete blood count (CBC) is a prerequisite for evaluating haematological parameters before initiating and following chemotherapy or mastectomy. Regular monitoring of changes in haematological parameters during treatment is crucial since abnormal parameters can negatively influence cancer outcomes. Blood cells, being among the most rapidly dividing cells in the body, are particularly sensitive to chemotherapy. Given the diverse effects of these treatment methods on the body's organ systems, this study aims to evaluate the impact of chemotherapy and mastectomy on haematological parameters in breast cancer patients across different age categories and to analyse the variations in these parameters among different age groups.

This work is structured into two main sections. The first section addresses the theoretical framework of our study, divided into four chapters: chapter one introduces the general concept of cancer, chapter two focuses on breast cancer, chapter three provides an overview of immunity and hematopoiesis, and chapter four explores chemotherapy and mastectomy. The second section outlines the materials and methodology used in this study, culminating in a general discussion and conclusion.

CHAPTER 1

GENERAL INSIGHT INTO CANCER

1.1 Generalities and epidemiology

The term "cancer" finds its roots in the work of the Greek physician Hippocrates (460-370 BC), often hailed as the "Father of Medicine." He used the terms "carcinos" and "carcinoma" to distinguish between non-ulcer forming and ulcer-forming tumors. The history of cancer traces back to 1761 when Giovanni Morgagni conducted the first autopsies, linking post-mortem pathological findings to patients' illnesses and laying the groundwork for the scientific study of cancer, known as oncology. Since then, cancer has remained a leading cause of death worldwide, with over 19.9 million new cases diagnosed and reported, resulting in approximately 9.9 million deaths in 2022, according to the Global Cancer Observatory GLOBOCAN 2022 **(Ferlay *et al*., 2024)**. The African continent had 1.2 million cases resulting in 763,843 deaths. Algeria accounted for 64,713 cases and 35,778 fatalities, while Kenya reported 44,726 cases, with 29,317 resulting in death. Additionally, Zimbabwe recorded 17,725 cases, with 11,739 resulting in mortality **(Ferlay *et al*., 2024)**.

The constant emerging incidences of cancer worldwide have caused millions of casualties annually bringing about the need and demand for developing potent pharmaceuticals for treating different cancers.

Numerous factors such as environmental influences, internal stress, or heredity are responsible for cancer. They vary from one patient to another depending on the type of cancer and geographical location, appropriate therapies have to be determined to meet the needs of each case.

Climate change as a result of industrialisation alongside with lifestyles and the food being consumed is considered as the major reason for increasing numbers of cancer incidences although a more convincing relation is yet to be established. The socio-economic status of a specific region impacts the availability of medical facilities and more effective expensive drugs. Recently according to **(Siegel *et al.*, 2023)** the corona virus disease 2019 (COVID-19) pandemic caused delays in the diagnosis and treatment of cancer due to closures of health care settings, disruptions in employment and health insurance, and paranoia of COVID-19 exposure. Although the impact was largest during the COVID-19 peak in mid-2020, the provision of health care has not fully recovered.

Additionally, the improper use of pesticides, industrial waste disposal practices, and pollution control policies indirectly contribute to the quality of healthy living. Increased prevalence in different genders and populace has been observed with the occurrence of a

particular kind of cancer. For example, breast cancer is majorly reported among women while prostate cancer among men at a global level **(Chhikara and Parang, 2020)**.

1.2 Cancer development phases

Cancer is characterised by unregulated cell division and multiplication. Through the bloodstream and lymphatic circulation, neoplastic cells can migrate and infiltrate other organs of the body. Cancer typically progresses through three phases at the cellular level: Initiation, promotion and progression.

The first phase begins when the deoxyribonucleic acid (DNA) molecule is damaged by either metabolic, genetic or cancerous factors such as chemicals, radiation and viruses. Carcinogens disrupt the DNA, leading to abnormalities through a process called carcinogenesis. This involves the activation of oncogenes and the inactivation of tumour suppressors, resulting in cell-cycle disruption and inhibition of cell death **(Sarkar *et al.*, 2013)**.

The promotion phase marks the subsequent stage in cancer development, commencing with the proliferation of abnormal cells from the initiation phase and culminating in the progression phase, characterized by the dissemination of cancerous cells that have proliferated **(Fig 1).** However, it is believed that a single genetic alteration is
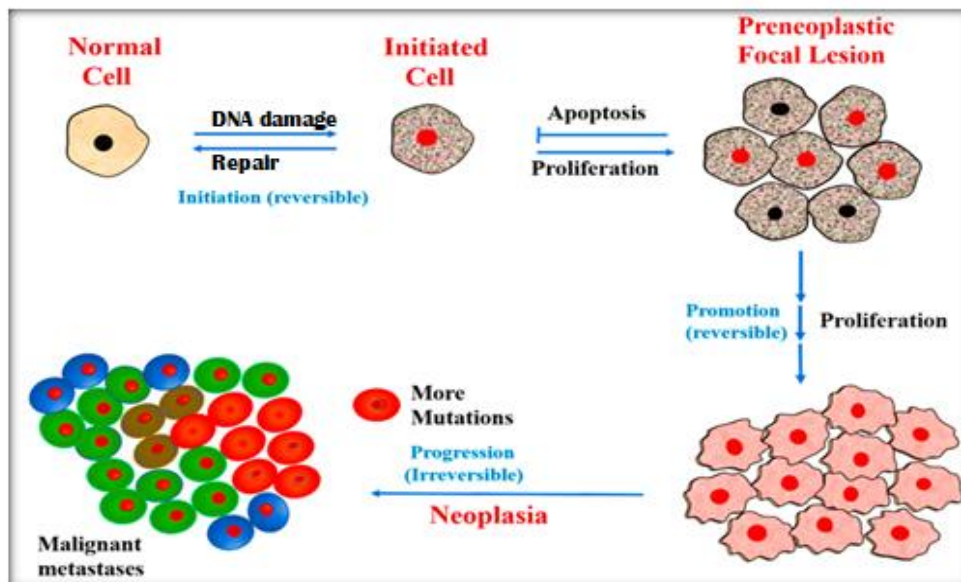
Figure 1: Stages of cancer development: From initiation to metastasis **(Anndleeb *et al*., 2021)**

insufficient to cause cancer hence various hypotheses state that cancer is caused by a build-up of genetic abnormalities in a cell's DNA **(Alzahrani *et al*., 2021)**.

1.3 Hallmark of cancer cells

Cancerous cells exhibit several differences from normal cells due to their less stringent regulation and uncontrolled proliferation resulting from defects in cellular regulatory processes. Cancer is characterized by numerous molecular and genetic changes, with common characteristics identified by Hanahan and Weinberg **(Fig 2)** which includes; lack of responsiveness to growth-suppressive signals, avoidance of cell death (apoptosis) processes, unlimited replicated capacity, prolonged angiogenesis, tissue infiltration, metastases, cellular metabolic reprogramming and immune system evasion (**Hanahan and Weinberg, 2000; Fimognari** *et al.***, 2011**).

Tumour growth affects multiple signal transduction pathways at the same time, including apoptosis, cell cycle, DNA repair, and redox balance. There are over 100 different
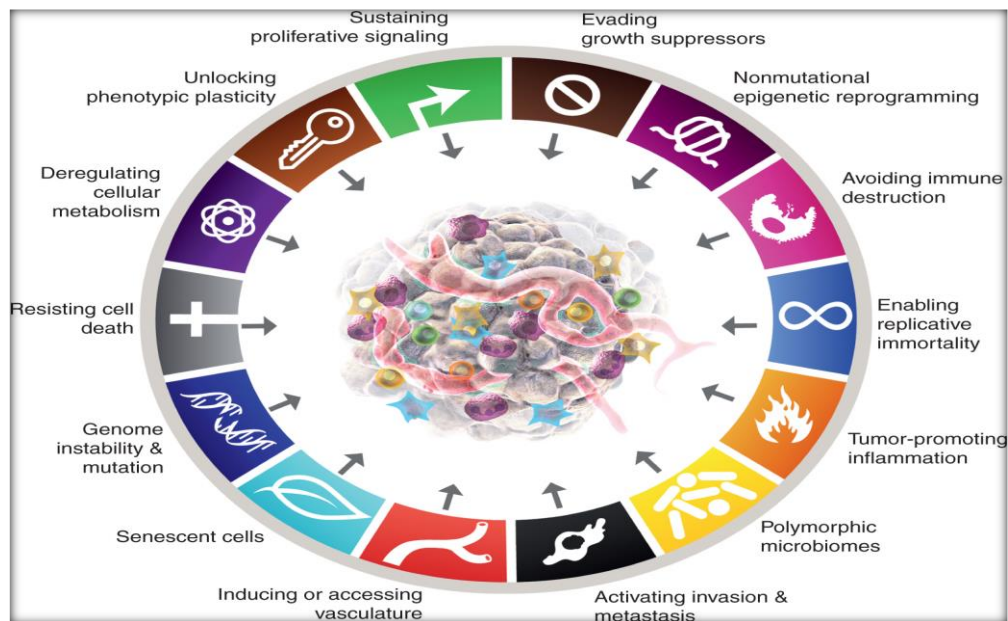
Figure 2: The fundamental traits of cancer (**Hanahan, 2022**)

forms of cancer arising from the unregulated proliferation of any of the body's cell types; each with its specific behaviour and therapeutic responses (**Cooper and Hausman, 2000).**

1.4 Tumours

The term 'tumor,' which denotes abnormal cell proliferation, can manifest as either benign or malignant. In cancer biology, various markers are utilized to differentiate between benign and malignant tumors, including rapid growth, increased cell turnover, aggressive expansion, metastasis, and infiltration of lymphatic and vascular channels. Benign tumors typically exhibit slow growth, lack invasiveness, are encapsulated within fibrous tissue, and

retain morphological resemblance to their cellular precursors. Timely diagnosis and treatment often result in a cure for benign tumors, provided they are not in close proximity to critical vascular or neural tissues.

On the other hand, malignant tumours rarely encapsulate (**Fig 3**), grow
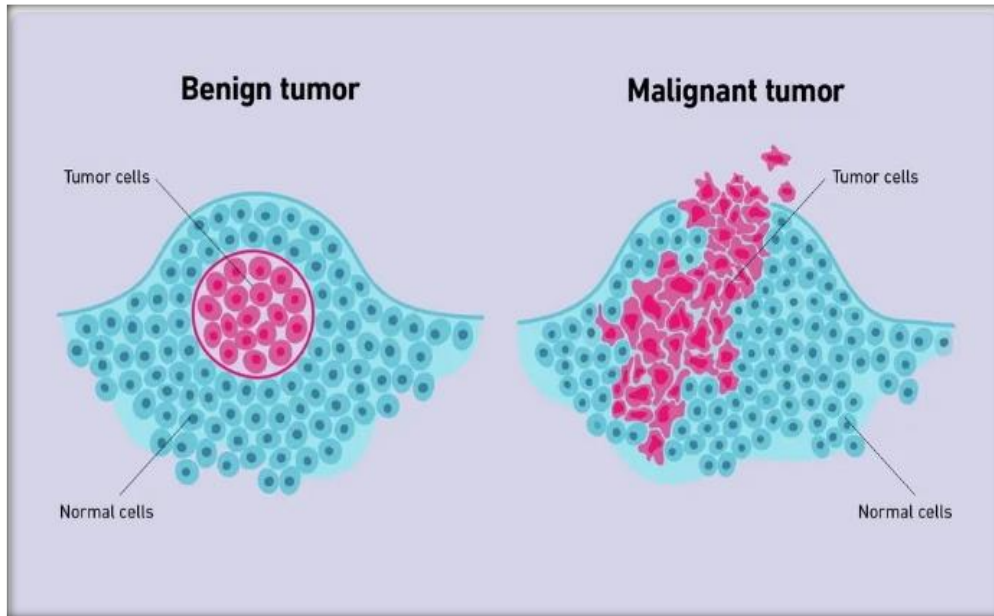
Figure 3: Benign and malignant tumour (**Whelan, 2022**)

rapidly, invade regional tissues (metastases), and display morphological abnormalities, making their tissue of origin difficult to detect (**Talmadge and Fidler, 2010**). Only malignant tumours are considered as cancers and their tendency to spread is what renders them deadly. Malignant tumours that spread to other parts of the body are typically resistant to treatments.

1.5 Forms of cancer

There are three forms of cancers that account for the majority of occurrences, namely sarcomas, carcinomas and leukaemia or lymphomas. The carcinomas are cancerous tumours that develop from epithelium tissues and account for 90% of all malignancies in humans. The sarcomas are infrequent solid tumours that develop from the bone and connective tissues (**Hoang *et al.*, 2018**). Cancers that arise from bone marrow stem cells (**Tab 1**) and cells of the immune system are known as leukaemia and lymphomas (**Davis *et al.*, 2014**).

Table 1: Cancer classification based on tissue types **(Alavi and Hamidi, 2019)**

Tumours are categorised depending on the kind of cells involved and the tissues of origin. Breast, bladder, colon/rectum, lungs, liver and uterine cancers, and also leukaemia and lymphomas account for more than 75% of all cancers. There are many different types of cancers but, very few are common. The breast, prostate, lung and colorectal cancer are the four most frequent kinds of cancers responsible for more than half of all cases of cancer.

1.6 Tissue homoeostasis and cancer progression.

Tissue homeostasis is governed by the regulation of cell longevity. Various mechanisms of cell cycle regulation coordinate the proliferation, growth, repair, or apoptosis of different cell types. The interplay among proliferating, quiescent, and senescent cells, poised for elimination, ensures the maintenance of tissue mass at a steady state. However, this balance between cell duplication and apoptosis is disrupted in cancer, leading to unrestrained tissue expansion due to uncontrolled cell growth. Consequently, the body loses nutrients and oxygen, resulting in physical exhaustion and ultimately death **(Diori karidio and Sanlier, 2021).**

Cells undergo daily genetic damages due to the environmental exposures such as ultra violet light as well as normal "wear and tear", faults during DNA synthesis and mitosis. These are regularly recognised and successfully corrected in cell-cycle checkpoints but if the damages are not corrected during sequence patching and nucleotide splicing, genetic alterations occur **(Loeb and Loeb, 2000)** Point mutations such as nucleotide deletions,

insertions, duplication and rearrangement as well as amplifications of segments that result in multiple gene copies and chromosomal rearrangements (gene transfer to some other promoter that results in an unregulated expression) that happen during cell division are examples of chromosomal abnormalities. The number, location, and severity of mutations impact the cell's ability to overcome selection boundaries and the likelihood of these mutations being inherited by daughter cells if a cell evades these boundaries. This results in genomic instability at the nucleotide sequences and at the chromosomal level, leading to the accumulation of numerous abnormalities (mutational set) and ultimately a changed genotype. When these abnormalities confer benefits for cellular development, differentiation, and lifespan, problems arise (**Loeb and Loeb, 2000**).

1.7 Cell cycle and cancer

Several critical events enable tumour cells and their progeny to multiply aberrantly and invade body organs. Among these, uncontrolled cell proliferation, coupled with suppressed apoptosis, paves the way for further neoplastic progression. A typical cell cycle comprises three key events which include growth, DNA synthesis and division. The duration of each event is meticulously controlled by the chemical signals provided or intrinsic to the cells. The transition between each phase is requisite of the exact chemical signals and timely responses and any slight adjustment to aforementioned can give rise to cancerous cells (**Malumbres and barbacid, 2009; Collins et al., 1997**).

The main phases of the cell cycle include G1 phase, S phase, G2 phase, and mitosis, each regulated by distinct checkpoints that halt cell division if specific requirements, such as DNA integrity and proper cell size, are not met. Progression to the division process only occurs when all checkpoints are satisfied. Cyclins and cyclin-dependent kinases (CDKs) play crucial roles during the cell cycle. CDKs, as catalytically active components, regulate the activity of other proteins by transferring phosphate groups (**Malumbres and barbacid, 2009**). However, their activity majorly depends on their interaction with cyclins forming cyclin/CDK complexes leading to CDK activation. Normal cell cycle requires cyclic patterns in the formation and degradation of cyclin/CDK complexes (**Chuphal, 2022**).

The G1 phase has a vital checkpoint START which decides appropriate time for the progression of a cell to phase S where it commences DNA replication. There are inhibitory proteins in phase G1 which after sensing abnormalities such as DNA damage can halt the cyclin/CDK complex thereby preventing the cell the S phase (**Chuphal, 2022**). In tumour cells, these checkpoints are deregulated usually as a result of abnormalities in

genetic machinery such as mutations in genes encoding cyclin or CDKs or by modified proteins during the cell cycle disruption. Normal cells are usually programmed to stop at START and confirm if all machinery is in a proper condition before proceeding for DNA replication. On the other hand, cells whose checkpoint is faulty move to the S phase without repairing the DNA damage (**Fig 4**). Over a period of time, these accumulated mutations cause further cell cycle deregulation, thus leading to the formation of aggressive cancerous cells (**Chuphal, 2022**).
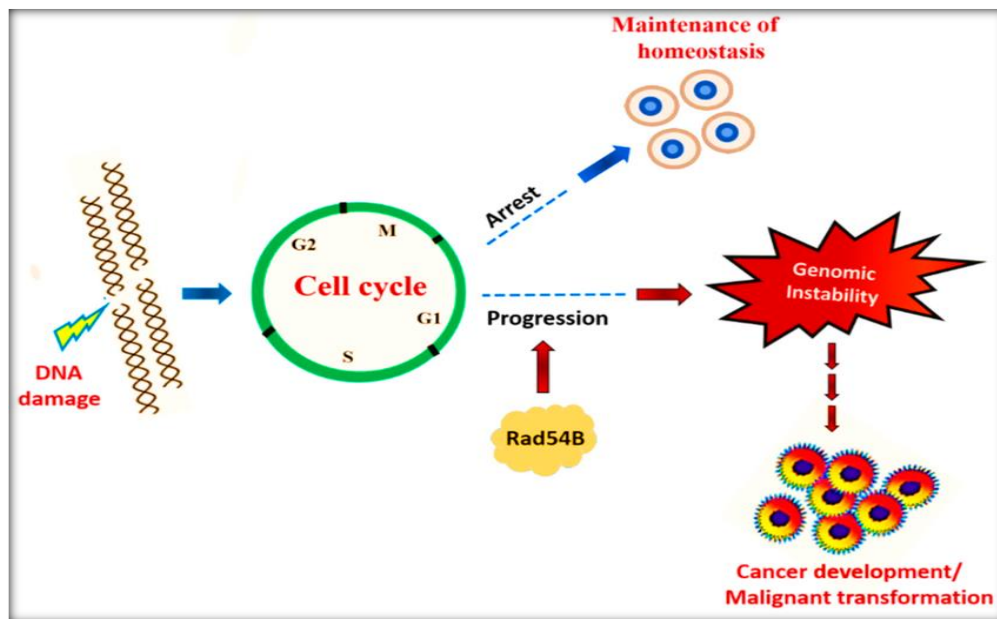
Figure 4: Cell cycle regulation in response to DNA damage(**Andleeb** *et al.*, **2021**)

1.8 Regulatory genes

There are two classes of genes that regulate cell proliferation and apoptosis which are;

1.8.1 Tumour suppressor genes

These genes encode proteins that help in slowing cell development process and promote apoptosis. The loss-of-function mutation either through insertion, deletion, epigenetic silencing and nonsense mutation leads to different cancer incidences. The tumour suppressor genes include tumour protein(p53), Phosphatase and Tensin genes(PTEN), Breast Cancer gene 1 & 2 (BRAC1 &BRAC2), Adenomatous polyposis coli (APC), neurofibromatosis type 1 (NF1) and cyclin dependant kinase inhibitor (p27^{Kip1}), Mutation

in any of these genes results to cancer development but a loss of function requires the alteration of both genes since the allele pairing is recessive **(Diori karidio and Sanlier; 2021; Chuphal, 2022)**.

1.8.2 Proto-oncogenes

These genes code for proteins involved in cell proliferation and differentiation. A gain of function through either gene amplification or chromosomal translocation can alter these genes and lead to the activation of oncogenes (which are mutated proto-oncogenes). There are many types of proto-oncogenes which include growth factors(GF), GF receptors, GF response transducers, and transcription factors. The oncogenes cause cancer development when present and they can be introduced either through viruses that cause cancer into the host cells or some genes in the host genome can undergo mutations to form oncogenes. These genes can code for proteins which result in stimulation of excessive proliferation of cells and can lead to enhanced cell survival **(Chuphal, 2022)**. The oncogenes are vulnerable to changes in expression since their allele pairs have genetic dominance and a mutation in one of them provides greater expression in the other **(Fig 5).**
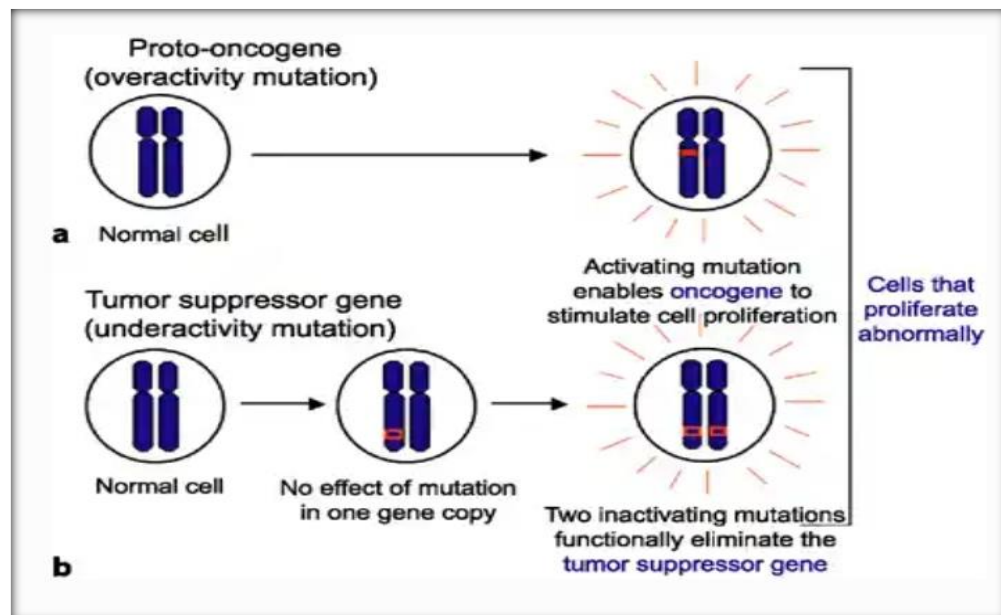
Figure 5: Mutations in proto-oncogenes and tumour suppressor genes.(**Wijnhoven *et al*., 2005)**

CHAPTER 2

BREAST CANCER

2.1 Preamble

Breast cancer is an uncontrolled hyper proliferation of the epithelial cells, incepting from the ducts or breast lobules. The pathology includes early noninvasive breast cancer such as ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS) and the late invasive BC that invades the surrounding breast stroma (Primary Invasive Breast Cancer) and is disseminated to the draining lymph nodes or distant organs like the liver, bone, brain and lungs (advanced or metastatic breast cancer **(Reilly, 2007)**.

Breast cancer is majorly diagnosed in women and it is considered as one of the leading causes of fatalities among the gender compared to men where it is infrequent and accounts for approximately less than 1% of the diagnosed cases **(Łukasiewicz *et al*., 2021)**.

2.2 Epidemiology

Global data from 2000 to 2018 reveals a rapid increase in the incidence of breast cancer in women, rising from 1.05 million cases in 2000 to 2.09 million in 2018. However, mortality cases fluctuated over this period. In 2000, global female breast cancer mortality cases were recorded at 370,000, peaking at around 520,000 in 2012, and then decreasing to 310,000 in 2018.In 2022, approximately 2.3 million cases were diagnosed globally, resulting in around 669,846 deaths. Within Africa, an estimated 198,831 new cases and 91,252 mortalities occurred. Specifically, Algeria reported 14,601 new incidence cases with 4,893 deaths, while Kenya had 7,243 new cases and 3,398 mortality cases. Zimbabwe reported 1,807 incidence cases and 915 deaths, according to GLOBOCAN 2022 **(Ferlay *et al*., 2024)**.

While the incidence rate was prominently reported in developed countries, Asia and Africa accounted for 63% of the total deaths in 2020. Recent projections indicate that by 2030, worldwide annual incidence cases may reach 2.7 million, with mortality cases totaling around 0.87 million. It's believed that women in high-income countries who develop breast neoplasms have higher survival chances compared to those in low- or middle-income countries. Furthermore, breast cancer incidence and mortality rates vary regionally across the globe due to lifestyle changes and disparities in access to diagnosis and treatment measures **(Łukasiewicz *et al*., 2021)**.

2.3 Risk factors associated breast cancer occurrence

The aetiology of BC includes both non-modifiable and modifiable factors.

