THE USE OF COMPLETE BLOOD COUNT AS INFLAMMATORY BIOMARKERS IN PATIENTS WITH COLORECTAL CANCERS IN ENUGU STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY TEACHING HOSPITAL, PARKLANE ENUGU

ABSTRACT

Full blood count is a prerequisite investigation requested from all colorectal cancer patients before treatment and during treatment and poor parameters adversely influences the outcome of cancers. This present study investigated the use of Complete Blood Count (CBC) and Erythrocyte Sedimentation Rate (ESR) as an assessment of inflammation in colorectal cancer subjects. The study comprised of 50 male and female subjects and controls with ages between 21-70 years. A longitudinal study method was used. The samples were collected from apparently healthy individuals as control, pre-treatment at diagnosis and the treatment samples at different stages of the treatment. Questionnaire used obtained other demographic information. The data was analyzed with IBM SPSS PC. Version 20.0; SPSS Inc., Chicago, III., USA. Results showed increased Neutrophil/Lymphocyte ratio(NLR) and decreased Lymphocyte/Monocyte ratio(LMR) were significantly associated with increased hazard ratio (HR) and decreased OS at $p<0.05$ while Platelet/Monocyte ratio(PLR) had no significant difference at $P>0.05$ in Colorectal Cancer. In CBC and ESR, control, pre-treatment and treatment period, RBC parameters and TWBC parameters showed a significant decrease at $p<0.05$ in treatment results compared to the pre-treatment and control results while others showed no significant increase at $p<0.05$ in treatment results compared to pre-treatment results. Treatment RDW and MPV observed a significant increase ($p<0.05$) compared to the control and pre-treatment results. Age group 31-40 years showed more susceptibility than other age groups with lowest mean±SD in CBC and ESR but with no significant difference at $p>0.05$.This present study, about 20% of the data supports the concept that biomarkers such as CBC and ESR can be used as a prognostic tool in early detection, treatment and monitoring the disease progression in these subjects

Keywords: *Colorectal cancer, inflammation, CBC.*

INTRODUCTION

"Hematopoietic stem cells (HSCs) maintain lifelong hematopoiesis via their ability to self-renew and differentiate into all blood cell lineages in humans" [1]. "HSCs are an extremely rare population of cells that usually reside in the highly organized bone marrow architecture (also called niche)" [2]. "Any perturbation of the bone marrow niche affects the hematopoiesis process" [3]. "Under physiological conditions, a small number (1%–5%) of hematopoietic stem and progenitor cells (HSPCs) regularly enter circulation and travel through peripheral blood" [4]. "HSPCs sense stress signals and are capable of converting environmental cues into versatile cytokine signals to regulate hematopoiesis" [5]. "Multiple factors, including growth factors, chemokines, and adhesion molecules, can influence HSPC circulation and activity. Extensive attention has been paid to the emergence and evolution of tumors, yet how the growth of malignant clones affect normal hematopoiesis is poorly understood. However, circulating HSPCs are highly enriched in tumor tissues and correlate with tumor progression" [6]. "Furthermore, tumor progression is manifested by alterations in intra- and extramedullary hematopoiesis

(EMH), which supports a systemic tumor-promoting myeloid response" [7]. "Therefore, understanding the process by which tumors interrupt normal hematopoiesis is an important question that is highly relevant to tumor progression. A reviewed work was done on normal hematopoiesis in the context of hematopoietic malignancies. In this review, it outlined the impact of solid tumors on hematopoiesis and summarizes their underlying mechanism. In clinical observations, the progression of different types of solid tumors has resulted in an increased peripheral neutrophil-to-lymphocyte ratio [8], [9] and circulating granulocyte–macrophage progenitors (GMPs)" [10]. " HSPCs, which are upstream of these cells, have been increasingly recognized as playing key roles in tumor growth and metastasis progression. It has been well-established that elevated levels of HSPCs correlate with higher tumor stage and decreased progression-free survival" [11]. "Tumors usually accumulate immune-suppressive hematopoietic lineages at primary sites. HSPC production and circulation are elevated in cancer patients before detectable metastases" [10]. "The number of circulating HSPCs decreases if tumor-mediated mobilization is inhibited, whereas the pharmacological mobilization of HSPCs increases metastasis" [11].