2.3.1 Non-modifiable factors

a) Female sex

It is non-negotiably one of the major factors linked with elevated BC cases simply because of the increased hormonal stimulation compared to the male sex, who have low oestrogen levels. Women possess breast cells that are highly sensitive to oestrogen and progesterone hormones as well as to any slight variation on their levels**. (Endogenous Hormones and Breast Cancer Collaborative Group *et al*., 2013).**

b) Senility

The accumulation of a vast number of cellular alterations and exposition to potential carcinogens results in an increase of carcinogenesis with time in aged women **(Łukasiewicz *et al*., 2021).** Approximately 80% of patients with breast cancer are aged >50 of which more than 40% are those more than 65 years old. A relationship between certain breast cancer molecular subtypes and a patient's age have been observed as well showing that aggressive resistant triple-negative breast cancer subtype is most commonly examined in groups under 40 age, while luminal A subtype is examined in patients >70 **(McGuire *et al*., 2015).** The breast cancer risk in relation to age increases as follows: 1.5% risk at age 40, 3% at age 50, and more than 4% at age 70 **(Łukasiewicz *et al*., 2021).**

c) Reproductive history

A relationship between exposure to endogenous hormones (oestrogen and progesterone) and heightened prevalence to breast cancer incidence has been confirmed through several studies showing that specific events such as pregnancy, first menstruation, breastfeeding and menopause along with their time frame and hormonal imbalances can hugely contribute to carcinogenic formations in breast microenvironment **(Łukasiewicz *et al*., 2021).**

The first full-term pregnancy at an early age (particularly in the early twenties) along with subsequent increasing number of births have been affiliated with a decreased risk of breast cancer occurrence **(Albrektsen *et al*., 2005).** Moreover, pregnancy provides protection against breast cancer occurrence **(Husby *et al*., 2018).**

An increased duration of the breastfeeding period also reduces the risk of both the estrogen receptor (ER)/ progesterone receptor(PR)-positive and -negative neoplasms **(Ursin *et al*., 2005)** and early menopause whether naturally or surgically induced confers protection against breast cancer development **(Titus-Ernstoff *et al*., 1998).**

d) Genetic mutations

Genetic mutations have been shown to be particularly correlated with a high risk of BC incidences. Two major genes BRCA1(located on chromosome 17) and BRCA2(located on chromosome 13) are mostly affiliated with the high incidence and high penetrance. The mutations within BRCA1 and BRCA2 are inherent in an autosomal dominant manner although sporadic mutations are also commonly reported. Other examples of high penetrant breast cancer genes include P53, cadherin 1 (CDH1), PTEN **(Shiovitz and Korde, 2015; Kechagioglou, 2014)**, and (STK11) serine /threonine kinase 11 **(Chen and Lindblom, 2000).** Besides the increased risk of breast cancer, carriers of such mutations are more prevalent to ovarian cancer as well **(Łukasiewicz *et al*., 2021).**

e) Breast tissue density

A greater breast tissue density has been associated with a greater risk of breast cancer among premenopausal and postmenopausal women. Given that the breast tissue remains inconsistent or evolves through lifetime 3 categories have been established; Low and high-density and fatty breasts **(Łukasiewicz *et al*., 2021).**

f) Racial disparities

Although there is emerging evidence supporting disparities in racial BC occurrences, there is a paucity of adequate molecular studies to offer responses related to confounding factors underlying interracial differences and comparison. Many research studies have been conducted in the United States with limited comparison between non-Hispanic white and black or African American women with major incidence rate being observed to be high among white non-Hispanic women and high mortality and low survival rates among black women **(Yedjou *et al*., 2019; Hill *et al*., 2019; Yap, 2023).**

2.3.2 Modifiable factors

a) Physical activity

Women under active physical activities have reduced susceptibility of developing BC compared to those who seldomly engage into physical activities. Although the insight into the mechanisms underpinning this phenomenon has not been clearly delineated, inflammation, sex steroid hormones and insulin/insulin growth factor system signalling, are among the major proposed causal pathways

Inflammation is associated with cancer growth and progression through various ways: stimulation of cell proliferation and influences the tumour microenvironment via the recruitment, proliferation, and function of pro tumorigenic auxiliary cells. Frequent physical

activity confers the potential of reducing inflammation via the production and release of myokines from skeletal muscle tissue, decreases the levels of body fat, slow age-related weight gain and reduces the production of pro-inflammatory cytokines by the immune system **(Łukasiewicz *et al*., 2021; Swain *et al.,* 2023).**

Studies have also shown that vigorous physical recreational activities have the potential to not only alter the regular menstruation cycle but also delay the onset of menarche. These are associated with reduced circulating oestrogen and androgen levels thereby diminishing exposure to sex steroid hormones and thus combating the risk of aberrant breast-cell proliferation.

Skeletal muscle insulin sensitivity in the uptake of glucose has a direct relation with increased physical activity thereby reducing insulin growth factors that engenders cell differentiation, proliferation and apoptosis **(McTiernan, 2008; Lynch *et al*., 2022).**

b) Pharmaceutical prescriptions

Certain drugs have been observed/correlated with high risk of Breast Cancer emergence such as hormone replacement therapy especially used in higher duration of 5-7 years is associated with the risk of BC **(Narod, 2011)** studies have also shown that the intake of chosen antidepressants, mainly paroxetine, tricyclic antidepressants, and selective serotonin reuptake inhibitors might be associated with a risk of breast cancer **(Wernli *et al.,* 2009)**.

c) Smoking

The uptake of carcinogens found in the tobacco either through active or passive smoking can invoke pro carcinogenic events such as the mutations of tumour suppressor genes or proto-oncogenes. A family history of breast cancer is majorly associated with a longer smoking duration and also smoking before the first full-term pregnancy among females **(Catsburg *et al*., 2015)**.

d) Alcohol consumption

The alcohol intake has been closely associated with elevated levels of oestrogen hormones (particularly linked with oestrogen-positive breast cancers) resulting in the hormonal imbalances thus affecting the risk of carcinogenesis within the female organs **(Łukasiewicz *et al*., 2021)**.

Alcohol also results in higher risks of corpulence with increased BMI (Body mass Index) levels which in turn increases the risk of BC. Likewise, the metabolism of alcohol

produces acetaldehyde and other reactive oxygen species (ROS) which are considered to induce DNA modifications, by generating protein adducts, chromosomal aberrations, and DNA point mutations **(Kotepui, 2016)**.

e) Inadequate vitamin supplementation

Vitamins have anticancer properties that protect women against the emergence of the Breast neoplasms especially Vit D. High serum 25-hydroxyvitamin D levels are associated with a lower incidence rate of breast cancer in premenopausal and postmenopausal women **(Estébanez *et al*., 2018)**. Intensified expression of vitamin D receptors was shown to be associated with lower mortality rates due to breast cancer. With the few findings, it is not yet confirmed to be highly effective.

f) Exposure to certain chemical compounds

Chronic exposure to chemicals has presented the plausibility of mutations on the breast microenvironment leading to breast tumours. Chemicals such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyl (PCB) are mostly investigated in terms of breast cancer since early exposure to those chemicals disrupts the development of mammary glands **(Łukasiewicz *et al*.,2021)**.

g) Highly processed foods ingestion

According to the World Health Organization (WHO), highly processed meat was categorised as a Group 1 carcinogen that might increase the risk of not only gastrointestinal malignancies but also breast cancer. Same observations were made in terms of an excessive intake of saturated fats **(Dandamudi *et al*., 2018)**. It was observed that a 10% increase of ultra-processed food in the diet is associated with an 11% greater risk of breast cancer **(Fiolet *et al*., 2018)**. Contrarily, a diet high in vegetables, fruits, legumes, whole grains, and lean protein is associated with a lowered risk of breast cancer **(Dandamudi *et al*., 2018)**. Generally, a diet that includes food containing high amounts of n-3 PUFA (polyunsaturated fatty acids), vitamin D, fibre, folate, and phytoestrogen might be beneficial as a prevention of breast cancer **(Kotepui, 2016)**. Green tea and turmeric have been observed to contain compounds that are anti-carcinogenic.

2.4 Breast anatomy and physiology

2.4.1 Breast anatomy

The breasts are milk-producing organs situated on the anterior part of the thorax. Their base extends superiorly to the second rib, inferiorly to the sixth coastal rib, medially to the sternum and laterally to the mid-axillary line where the axillary tail of Spence is located. They are anchored on the pectoralis major fascia by the Cooper ligaments also called suspensory ligaments **(Fig 6)**.

The breast has two main tissues which are;

➢ Glandular tissue which has approximately 15-20 lobes that radially surround the
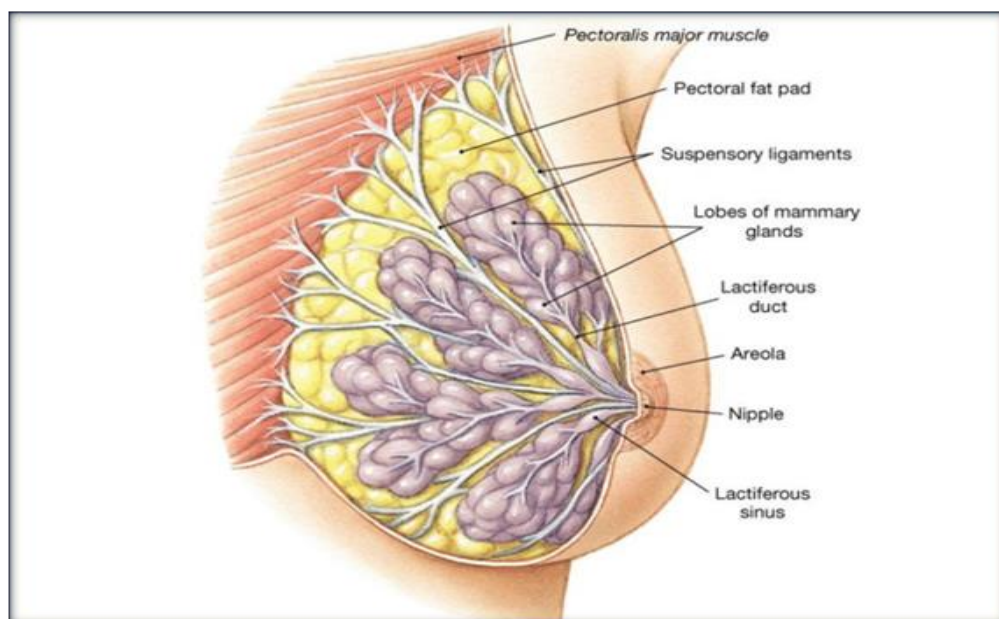
Figure 6: Breast structure.**(Jabbar, 2016)**

nipple. Each lobe consists of lobules that enclose the alveolar clusters responsible for milk production via the mammary secretory epithelial cells. Alveolar clusters are connected to small ducts that drains into larger ducts, eventually leading to the main duct of the lobe. Ducts widen at the lactiferous sinuses and narrows down at the nipple, centrally located above the inframammary crease. Surrounding the nipple is the areola, a hyper-pigmented area of the skin, forming the nipple areola complex (NAC) **(Bistoni and Farhadi, 2015).**

➢ The stromal tissue which is composed of fatty and fibrous connective tissues of the breast which cushions and supports the glandular tissue. The breast is as well

endowed with the immune system and the lymphatic system which evacuates cellular wastes and fluids.

The lymphatic channels gather under the areola to form Sappey's plexus and the lymph from the breast tissue drain into these lymphatic nodes. There are three types of lymph nodes: •Axillary nodes (lymph drains here 75% of the time **(Fig 7)**.

  •Supraclavicular nodes (20%)

• Internal mammary nodes (5%) **(Rinaldi *et al*., 2023).**
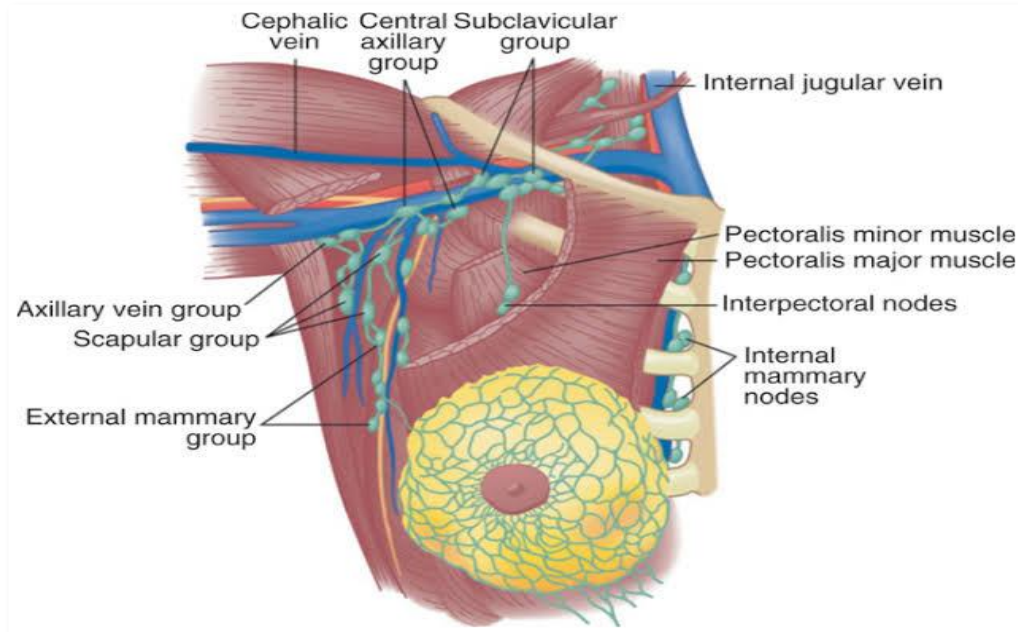
Figure 7: Breast's lymphatic system anatomy.**(McGuire, 2019)**

Axillary lymph nodes are classified into:

- Level I – nodes situated lateral to the side margin of the pectoralis minor muscle **(Fig 8)**.

- Level II – nodes situated below the pectoralis minor muscle.

- Level III – nodes situated medial and superior to the pectoralis minor muscle up to the clavicle **(Kantharia, 2023).**
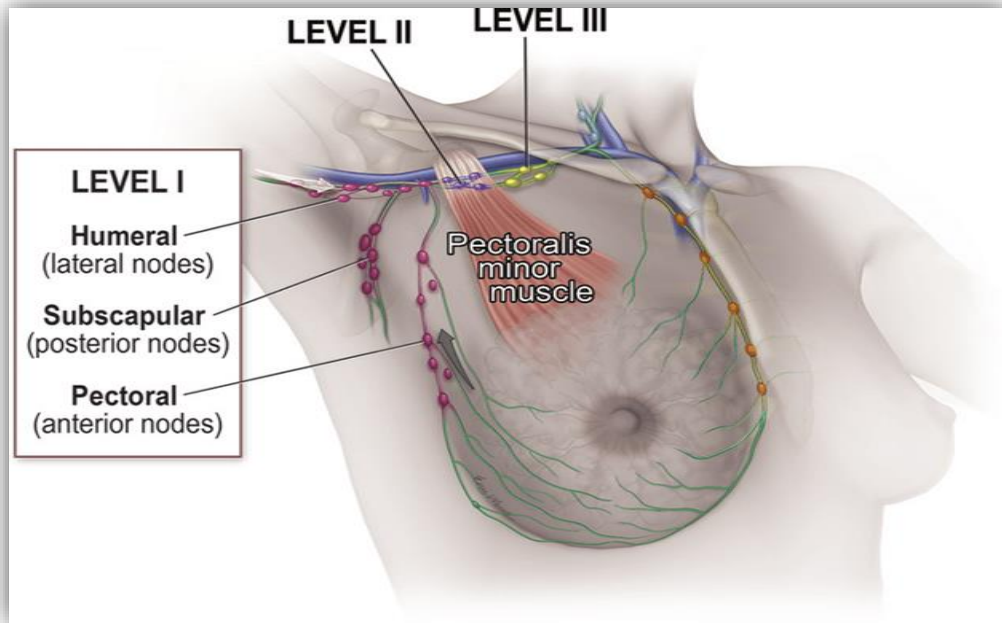
Figure 8: Axillary nodal anatomy (**Zhang et al., 2020**)

The shape and the size of the breast are influenced by the age, racial factors, genetics, dietary factors and menopause state of the individual. They can be conical, hemispherical, slightly pendulous, thin and flattened.

2.4.2 Breast physiology

Functionally, breasts are essential for lactation, the process that involves production, storage and secretion of milk by the mammary glands for infant consumption and nourishment. Breasts although present in both sexes are functional in female gender only. The function of the mammary glands is regulated by various hormones:

- Estrogen, secreted by the ovaries, plays a crucial role in stimulating the development of glandular tissue in the female breast during puberty. Additionally, it promotes breast enlargement by accumulating adipose tissue.

- Progesterone, released by the ovaries, stimulates the development of ducts in the breast.

- Prolactin stimulates the production of milk by the glandular tissue and is secreted by the acidophilic cells of the anterior pituitary gland.

- Oxytocin is synthesised in the hypothalamus and released by the posterior pituitary gland and stimulates milk ejection from the glands (**SEER Training Modules, 2024; Bistoni and Farhadi, 2015).**

2.5 Breast cancer tumour microenvironment (TME)

A tumour doesn't only consist of neoplastic cells rather it is a combined collection of infiltrating and resident host cells, secreted factors and extracellular matrix (ECM) which over a period of time evolves to form an ecosystem that supports proliferation, progression, metastasis and immune suppression. This ecosystem which is termed as the tumour microenvironment can be categorised into cellular components, soluble factors and physical properties (potential of hydrogen (pH) and oxygen levels of the microenvironment).

2.5.1 Cellular components

They can be grouped into local (intratumoral), regional (in the breast) and metastatic chambers. The local compartment is further grouped into the biological features of neoplastic cells and the tumour infiltrating inflammatory cells such as lymphocytes, plasma cells, dendritic cells, macrophages and neutrophils. The regional consists of the interaction between the tumour cells and the surrounding cells in the stroma normally, at the infiltrating edge, involving stromal fibroblasts, myoepithelial cells, adipocytes and endothelial and vascular/lymphatic endothelial cells **(Soysal *et al*., 2015)**. Finally, metastatic compartment is comprised of host cells at lymph nodes(lymphocytes) and distant organs which can be the metastatic targets (lungs).

2.5.2 Soluble factors

These are comprised of all factors that interact in the TME through communication between cells to allow for different processes in the TME like angiogenesis, proliferation, immunosuppression, recruitment of adaptive immune response, etc. And these include cytokines, enzymes and growth factors.

2.6 Types of breast cancer

The types of breast cancer are categorised into histological types and molecular subtypes.

2.6.1 Histological types

Breast cancer histological types are categorised based on the site of origin, with affected cells originating from the ducts, lobules (glandular), or surrounding tissue (stromal). These cancers are classified as either carcinomas or sarcomas depending on the cell of origin. Carcinomas, originating from the cells lining the ducts and lobules, are more prevalent than sarcomas. Sarcomas arise from the stroma, including myofibroblasts and blood vessels. Within carcinomas, various types of breast cancer are categorized based on their

invasiveness from their site of origin. There are three major groups of breast cancer histological types: noninvasive (in situ), invasive, or metastatic breast cancers. Noninvasive Breast Cancer: Neoplasms are confined to the ducts or lobules and do not invade surrounding fatty and connective tissues of the breast. The frequently occurring types include:

- The ductal carcinoma in situ (DCIS): DCIS is the most frequently occurring type of noninvasive breast cancer (90%). Ductal comedo carcinoma for example, is a DCIS **(Sharma *et al*., 2010).**

- The lobular carcinoma in situ (LCIS): LCIS is a high augmentation in the number of cells within the lobules of the breast. It is taken as a marker for augmented risk for breast cancer and it is less frequently occurring.

Invasive Breast cancer: The neoplasms spread to other parts of the body and they include:

- Infiltrating ductal carcinoma (IDC): IDC also called invasive ductal carcinoma starts from the milk ducts of the breast infiltrating the wall of the duct and the fatty tissue of the breast. It can also infiltrate other body parts. IDC accounts for 80% of breast cancer diagnoses therefore it is the most frequently occurring type of breast cancer.

- Infiltrating lobular carcinoma**:** ILC also called invasive lobular carcinoma starts from the lobules of the breast and usually spreads to other body parts. Ten percent to fifteen percent of breast cancers are attributed to ILC.

Less frequently occurring Breast cancers

Medullary carcinoma (5%), Mutinous carcinoma (colloid carcinoma), Tubular carcinoma (2%), Inflammatory breast cancer, Paget's disease of the nipple, phylloides tumour **(Sharma *et al*., 2010)**

2.6.2 Molecular subtypes of breast cancer

Molecular subtypes are categorised according to the degree of the presence of hormone receptors (oestrogen and progesterone receptors) and the human epidermal growth factor receptor. The molecular subtypes are important in breast cancer prognosis and treatment plans.

Luminal A breast cancer:

This subtype is marked by the presence of ER, PR, the absence of human epidermal growth factor receptor 2 (HER 2) and low expression of cell replication marker Ki-67.

Additionally, they amount for 40% of all breast cancer occurrences. They have the best prognosis, less incidence of relapse and high survival rate **(Orrantia-Burunda, 2022)**.

Luminal B breast cancer

Almost 20% of all breast cancers fall under this subtype, consisting of ER, PR, high levels of Ki-67 and sometimes HER2 **(Sharma *et al*., 2010).** Luminal B breast cancers grow marginally faster than luminal A cancer with a marginally worse prognosis **(Miricescu et al., 2020)**.

HER2-enriched breast cancer

This subtype is ER and PR negative and shows high levels of HER2 making up 10% to 15% of breast cancers. HER2- enriched cancers grow more rapidly than luminal cancers and normally have a worse prognosis. They can still be treated effectively with targeted therapies focused on the HER2 protein, like Herceptin. It is to be noted that the HER2-enriched subtype is not the same as clinically HER2-positive breast cancer although almost 50% of clinical HER2-positive breast cancers are HER2-enriched.

Triple-negative breast cancer (TNBC, basal like)

This subtype is characterised by the absence of ER, PR and HER2. It makes up 20% of all breast cancers and has the worst prognosis. TNBC is more common in younger black women specially those who are less than 40 years old. It is considered a high-grade breast cancer as it is more destructive than other types of breast cancer.

Normal-like breast cancer

This subtype is rare, marked by the presence of ER, absence of HER2 and PR can be either present or absent. Although it resembles the luminal A cancer, its prognosis is marginally worse.

2.7 Mechanisms of breast cancer development

There are 3 major signalling pathways by which breast cancer develops which are ER signalling, HER2 signalling and PR signalling.

2.7.1 ER signaling

Oestrogen receptors (ERs) are membrane receptors (usually G protein-coupled receptors) and nuclear oestrogen receptors (ERa, ERb). The latter are transcriptional factors; they activate or repress the expression of target genes immediately after binding to the ligand. ERa and ERb have similar structural and functional features for example the DNA-binding domain (DBD) and some slight differences. They both have the ability to form heterodimers. The DBD on the ER dimers is responsible for their interaction with the oestrogen response elements(EREs) which are specific DNA sequences of target genes. ERs can also regulate transcription without interacting with EREs. This process involves numerous co-activators and co-repressors having essential roles such as BRAC1, a tumour suppressor which inhibits ERa signalling partially. About 75% of breast cancers are ERa positive showing that it plays an important role in breast cancer development **(Ciruelos-Gil, 2014).** The interaction of ERa with cyclin D1 contributing to the growth of breast tumour cells is one of its typical mechanisms. Cyclin D1 activates cyclin-dependent kinases (CDKs) 4 and 6, which causes the cell cycle to change from G1 to S phase in numerous cancer cells **(Fig 9)**. This interaction between ERa and cycin D1 could elucidate the mechanism of antiestrogen therapy resistance, the use of selective CDK4/6 inhibitors and hormonal therapy agents in patients who are ERb positive.
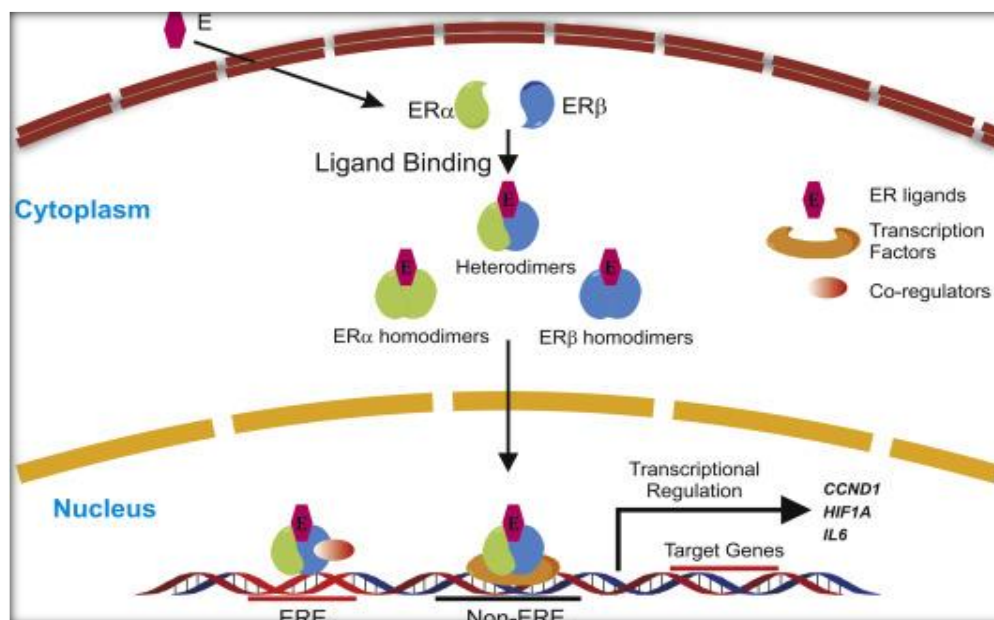
Figure 9: ER signalling pathway **(Feng et *al*., 2018)**

Many isoforms of ERa like ERa36 **(Wang and Yin, 2015)** also influence breast cancers and their therapy contingent on their structural domains. The regulation of ERa signalling is affected by the presence of these isoforms. High levels of ERa36 were discovered to be linked with metastatic phenotype and poor prognosis in breast cancer patients **(Feng *et al*., 2018).**

2.7.2 HER2 signalling

Human epidermal growth factor receptors (EGFRs, or HERs) are tyrosine kinase receptors found in cell membranes in normal tissues and in several types of cancers. HERs are grouped from 1 to 4. Human epidermal growth factor receptor-2 (HER2) is part of the EGFRs **(Kavarthapu et *al*., 2021)**. As aforementioned HER2 is a receptor tyrosine kinase, made up of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain. The active form of HER2 forms dimers with other molecules thus making HER2 capable of affecting many functions of cells through different pathways. The phosphorylation of tyrosine residues in the intracellular domain of HER2 is initiated by ligand binding and dimerisation. These are preceding processes to the activation of several downstream signalling pathways such as the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 4,5-bisphosphate 3-kinase (PI3K) pathways which are closely linked to breast tumorigenesis. HER2 is highly expressed in different human breast cancer cell lines **(Fig 10)**.

The amplification of HER2 signalling leads to HER2 protein overexpression which is associated with the tumour cell proliferation and cancer progression.

The precancerous effect of HER2 was discovered to be associated with inflammation and the proliferation of cancer stem-like cells (CSCs) in breast cancer.

Breast cancer cells that are HER2 positive are liable to progress to metastasis. Therefore, HER2 testing is used to choose patients appropriate for what is possibly resistant
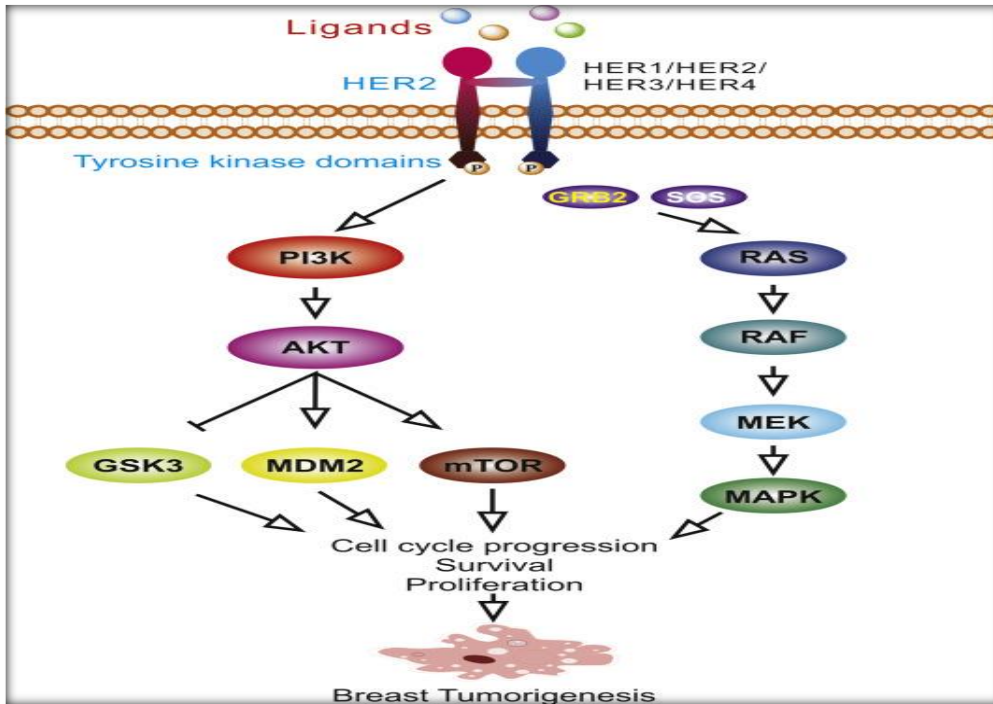
Figure 10: HER2 signalling pathway (**Feng et al., 2018**)

and costly therapy. In order to have more specific and effective treatment, HER2 molecular analysis has become an essential part of the diagnostic breast cancer patient workup (**Feng** *et al***., 2018).**

2.7.3 PR pathway

Progesterone is a steroid hormone consisting of 21 carbon atoms which binds to PRs hence playing an important role in processes like the menstrual cycle, pregnancy and embryogenesis in humans. A breast mouse model and normal human breast culture were used for studies. Together with clinical studies the aforementioned showed that oestrogen and progesterone are the main hormones for proliferation in the mammary epithelium, signalling the mammary gland development **(Zhuo *et al.*, 2022)**. PR signalling by progesterone are involved in differentiation of the alveoli in the last stages of pregnancy then ceases at term to prevent terminal differentiation and to allow lactation to take place. Cells with steroid receptors can produce both PR and ER. **(Fig 11)**
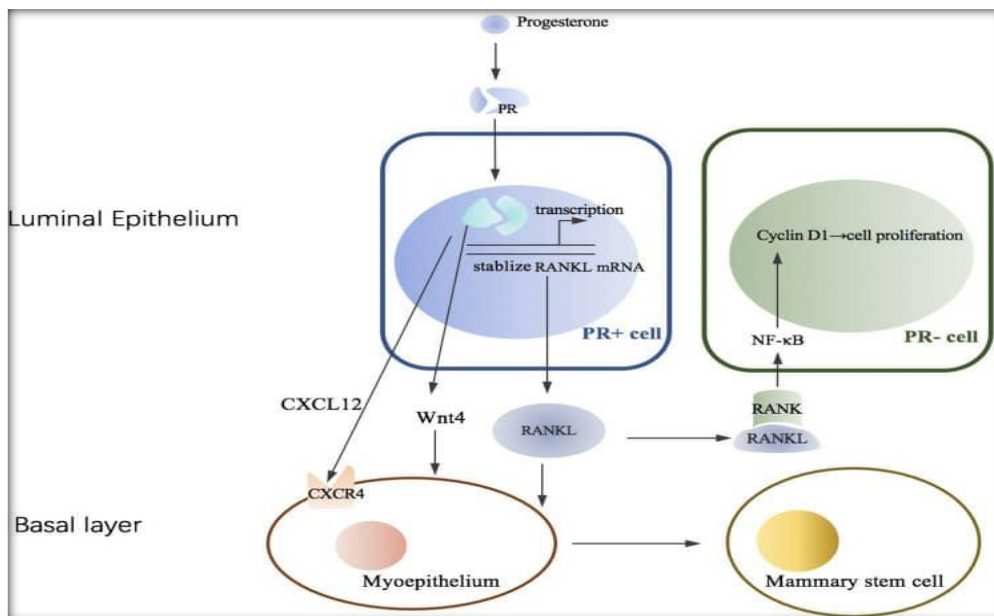
Figure 11: PR signaling pathway **(Zhuo *et al*., 2022)**

The synthesis of PR in both normal and cancer cells is regulated by oestrogen and ERs. PR is not only a gene target induced by ER but also a protein that regulates ER behaviour **(Mohammed et *al*.,2015).** From the studies on the mice models, it was shown that ER is essential at an earlier stage to initiate ductal elongation and PR is necessary for development of the lobules. PR is found on a few luminal epithelial cells in the mammary gland of an adult mouse and the basal epithelium does not have PR at all. Progesterone acts on some mammary epithelial cells causing growth of the alveoli by a paracrine mechanism. Progesterone, by binding to its receptor in breast luminal cells that has PR, it increases the expression of receptor activator of NF-Kb ligands (RANKL). RANKL then binds to the

receptor activator of NK-Kb (RANK) found on the cell membrane of the surrounding luminal or basal cells which do not have PR, initiating downstream pathways of the proliferation and survival of cells **(Zhuo *et al*., 2022)**.

2.8 Breast cancer staging

Breast cancer staging is determined by tumour size(T), nodal involvement (N), the presence of distant metastases (M), **(Tab 2)** and specific biomarkers such as ER, PR, and the HER2 **(Trayes and Cokenakes, 2021).** The stages include:

Stage 0: It is comprised of noninvasive breast cancers.

Stage I: It is made up of two categories which are IA and IB stage. 1A describes the tumour which measures up to 2 cm and there is no involvement of the lymph nodes whereas stage 1B describes the tumour larger than 0.2 mm and it involves lymph nodes. These are early invasive breast cancers.

Stage II: It is comprised of two categories IIA and IIB. In Stage IIA the tumour is found in axillary lymph nodes. The tumour can be smaller or larger than 2 cm but not more than 5 cm while in stage IIB the tumour may be larger than 5 cm but doesn't get to the axillary lymph nodes

Stage III: This stage is comprised of three categories IIIa, IIIb, IIIc and it consists of locally advanced breast cancers.

Stage IV: metastatic breast cancer **(Akram *et al*., 2017).**

Table 2 : Description of breast cancer staging (**Simos et *al.*, 2015)**

Stage	Description
Noninvasive	
0	• There is no evidence of cancer cells or invasion of the basement membrane of the duct or adjacent normal tissue; includes ductal carcinoma in situ.
Invasive	
IA	• Tumour ≤ 2 cm AND • There is no metastasis beyond the breast; no lymph nodes involved
IB	• No tumour in the breast, but microscopic metastases (> 0.2 mm but ≤ 2 mm) present in axillary lymph nodes OR • Tumour present in the breast, ≤ 2 cm, with lymph nodes involvement.
IIA	• No tumour in the breast, but macroscopic cancer (> 2 mm) in 1–3 axillary lymph nodes OR • Tumour ≤ 2 cm, with spread to axillary lymph nodes OR • Tumour > 2 cm but ≤ 5 cm, with no spread to axillary lymph nodes
IIB	• Tumour > 2 cm but ≤ 5 cm, with spread to 1–3 axillary lymph nodes OR • Tumour > 5 cm, with no spread to axillary lymph nodes
IIIA	• No tumour in the breast or presence of a breast tumour of any size associated with metastases in 4–9 axillary lymph nodes or in internal mammary nodes OR • Tumour > 5 cm, with spread to axillary and/or internal mammary nodes
IIIB	• Tumour of any size, with spread to the chest wall and/or skin of the breast; may also have spread to axillary or internal mammary nodes.
IIIC	• Tumour of any size, with a spread to ≥ 10 axillary lymph nodes OR • Spread to lymph nodes above or below the collarbone (supraclavicular nodes) OR • Spread to both axillary lymph nodes and internal mammary nodes.
Metastatic	
IV	• Spread of cancer to other parts of the body such as liver, lungs or bone

CHAPTER 3

IMMUNE SYSTEM

3.1 Elementary knowledge about immune system

The immune system is a biological body system composed of organs, cells, and bioactive molecules responsible for defending the body against exogenous, endogenous, and neo-antigens. It is classified into innate immunity, which is nonspecific and initiates immediate responses, and adaptive immunity, which is highly specific to the antigen and long-lasting **(Goff and Danforth, 2020).**

The innate immune response serves as the body's first line of defense, capable of distinguishing between self and non-self, using the toll-like receptors (TLRs), which detect specific pathogens or danger-associated molecular patterns (PAMPs or DAMPs). Additionally, the innate immune system exerts its effects through cytokines and proteins of the complement system.

The major cellular components of the innate immune system include phagocytes and natural killer (NK) cells. Phagocytes, such as monocytes, neutrophils, and macrophages, are capable of engulfing cells expressing foreign or abnormal self-antigens, killing them via phagocytosis. NK cells secrete perforins and granzymes, inducing cell apoptosis in cases where cells exhibit abnormal major histocompatibility complex class 1 (MHC1) expression due to oncogenic mutations or pathogens.

On the other hand, mast cells, basophils and eosinophils induce their function by producing and secreting pro-inflammatory mediators that induce immune cell infiltration to the inflammation or wounded sites **(Goff and Danforth, 2021).**

The adaptive immune system, highly specific and capable of developing immunological memory, is not immediate, as naïve T and B cells require ample time to differentiate into T effector cells and plasma cells upon encountering an antigen **(Fig 12)**. T cells are categorized according to the type of receptors: αβ T cells and γδ T cells. αβ Tcells
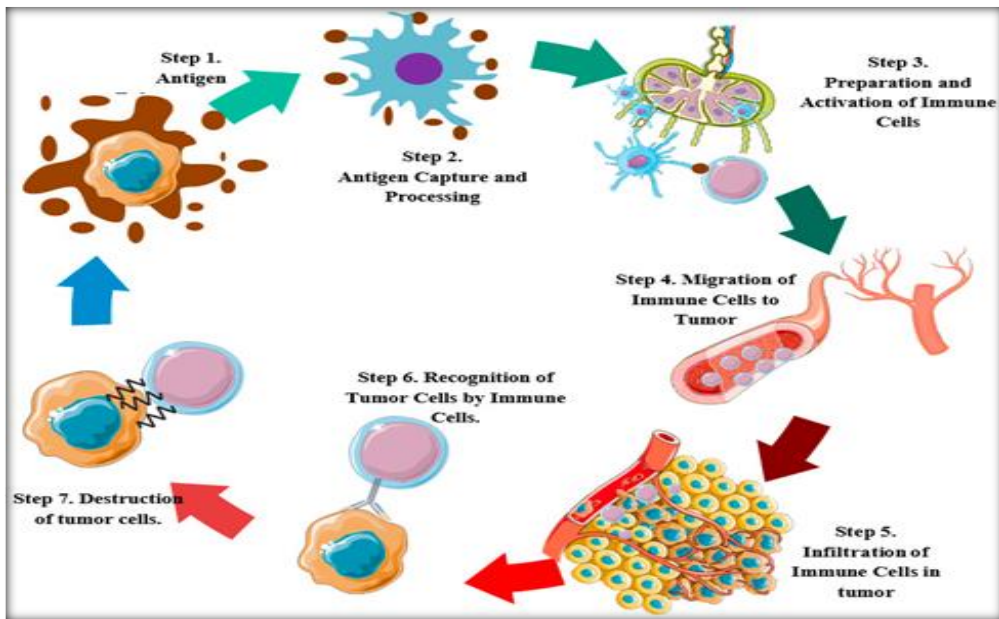
Figure 12: Main processes of the anti-tumour cellular adaptive immune response system(**Avila *et al*., 2023)**

require MHC-mediated antigen presentation, while γδ T cells recognize antigens based on patterns **(Edechi *et al.,* 2019; Goff & Danforth, 2021).**

The immune cells are present within the epithelium of the breast lobules and they comprise both the innate and adaptive immune cells **(Fig 13).** The progression from normal breast tissue to breast cancer via the carcinogenic pathway is followed by qualitative and
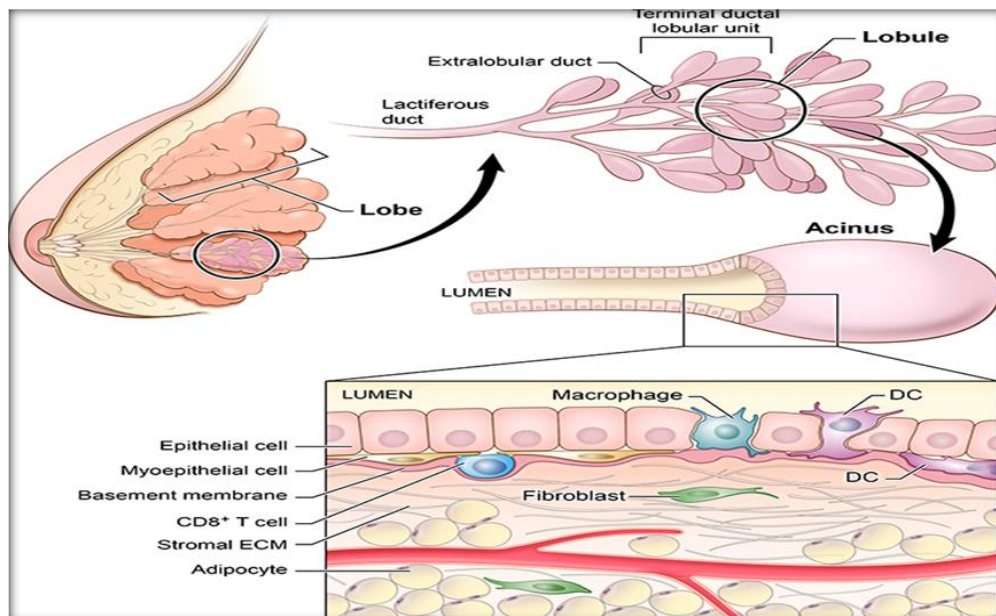
Figure 13: Characterisation and localisation of immune cells in healthy breast tissue (**Goff and Danforth, 2020)**

quantitative changes on the nature and location of the immune cells population which are all brought about by inflammation and the need to eradicate tampered cells (immunosurveillance) i.e. increase in immune cells population in the parenchymal and stromal compartments **(Goff & Danforth, 2020).**

During the early stages of cancer growth, neoplasms are detected and eradicated by the innate immune system through a process called immunosurveillance. The atypical growth of cancer cells can stimulate the secretion of tissue damage signals such as interferon gamma (IFN-γ), by adjacent cells, triggering and recruiting the NK cells, which are key components of immunosurveillance.Through this mechanism cancerous cells can be eliminated even before the tumour develops but in frequent times, it is believed that the pressure exerted by the host's immune system can cause tumour growth through certain mutations thus creating an immune-evasive cancer **(Edechi *et al*., 2019)**.

3.2 Haematopoiesis

The blood is composed of various cells that need replacement when their lifespan ends or when there is increased demand. This replacement process, known as hematopoiesis, predominantly occurs in the bone marrow but can also take place in the spleen and other organs depending on the developmental stage. Hematopoiesis is driven by hematopoietic stem cells (HSCs), which divide and either self-renew or differentiate into lineage-committed progenitors. These progenitors continue to differentiate into mature blood cells in response to signals from cytokines and other bioactive molecules.

The HSCs differentiate into myeloid and lymphoid progenitor cells **(Fig 14)** and afterwards myeloid progenitor cells differentiate into erythrocytes and platelets (through fragmentation of megakaryocytes), neutrophils, monocytes, basophils, and eosinophils while lymphoid progenitor cells differentiate into B lymphocytes, T lymphocytes, NK cells, and a population of dendritic cells **(Raza *et al*., 2021)**.

The immune cells both from Myeloid and Lymphoid progenitor cells carry out different functions as shown on the **(Tab 3).**

Table 3: Blood cells and functions **(Devine et al., 2010)**

LINEAGE	PROGENITOR	BLOOD CELL	FUNCTION
Lymphoid	Common lymphoid	T lymphocytes	Participate in delayed-type hypersensitivity, directly kill infected cells, and assist with B cell function
Lymphoid	Common lymphoid	B lymphocytes	Synthesize and secrete immunoglobulins and become plasma cells
Lymphoid	Common lymphoid	Natural killer	Kill selected tumor cells without having to be activated or immunized against a tumor cell; participate in early host defenses against intracellular organisms and against viral infections
Myeloid	Granulocyte-monocyte	Granulocytes Monocytes	Produce neutrophils, basophils, and eosinophils Assist in the facilitation of monocytes leaving the blood and entering tissues, thus turning into macrophages
		Dendritic cells	Involved in antigen processing and presentation to T cells of the immune system
Myeloid	Megakaryocyte-erythrocyte	Megakaryocytes	Fragment to form platelets just before release into the circulation, assisting with coagulation
		Erythrocytes	Tissue nourishment, oxygenation, and blood viscosity
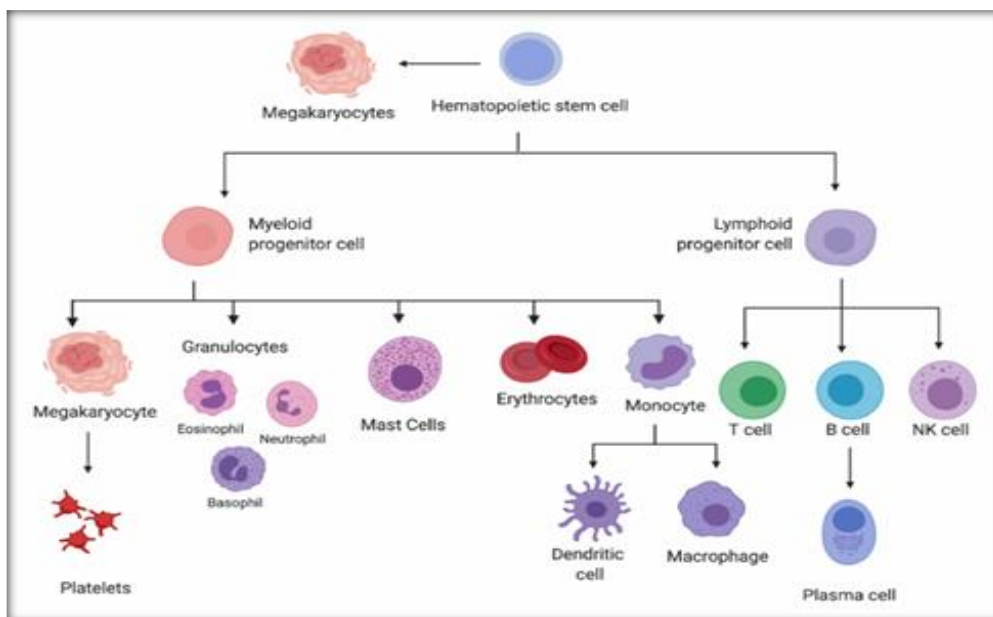
Figure 14: Deciphering hematopoietic differentiation: From stem cells to specialised blood cells **(Raza et al., 2021)**

3.3 Immunoediting

The immune system's mechanism of eradicating neoplasms occurs in three phases:

- Elimination Phase: Complete eradication of tumor cells.

- Equilibrium Phase: The immune system controls tumor growth without completely eliminating the cells.

- Escape Phase: Cancerous cells resist the immune system due to selection pressure, leading to a failure in immune-mediated cancer control **(Fig 15).**

Additionally, cancers develop resistance by expressing decreased levels of MHC 1 molecules and costimulatory molecules **(Edechi *et al*., 2019)**. This explains the need of exogenous treatment methods that can help suppress the cancerous cells.
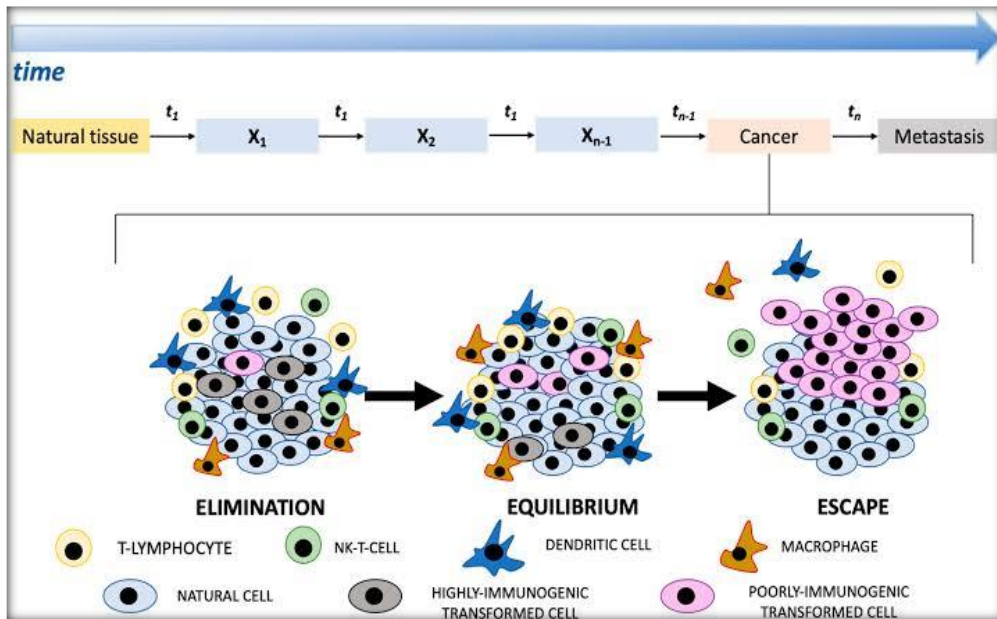
Figure 15: The three phases of cancer immunoediting **(Borroni and Grizzi, 2021)**

CHAPTER 4

BREAST CANCER THERAPIES

4.1 Evolution of breast cancer and therapy

The history of breast cancer dates back to 3500 BCE, as documented in ancient Egyptian papyri, notably the Edwin Smith Surgical Papyrus, named after an American antiquarian in Cairo. The papyrus describes 48 cases of breast tumors, detailing their physical characteristics and noting the lack of effective treatments, although cauterization with a fire stick had been attempted in one case. In the following centuries, between 400 BCE and 200 BCE, physicians Hippocrates and Galen hypothesized that breast cancer was associated with humors, specifically black bile. They recommended treatments such as opium, castor oil, lycorine, and sulfur. Galen also suggested a surgical approach, lumpectomy, which involved the incision and removal of the tumor **(Lukong, 2017)**.

The advent of modern therapeutic measures brought promising developments in 1882, when William Halsted introduced the radical mastectomy for breast cancer. This procedure involved the removal of the entire breast, underlying chest muscles (pectoralis major and minor), axillary components, and later extended to the removal of lymph nodes in the supraclavicular region. Although it did not guarantee overall survival, it remained the standard operation throughout the 19th and 20th centuries. The introduction of novel anesthetics (ether) and aseptic measures (introduced and commended in 1846) made the procedure more bearable for patients.

Extensive studies and numerous hypotheses led to the invention of novel therapeutic measures for breast cancer **(Fig 16).** These include radiotherapy in 1896, hormone therapy in 1980 using tamoxifen (a partial antiestrogen), chemotherapy (5-fluorouracil) in 1957, targeted therapy (Trastuzumab also named Herceptin which targets HER2) in 1998 and immunotherapy **(Lukong, 2017)**.
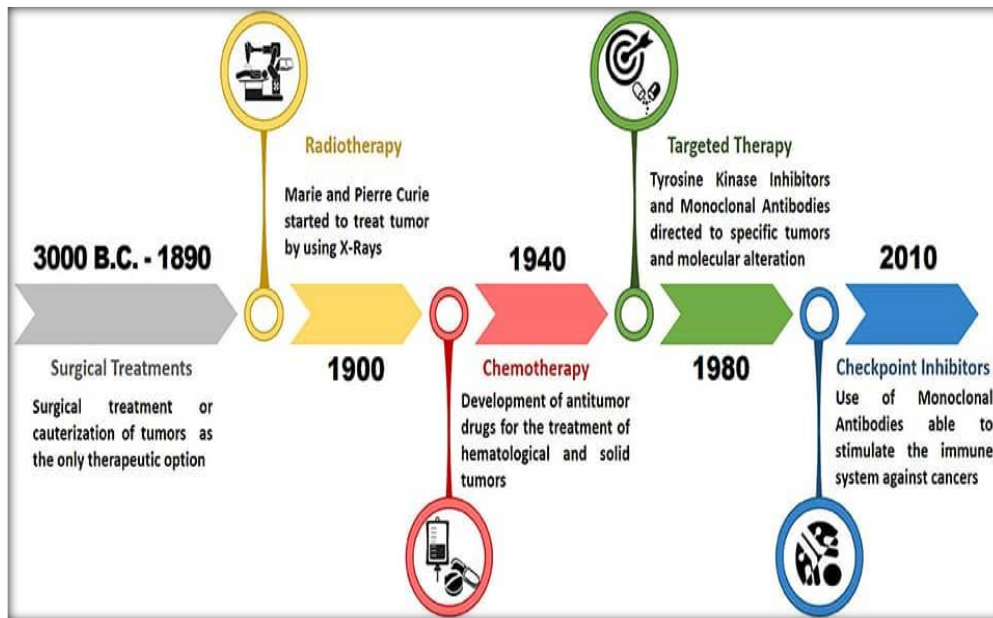
Figure 16: Evolution of breast cancer **(Falzone *et al*., 2018)**

4.2 Surgery

It's a branch of medicine that deals with treatment of bruises, maladies and disorders through manually conducted procedures rather than administration of drugs. It involves the utilisation of specialised apparatuses such as scalpels to lacerate tissue, forceps to maintain blood vessels closed or hold and manipulate structures, clamps to secure tissues in place or instruments, gauze sponges to soak up fluids and keep an area dry, retractors to hold incisions open and curved needles to stitch them closed. Surgeries are conducted by professional personnel known as surgeons **(Britannica, 2024).**

There are 4 types of surgery that are conducted in the modern world:

- Wound treatment: It is performed to ensure proper wound healing and to prevent infections.