"The relationship between cancer and the immune system has been increasingly recognized over the past three decades. While immune-surveillance is a strong line of defense by which transformed cells are cleared by cells like lymphocytes and natural killer cells, chronic inflammation is an established risk factor for developing several types of cancer including colon cancer, hepatocellular carcinoma and gastric cancer" [12]. "In addition, the tumor microenvironment is infiltrated by a heterogeneous population of immune cells, each playing a different role in the cross-talk between cancer cells and the host, either favoring or suppressing tumor progression. For example, a subset of myeloid cells which is expanded in cancer patients are myeloid-derived suppressor cells (MDSCs). These are immature myeloid cells of granulocytic or monocytic lineages are elevated in cancer. MDSCs are capable of suppressing anti-tumor T cell activity and promoting tumor angiogenesis" [13]. "In fact, higher numbers of circulating MDSCs is a poor prognostic indicator in solid cancers especially colorectal, esophageal, gastric and pancreatic cancers" [14]. "On the other hand, higher lymphocyte infiltration in the tumor (tumor-infiltrating lymphocytes, TILs) is a good prognostic indicator in head and neck cancer (HNC)" [15]. "In turn, cancer cells modify the behavior of neutrophils by inducing the release of cytokines and metalloproteinases, increasing their chemotactic potential and inhibiting apoptosis, which perpetuates cancer-associated inflammation. This suggests that different subsets of the inflammatory arsenal play opposing roles in shaping cancer behavior" [16]. It is evident that components of the CBC can provide valuable prognostic information in solid tumors and haematologic malignancies that are not only limited to survival predictions or assessment of disease progression, but also are important tools when evaluating response to treatment. Thus, true assessment of the utility of the CBC as an inexpensive, established, and globally accessible prognostic factor in many malignancies requires careful studies of the sample results obtained. It is likely that future prospective studies examining the biology behind the prognostic value of the different components of the CBC would later yield significant therapeutic progress and a thorough understanding of disease pathogenesis.

AIM

The aim of this study is to use peripheral blood cells as an inflammation biomarker in colorectal cancer in patients attending Surgery Department at ESUT Teaching Hospital, Parklane Enugu.

SPECIFIC OBJECTIVE

1. To determine the complete blood count and erythrocyte sedimentation rate of the subjects at their pre-treatment and treatment period.
2. To calculate the neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR) and platelet to lymphocyte ratio (PLR) in the subjects and access their use as prognostic biomarkers.
3. To determine age group susceptibility in these solid cancers for proper treatment and management in these patients.

JUSTIFICATION OF THE STUDY

Though biomarkers are organ specific, most are not cost effective and takes days for result to be available. Interestingly, recent studies shows that NLR, LMR, PLR has predictive value in accessing inflammation in systemic disorders. These indices can be obtained from simple complete blood counts. The relevance of this study lies in exploring the possible use of these simple, costs effective and less laborious indices in the diagnosis and treatment monitoring of cancer cases especially in resource poor settings.

SCOPE OF THE STUDY

The economic and psychological challenges posed by cancer is profoundly overwhelming in our society nowadays, so this work focused on the use of peripheral blood cells for the assessment of inflammation in some solid cancers studied in this work. The assessment of inflammation involved the use of these ratios: NLR, LMR and PLR simple and cost effective biomarkers for the prediction of hazard ratios and overall survival in these patients. The CBC result obtained using autohaemoanalyzer monitored the efficacy of the chemotherapeutic plans and treatment during the different stages of these solid cancers. This work obtained the specific age group susceptibility of these patients in some solid cancer while in some specific age group susceptibility was not ascertained. This specific age group susceptibility creates better awareness, treatment and management for the patients, their families and society at large.