- Extirpative surgery: It is carried out with a purpose of removing defected or diseased body tissues and organs and it is majorly conducted on cancer patients i.e. mastectomy (removal of the breast), hysterectomy (removal of the uterus), cholecystectomy (removal of gall bladder).

- Reconstructive surgery: It is principally effectuated to replace defected organs or tissues due to burns, etc.

- Transplantation Surgery: Involves replacement of diseased organ with organs derived from another individual i.e. kidney transplant (**Britannica, 2024**).

4.2.1 Mastectomy

It's a type of breast cancer treatment measure aimed at removal of either the whole breast or some breast tissues to facilitate recovery and hinder the spread of cancerous cells to other critical body organs (**Goethals and Rose, 2024**).

Types of mastectomy and techniques

- **Halsted's radical Mastectomy**

It's the type of mastectomy which entails the removal of the breast tissue, pectoralis major, minor muscles and all axillary tissues. It is frequently opted for if the cancer cells have spread rampantly on the chest wall muscles (**Plesca et *al*., 2016**)

- **Patey's mastectomy (modified radical mastectomy)**

This type of mastectomy involves the removal of breast tissue, pectoralis minor and axillary nodes. It is mostly performed on patients with the invasive type of breast tumours associated with the spread to the axillary nodes (**Goethals and Rose, 2024**).

- **Total or simple mastectomy**

This is the most common type of procedure performed on breast cancer patients who cannot undertake the breast-conserving surgery (lumpectomy) and it involves extirpation of the breast tissue and the associated skin with or without axillary surgery while conserving the pectoralis fascia. However, axillary tail is eliminated leaving the patient with two drains in situ (**Lazaraviciute and Chaturvedi, 2017**).

- **Skin sparing mastectomy or nipple sparing mastectomy**

Skin sparing mastectomy involves the removal of the breast tissue while preserving the skin overlying the breast. Furthermore, nipple sparing mastectomy allows for conservation of the skin envelope and the nipple-areola complex giving way into breast reconstruction (**Tokin et *al*., 2012**).

- **Bilateral prophylactic mastectomy**

Mostly performed on women with germline (BRCA1 and BRCA2) mutations to reduce the risk of breast cancer occurrence. It involves the removal of both breasts prescribed with any technique depending on the patient's preferences (**Lazaraviciute and Chaturvedi, 2017**).

4.2.2 Effects of surgery on hematological parameters

Leucocytes

The leucocytes refer to the total immune cell population that defend the body against foreign substances.

Implications incurred to the population of the leucocytes following surgical procedures can either be associated with post-operative acute infections or a normal response to surgical trauma. Leukocytosis (term that describes white blood cells count beyond normal levels) is mostly observed in patients during the early post-operative period (**Jung** *et al.***, 2019**) ranging from the operation day to the 3rd day after the surgery when the levels begin to normalise following a normal response to trauma, this also happens when there is an outbreak of an infection (**Elbromboly** *et al.***, 2023).**

Neutrophils

Neutrophils are the immune cells recruited as the first line of defence against antigens during intraoperative and post-operative period. Neutrophils are produced in bone marrow in response to granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-6 and IL-8. The total number of circulating neutrophils is regulated by an equilibrium of various factors such as granulopoiesis, bone marrow storage and release, intravascular margination, clearance, apoptosis and destruction (**Inagi** *et al.***, 2016).**

Neutrophil count increases after the surgical incision up to the third day after the operation and begins to gradually decrease to pre-operative levels. The increase of the neutrophil levels is correlated to surgical trauma following the pro-inflammatory factors released in the injury site that causes neutrophil chemotaxis, demargination and production in the bone marrow (**Tabuchi** *et al.***, 1989).**

Thrombocytes

Platelets play a crucial role in wound healing through the process of blood clotting which helps to defend the patient from infections.

Following surgical intervention, platelet count fluctuates in the peripheral blood due to hemodilution, increased platelets consumption caused by surgical haemostasis. Thrombocytopenia is observed minutes to hours after the surgical resection and continues for 4 days after which thrombopoietin responds by increasing platelet production through the bone marrow megakaryocytes. The platelets count increases three times more than the pre-operative count on the 14th day past the surgery and standardises to the patient's normal

range. This phenomenon is well elucidated by the delay between an elevation of thrombopoietin release and the release of new platelets from the bone marrow (**Skeith** *et al.*, **2020).**

Erythrocytes and their indices

The erythrocytes and their indices (MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC(mean corpuscular hemoglobin concentration), hematocrit and hemoglobin largely fluctuate after surgery procedures leading to anaemia.

These fluctuations are implicated in pre-operative anaemia, intra-operative and perioperative blood loss (phlebotomy) and post-operative decreased erythropoiesis due to surgery-associated inflammation. The inflammatory cytokines released after surgery induce a cascade of events where hepcidin binds to the iron transporter ferroportin causing its degradation and sequestration of iron in macrophages. Inflammation diminishes iron uptake from the gastrointestinal tract and a decreased erythroid response to erythropoietin. This culminates to delayed recovery of haemoglobin postoperatively.

Cancer patients undergoing surgery are particularly at a higher risk of anaemia due to the activation of antifibrinolytic pathways and pro-coagulants, anaemia associated with chemotherapy, thrombocytopenia and endothelial dysfunction, the excursion of hyper vascularised tumours and hemodilution emerging from the administration of excess fluids during the perioperative period (**Kalra** *et al.*, **2021).**

4.2.3 Effects of mastectomy on haematological parameters

Haemoglobin (Hb), haematocrit(Ht) and erythrocytes count fluctuates after the surgical procedure according to the study conducted by (**Ufelle e***t al.*, **2012**) and neutrophils, platelets and leucocytes increases for the first 3 days after mastectomy and later decreases according to the study conducted on canine mammary cancer (**Karayannopoulou** *et al.*, **2022).**

These effects arise due to surgical trauma, excessive bleeding and neo-adjuvant chemotherapy that may kill the immune cells and affect their peripheral blood levels (**Ufelle** *et al.*, **2012**). General anaesthesia is also thought to negatively alter the immune cell population by immunosuppressive process.

4.2.4 Mechanisms through which surgical operations suppress the immunity.

Surgical excision is the efficient measure of eliminating primary tumours and metastatic lymph nodes. However, it doesn't achieve complete extirpation of tumour cells and micro metastases which can be detrimental to the patient in the case when their immune system is compromised.

The surgical procedure comprises perioperative period which includes pre-operative, intra-operation and post-operative periods. During this perioperative period the patient might undergo surgical trauma, receive anaesthesia and analgaesic induction which can thereby negatively affect the immune function **(Kim, 2018).**

The incision of the damaged tissue generates corresponding local and systemic trauma responses which evoke mechanisms such as local inflammation, activation of the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic nervous system (SNS) to restore the tissue and cellular homoeostasis due to tissue damage. The activation of these systems engenders the secretion of catecholamines and glucocorticoids which trigger prostaglandins (PGE2) and other soluble factors **(Fig 17)**. These immunomodulatory factors can induce immune system suppression thus resulting in fluctuations of natural killer cell
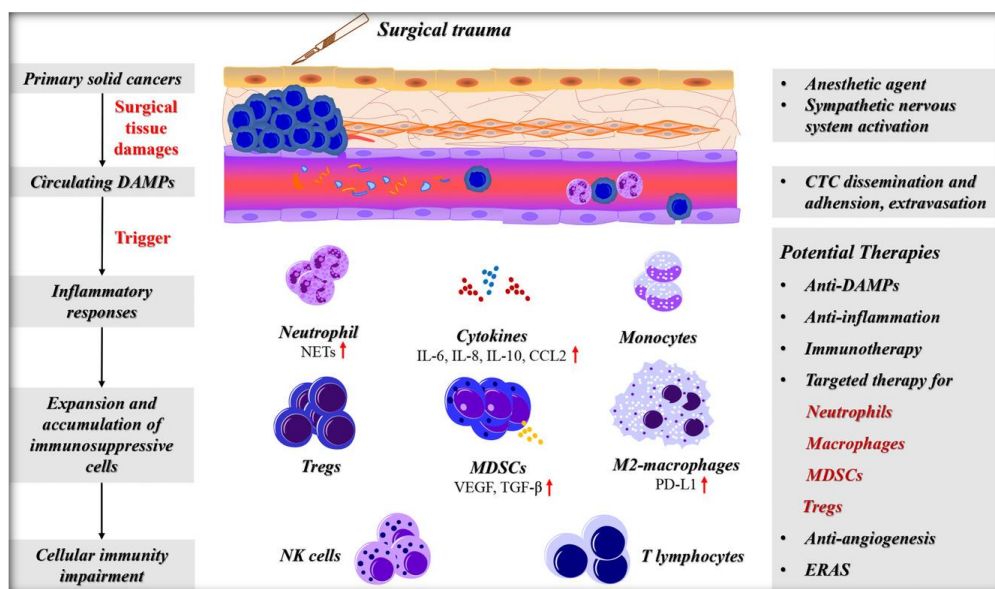
Figure 17: Surgical trauma and the cellular immunity dysfunction.**(Tang et al., 2023)**

activity which plays a pivotal role in preventing cancer cells metastasis and affect the expression of HLA-DR which is located on the monocytes and thereby rendering them ineffective in defending the body against infections **(Kim, 2018; Pinheiro *et al*., 2023).**

4.3 Chemotherapy

Chemotherapy is a treatment method that uses drugs to halt the growth of neoplastic cells by either disrupting their division cycle or killing them. Chemotherapy is systemic since it is administered intravenously or orally and this type of drug administration helps to eliminate all cancerous cells in the body. It is administered synergistically with other treatment strategies like surgery, radiotherapy and hormone therapy depending on the various patient's pathological factors to enhance chances of survival and prevent tumour recurrence **(Amjad *et al*., 2023).**

4.3.1 Categories of chemotherapy drugs

- Alkylating Agents

Nitrogen mustard - Cyclophosphamide.

Platinum analogues - Carboplatin.

Alkyl sulphonate, Ethylenediamine and Triazenes.

Mechanism of action

They inhibit DNA replication and transcription by yielding an unstable alkyl group (R-CH2+) which combines with nucleic acids and nucleophilic centres **(Colvin, 2003).**

- Antimetabolites

Pyrimidine analogues (fluorouracil (5-FU)

The cytidine analogues (gemcitabine).

Purine analogues

Folate antagonists.

Mechanism of action

They inhibit DNA replication. They act as false metabolites similar to those essential for cell growth and upon their uptake by the cells their division cycle is disrupted. They are classified according to the metabolites they mimic **(Amjad *et al*., 2023).**

- **Topoisomerase inhibitors**

These are chemotherapeutic agents that interfere with Topoisomerase enzymes (I and II) activity. They block the ligation step of the cell cycle which produces DNA double and single strand causing apoptotic cell death.

Topoisomerase I inhibitors include irinotecan, topotecan, and camptothecin.

Topoisomerase II inhibitors include etoposide, doxorubicin and epirubicin **(Kuroda *et al*., 2014).**

- **Plant alkaloids (antimicrotubule agents)**

Plant alkaloids are derived from plants and inhibit the mitotic phase of cell division by either disrupting the polymerisation or depolymerisation of the spindle fibres.

Taxanes (paclitaxel and docetaxel) exert their action by binding to the microtubules and impede their depolymerisation while Vinca alkaloids bind to tubulin dimers and impede their polymerisation (**Bharti** *et al.***, 2018).**

4.3.2 Chemotherapy regimens

Administration of chemotherapy to patients largely depends on the stage of the disease, tumour size and age. Chemotherapy can be categorised into:

Neo-adjuvant

It is administered to shrink the tumour that cannot be operated for the first time of diagnosis especially when cancer has infitrated many lymph nodes or if it is inflammatory breast cancer.

It is also given to avoid extensive surgery (**Larissa** *et al.***, 2021).**

The standard neo adjuvant regimen is adriamycin (doxorubicin) and cyclophosphamide (AC) followed by a taxane (**Shien and Iwata, 2020).**

Adjuvant

This regimen is administered to the patient to impede cancer recurrence by destroying neoplastic cells that could have remained or any cancerous cells that have spread that are undetectable (**Tab 4).**

Table 4: Chemotherapy drugs regimen and toxic effects (**Hernandez-Aya and Ma, 2016; Hanna and Mayden, 2021**)

Chemotherapy Agent/regimen	Toxic effects
Doxorubicin	Cardiotoxicity, myelosuppression, hypersensitivity, extravasation
Paclitaxel	neuropathy, myelosuppression, hypersensitivity, extravasation
Capecitabine	Oedema, fatigue, diarrhoea, hypersensitivity, cardiotoxicity
Doxorubicin plus cyclophosphamide	Myelotoxicity, cardiotoxicity, hepatic or renal dysfunction
Sequential fluorouracil, epirubicin, and cyclophosphamide (FEC) followed by weekly paclitaxel	Myelotoxicity, gastrointestinal toxicity, neurotoxicity, cardiotoxicity
Gemcitabine plus paclitaxel	Neutropenia, fatigue, neuropathy
Atezolizumab plus nanoparticle albumin-bound paclitaxel	Myelotoxicity, peripheral neuropathy, hepatotoxicity, immune-related adverse events.

Patients receive anthracycline (A) containing regimen or docetaxel(Taxotere) plus cyclophosphamide (TC) regimen or AC followed by taxane (docetaxel or paclitaxel) regimen as adjuvant chemotherapy **(Shien and Iwata, 2020)**.

4.3.3 Adjuvant and neoadjuvant chemotherapy drugs

Anthracyclines, such as doxorubicin (Adriamycin) and epirubicin (ellence)

Taxanes, such as paclitaxel (Taxol) and docetaxel (Taxotere)

5-fluorouracil (5-FU) or capecitabine (Xeloda)

Cyclophosphamide (Cytoxan)

Carboplatin (Paraplatin) **(Shien and Iwata, 2020)**

Chemotherapy drugs are administered in cycles after every 2-3 week interval for a total of 3 months for neo-adjuvant and 6 months for adjuvant. Metastatic drug's duration depends on its effectiveness and tolerance. Despite its effectiveness in killing cancer cells, this treatment presents numerous side effects that can be detrimental to the host's cells that are actively dividing like hair follicles, bone marrow, etc. affecting the patient's blood cells count **(Colleoni *et al*., 2002; Asaoka *et al*., 2020).**

4.3.4 Effects of breast chemotherapy on haematological parameters

Chemotherapy is the most effective treatment strategy in eradicating cancerous cells and has been proven to increase life expectancy. Chemotherapy functions by killing cancer cells that are actively dividing and can also damage other rapidly dividing healthy cells e.g hair follicles and blood cells in the bone marrow causing neutropenia, anaemia and even leukopenia. This arises due to extreme depletion and acute myelosuppression of haematopoietic progenitor cells in the bone marrow. Once haematopoiesis is altered, the self-renewal capacity of hemopoiesis stem cells is impaired **(Fathoni *et al*., 2021).**

Leucocytes, neutrophils, platelets, red blood cells (and their indices) and haemoglobin decrease after administration of breast chemotherapy. According to Rhamaniar, haematological parameters fluctuated after every cycle of chemotherapy with a great difference from the pretreatment values **(Rahmaniar *et al*., 2024)**. The test results were lower in middle aged patients than the elderly due to the administration of stronger and higher doses of chemotherapy because of their strong physical capacity, fewer comorbidities and faster drug metabolism. Studies have also shown that cancer in middle aged group is more aggressive than in older patients thereby requiring high doses of chemotherapy to achieve better results.

CHAPTER 5

MATERIALS AND METHODS

5.1 Research problematic question.

Mastectomy and chemotherapy, common treatments for breast cancer, present significant challenges. The removal of one or both breasts can lead to physical changes and emotional distress, including body image issues, loss of self-esteem, and anxiety. Additionally, mastectomy carries surgical risks such as infection, bleeding, and anesthesia complications, while breast reconstruction can result in further complications and multiple surgeries.

Chemotherapy further complicates recovery by weakening the immune system, making patients more vulnerable to infections and delaying recovery. This increased susceptibility to opportunistic infections can prolong the overall recovery process for mastectomized patients. Addressing these issues requires a multidisciplinary approach, including improved surgical techniques, effective side effect management, immune system support, psychological support, and exploration of less invasive treatments. Ongoing research and patient-centered care strategies are essential to enhance outcomes and quality of life for breast cancer patients

5.2 Background

Chemotherapy is a systemic treatment method full of cytotoxic compounds capable of not only destroying cancerous cells but also healthy cells and have been associated with many complications on the patients such as decrease in the immune cells and red blood cells which further lead to anaemia and infections.

Similarly, mastectomy, though effective in removing the tumour and surrounding lymph nodes that are invaded by cancerous cells, is also linked to complications like anaemia and infections due to immune suppression. Our study aims to understand the implications incurred by these two methods of treatment on BC patient's haematological parameters using complete blood count automatic analyzer.

5.3 Objective

The objective of this project was to assess the variation and the levels of haematological parameters in BC patients undergoing chemotherapy treatment and those who had undergone mastectomy.

5.4 Place of study and the population

This work was realized at the Mostaganem University Hospital oncology center from 28 February to 31st May 2024.

The sample comprised of 70 patients who had received chemotherapy and 28 patients who had undergone mastectomy and had all their CBC test results before and after undergoing the treatment.

The age of the patients ranged from the age of 27 to 84 years old.

This study constituted the utilisation of the patient's files dating from the year 2019 to 2024.

a)   Criteria of inclusion

Our study included all patients who had received either adjuvant or neo-adjuvant chemotherapy whose files had haematological test results for both before and after treatment. It also included patients who had undergone mastectomy whose files had haematological test results for both before and after surgery.

b)   Criteria of exclusion

Any file that didn't have either personal or family history and didn't include test results taken before treatment and had not received up to four cycles of chemotherapy treatment were excluded.

5.5 Materials

Sysmex xn 350 automatic CBC analyzer, ethylene diamine tetra acetic (EDTA) coated tubes, CBC reagents, sterile gloves and gowns, antiseptic, needles, No. 15 scalpel, forceps, sterile sponges, suction system, sterile irrigation solution (water and normal saline), standard mastectomy tray, skin hooks, retractors, sutures and ties.

5.6 Principle of Sysmex XN-350 CBC

Hematological analyzers can use either volumetric impedance or light-scatter technique to analyze the cells (size, number, internal components and etc.).

The Sysmex XN-350 uses the light scattering method with fluorescence flow cytometry, examining cells as they flow through a sheath of fluid.

The aspirated blood sample is divided into aliquots then diluted to a desired ratio and labelled with fluorescence marker which binds specifically to nucleic acids. The sample is then translocated to the flow cell where it is illuminated by semiconductor laser beam, which separate the cells using three different signals:

- Forward-scattered light-indicates the cell size.

- Side-scattered light -examines the internal cell structure and its content, such as nucleus.

- Side-fluorescence light -gives information about the amount of nucleic acids present in the cell.

Cells which have similar chemical and physical characteristics form a cluster in a graph and show the distribution of different blood cells.

a)   Complete blood count procedure

•Phlebotomist draws 4-5 micro liters of blood from the patient using a test tube coated with EDTA.

•Mix the blood well with the anticoagulant while avoiding vigorous shaking.

•Program the analyzer and key in the patient's code on the screen and then aspirate the blood sample directly from the test tube into the analyzer.

•Note down or print the results shown on the screen and repeat the steps for all the patients.

5.7 Chemotherapy administration methods

The CBC test and biochemical parameters test is done in at least 48h before the patients receives the chemotherapy. (If the results are normal the chemotherapy treatment will be done on the planned date.

On the contrary if the results are not within the normal healthy range, the patients are assigned another date for treatment).

The patients are obligated to come on the day assigned for them and eat well before they come for the treatment.

a)   Preparation of the solution

We injected the medications into the salt and glucose serum with a precise dose prescribed by the doctor.

b) Injection

c) Pre medication

Before the chemotherapy session, the patient must be pre medicated for a minimum of 30 minutes to avoid secondary effects like nausea and vomiting.

- 250 ccs of salt and glucose serum 15% + 8mg ONDANSTRON for 15 minutes.

- 120mg of SOLUMEDROL through direct intravenous injection (DIV).

- 01 AMP of AZANTAC in IVD.

- 100 mg of hydrocortisone (HHC) through DVI

c) Chemotherapy session

•The session commences by disinfecting the patient's arm with alcohol (antiseptic) then pricking the vein using the catheter to administer the medication.

• The duration of the session depends with the dose, the type of the medication and the age of the patient varying from 1h to 4h.

•The next session will be after 15-21 days.

5.8 Mastectomy protocol

Pre requisites

•Diagnosis of histological type of cancer using needle biopsy.

•Breast imaging to know the extent of the lesion.

•The patient should give a complete medical

history and have proper physical examination.

•An informed discussion with the patient must be done concerning all types of mastectomy including the risks and benefits of each type.

a)   The procedure

•The patient first receives general / local anaesthesia and then held in supine position with arms abducted at 90 degrees depending on the operation plan. Prophylactic antibiotics are given prior to induction.

•The surgery procedure is done according to the type of mastectomy the patient is undergoing.

Modified radical (Patey's) mastectomy procedure

•Measure the elliptical incisions, horizontal and oblique.

• Dissect the superior skin flap with the scalpel while retracting the breast with the left hand inferiorly and the assistant surgeon retracting the skin upward for an easy and blood free dissection.

• Dissect the breast tissue off the pectoralis major muscle.

• Dissect the inferior skin flap following the traction and counter traction technique.

•Hold the pectoralis major muscle upward with the forceps to clear the way easily access the pectoralis minor and the axillary lymph nodes.

•Dissect the pectoralis minor and the lymph nodes where cancer has spread and remove them together with the breast tissue.

• Irrigate the wound, perform haemostasis and close the wound **(Fig 18).**

5.9 Statistical analysis

Obtained data were rigorously analysed using a completely randomized design framework, employing Analysis of Variance (ANOVA) to identify significant differences across the studied population groups and haematological parameters. Specifically, this approach will facilitate the examination of the impacts of mastectomy and chemotherapy on blood parameters of breast cancer patients. Further analysis will involve Duncan's multiple range test for post-hoc comparisons, enabling detailed evaluation of significant differences highlighted by ANOVA (single degree of freedom contrasts was utilised to explore the specific effects). A p-value of less than 0.05 will denote statistical significance throughout the analysis, ensuring the reliability of our findings (SAS, 2008).
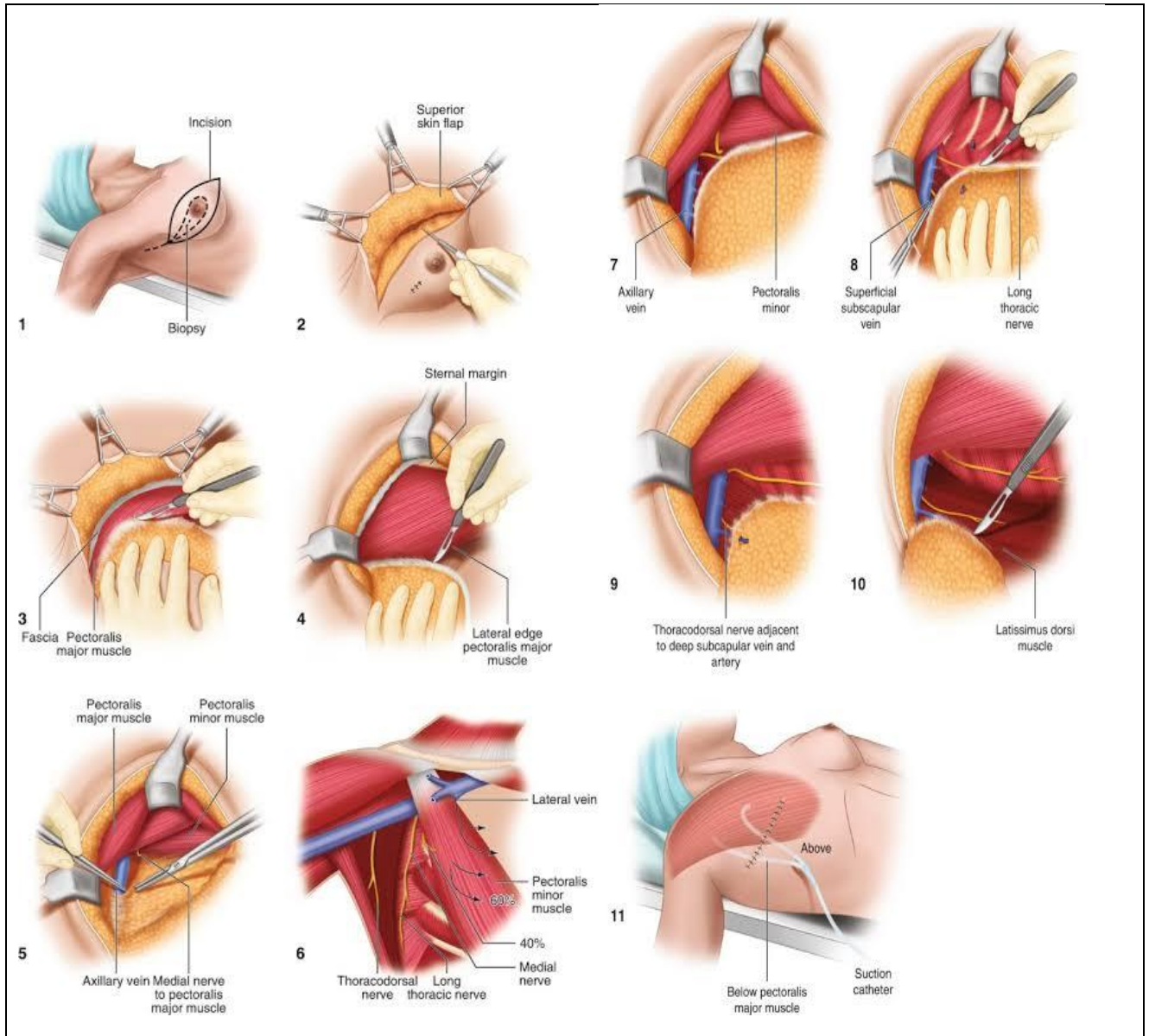
Figure 18: Modified radical mastectomy's sequential protocol **(Perrins *et al*., 2020)**

CHAPTER 6

RESULTS AND DISCUSSION

6.1 General characterization of patients under chemotherapy treatment.

Our study sample comprised of 70 patients under chemotherapy treatment who adhered to inclusion criteria from the year 2019 to 2024 partitioned based on their age and their menopause status at the time of cancer diagnosis **(Tab 5)**. Majority of the patients aged 50 years and above and 60% of the patients were still in their menses.

Table 5: Patient's general characteristics

Age categories	20-29:5 (7%) 30-39:13 (19%) 40-49:25 (36%) 50 and above:27 (38%)
Menopause status	- Menopause:28 (40%) - Non-menopaused :42 (60%)
Cancer stage	- I-1(2%) - II-45(%) - III-24(%)
Positive receptors	- ER-55 - PR-50 - ER/PR-50 - HER2-24

6.1.1 Repartition of patients on the bases of the types and the stages.

Majority of the patients in our sample had been diagnosed with stage II invasive carcinoma NST(no special type) (22) **(Fig 20)** and luminal A (19) **(Fig 19)** molecular subtype. TNBC cases were 17 both in stage II and III and only one patient was diagnosed with stage I BC.
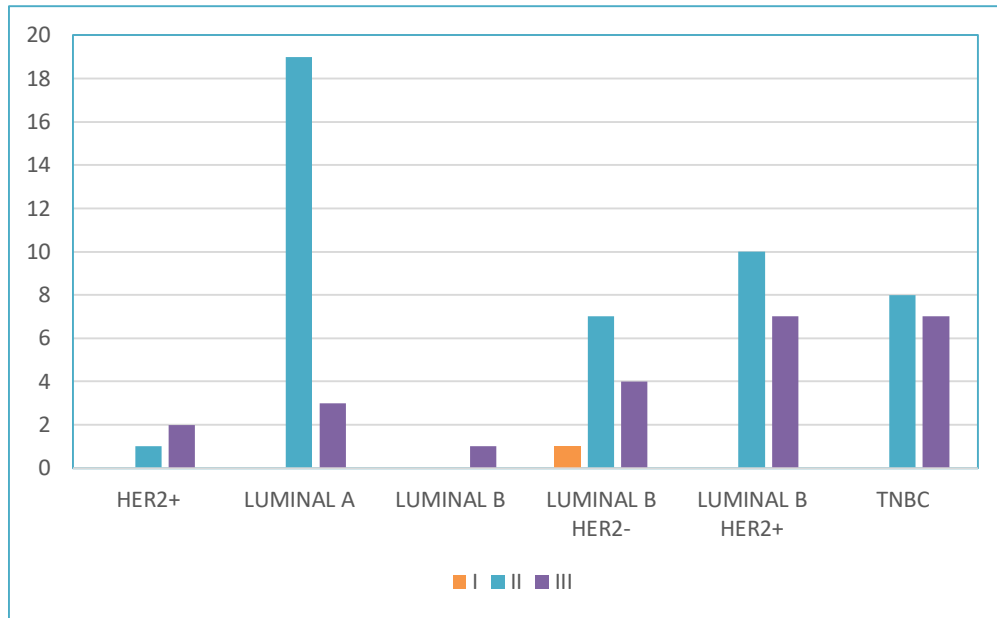
Figure 19: Distribution of patients in relation to molecular subtypes and BC
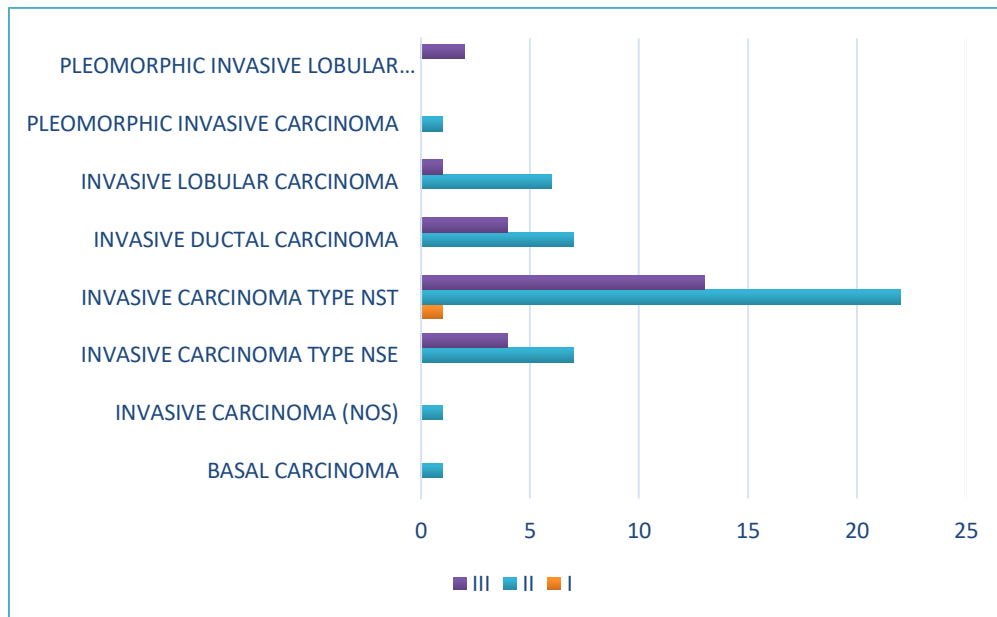
Figure 20:Histological types vs BC stages

6.1.2 Chemotherapy regimen and type of treatment

A high percentage of the patients were under adjuvant chemotherapy treatment, 16 of them were administered with FEC/T regimen and a small number of patients had received AC/TC regimen. Only 4 patients had received both neo-adjuvant and adjuvant treatment **(Fig 21).**
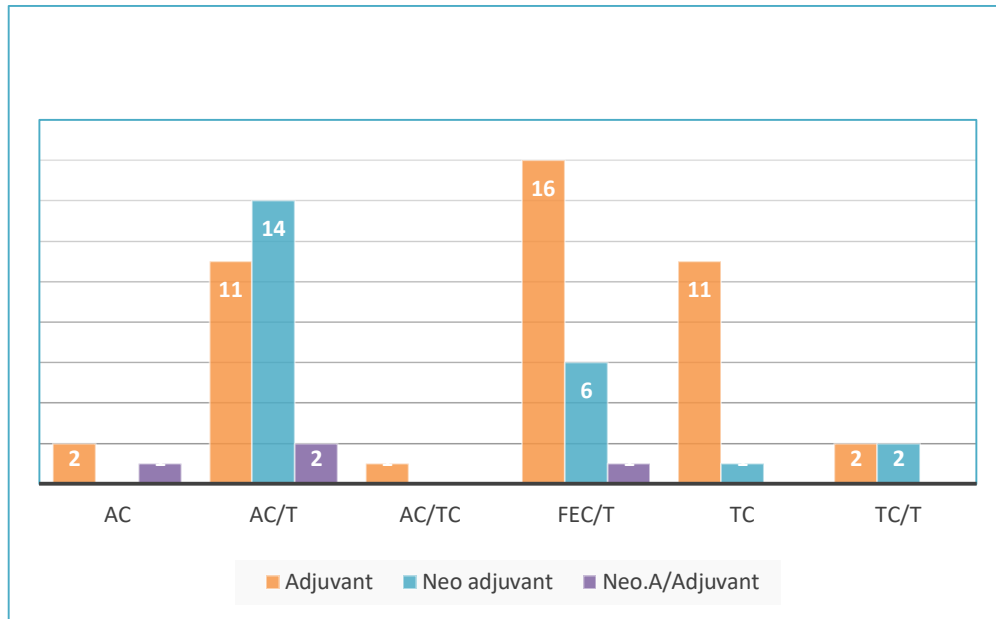
Figure 21: Types of chemotherapy regimen and mode of treatment

6.2 Levels and variations of haematological parameters before and after chemotherapy treatment

Leucocytes

The results and the variations of the leukocytes values are illustrated on the **(Fig 22)**.
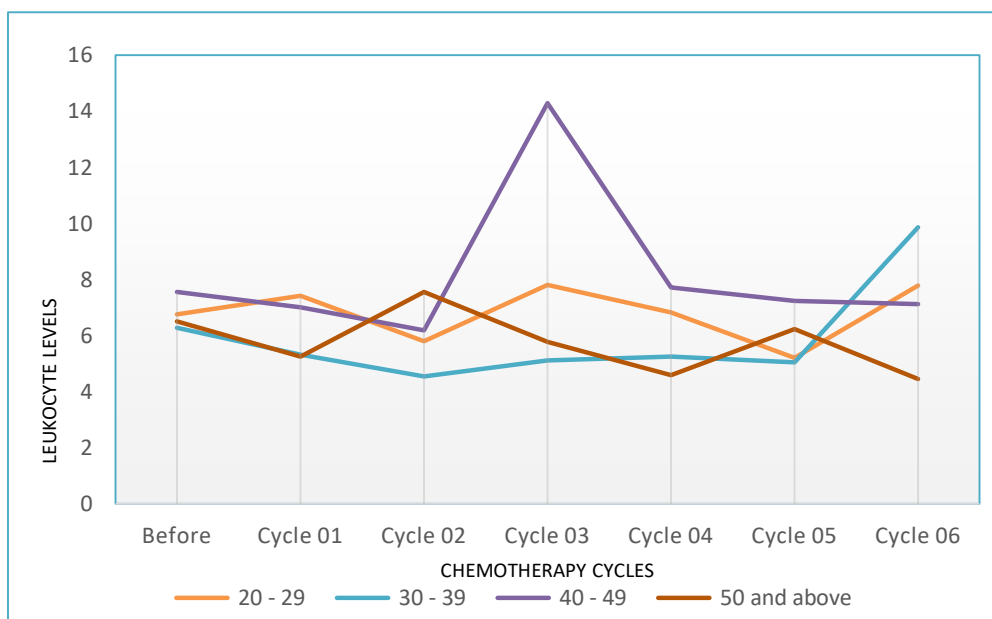
Figure 22: Leukocytes variation before and after chemotherapy in relation to age group

According to the study no changes were observed before the onset of the chemotherapy in all age groups but after the commencement the levels for the age group 30 – 39 and also 50 and above slightly reduced to 5.32 ± 1.76 and 5.26 ± 2.28 respectively and recovered in subsequent cycles. Leukocytes levels for the age group 40 –49 significantly increased with a SD of ±14.41 in the 3rd cycle while the age group 20-29 exhibited standard levels with slight variations in their mean values**.** The normal mean value is 7.75 ± 3.25. All the mean and SD values are presented on the **(Appx A).**

Erythrocytes

The study findings and the variations of the erythrocytes are illustrated on the **(Fig 23)** and the average values with their SD values on the **(Appx B).**
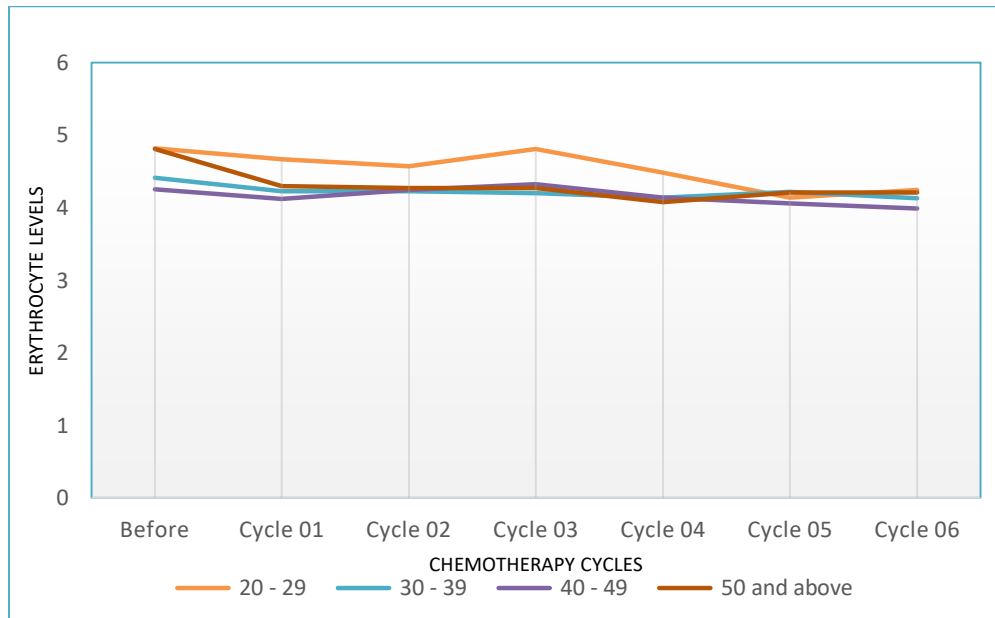
Figure 23:Variations in erythrocyte levels across different age groups

Erythrocytes normal mean value is 4.75 ±0.25 mm^6.

A slight decrease in erythrocyte values was observed in all age groups compared to the values before the chemotherapy session commenced**.** The age groups between 20- 29 and 40- 49 had low values before they started the treatment and the latter had a significant decrease from the normal interval to 3.99±0.45 in the 6th cycle.

.

Thrombocytes

The analysed data of the thrombocytes values is depicted on the **(Fig 24).** The mean and SD values are on the **(Appx C)**

Healthy values vary between 150-450mm^3. (300±150) **.**
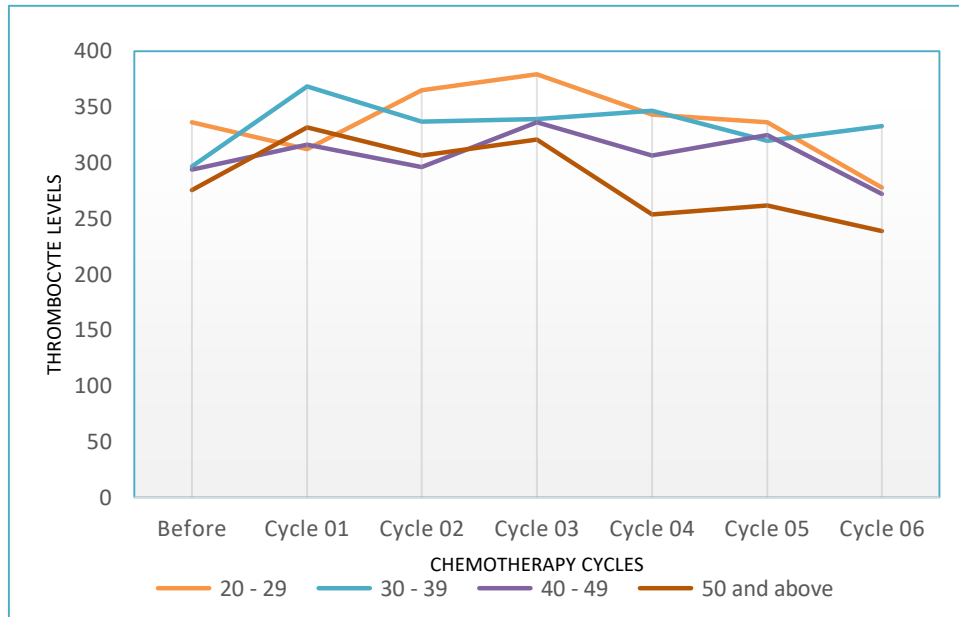
Figure 24: Illustration of pre and post chemotherapy thrombocyte levels

- Healthy values vary between 150-450mm^3. (300±150)

A healthy range of values prior to chemotherapy treatment was observed in all age categories with a slight increase throughout the cycles. The values in the 6th cycle closely resembled the pretreatment values with a small difference**.** However, the second cohort had significant SD values in the 1st,3rd and 4th cycles that later stabilized after the 6th cycle and the 1st age group (20- 29) had a slight increase after the 2nd and 3rd cycles which later stabilized after the 6th cycle. A notable decrease in the 4th age group is depicted in the graph with slightly fluctuating values from the 4th cycle to the 6th cycle although within a healthy range**.** Healthy values vary between 150-450mm^3. (300±150) **.**

Haemoglobin

The study findings of the hemoglobin are shown in the **(Fig 25)** and the mean and SD values are in the **(Appx D).**

The standard interval varies from 12-15 g/dl.
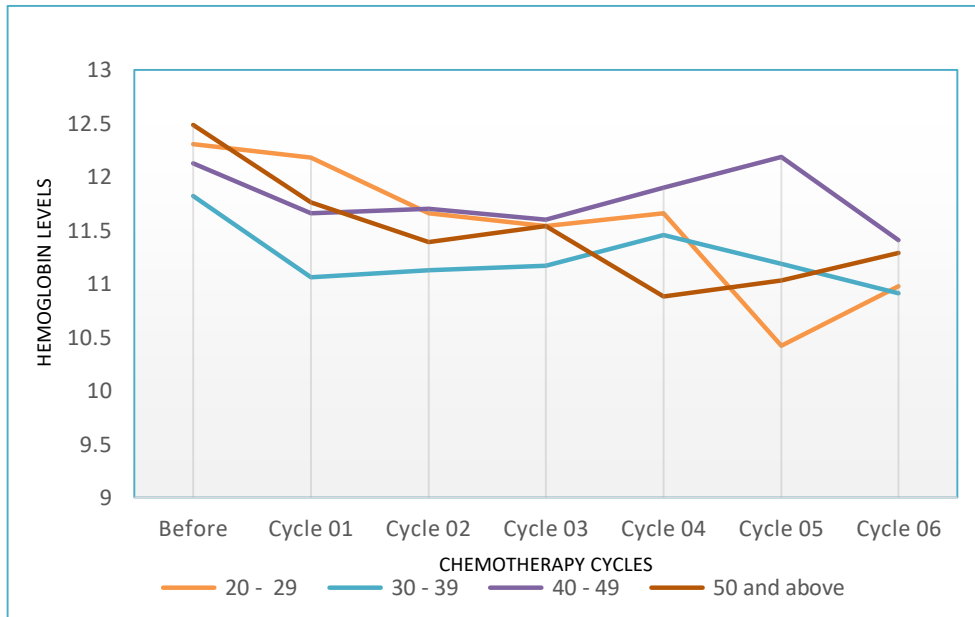
Figure 25: Hemoglobin levels pre and post chemotherapy analysis

The study showed a notable decrease in the haemoglobin mean values after the administration of chemotherapy in each age group i.e all the curves tend towards the lowest values. A sample cohort between age 30-39 had the lowest pre and post chemotherapy haemoglobin levels along with age group 20-29 that had low mean values after the 5th and 6th cycles of 10.42±1.36 and 10.98±1.58 accordingly.A low mean value of 10.88±1.02 in the age group of 50 and above after the 4th cycle was also observed.

Neutrophils

The statistically analysed data is demonstrated in **(Fig 26)** and the values in **(Appx E).**
The control values for neutrophils vary from 40%-60%.
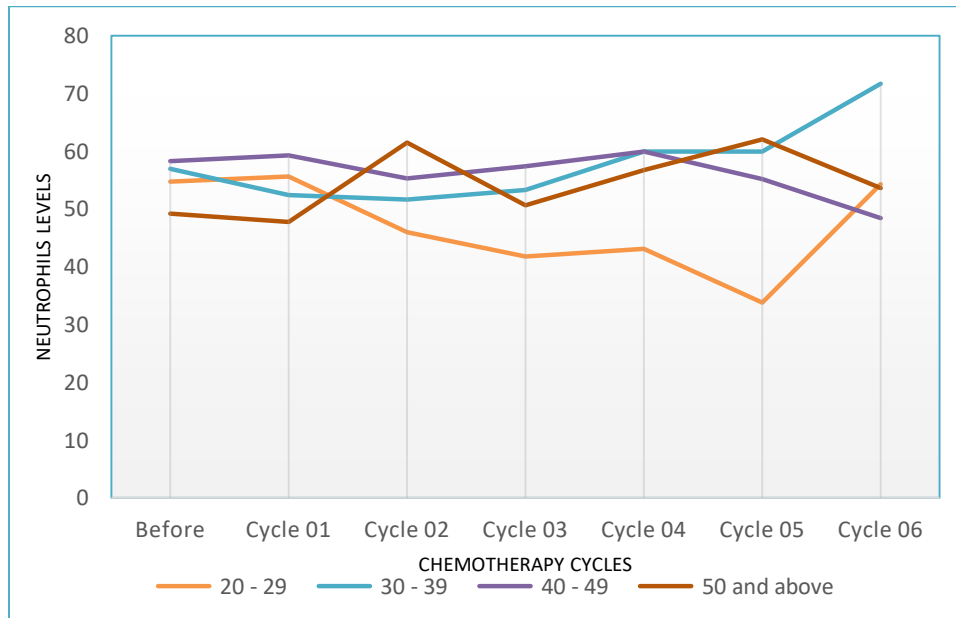
Figure 26: Neutrophils dynamics following chemotherapy treatment

Slight variations were observed from the pretreatment values although they were still within the healthy range with the exception of a cohort group (20-29) where they had decreased to 33.86 ± 17.18 after the 5th cycle and (30-39) where the value increased significantly to 71.66 ± 14.6 after the 6th cycle. Moreover, the values in the age group of 50 and above had a marginal increase after the 2nd and 5th cycles of 61.49 ± 12.96 and 62.1 ± 14 respectively.

Haematocrit

The variations of the hematocrit values are shown in **(Fig 27)** and the average/SD values in **(Appx F)**

The normal values range from 36%-45%.
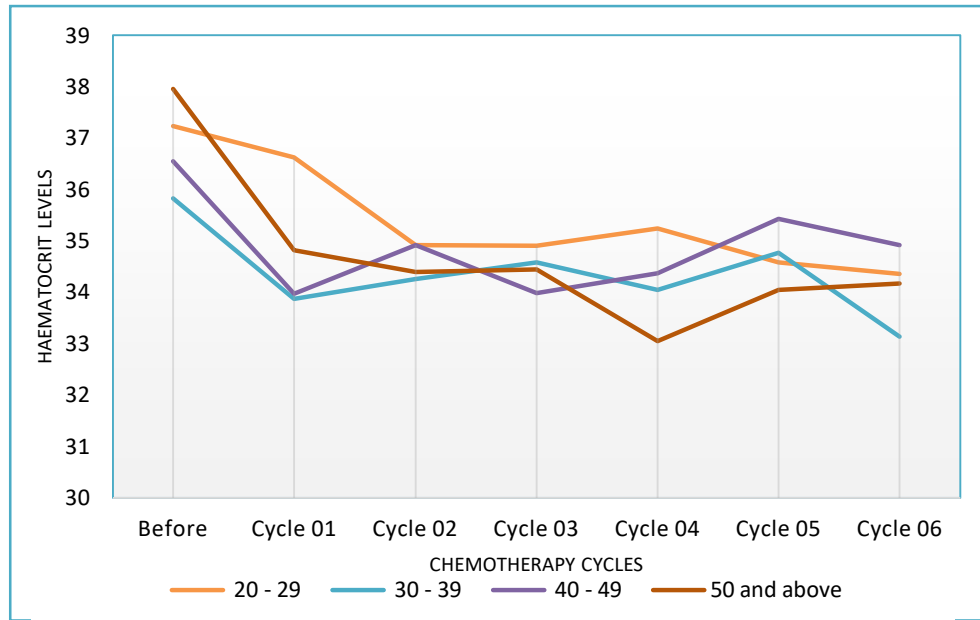
Figure 27: A linear representation of haematocrit levels: before and after chemotherapy

The haematocrit mean values before the commencement of chemotherapy were all within the healthy range for all age groups but a marginal decrease was observed after the 1st cycle in all age groups except a sample cohort of 20- 29. The mean values remained below the normal interval across the age groups after the chemotherapy cycles.