STATEMENT OF PROBLEMS

The encumbrances in the early detection, diagnosis and treatment of cancer has led to early deaths, so employing simple, cost effective, fast and non-invasive method will timely eradicate the delays in treatment hence ensuring chances of long time survival. Also determining the specific age group that is more susceptible will increase awareness and inform screening of the age group.

LIMITATION OF STUDY

Some of the subjects were lost to death during the course of this work. Follow-up proved difficult due to subjects' unavailability for their scheduled chemotherapy due to economic constraints.

LITERATURE REVIEW

"Colorectal cancer (CRC), also known as bowel cancer and colon cancer, is the development of cancer from the colon or rectum (parts of the large intestine). Colorectal cancer is characterized by uncontrolled cell growth in either the large intestine or the appendix, and is the fourth most prevalent cancer" [17]. "Signs and symptoms may include blood in the stool, a change in bowel movements, weight loss, and feeling tired all the time. Most colorectal cancers are due to old age and lifestyle factors, with only a small number (10%) of cases due to underlying genetic disorders" [18]. " Other risk factors include diet, obesity, smoking, and lack of physical activity. Dietary factors that increase the risk include red meat, processed meat, and alcohol" [19]. . "Another risk factor is inflammatory bowel disease, which includes Crohn's disease and ulcerative colitis. Some of the inherited genetic disorders that can cause colorectal

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cancer include familial adenomatous polyposis and hereditary non-polyposis colon cancer; however, these represent less than 5% of cases" [18]. "It typically starts as a benign tumor, often in the form of a polyp, which over time becomes cancerous. Bowel cancer may be diagnosed by obtaining a sample of the colon during a sigmoidoscopy or colonoscopy" [20]. "This is then followed by medical imaging to determine if the disease has spread. Screening is effective for preventing and decreasing deaths from colorectal cancer. Screening, by one of a number of methods, is recommended starting from the age of 50 to 75. During colonoscopy, small polyps may be removed if found. If a large polyp or tumor is found, a biopsy may be performed to check if it is cancerous. Aspirin and other non-steroidal anti-inflammatory drugs decrease the risk" [21]. "Their general use is not recommended for this purpose, however, due to side effects. Treatments used for colorectal cancer may include some combination of surgery, radiation therapy, chemotherapy and targeted therapy. Cancers that are confined within the wall of the colon may be curable with surgery, while cancer that has spread widely are usually not curable, with management being directed towards improving quality of life and symptoms" [22]. "Improved affluence leads to increased consumption of meat and poultry products. Unfortunately, poor electricity supply in most rural and urban areas discourages refrigeration of meat, thus many households smoke or deep-fry meat and fish for preservation and consumption. Most of the soups that are popular in Nigeria are incomplete without garnishing with smoked fish, and this has been shown (together with smoked and fried meat) to contain high levels of PAHs (polycyclic aromatic hydrocarbons)". [23]. "Offal, examples, animal entrails is fatty, and there are many Nigerians who like abodi (rectum), shaki (stomach) and ifun (intestines) more than beef. Since bile is required for the digestion of fat, the fatter one consumes, the higher the production of bile. Excess bile that spills over into the colon is acted upon by colonic bacteria to form the secondary bile acids, namely deoxycholic and lithocholic acids which are carcinogenic .Another delicacy that is peculiar to the Western part of Nigeria is the cowhide popularly known as "Ponmo". In a 2009 study from the University of Lagos on the dangers of flame-processed meat and hide, some serious public health concerns were voiced against ponmo; the poor man's meat" [24].