Mean corpuscular volume

The results of the are represented in **(Fig 28)** and **(Appx G).**

Normal values range from 80 – 100 femtoliter (fL)
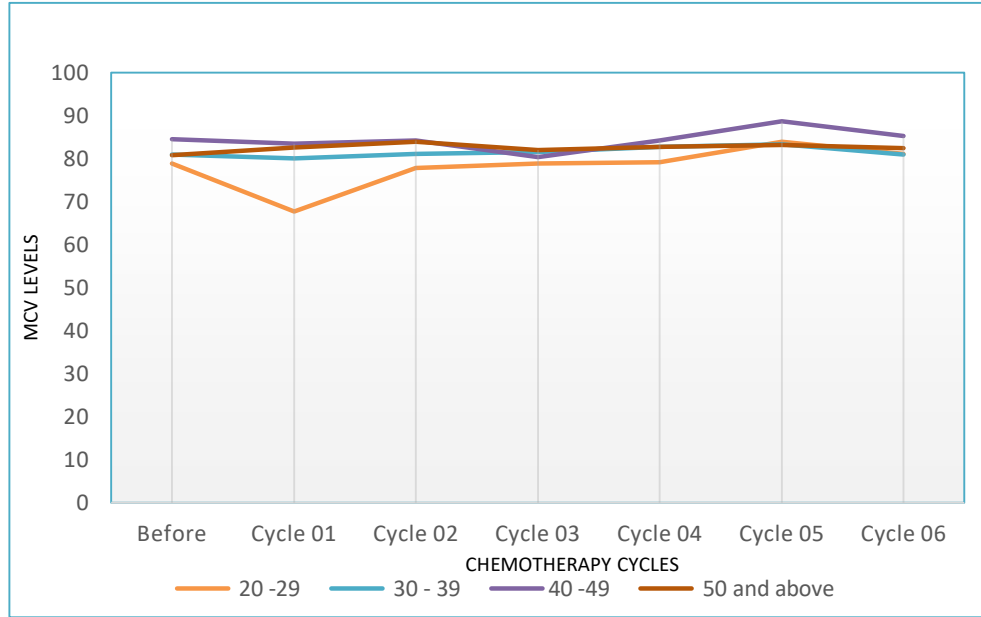
Figure 28: MCV variations before and after chemotherapy

The MCV mean level for the first age group (20-29) 78.88±10.8 was slightly lower before the 1st chemotherapy session compared to other age groups and there was a subsequent notable fluctuations of the mean values in this age group which stabilized after the 5th cycle and the 1st cycle stood out in all age groups with the lowest value of 67.71±24.2. The 2nd 3rd and 4th age group exhibited positive mean values falling within the normal interval**.**

Mean corpuscular hemoglobin

The findings are demonstrated in **(Fig 29)** and in **(Appx H).**

The MCH standard values vary from 27–31 picograms/cell (pg).
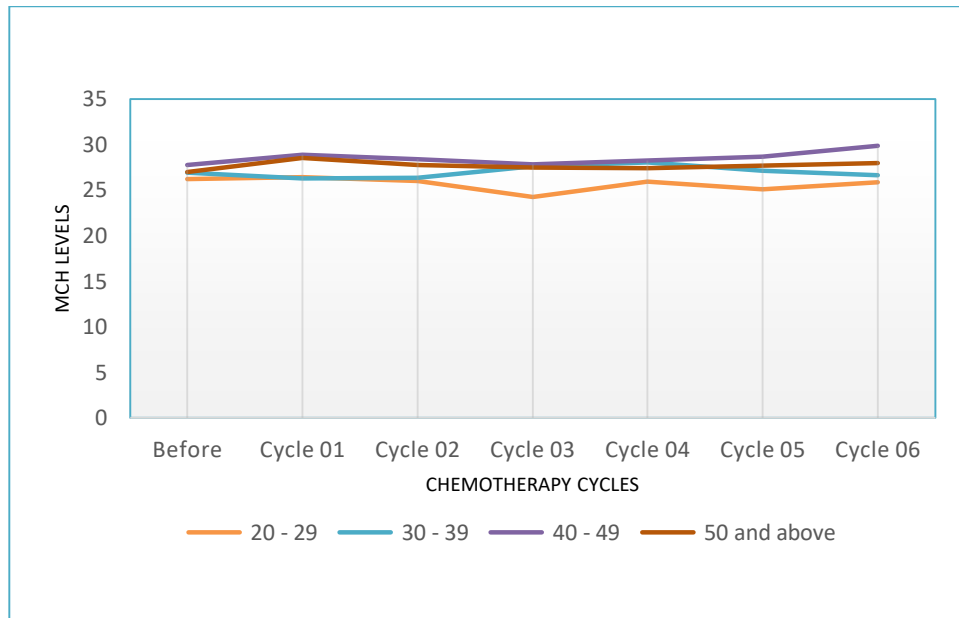
Figure 29: MCH levels: comparison before and after chemotherapy

According to the study, all groups with the exception of the 4th cohort had low MCH mean values before the onset of the chemotherapy. The MHC values of the first group exhibited a decreasing trend, reaching the lowest mean value of 24.28 with a standard deviation of ±4.17. Conversely, other age groups maintained standard values within the healthy range, with approximate standard deviation values**.**

Mean corpuscular haemoglobin concentration

The findings of this study are depicted in **(Fig 30)** and in **(Appx I)**.

Normal values range from 32-36 grams/deciliter (g/dl).
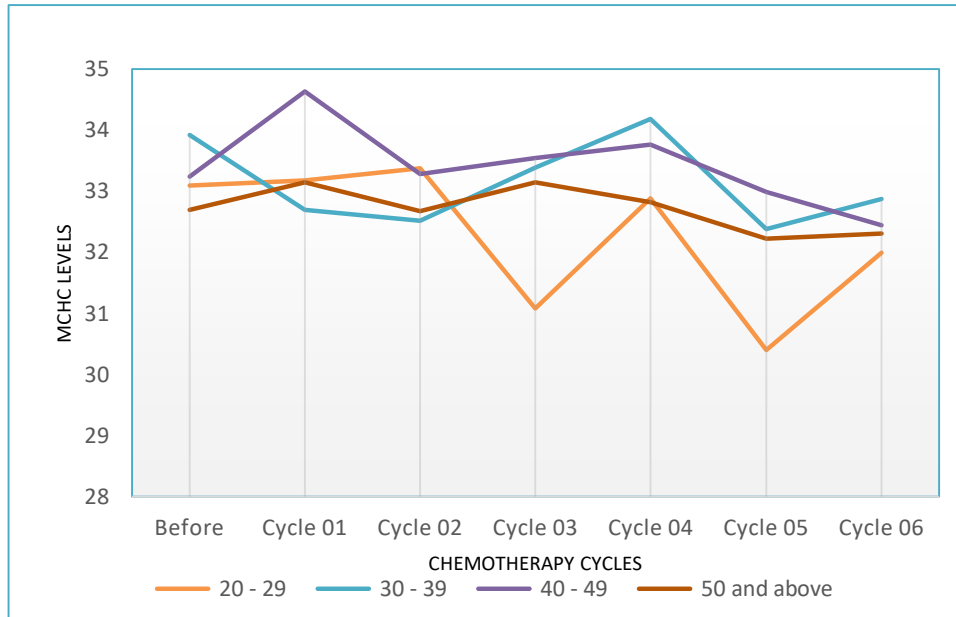
Figure 30: Visualising the impact of chemotherapy on MCHC mean
values prior and after chemotherapy

Basing on the findings, the MCHC levels in the first age group formed a sharp zigzag curve with the lowest value of 30.4±3.01. Majority of the mean levels were slightly below or within the lowest normal value of 32. The first age group was significantly affected than the other age groups**.**

6.3 Mastectomy

The study population consisted of 28 patients, with 26 receiving neo-adjuvant chemotherapy and 2 receiving neo-adjuvant hormone therapy. Patient ages ranged from 35 to 84, with the majority diagnosed with stage II breast cancer. A high percentage of patients underwent modified radical mastectomy, while a few remained undetermined. Prior to the surgical resection procedure, patients received intravenous injection of general anesthesia, and hemostasis was achieved after the operation **(Fig 31).**
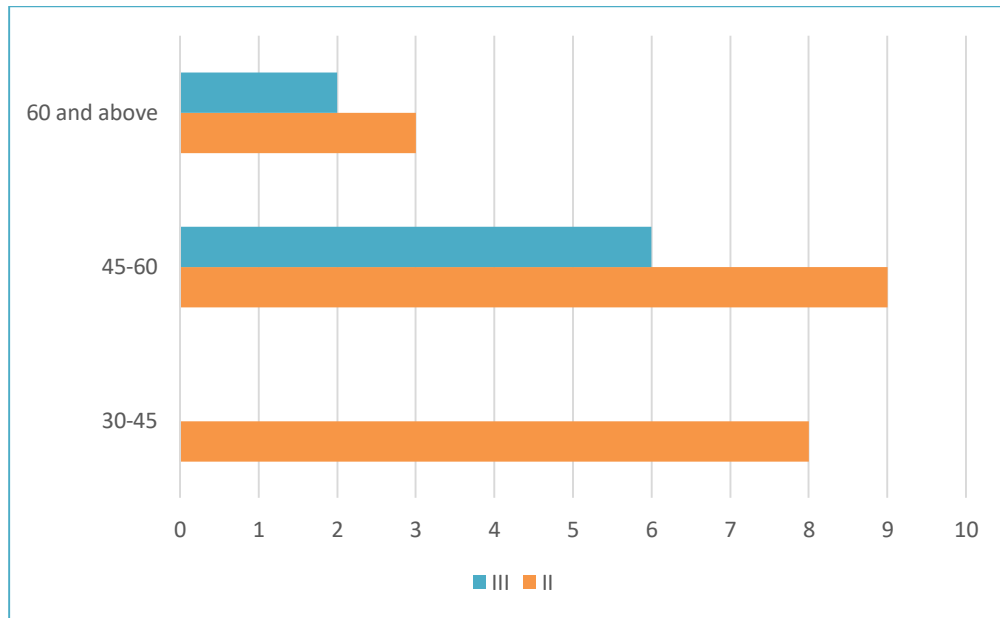
Figure 31: The partitioning of patients according to their age groups and BC stage

6.3.1 Hematological parameters variations pre and post mastectomy
Leucocytes

The erythrocytes test results of our patients distributed according to age group before and after mastectomy are illustrated in **(Fig. 32).**
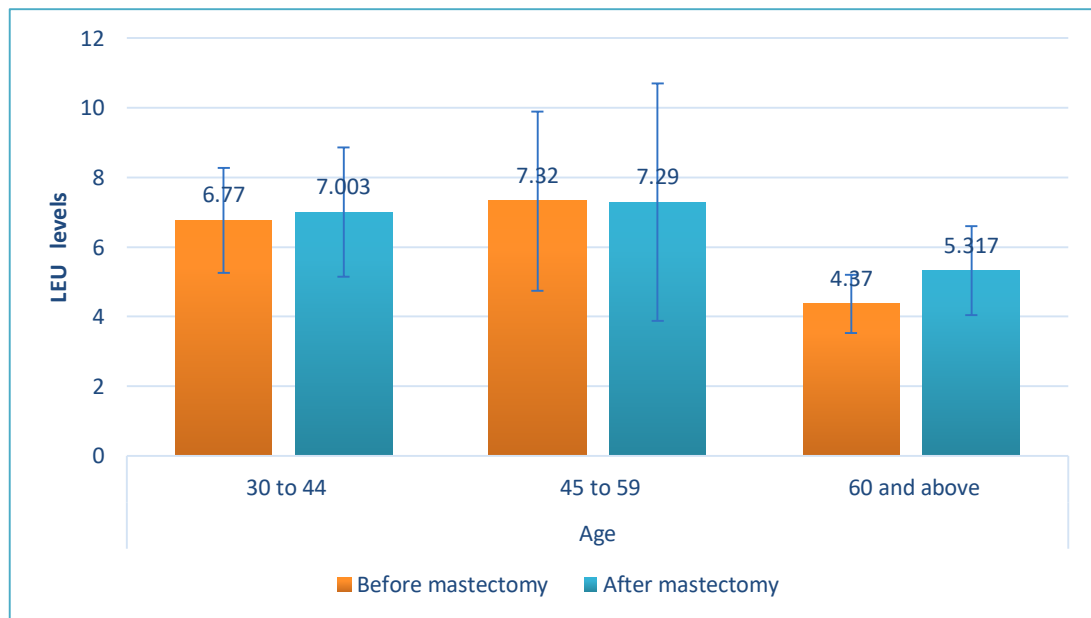
Figure 32: Mean leukocyte values before and after mastectomy

The statistical study of shows sufficient leucocytes (4-11µL normal range of values) in age group 30-44 with mean values of 6,77µL and 7,003µL showing just a marginal difference before and after mastectomy respectively. The 45-60 age group have normal values, 7,32 µL and 7,29µL with almost no difference before and after mastectomy accordingly. The 60 and above age group had sufficient leucocytes ,4,37µL before and 5,317µL after mastectomy. A relatively significant increase is observed though after mastectomy.

Erythrocytes

The erythrocytes test results of our patients distributed according to age group before and after mastectomy are illustrated in **(Fig 33).**
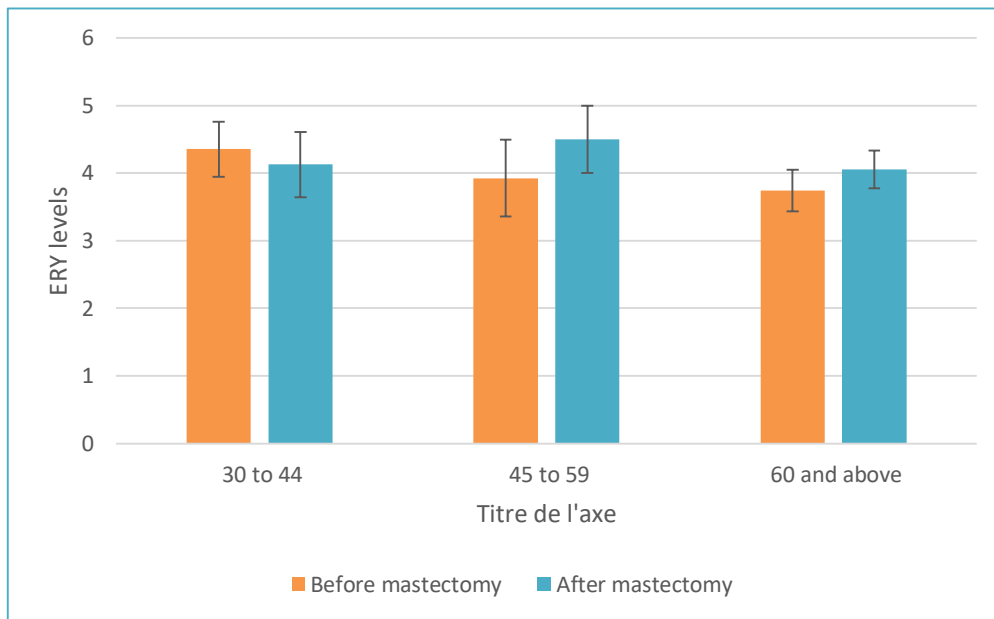
Figure 33: Erythrocyte variations before and after surgical intervention

Note: Normal erythrocyte values are 4.0-5.0μL

The statistical study of indicates sufficient erythrocytes in the 30-45 age group with mean values of 4.353μL and 4.126μL before and after mastectomy respectively although there was a slight decrease after mastectomy. The age groups 45-59 and 60 and above had insufficient erythrocytes before mastectomy with mean values of 3.923μL and 3.739μL. Their erythrocyte mean values both increase to the range of normal values 4.5μL and 4.057μL respectively after mastectomy.

Thrombocytes

The variations of the thrombocytes values are illustrated in (**Fig 34**).

	30 to 44	45 to 59	60 and above
Before mastectomy	263.571	302.586	289.714
After mastectomy	334.429	362.486	229.286
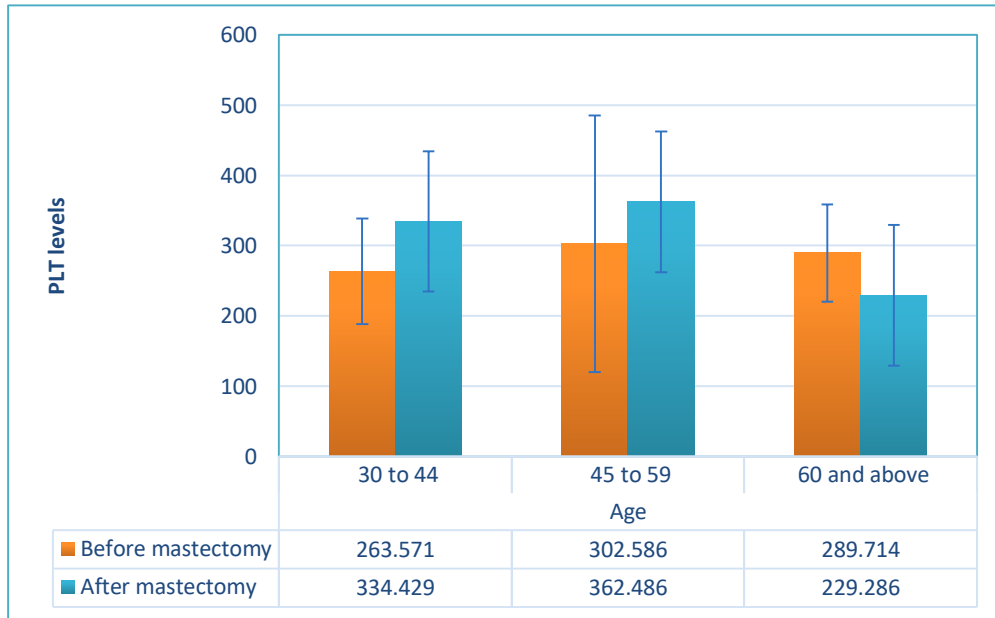
Figure 34: Thrombocyte count before and after mastectomy

Note: The acceptable values for platelets are 150 -450 μL

The statistical study of reveals sufficient levels of platelets in age groups 30-44 and 45-59 with mean values 263.571μL and 302.586μL before mastectomy and; 334.429μL and 362.486μL after mastectomy signifying an increase in the platelet count. The age group 60 and above hand sufficient platelet count before and after mastectomy with mean values of 289.714μL before mastectomy and 229.286μL after mastectomy signifying a decrease.

Haemoglobin

The hemoglobin results of our patients before and after mastectomy according to age group are shown in **(Fig 35).**
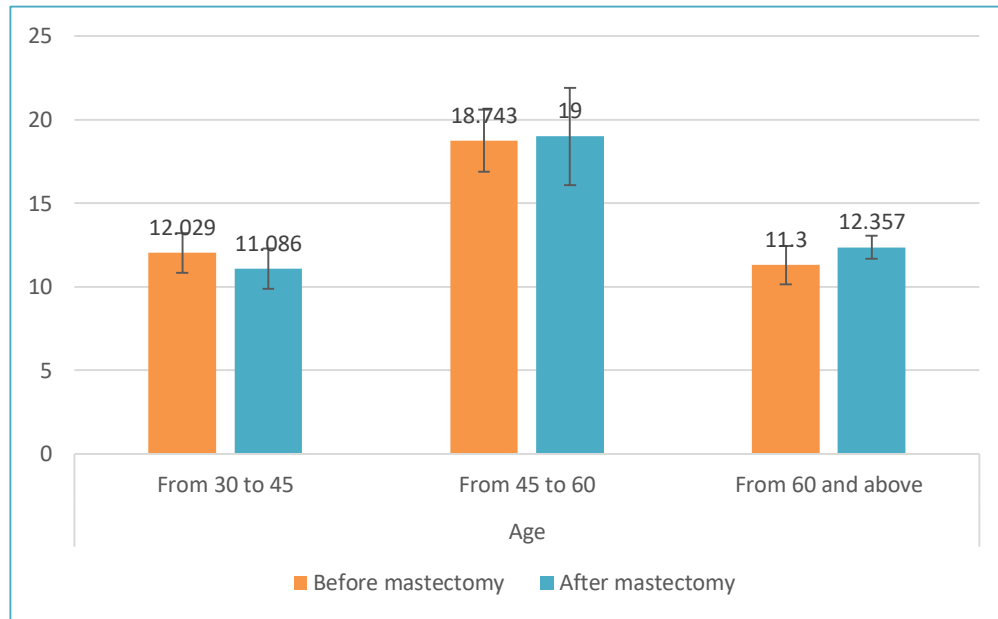
Figure 35: Mean hemoglobin values before and after mastectomy for each age group

.

Note: Normal Hb values are 12-15g/mL

The statistical analysis presented on shows normal values of Hb for the 30-44 age group before mastectomy with a mean value of 12.029g/mL, there is a decrease post mastectomy with a mean value of 11.086g/mL. The 45-59 age group had elevated Hb levels showing a slight increase with mean values of 18.743g/mL and 19g/mL pre and post mastectomy respectively. Low Hb levels are shown in the 60 and above age group before mastectomy with a mean value of 11.3gmL, the Hb level increased after mastectomy with mean value of 12.357g/mL.

Neutrophils

The results of the neutrophil count of our patients grouped according to age are illustrated in (**Fig 36**).
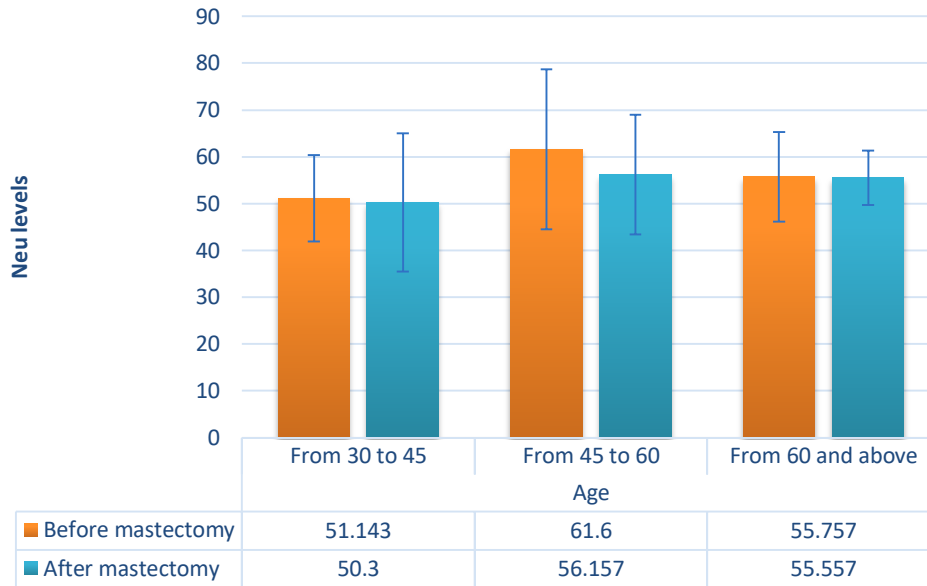
Figure 36: Neutrophil count: pre and post mastectomy comparison

Note: Normal neutrophil count values are 40-60%

The statistical study presented in **(Fig 36)** shows normal neutrophil levels in the age group 30-44 with mean values of 51.143% and 50.3% before and after mastectomy indicating a small decline. The age group 45-59 has sufficient neutrophil levels before and after mastectomy with mean values of 61.6% and 56.157% respectively stipulating a slight diminish after mastectomy. Normal neutrophil levels are shown in the 60 and above age group with mean values of 55.757% and 55.557 before and after mastectomy accordingly showing no significant change.

Hematocrit

The hematocrit (Ht) results of our patients distributed according to age group before and after mastectomy are shown in (**Fig 37**).
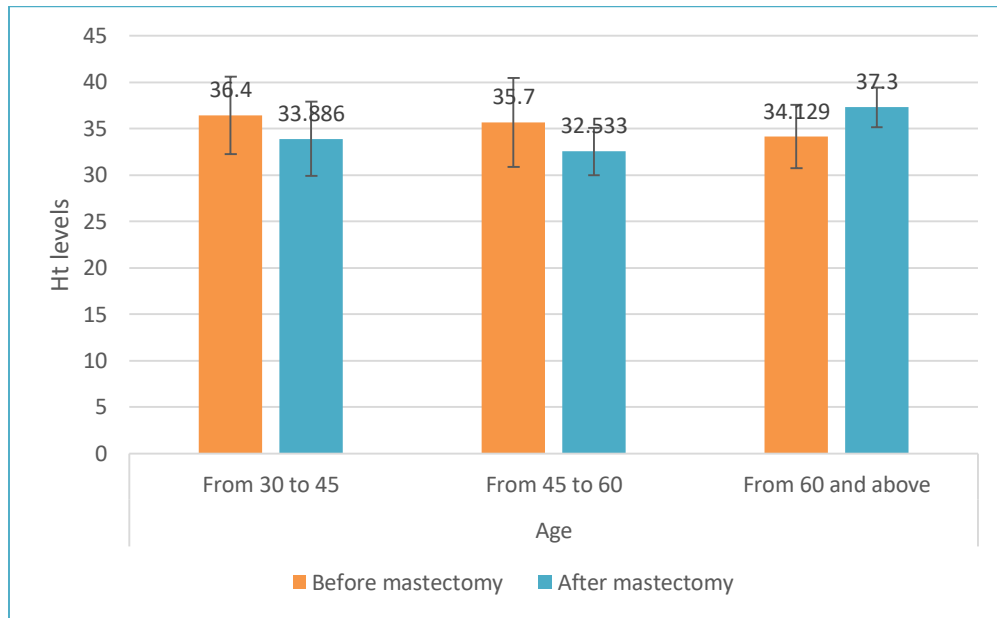
Figure 37: Changes in hematocrit levels following mastectomy

Note: Standard Ht values are 36-45%

The statistical analysis depicted on (**Fig 37**) reveals a decrease in Ht level below normal range following mastectomy in the 30-44 and 45-59 age groups with mean values of 36.4% and 35.7% before mastectomy and 33.886% and 32.533% post mastectomy accordingly. The 45-59 age group had low Ht levels pre mastectomy with mean value of 34.129%, an increase into normal range value is shown after mastectomy with mean value of 37.3%.

Mean corpuscular volume

The patients' MCV test results before and after mastectomy for each age group are shown in (**Fig 38**).

65

	30 to 44	45 to 59	60 and above
Before mastectomy	83.671	89.157	91.314
After mastectomy	82.714	87.171	91.441
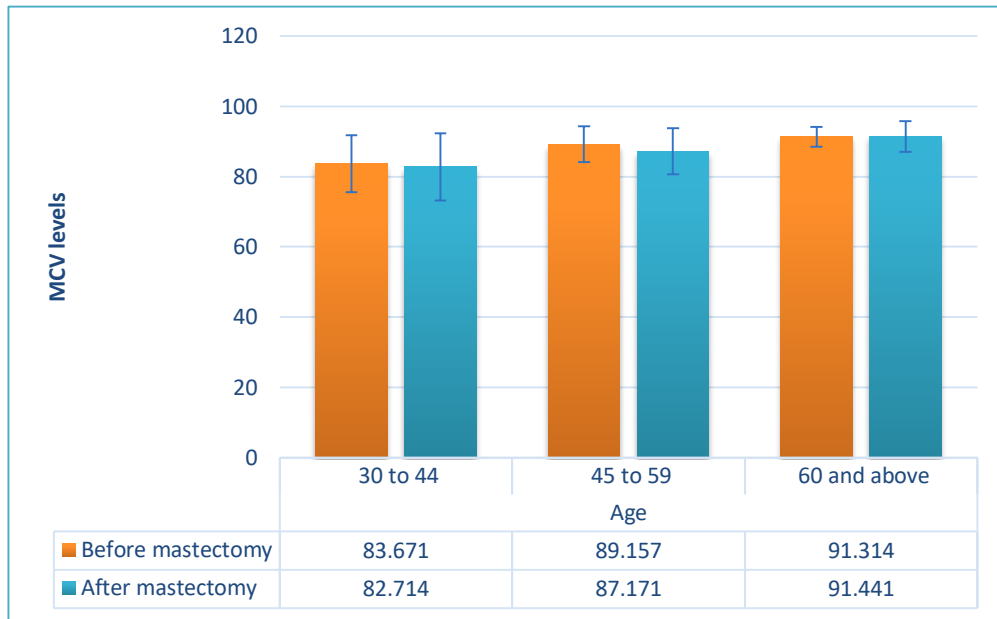
Figure 38: MCV mean values before and after mastectomy

Note: Normal MCV values are 80-100fL

The statistical analysis presented in **(Fig 38)** illustrates normal mean corpuscular volume levels in the 30-44 age group, with mean values of 83.671fL and 82.714fL before and after mastectomy, respectively, indicating no significant difference. In the 45-59 age group, acceptable MCV levels were observed, with mean values of 89.157fL and 87.171fL before and after mastectomy, respectively, indicating a slight decrease. Similarly, sufficient MCV levels were noted in the 60 and above age group, with mean values of 91.314fL and 91.441fL before and after mastectomy, respectively, showing no notable difference

Mean corpuscular haemoglobin

The MCH test results of our patients distributed according to age group before and after mastectomy are illustrated in (**Fig 39**).
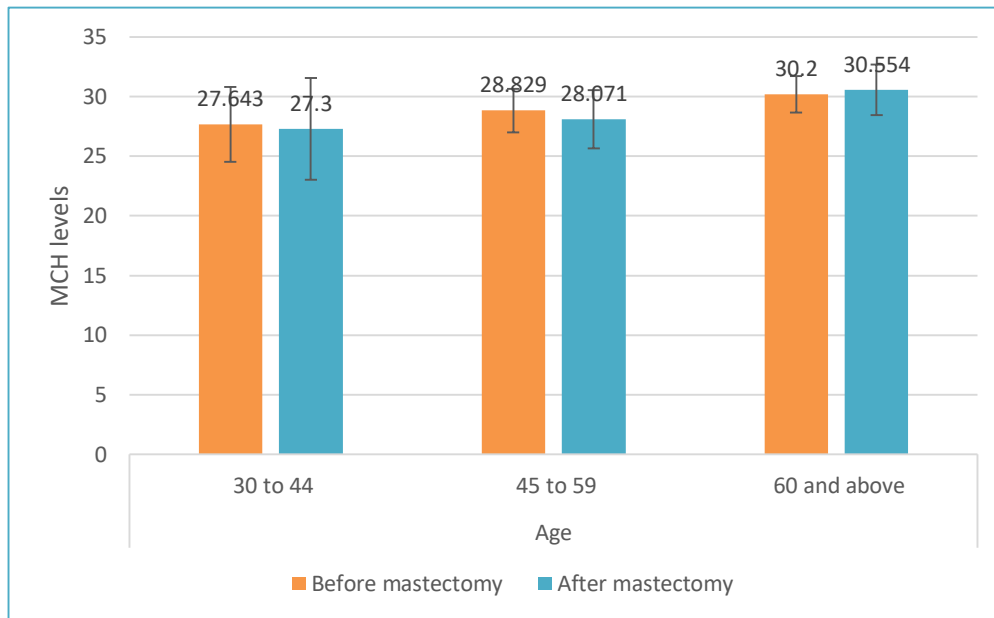
Figure 39: MCH values prior and after mastectomy

Note: Normal MCH values are 27-31pg

Analysis of **(Fig 39)** reveals that in the 30-44 age group, mean corpuscular volume levels remained within the normal range,with mean values of 27.643pg and 27.3pg before and after mastectomy, respectively, showing no significant difference. For the 45-59 age group, MCV levels were initially sufficient, with mean values of 28.829pg before mastectomy, decreasing slightly to 28.071pg after the procedure. Similarly, in the 60 and above age group, MCV levels remained normal, with average values of 30.2pg and 30.554pg before and after mastectomy, respectively, indicating no significant change.

Mean corpuscular haemoglobin concentration

The MCHC results for our patients' age groups before and after mastectomy are displayed in (**Fig 40**)
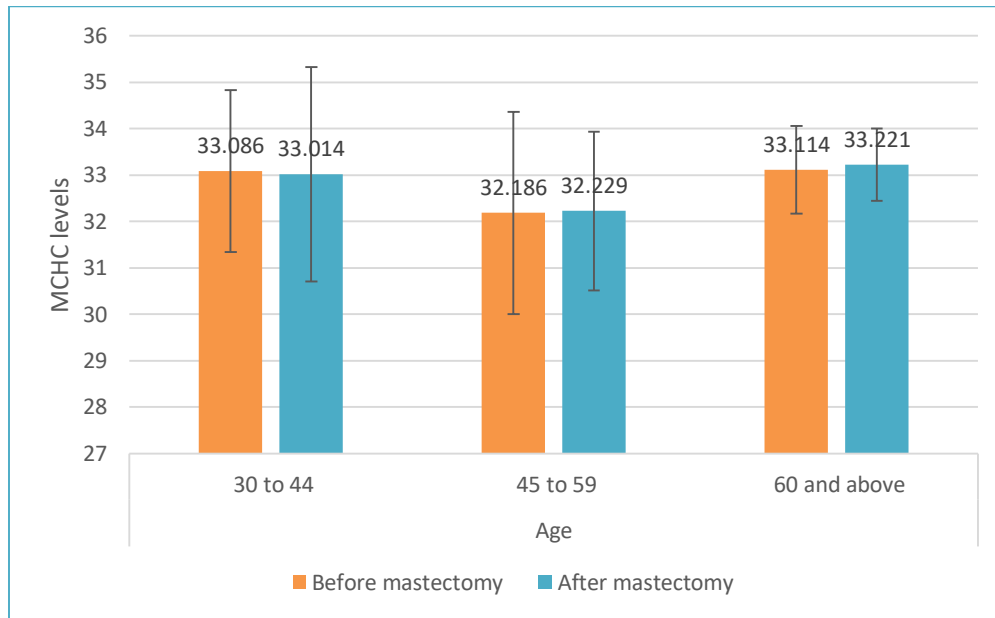
Figure 40: Before and after mastectomy MCHC variations

Note: Normal MCHC values are 32-36 g/dL

Fig 40 presents the statistical analysis of mean corpuscular haemoglobin concentration levels across different age groups before and after mastectomy. The findings indicate normal MCHC levels in all age groups, with mean values of 33.086 and 33.014 in the 30-44 age group, 32.186 and 32.229 in the 45-60 age group, and 33.114 and 33.221 in the 60 and above age group, before and after mastectomy, respectively. Notably, the mean values for the 45-59 age group were slightly lower than those of the other age groups, although the differences were not statistically significant.

6.4 General discussion

Breast cancer is a major global concern associated with a high mortality rate due to complications in many organ systems, including haematological abnormalities observed both before and after treatment. While not exclusively age-related, breast cancer is more prevalent in women aged 50 and above, likely due to age-related comorbidities, accumulated mutations, and prolonged exposure to oestrogen and progesterone, known contributors to breast cancer development. In our study, 60% of participants were in their menstrual cycle and 40% had attained menopause, reflecting the impact of hormonal exposure on breast cancer susceptibility. The

majority of patients were diagnosed with stage II invasive carcinoma NST, predominantly ductal carcinoma. Nineteen patients were diagnosed with the luminal A molecular subtype, while 12 had TNBC. This indicates that most patients tested positive for ER and PR receptors (50 patients), with fewer testing positive for HER2 receptor (24 patients).

Breast chemotherapy drugs contain cytotoxic compounds capable of destroying both cancerous and rapidly dividing cells, making treatment challenging for patients under both neoadjuvant and adjuvant regimens. Haematological abnormalities such as neutropenia, anaemia, leucopenia, and thrombopenia often arise due to chemotherapeutic drugs. A high percentage of our patients were on taxane-containing drugs, which are associated with myelosuppression, thus slowing down haematopoiesis in the bone marrow. In our study, post-chemotherapy anaemia was observed across all age cohorts, with slight decreases in erythrocytes and their indices, haematocrit, and haemoglobin. Exceptionally low values of MCV, haemoglobin, MCH and MCHC were observed in the 20-29 age group, indicating anaemia related with microcytosis, iron metabolism disruption due to inflammation and low iron uptake. The outcome of our study resembled with the study conducted by (**Aynalem *et al.,* 2022; Rahmaniar *et al*., 2024).**

There was no significant deviation from the normal range in leukocyte levels across all age groups, except for the 40-49 group after the third cycle of chemotherapy. We also observed increased neutrophil levels, which varied according to age and chemotherapy cycle. For instance, the 20-29 age group showed decreased levels from cycles 2 to 5, which stabilized in cycle 6, while other cohorts exhibited varied fluctuations. Most groups showed slight variations, likely due to the 2-3 weeks interval that allowed proper cell count recovery after treatment.

The unique values for the 20-29 and 40-49 age groups may be due to dense-dose chemotherapy extending its effects to the next cycle or other minimal factors. Our observations differed from those of (**Aynalem *et al.,* 2022)**, who reported decreased leukocyte and neutrophil levels after treatment. Thrombocytosis was particularly noted in the 30-39 age group, with SD values of 119.33 and 115.24, while other age cohorts showed slight fluctuations after the cycle, consistent with the findings of (**Rahmaniar *et al*., 2024)** in Indonesia, where thrombocytopenia was observed in the 40-59 age cohort.

Mastectomy patients had most of their axillary nodes removed due to the nature of their surgery and received general anaesthesia, which can cause immunosuppression through the

sympathetic nervous system. Most patients had anaemia with low levels of erythrocytes, haemoglobin, haematocrit, and MCV both before and after mastectomy. MCH and MCHC levels were normal before and after surgery. The only group with high haemoglobin levels was the 45-59 age group, both before and after mastectomy. Leukocyte levels were low before surgery in the 30-39 and 60+ age groups but remained within the normal range. Neutrophil levels were within healthy limits for the 20-29 and 60+ age groups and showed no changes before and after mastectomy, but the 45-59 group exhibited increased levels that decreased after surgery, which is unique considering that most patients were under chemotherapy. However, since most patients were receiving haematopoiesis boosters this could be the core reason behind the elevated levels beside the infections that are related with increased neutrophils levels. Perioperative blood loss and surgical trauma likely triggered the HPA axis and SNS to restore tissue homeostasis. Post-surgery, thrombocyte numbers increased in the 20-29 and 45-59 age groups and decreased in the 60+ age group. The increase in platelet count may be due to a transient overshoot caused by delayed thrombopoietin release related to surgical haemostasis, while the low levels in the 60+ group could be attributed to age-related reduced haematopoiesis and increased platelet demand for coagulation.

Studies by **(Ufelle et al., 2012)** in Enugu, Nigeria, showed significant decreases in haematological parameters pre- and post-surgery, similar to our findings. However, most patients in our study showed increased thrombocyte levels, contrary to their study. **(Skeith *et al*., 2020)** observed thrombopenia in cardiac patients post-surgery, followed by a significant increase, aligning with our observations. Our study noted increased anaemia due to chemotherapy drugs and mastectomy. Leucopenia, neutropenia, and thrombopenia were age-group-related and fluctuated more than erythrocytes and their indices. Medications administered to patients with haematological parameter fluctuations post-chemotherapy could have stimulated production to restore peripheral blood levels, and the interval between chemotherapy cycles likely aided in blood cell recovery.

This study had limited provisions of immediate tests after short chemotherapy sessions and mastectomy due to its retrospective design. However, chemotherapy and mastectomy negatively affect haematological parameters to levels detrimental to patient well-being, necessitating immune-boosting supplements alongside the regimen.

6.5 Conclusion

In conclusion, breast cancer chemotherapy and mastectomy significantly impact haematological parameters, often resulting in anaemia, neutropenia, leucopenia, and thrombocytopenia. These haematological changes can adversely affect patient well-being and complicate recovery. Our study highlights the need for vigilant clinical monitoring and timely interventions to manage these side effects. The use of haematopoiesis boosters and maintaining a well-balanced nutritional regimen are critical strategies for mitigating the adverse effects of treatment.

Furthermore, our findings underscore the importance of personalized treatment plans that consider patient-specific factors such as age, hormonal status, and baseline haematological parameters. Tailoring interventions to individual patient needs can improve treatment outcomes and enhance quality of life during and after breast cancer therapy.

Future prospective studies should focus on identifying the primary causes of chemotherapy-associated anemia, whether stemming from myelosuppression or hemolysis. Understanding these mechanisms will aid in developing more targeted and effective treatment methods. Additionally, investigating the role of different chemotherapy regimens and surgical techniques on haematological health can provide insights into optimizing breast cancer treatment protocols.

Overall, a comprehensive approach that includes early detection, personalized treatment, supportive care, and ongoing research is essential for improving patient outcomes and managing the haematological side effects associated with breast cancer treatment.

References

References

Akram, M., Iqbal, M., Daniyal, M. et al. (2017) Awareness and current knowledge of breast cancer. Biol Res 50, 33. https://doi.org/10.1186/s40659-017-0140-9

Alavi, M., & Hamidi, M. (2019). Passive and active targeting in cancer therapy by liposomes and lipid nanoparticles. Drug Metabolism and Personalized Therapy, 34(1), 20180032. https://doi.org/10.1515/dmpt-2018-0032.

Albrektsen, G., Heuch, I., Hansen, S., & Kvåle, G. (2005). Breast cancer risk by age at birth, time since birth and time intervals between births: exploring interaction effects. *British journal of cancer*, 92(1), 167–175. https://doi.org/10.1038/sj.bjc.6602302

Alzahrani, S.M., Al Doghaither, H.A., & Al-Ghafari, A.B., (2021). General insight into cancer: an overview of colorectal cancer. Mol. Clin. Oncol. 15 (6), 1–8.

Amjad, MT., Chidharla, A., & Kasi, A. (2023). Cancer Chemotherapy In: StatPearls. Treasure Island (FL): StatPearls Publishing; https://www.ncbi.nlm.nih.gov/books/NBK564367/

Andleeb, A., Andleeb, A., Asghar, S., Zaman, G., Tariq, M., Mehmood, A., Nadeem, M., Hano, C.,

Lorenzo, J.M & Abbasi, B.H. (2021).A Systematic Review of Biosynthesized Metallic Nanoparticles as a Promising Anti-Cancer-Strategy,Cancers 2021, 13, 2818,22.

Asaoka, M., Gandhi, S., Ishikawa, T., & Takabe, K. (2020). Neoadjuvant Chemotherapy for Breast Cancer: Past, Present, and Future. Breast cancer : basic and clinical research, 14, 1178223420980377. https://doi.org/10.1177/1178223420980377.

Avila J.P., Carvalho B.M., & Coimbra EC. (2023) A Comprehensive View of the Cancer-Immunity Cycle (CIC) in HPV-Mediated Cervical Cancer and Prospects for Emerging Therapeutic Opportunities. Cancers. 15(4):1333. https://doi.org/10.3390/cancers15041333

Aynalem, M., Adem, N., Wendesson, F., Misganaw, B., Mintesnot, S., Godo, N., Getawa, S., Adane, T., Woldu, B., & Shiferaw, E. (2022). Hematological abnormalities before and after initiation of cancer treatment among breast cancer patients attending at the University of Gondar comprehensive specialized hospital cancer treatment center. PloS one, 17(8), e0271895. https://doi.org/10.1371/journal.pone.0271895.

References

Baghban, R., Roshangar, L., Jahanban-Esfahlan, R. et al. (2020)Tumor microenvironment complexity and therapeutic implications at a glance. Cell Commun Signal 18, 59. https://doi.org/10.1186/s12964-020-0530-4.

Bharti, A.C., Vishnoi, K., Singh, S.M., & Aggarwal, B.B. (2018) Pathways Linked to Cancer Chemoresistance and Their Targeting by Nutraceuticals. In Cancer Sensitizing Agents for Chemotherapy, Role of Nutraceuticals in Cancer Chemosensitization, 1st ed.; Bharti, A.C., Aggarwal, B.B., Eds.; Academic Press: Cambridge, MA, USA; Volume 2, pp. 1–30. https://doi.org/10.1016/B978-0-12-812373-7.00001-2.

Bistoni, G & Farhadi, J. (2015). Anatomy and Physiology of the Breast. https://doi:10.1002/9781118655412.ch37

Britannica T. Editors of Encyclopaedia (2024). surgery. Encyclopedia Britannica. https://www.britannica.com/science/surgery-medicine.

Catsburg C., Miller A.B.,& Rohan TE.(2015) Active cigarette smoking and risk of breast cancer. *Int J Cancer*.;136(9):2204-9. Epub 2014 Oct 23. PMID: 25307527. https://doi: 10.1002/ijc.29266.

Chen, J., & Lindblom, A. (2000). Germline mutation screening of the STK11/LKB1 gene in familial breast cancer with LOH on 19p. Clinical genetics, 57(5), 394–397. https://doi.org/10.1034/j.1399-0004.2000.570511.x

Chhikara, B. S., & Parang, K. (2023). Global Cancer Statistics 2022: the trends projection analysis. Chemical Biology Letters, 10(1), 451.

Chuphal, B. (2022). Molecular Genetics of Cancer. In: Kar, D., Sarkar, S. (eds) Genetics Fundamentals Notes. Springer, Singapore. https://doi.org/10.1007/978-981-16-7041-1_18

Ciruelos Gil E. M. (2014). Targeting the PI3K/AKT/mTOR pathway in estrogen receptor-positive breast cancer. *Cancer treatment reviews*, 40(7), 862–871. https://doi.org/10.1016/j.ctrv.2014.03.004.

Colleoni, M., L., Castiglione-Gertsch, M., Sauerbrei, W., Gelber, R. D., Bonetti, M., Coates, A. S., Schumacher, M., Bastert, G., Rudenstam, C. M., Schmoor, C., Lindtner, J., Collins, J., Thürlimann, B., Holmberg, S. B., Crivellari, D., Beyerle, C., Neumann, R. L & Goldhirsch, A. (2002) International Breast Cancer Study Group, German Breast Cancer Study Group. Duration of adjuvant chemotherapy for breast cancer: a joint analysis

References

of two randomised trials investigating three versus six courses of CMF. *British journal of cancer*, 86(11), 1705–1714. https://doi.org/10.1038/sj.bjc.6600334

Collins K., Jacks T & Pavletich N.P(1997) The cell cycle and cancer. Proc Natl Acad Sci USA (1997) 7:2776–8. https://doi: 10.1073/pnas.94.7.2776

Colvin M. (2003). Alkylating Agents. In:Holland-Frei Cancer Medicine. Kufe DW, Pollock RE, Weichselbaum RR, et al., editors. 6th edition. Hamilton (ON): BC Decker; Available from: https://www.ncbi.nlm.nih.gov/books/NBK12772/

Cooper, G.M & Hausman, R.(2000). A molecular approach. In: The Cell, second ed. Sinauer Associates,Sunderland, MA.

Dandamudi, A., Tommie, J., Nommsen-Rivers, L.; & Couch, S.(2018) Dietary Patterns and Breast Cancer Risk: A Systematic Review.Anticancer. Res. 2018, 38(6) 3209–3222. https://doi.org/10.21873/anticanres.125

Daniel, C & Daniel A. H.(2010),Basic Principles Of Cancer Genetics,In: Principles of clinical Cancer Genetics: A Handbook from the Massachusetts General Hospital,Springer Science & Business Media,Springer New York Dordrecht Heidelberg London,1-22.

Davis, A. S., Viera, A. J., & Mead, M. D. (2014). Leukemia: an overview for primary care. American family physician, 89(9), 731–738.

Devine H, Tierney D, Schmit-Pokorny K & McDermott, K. (2010). Mobilization of Hematopoietic Stem Cells for Use in Autologous Transplantation. *Clinical journal of oncology nursing*. 14. 212-22. https://10.1188/10.CJON.212-222.

Diori Karidio, I.,& Sanlier, S.H. (2021). Reviewing cancer's biology: an eclectic approach. J Egypt Natl Canc Inst 33, 32 https://doi.org/10.1186/s43046-021-00088-y

Edechi, C. A., Ikeogu, N., Uzonna, J. E., & Myal, Y. (2019). Regulation of Immunity in Breast Cancer. Cancers, 11(8), 1080. https://doi.org/10.3390/cancers11081080

Elbromboly, Y & Esawy, M.A.(2023) Post-operative C-reactive protein and white blood cells changes pattern following spinal deformity surgery and its clinical correlation. J Orthop Surg Res 18, 790 https://doi.org/10.1186/s13018-023-04288-1.

Endogenous Hormones and Breast Cancer Collaborative Group, Key, T. J., Appleby, P. N., Reeves, G. K., Travis, R. C., Alberg, A. J., Barricarte, A., Berrino, F., Krogh, V., Sieri, S., Brinton, L. A., Dorgan, J. F., Dossus, L., Dowsett, M., Eliassen, A. H., Fortner, R.

References

  T., Hankinson, S. E., Helzlsouer, K. J., Hoff man-Bolton, J., Comstock, G. W., & Vineis, P. (2013). Sex hormones and risk of breast cancer in premenopausal women: a collaborative reanalysis of individual participant data from seven prospective studies. The Lancet. Oncology, 14(10), 1009–1019. https://doi.org/10.1016/S1470-2045(13)70301-2

Fathoni, M.I.A., Adi-Kusumo, F., Gunardi, G. & Hutajulu, S.H.(2021)"Dynamics of a Breast Cancer Model for Neutropenia Case due to Chemotherapy Effects", *International Journal of Differential Equations*, Article ID 3401639, 8 pages. https://doi.org/10.1155/2021/3401639.

Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., Ji, X., Liu, W., Huang, B., Luo, W., Liu, B., Lei, Y., Du, S., Vuppalapati, A., Luu, H. H., Haydon, R. C., He, T. C., & Ren, G. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes & diseases, 5(2), 77–106. https://doi.org/10.1016/j.gendis.2018.05.001.

Ferlay J., Ervik M, Lam F, Laversanne M, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F (2024).Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.who.int/today

Ferlay, J., Ervik, M., Lam, F., Laversanne, M., Colombet, M., Mery, L., Piñeros, M., Znaor, A., Soerjomataram, I., &Bray F (2024).Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. https://gco.iarc.who.int/today.

Fiolet, T.,Srour, B., Sellem, L., Kesse-Guyot, E., Allès, B., Méjean, C., Deschasaux, M., Fassier, P., Latino-Martel, P., Beslay, M.; et al.,Consumption of ultra-processed foods and cancer risk: Results from Nutri Net-Santé prospective cohort. BMJ 2018, 360, k322.

Garay, R.P., Viens, P., Bauer, J., Normier, G., Bardou, M., Jeannin, J.-F & Chiavaroli, C. (2007). Cancerrelapse under chemotherapy: why TLR2/4 receptor agonists can help. Eur. J. Pharmacol. 563 (1–3),1–17.

Goethals A & Rose J. (2024) Mastectomy.In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing: https://www.ncbi.nlm.nih.gov/books/NBK538212/

References

Goff, S. L., & Danforth, D. N. (2021). The Role of Immune Cells in Breast Tissue and Immunotherapy for the Treatment of Breast Cancer. Clinical breast cancer, 21(1), e63–e73. https://doi.org/10.1016/j.clbc.2020.06.011

Hanahan D & Weinberg RA (2000). The hallmarks of cancer. Cell 2000;100:57–70.

Hanahan D(2022).Hallmarks of Cancer: New Dimensions. Agora Translational Cancer Research Center. Rue du Bugnon 25A, Lausanne CH-1011, Switzerland.12 (1): 31–46.

Hanahan, D &Weinberg, R.A(2011). Hallmarks of cancer: the next generation. Cell 144 (5), 646–674.

Hanna, K & Mayden, K. (2021). Chemotherapy Treatment Considerations in Metastatic Breast Cancer. *Journal of the advanced practitioner in oncology*, 12(Suppl 2), 6–12. https://doi.org/10.6004/jadpro.2021.12.2.11

Hernandez-Aya, L. F., & Ma, C. X. (2016). Chemotherapy principles of managing stage IV breast cancer in the United States. Chinese Clinical Oncology, 5(3), 13.

Hiam-Galvez, K.J., Allen, B.M., & Spitzer, M.H. (2021) Systemic immunity in cancer. Nat Rev Cancer 21, 345–359. https://doi.org/10.1038/s41568-021-00347-z.

Hill, D. A., Prossnitz, E. R., Royce, M., & Nibbe, A. (2019). Temporal trends in breast cancer survival by race and ethnicity: A population-based cohort study. PloS one, 14(10), e0224064. https://doi.org/10.1371/journal.pone.0224064

Hoang, N. T., Acevedo, L. A., Mann, M. J., & Tolani, B. (2018). A review of soft-tissue sarcomas: translation of biological advances into treatment measures. Cancer management and research, 10, 1089–1114. https://doi.org/10.2147/CMAR.S159641

Hopper, J.L., Dite, G.S., MacInnis, R.J. et al. (2018) Age-specific breast cancer risk by body mass index and familial risk: prospective family study cohort (ProF-SC). Breast Cancer Res 20, 132. https://doi.org/10.1186/s13058-018-1056-1.