"The five-year survival rate in the United States is around 65%. The individual likelihood of survival depends on how advanced the cancer is, whether or not all the cancer can be removed with surgery and the person's overall health. Globally, colorectal cancer is the third most common type of cancer, making up about 10% of all cases. In 2012, there were 1.4 million new cases and 694,000 deaths from the disease. It is more common in developed countries, where more than 65% of cases are found. It is less common in women than men" [26]. "The incidence varies according to geographical location with the highest rates reported from Australia, New Zealand, and Northern and Western Europe. Although the highest incidence of colorectal carcinoma in United States is seen among African Americans, colorectal carcinoma is an uncommon malignancy in Africans and most of the other developing countries". [27].

"Several epidemiological studies done in different parts of Nigeria, mostly hospital-based, have highlighted the relative incidence, age and sex distribution, and pathological characteristics of colorectal carcinoma" [28]. "However, it is difficult to ascertain the national incidence and mortality for colorectal carcinoma in Nigeria due to lack of a reliable population statistics and absence of population-based cancer registries. The average of eight cases of colorectal carcinoma per year during the 30-year study is lower compared with reported cases at Jos, an area within the same North-Central region of Nigeria. However, considering the last 5

years (2004-2008) of the study period alone, with 93 cases (38.6%), the annual incidence increased to an average of 19 cases per year" [29].

"Colorectal carcinomas (CRC) have been described in all age groups. Sporadic cases occur over the age of 50 years, whereas colorectal carcinomas occurring at a younger age increase the likelihood of genetic abnormality" [30]. "CRC is uncommon before the age of 40; the incidence begins to increase significantly between the ages of 40 and 50, and age-specific incidence rates increase in each succeeding decade thereafter". [31]. "Some registries report a rising incidence of CRC even among young adults 20 to 39 years of age, although the absolute incidence in this age group remains far lower than for adults aged 50 or over" [32]. "Over 86 percent of those diagnosed with CRC under the age of 50 are symptomatic, and the disease is being diagnosed at later stages, suggesting that the increased incidence is real and not representative of a shift in age at diagnosis attributable to earlier detection" [33]. "The reason(s) underlying this trend is unclear. At least some data associate prolonged sedentary television viewing time (independent of exercise and obesity) with an increased risk of young-onset CRC, particularly of the rectum" [34]. "Ingesting hides processed with a flame fuelled by firewood and spent engine oil may contain toxic organic compounds such as polyaromatic hydrocarbons (PAHs), dioxins, furan, and benzene which can potentiates the onset of CRC in young adults between the age's 30-40years" [25]. "Interestingly, these trends have also been observed in developing countries, including "low-resource" countries traditionally thought of as having low rates of CRC compared with Western countries" [35]. "Current efforts to reduce young adult CRC incidence and mortality are focused on identifying those eligible for earlier age surveillance, based on family history, and promoting both physician and consumer awareness of the potential cancer risk of symptoms, such as persistent rectal bleeding at any age" [36].

"There is slight male predominance of colorectal carcinoma with a male: female ratio of 1.5:1. These are similar to findings from other studies on colorectal carcinomas in Nigeria and other countries. Even though no agreeable reason has been attributable to this male preponderance worldwide, it may be due to higher frequency of abdominal obesity, cigarette smoking, and alcohol consumption in men". [37]

"One of the earliest papers from Nigeria on CRC emanated in 1967 from Ibadan, where 166 cases were seen over 8 years; an average of 21 cases per year" [38]. "From Ibadan, between 1971 and 1979, the incidence ranged from 12 to 14 patients annually; from 1980 to 2000, there was an annual average of 18-26 patients. Ibadan in the core west showed 27 patients per year from 1995 to 2004" [39]. "Lagos and Sagamu accrued 402 cases over 12 years from five centers giving about 34 cases per year" [28]. "Ife showed an average of 10-13 cases per year between 1991 and 1994. Jos in the middle belt of Nigeria saw a moderate increase from nine cases a year in 1990 to 1994, to 16 in 2003"[40]. "Sites from the Northern parts of Nigeria showed seven cases per year in Zaria, and 12 per year in Kano" [41]. "In Benin City, the capital of Edo state, 106 cases were encountered over a period of 20 years giving an average of <6 cases per year". [42] ."Enugu in the eastern part of the country saw between three and five patients yearly from 1975 to 1980". [43]. "In the Riverine area of Nigeria, reports from Port Harcourt reveal rates of 2-4 patients per year".[44], "Calabar which is home to both Efik and Ibibio, showed figures of about five cases of CRC per year, indeed in the report from the Calabar cancer registry covering the period from 2004 to 2009, the top seven cancers did not include CRC". [45].