Husby, A., Wohlfahrt, J., Øyen, N., & Melbye, M. (2018). Pregnancy duration and breast cancer risk. Nature communications, 9(1), 4255. https://doi.org/10.1038/s41467-018-06748-3

Inagi, T., Hoshina, H., Suzuki, M. et al. (2016). Remifentanil-induced alterations in neutrophil numbers after surgery. JA Clin Rep 2, 5 https://doi.org/10.1186/s40981-016-0031-z.

Jabbar, A.S. (2016). Introduction to Human Physiology.Dar Wael publishers.

References

Jung, G. H., Hwang, H. K., Lee, W. J., & Kang, C. M. (2019). Extremely high white blood cell counts on postoperative day 1 do not predict severe complications following distal pancreatectomy. Annals of hepato-biliary-pancreatic surgery, 23(4), 377–384. https://doi.org/10.14701/ahbps.2019.23.4.377

Kalra, S.K., Thilagar, B., Khambaty, M. & Manjarrez, E. (2021). Post-operative Anemia After Major Surgery: a Brief Review. Curr Emerg Hosp Med Rep 9, 89–95 . https://doi.org/10.1007/s40138-021-00232-x

Kantharia, S., Gadgil, A., Cherian, S., Basu, P., & Lucas E (2023). Atlas of breast cancer early detection: IARC CancerBase No. 17 . Lyon, France: International Agency for Research on Cancer. https://screening.iarc.fr/atlasbreast.php.

Karayannopoulou B, Anagnostou T, Margariti A, Kritsepi-Konstantinou M, Psalla D, Angelos-Lauris T & Savvas I (2022).Effect of major versus minor mastectomy on host immunity in canine mammary cancer,Veterinary Immunology and Immunopathology.Article ID 110403, Volume 246.https://doi.org/10.1016/j.vetimm.2022.110403.

Kavarthapu, R., Anbazhagan, R., & Dufau, M. L. (2021). Crosstalk between PRLR and EGFR/HER2 Signaling Pathways in Breast Cancer. Cancers, 13(18), 4685. https://doi.org/10.3390/cancers13184685.

Kechagioglou, P., Papi, R. M., Provatopoulou, X., Kalogera, E., Papadimitriou, E., Grigoropoulos, P., Nonni, A., Zografos, G., Kyriakidis, D. A., & Gounaris, A. (2014). Tumor suppressor PTEN in breast cancer: heterozygosity, mutations and protein expression. Anticancer research, 34(3), 1387–1400.

Kim R. (2018). Effects of surgery and anesthetic choice on immunosuppression and cancer recurrence. Journal of translational medicine, 16(1), 8. https://doi.org/10.1186/s12967-018-1389-7

Kotepui, M. (2016). Diet and risk of breast cancer. Contemporary Oncology/Współczesna Onkologia, 20(1), 13-19. https://doi.org/10.5114/wo.2014.40560.

Kuroda, S., Kagawa, S., & Fujiwara, T. (2014).Selectively Replicating Oncolytic Adenoviruses Combined with Chemotherapy, Radiotherapy, or Molecular Targeted Therapy for

Treatment of Human Cancers In:Gene Therapy of Cancer,3rd ed.; Lattime, E.C, Gerson, S.L.;Pages 171-183.https://doi.org/10.1016/B978-0-12-394295-1.00012-3.

Larissa A., Korde Mark R., Somerfield, Lisa A., Carey, Jennie R., Crews, Neelima Denduluri, E., Shelley H., Seema A. K., Sibylle L., Elizabeth A.M., Alejandra P., Meredith M. R., Patricia A. S., Preeti K. S., Fraser Symmans W., Rachel L. Y., Brittany E. H & Dawn L.H. (2021). Neoadjuvant Chemotherapy, Endocrine Therapy, and Targeted Therapy for Breast Cancer: ASCO Guideline.*Journal of Clinical Oncology* 39:13, 1485-1505.DOI:https://10.1200/JCO.20.03399

Lazaraviciute, G & Chaturvedi, S (2017) Mastectomy—A Critical Review. *Open Journal of Clinical Diagnostics*, 7, 58-66. https://doi: 10.4236/ojcd.2017.72006.

Li, Z., Wei, H., Li, S., Wu, P., & Mao, X. (2022). The Role of Progesterone Receptors in Breast Cancer. Drug design, development and therapy, 16, 305–314. https://doi.org/10.2147/DDDT.S336643.

Loeb.K.R & Loeb.L.A. (2000) Significance of multiple mutations in cancer, Carcinogenesis, Volume 21(3), 379–385.

Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanisławek, A. (2021). Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies-An Updated Review. Cancers, 13(17), 4287. https://doi.org/10.3390/cancers13174287

Lukong K. E. (2017). Understanding breast cancer - The long and winding road. BBA clinical, 7, 64–77. https://doi.org/10.1016/j.bbacli.2017.01.001

Lynch BM, Milne RL, English DR, Brown KA, Drummond AE, Swain CTV, et al..(2022) Linking physical activity to breast cancer: text mining results and a protocol for systematically reviewing three potential mechanistic pathways. Cancer Epidemiol Biomarkers Prev 2022;31:11–5.

Malumbres, M & Barbacid, M. (2009). Cell cycle, CDKs and cancer: a changing paradigm. Nature reviews. Cancer, 9(3), 153–166. https://doi.org/10.1038/nrc2602

Mandal, A. (2023). Chemotherapy Types. News-Medical. Retrieved on April 23, 2024 from https://www.news-medical.net/health/Chemotherapy-Types.aspx.

References

Martini V., D'Avanzo F., Maggiora P., Varughese F, Sica A., & Gennari A. (2020). Oncolytic virotherapy: New weapon for breast cancer treatment. ecancermedicalscience. 14. https://10.3332/ecancer.2020.1149.

McGuire, A., Brown, J. A., Malone, C., McLaughlin, R., & Kerin, M. J. (2015). Effects of age on the detection and management of breast cancer. Cancers, 7(2), 908–929. https://doi.org/10.3390/cancers7020815

McTiernan A. (2008) Mechanisms linking physical activity with cancer. Nat Rev Cancer;8:205–11.

Miricescu, D., Totan, A., Stanescu-Spinu, I. I, Badoiu, S. C., Stefani, C., & Greabu, M. (2020). PI3K/AKT/mTOR Signaling Pathway in Breast Cancer: From Molecular Landscape to Clinical Aspects. *International journal of molecular sciences*, 22(1), 173. https://doi.org/10.3390/ijms22010173.

Mohammed, H., Russell, I. A., Stark, R., Rueda, O. M., Hickey, T. E., Tarulli, G. A., Serandour, A. A., Birrell, S. N., Bruna, A., Saadi, A., Menon, S., Hadfield, J., Pugh, M., Raj, G. V., Brown, G. D., D'Santos, C., Robinson, J. L., Silva, G., Launchbury, R., Perou, C. M., & Carroll, J. S. (2015). Corrigendum: Progesterone receptor modulates ERα action in breast cancer. Nature, 526(7571), 144. https://doi.org/10.1038/nature14959

Narod S. A. (2011). Hormone replacement therapy and the risk of breast cancer. *Nature reviews*. Clinical oncology, 8(11), 669–676. https://doi.org/10.1038/nrclinonc.2011.110

Nicholson L. B. (2016). The immune system. Essays in biochemistry, 60(3), 275–301. https://doi.org/10.1042/EBC20160017

Nicole M., Anderson, M.,& Celeste Simon.(2020).The tumor microenvironment, Current Biology,30(16), R921-R925,0960-9822. https://doi.org/10.1016/j.cub.2020.06.081.

PDQ Adult Treatment Editorial Board, (2024). Breast Cancer Treatment (PDQ®): Patient Version.In: PDQ Cancer Information Summaries. Bethesda (MD): National Cancer Institute (US); 2002-. https://www.ncbi.nlm.nih.gov/books/NBK65969/

Perrins, S., Moon, S & Blake, C. (2020). Modified Radical Mastectomy. In: Rosenthal, R., Rosales, A., Lo Menzo, E., Dip, F. (eds) Mental Conditioning to Perform Common Operations in General Surgery Training. Springer, Cham. https://doi.org/10.1007/978-3-319-91164-9_5.

References

Pinheiro, A.V., Petrucci,G.N., Dourado, A & Pires, I. (2023)Anaesthesia in Veterinary Oncology:The Effects of Surgery, Volatile and Intravenous Anaesthetics on the Immune System and Tumour Spread.Animals, 13, 3392. https://doi.org/10.3390/ani13213392.

Plesca, M., Bordea, C., El Houcheimi, B., Ichim, E., & Blidaru, A. (2016). Evolution of radical mastectomy for breast cancer. *Journal of medicine and life*, 9(2), 183–186.

Rahmaniar, S., Benny Kusuma, & Irfanuddin. (2024). Analysis of the Role of Age Factors on Hemoglobin, Leukocyte and Platelet Levels in Breast Cancer Patients Post Chemotherapy 3 Cycles at Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia. Sriwijaya. *Journal of Surgery*, 7(2), 629-637. https://doi.org/10.37275/sjs.v7i2.103

Raza Y., Salman H & Luberto C. (2021). Sphingolipids in Hematopoiesis: Exploring Their Role in Lineage Commitment. Cells. https://10. 2507. 10.3390/cells10102507.

Reilly, R.(2007). Breast Cancer.Editor(s): S.J. Enna, David B. Bylund,xPharm: The Comprehensive Pharmacology Reference,Elsevier,Pages 1-9,ISBN 9780080552323.https://doi.org/10.1016/B978-008055232-3.60809-8.

Rinaldi, R.M., Sapra, A., & Bellin LS. .(2023)Breast Lymphatics. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553093/

Sarkar, S., Horn, G., Moulton, K., Oza, A., Byler, S., Kokolus, S., & Longacre, M. (2013). Cancer development, progression, and therapy: an epigenetic overview. *International journal of molecular sciences,* 14(10), 21087–21113. https://doi.org/10.3390/ijms141021087.

SAS (2008) Statistical analysis systems user's guide '2008 version 9.2. 2nd edition,, I. SAS Institute, Cary, NC, Editor. 2008.

Scoccianti, C., Lauby-Secretan, B., Bello, P. Y., Chajes, V., & Romieu, I. (2014). Female breast cancer and alcohol consumption: a review of the literature. *American journal of preventive medicine*, 46(3 Suppl 1), S16–S25. https://doi.org/10.1016/j.amepre.2013.10.031.

Scott-Conner, C.E.H. (2022). Mastectomy: Simple (Total), Modified, and Classical Radical. In: Scott-Conner, C.E.H., Kaiser, A.M., Nguyen, N.T., Sarpel, U., Sugg, S.L. (eds) Chassin's Operative Strategy in General Surgery. Springer, Cham. https://doi.org/10.1007/978-3-030-81415-1_119

References

SEER Training Modules, Mammary Glands. U.S. National Institutes of Health, National Cancer Institute.(2024).https://training.seer.cancer.gov/anatomy/reproductive/female/glands.html.

Shang, C., & Xu, D. (2022). Epidemiology of Breast Cancer. Oncologie, 24(4), 649–663.https://doi.org/10.32604/oncologie.2022.027640

Sharma, G. N., Dave, R., Sanadya, J., Sharma, P., & Sharma, K. K. (2010). Various types and management of breast cancer: an overview. *Journal of advanced pharmaceutical technology & research*, 1(2), 109–126.

Sharma, G. N., Dave, R., Sanadya, J., Sharma, P., & Sharma, K. K. (2010). Various types and management of breast cancer: an overview. *Journal of advanced pharmaceutical technology & research*, 1(2), 109–126.

Shien, T & Iwata, H. (2020), Adjuvant and neoadjuvant therapy for breast cancer, *Japanese Journal of Clinical Oncology*, Volume 50, Issue 3, Pages 225–229, https://doi.org/10.1093/jjco/hyz213

Shiovitz, S., & Korde, L. A. (2015). Genetics of breast cancer: a topic in evolution. Annals of oncology : *official journal of the European Society for Medical Oncology*, 26(7), 1291–1299. https://doi.org/10.1093/annonc/mdv022

Siegel, R. L., Miller, K. D., Wagle, N. S., & Jemal, A. (2023). Cancer statistics, 2023. CA: *a cancer journal for clinicians*, 73(1), 17–48.

Simos, D., Catley, C., van, W.C., Arnaout, A., Booth, C., Mcinnes, M., Fergusson, D., Dent, S., & Clemons, M. (2015). Imaging for distant metastases in women with early-stage breast cancer: A population-based cohort study. *Canadian Medical Association Journal*. 187. 10.1503/cmaj.150003.

Skeith, L., Baumann Kreuziger, L., Crowther, M. A., & Warkentin, T. E. (2020). A practical approach to evaluating postoperative thrombocytopenia. Blood advances, 4(4), 776–783. https://doi.org/10.1182/bloodadvances.2019001414.

Soysal, S. D., Tzankov, A., & Muenst, S. E. (2015). Role of the Tumor Microenvironment in Breast Cancer. Pathobiology : *journal of immunopathology, molecular and cellular biology*, 82(3-4), 142–152. https://doi.org/10.1159/000430499

References

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a cancer journal for clinicians, 71(3), 209–249. https://doi.org/10.3322/caac.21660

Swain, C. T. V., Drummond, A. E., Milne, R. L., English, D. R., Brown, K. A., Lou, M. W. C., Boing, L., Bageley, A., Skinner, T. L., van Roekel, E. H., Moore, M. M., Gaunt, T. R., Martin, R. M., Lewis, S. J., & Lynch, B. M. (2023). Linking Physical Activity to Breast Cancer Risk via Inflammation, Part 1: The Effect of Physical Activity on Inflammation. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 32(5), 588–596. https://doi.org/10.1158/1055-9965.EPI-22-0928

Tabuchi, Y., Shinka, S., & Ishida, H. (1989). The effects of anesthesia and surgery on count and function of neutrophils. *Journal of anesthesia*, 3(2), 123–131. https://doi.org/10.1007/s0054090030123

Talmadge, J. E., & Fidler, I. J,(2010). AACR centennial series: the biology of cancer metastasis:Historical perspective. Cancer research, 70(14), 5649–5669.

Tang F., Tie Y., Tu C & Wei X.(2020;)Surgical trauma-induced immunosuppression in cancer: Recent advances and the potential therapies.Clin Transl Med.10:199–223.https://doi.org/10.1002/ctm2.24

Titus-Ernstoff, L., Longnecker, M. P., Newcomb, P. A., Dain, B., Greenberg, E. R., Mittendorf, R., Stampfer, M., & Willett, W. (1998). Menstrual factors in relation to breast cancer risk. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 7(9), 783–789.

Tokin, C., Weiss, A., Wang-Rodriguez, J., & Blair, S. L. (2012). Oncologic safety of skin-sparing and nipple-sparing mastectomy: a discussion and review of the literature. *International journal of surgical oncology*. https://doi.org/10.1155/2012/921821

Tomaska, J. (2015). Differences in Breast Cancer Tumor Size, Stage, and Survival by Socioeconomic Position in Young Women..https://DOI:10.13140/RG.2.1.2833.1361.

References

Trayes, K. P., & Cokenakes, S. E. H. (2021). Breast Cancer Treatment. American family physician, 104(2), 171–178.

Ufelle, S.A., Ukaejiofo, E.O., Neboh E.E *et al.,* (2012). Some haematological parameters in pre- and post-surgery breast cancer patients in Enugu, Nigeria. Int J Current Biological Medical Sci, 2, 188-190.

Ursin, G., Bernstein, L., Lord, S. J., Karim, R., Deapen, D., Press, M. F., Daling, J. R., Norman, S. A., Liff, J. M., Marchbanks, P. A., Folger, S. G., Simon, M. S., Strom, B. L., Burkman, R. T., Weiss, L. K., & Spirtas, R. (2005). Reproductive factors and subtypes of breast cancer defined by hormone receptor and histology. *British journal of cancer*, 93(3), 364–371. https://doi.org/10.1038/sj.bjc.6602712

Wernli, K. J., Hampton, J. M., Trentham-Dietz, A., & Newcomb, P. A. (2009). Antidepressant medication use and breast cancer risk. Pharmacoepidemiology and drug safety, 18(4), 284–290. https://doi.org/10.1002/pds.1719

Whelan S. (2022) ,Bening vs malignant tumour.Technology networks.https://www.technologynetworks.com/benign-vs-malignant-tumors-364765.

Yap YS. (2023) Outcomes in breast cancer- Does ethnicity matter? ESMO. https://doi: 10.1016/j.esmoop.2023.101564

Yedjou, C. G., Sims, J. N., Miele, L., Noubissi, F., Lowe, L., Fonseca, D. D., Alo, R. A., Payton, M., & Tchounwou, P. B. (2019). Health and Racial Disparity in Breast Cancer. Advances in experimental medicine and biology, 1152, 31–49. https://doi.org/10.1007/978-3-030-20301-6_3

Zarrilli, G., Businello, G., Dieci, M.V., Paccagnella, S., Carraro, V., Cappellesso, R., Miglietta, F., Griguolo, G., Guarneri, V., Lo Mele, M., & Fassan M, (2020). The Tumor Microenvironment of Primitive and Metastatic Breast Cancer: Implications for Novel Therapeutic Strategies. *International Journal of Molecular Sciences*.21(21):8102. https://doi.org/10.3390/ijms21218102

Zhang, M., Ahn, R., Hayes, J., Seiler, S., Mootz, A & Porembka, J. (2022). Axillary Lymphadenopathy in the COVID-19 Era: What the Radiologist Needs to Know. RadioGraphics. 42. https://DOI:10.1148/rg.220045.

References

Zhao-Yi,W & Li Yin. (2015). 'Estrogen Receptor Alpha-36 (ER-A36): A New Player in Human Breast Cancer'. *Molecular and Cellular Endocrinology* 418 Pt 3 (December):193–206. 5

APPENDICES

Appendices

Appendix A: Leukocytes' mean and SD values for each age group: before and after chemotherapy cycles

Chemotherapy	Age groups			
	20 - 29	30 - 39	40 - 50	50 and above
Before	6.75±2.21	6.29±1.81	7.55±3.78	6.5±1.87
C1	7.43±3.1	5.32±1.76	7.01±2.65	5.26±2.28
C2	5.81±1.35	4.55±1.19	6.19±2.54	7.56±4.36
C3	7.81±1.97	5.11±1.86	14.3±14.41	5.77±3.37
C4	6.82±2.32	5.25±2.81	7.72±2.04	4.6±0.86
C5	5.22±2.75	5.05±2.13	7.24±2.37	6.24±2.65
C6	7.78±2.4	9.86±6.04	7.12±1.95	4.45±2.05

Appendix B: Erythrocyte metrics pre and post chemotherapy treatment

Chemotherapy	Age groups			
	20 - 29	30 - 39	40 - 49	50 and above
Before	4.82±0.81	4.41±0.37	4.25±0.67	4.81±0.61
C1	4.67±0.49	4.23±0.42	4.12±0.71	4.3±0.53
C 2	4.57±0.67	4.23±0.29	4.24±0.59	4.27±0.71
C 3	4.81±0.5	4.2±0.57	4.32±0.5	4.27±0.66
C 4	4.48±0.37	4.14±0.46	4.14±0.38	4.08±0.69
C 5	4.14±0.56	4.22±0.53	4.06±0.36	4.21±0.6
C 6	4.24±0.4	4.13±0.32	3.99±0.45	4.21±0.45

Appendix C: Thrombocytes mean and SD levels for each group (before and after chemotherapy sessions)

Chemotherapy	Age groups			
	20 -29	30 - 39	40 - 49	50 and above
Before	336.11±49.54	296.89±95.86	293.67±88.22	275.22±106.25
C 1	312.44±61.88	368.22±119.33	316.44±113.77	331.56±61.43
C 2	364.78±33.81	336.89±87.06	296±69.88	306.44±98.87
C 3	379.44±81.01	339.11±115.24	336.22±112.99	320.78±42.85
C 4	343.22±56.84	346.44±102.73	306.56±73.53	253.78±49.86
C 5	336.33±74.76	319.33±87.56	324.67±96.94	261.44±73.64
C 6	277.67±80.38	333±125.66	272±79.55	238.64±104.04

Appendix D: Pre and post mean and SD hemoglobin values for patients under chemotherapy

Chemotherapy	Age groups			
	20 - 29	30 - 39	40 - 49	50 and above
Before	12.31±0.94	11.82±0.96	12.13±1.71	12.49±1.28
C 1	12.18±1.63	11.06±1.35	11.66±1.07	11.76±0.85
C 2	11.66±1.23	11.13±1.54	11.7±1.18	11.39±1.07
C 3	11.54±1.38	11.17±1.19	11.6±1.03	11.54±0.68
C 4	11.66±1.58	11.46±1.32	11.9±1.11	10.88±1.02
C 5	10.42±1.36	11.19±0.94	12.19±0.98	11.03±1.02
C 6	10.98±1.58	10.91±0.97	11.41±1.91	11.29±1.22

Appendix E: Pre and post chemotherapy neutrophils values

Chemotherapy	Age groups			
	20 - 29	30 - 39	40 - 49	50 and above
Before	54.71±11.99	56.99±9.22	58.31±9.12	49.19±19.52
C 1	55.66±16.68	52.44±9.85	59.25±16.31	47.82±19.22
C 2	46.02±6.96	51.63±12.26	55.28±16.34	61.49±12.96
C 3	41.76±21.93	53.37±17.77	57.45±11.43	50.69±16.65
C 4	43.16±22.07	59.99±17.6	59.94±17.97	56.77±11.51
C 5	33.86±17.18	59.96±21.41	55.23±16.01	62.1±14
C 6	54.33±27.44	71.66±14.6	48.42±29.16	53.69±15.91

Appendix F: Haematocrit values for each age cohort prior to and after every chemotherapy cycle

Chemotherapy	Age groups			
	20 - 29	30 - 39	40 - 49	50 and above
Before	37,24±2,72	35.83±3.59	36.55±5.06	37.96±3.55
C 1	36,62±3,33	33.87±4.24	33.97±4.54	34.82±1.74
C 2	34.92±3.77	34.26±2.83	34.92±3.97	34.4±2.03
C 3	34.9±3.94	34.58±4.17	33.98±4.27	34.45±1.46
C 4	35.24±4.59	34.04±3.67	34.37±4.14	33.05±1.73
C 5	34.58±6.11	34.77±2.68	35.43±4.26	34.05±3.14
C 6	34.36±5.38	33.14±1.77	34.92±5.79	34.17±2.47

Appendix G: MCV values pre and post chemotherapy

Chemotherapy	Age groups			
	20 - 29	30 - 39	40 - 49	50 and above
Before	78.88±10.86	80.94±7.08	84.5±8.84	80.81±9.03
C 1	**67.71±24.2**	80.11±8.52	83.49±9.09	82.59±9.71
C 2	77.86±12.81	81.01±7.12	84.28±9.95	83.86±10.29
C 3	78.83±12.99	81.52±7.29	80.3±12.54	81.97±10.27
C 4	79.11±11.83	82.72±7.36	84.27±9.51	82.68±10.18
C 5	83.98±13.39	83.34±7.65	88.6±3.13	83.1±10.79
C 6	81.12±11.35	80.86±7.18	85.2±5.92	82.49±10.43

Appendix H: MCH values: pre and post chemotherapy analysis

Chemotherapy	Age groups			
	20 to 29	30 to 39	40 to 49	50 and above
Before	26.22±4.75	26.94±3.26	27.78±3.12	26.99±3.11
C 1	26.39±4.77	26.31±2.98	28.9±4.25	28.5±4.82
C 2	25.99±4.4	26.32±3.34	28.42±4	27.73±3.72
C 3	24.28±4.17	27.61±3.95	27.84±3.7	27.49±4.51
C 4	25.92±3.58	28.03±4.21	28.23±2.7	27.4±4.42
C 5	25.1±2.79	27.11±4.3	28.65±0.96	27.71±4.28
C 6	25.88±3.12	26.67±3.8	29.88±0.84	27.97±4.67

Appendix I: Pre and post chemotherapy MCHC mean and SD values

Chemotherapy	Age groups			
	20 - 29	30 - 39	40 - 49	50 and above
Before	33.09±2	33.92±2.72	33.24±1.62	32.69±1.92
C 1	33.18±2.34	32.69±1.02	34.63±3.4	33.14±2.28
C 2	33.37±0.31	32.52±3.06	33.28±1.82	32.67±2.39
C 3	31.08±4.25	33.39±3.52	33.54±3.93	33.14±2.35
C 4	32.88±0.77	34.18±2.37	33.76±1.2	32.82±2.45
C 5	30.4±3.01	32.38±2.55	32.99±1.27	32.22±2.93
C 6	31.99±1.01	32.87±2.23	32.44±3.29	32.31±3.26

Appendix J: Patients' information form

NUMBER: [ ]

• AGE [ ]	• SEX: MALE [ ] FEMALE [ ]
3) Date of cancer diagnosis. _____	4) ORIGINE _____
5) Family history	
6) Histological type of breast cancer: _____	7) cancer stage diagnosed I.[ ] II. [ ] III. [ ] IV. [ ]
8) Hormonal receptors (-ve\+ve)	o Œstrogen receptors o Progesterone receptors o HER2 o TNBC
9) Probable risk factors	
10) Types of treatment received :	• Chemotherapy (the precise regime): • Mastectomy:
11) Treatment duration :	
12) First day of treatment. ____/___/_____	Last day of treatment. ____/___/_____

Post-treatment follow-up

Haematological parameters
Before and after treatment :
  o   Leukocytes
  o   Erythrocytes
  o   Thrombocytes
  o   Haemoglobine
  o   Neutrophils
  o   Haematocrit
	o Erythrocyte indices :
	MCV, MCH, MCHC