COLORECTAL CANCER IN CBC AND ESR

"Patients with cancer may develop anaemia secondary to poor nutrition in general or due to reduced function in the gastrointestinal (GI) tracts to absorb nutrients" [46]. "Folate deficiency

may develop in anorexic patients with cancer, while vitamin B_{12}deficiency can arise in patients who have undergone gastric or small bowel resection or bypass or have atrophy of stomach parietal cells, which produce intrinsic factor necessary for vitamin B_{12} absorption" [47]. "Iron deficiency anaemia due to blood loss or the inability to absorb iron in the GI tract often occurs in patients with malignancies of the GI tract, including colorectal cancers". [48]. "Nutrient deficiencies in folate, vitamin B_{12} or iron may lead to anaemia because all of these nutrients are essential to red blood cell (RBC) production and development". [49].

"Some researchers reported anaemia in solid cancer patients. They suggested that this anaemia which is mostly of iron deficiency may be due to the malignancy itself or a direct consequence of the treatment due to the decreased value in red blood indices. They also reported that this anaemia in these cancers may be evident at initial diagnosis and develops due to activation of immune system which appears to be the driving force of global diminution of erythropoiesis" [50]. "Other researchers' hypothesed that this immune system once activated stimulates the production of inflammatory cytokines that impedes erythropoiesis hence leading to insufficient differentiation and proliferation of erythroid precursors leading to anaemia" [51]. "Reports were made that these inflammatory cytokines also impairs iron metabolism which results in reduced serum iron levels and iron retention within the reticuloendothelial system (RES)" [52]. "Some scientists in their work reported that, these cytokines can be produced by the cancer cells themselves which then induces iron sequestration, thereby decreasing RBC production and over expression of these inflammatory cytokines causes shortened RBC survival", [53]. "Some other reported works all observed that chronic blood loss at common sites can exacerbate anaemia from bone marrow invasion by these solid cancers causing myelophthisis resulting from bone marrow replacement causing pancytopenia" [54], [55], [56]. "Studies done by other researchers reported leucocytosis in solid cancer subjects" [57], [58], [59]. "Several studies had attempted to identify the association between TWBC and solid cancer risk, but no consistent evidence has been found most reports were done on neutrophils/ lymphocytes ratios" [60], [61]. "In differential TWBC, most data were available for the ratio of NLR. The role of neutrophils in cancer is multifactorial and not fully understood. Another researcher reported that neutrophils participate in different stages of the oncogenic process including tumor initiation, growth, proliferation or metastatic spreading. The various roles of neutrophils in cancer development and progression by several researchers have recently explored the role of neutrophils and other markers of host inflammation on clinical outcomes" [62]. "Other scientists in their work reported also that an elevated absolute neutrophil count is an adverse prognostic factor incorporated in a contemporary prognostic score for metastatic carcinoma treated with targeted therapy" [63]. "A lot of controversies in neutrophil counts have been involved in neutrophil count report. Another researcher reported that the expression of neutrophils in the tumour had been linked with detrimental outcome in some cancer examples include: head and neck cancer, esophageal cancer whereas in other cancers, it has been associated with better survival" [64]. "However, some works reported that neutrophils assessment can be used as indirect measurement of the tumor inflammation outcome" [65].

"Some works reported that peripheral bloods neutrophils are increased in subjects with solid cancer before treatment. They suggested that this is because tumors at initial stage produces granulocyte colony – stimulating factor (G-CSF) which skews the neutrophil retention or release balance in bone marrow, leading to this increase in circulating neutrophil" [66]. "However during treatment, works reported reduce neutrophil count are seen in these subjects showing to be beneficial to the survival of the subjects and this may of course just be a reflection of adequate

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toxicity of the drug being achieved as it kills tumour cells. The direct effect of toxicity during therapy on neutrophils should be closely monitored in order to prevent the occurrence of neutropenia" [67].

"Quite recently, red cell distribution width (RDW), have also been shown to associate with survival of solid tumors" [68]. "Growing evidence indicated that high RDW is associated with systematic inflammation and elevated RDW harbored the potential to predict poor survival in a variety of human cancers, consisting of breast cancer, lung cancer, prostate cancer, endometrial cancer, colon, esophageal cancer and upper tract urothelial carcinoma" [69], [70]. "Works done in 2003 studied 127 patients who had colon cancer. Among them, 107 (84%) patients had an elevated RDW, and it was revealed that the elevated RDW had high sensitivity (0.84) and specificity (0.88) for identifying right-sided colon cancer. Therefore, they inferred that RDW may be useful as a cost-effective screening tool for colon cancer" [71]. "Another work analyzed 115 patients with colon polyps and 30 with colon cancer and found that RDW values were significantly higher in patients with colon cancer compared to those with colonic polyps. They proposed that RDW may be used as an early-warning biomarker for solid colon tumors" [72]. "Other works done in 2012, reported that there were high platelet counts at presurgery of colorectal cancer and renal cancer are related to poor prognosis" [73]. They observed a significant increase in MPV in patients with metastases (mCRC) as compared to nonmetastatic colorectal cancer patients (non-mCRC), suggesting that these differences result from considerable enhancement of the inflammatory process and platelet activation in more advanced metastatic disease.. They also showed that patients with decreased MPV pre-treatment responded much better to the therapy applied, achieving longer remission. [74], examined "the effect of chemotherapy treatment on MPV levels in colorectal cancer patients. Pre-treatment MPV findings were similar in the whole study group, but decreases during treatment. They indicated that changes in MPV could be due to the effect of chemotherapy on the formation of blood platelets and cyclic drug administration".

" Recently, there has been intense interest in the prognostic value of peripheral blood biomarkers in colorectal cancer (CRC). Inflammation has been reported to be involved in carcinogenesis and disease progression and local cancer-related inflammation can be reflected by a systemic inflammatory response (SIR)" [75]. "Nearly a third of cancer patients have thrombocytosis at diagnosis and aberrant activation of platelets has been shown to be associated with CRC" [73]. "Lymphocytes are essential components of the tumor microenvironment, which contributes to carcinogenesis" [76]. "Monocytes have been reported to influence CRC progression and can be used to predict prognosis" [77]. Therefore, a comprehensive evaluation of the literature is warranted. Routine peripheral blood counts may be useful prognostic factor for evaluating the accuracy of risk stratification in patients with solid cancers. Since chemotherapy and other solid cancer treatment affect the full blood count, it is important to know the extent of these effects by comparing the full blood count results before and during treatment in these subjects.

"Elevated ESR is frequently encountered in patients with cancer. The outcome in various malignancies depends on the type of the underlying disorder, the stage and duration of the disease, and the regimen and intensity of the antitumor treatment" [78]. "In addition, an elevated ESR level has also been identified as a prognostic factor adversely affecting survival in cancer patients" [79]. "A number of studies indicated that an increased ESR level is associated with worse survival; patients with higher ESR values in various malignancies, including colorectal cancer [80] , renal cell cancer [81], head and neck cancer [82], soft tissue sarcoma [78], breast

cancer [83], and prostate cancer [84], had a shorter survival compared with those with normal ESR levels. However, serum CRP levels are not routinely assessed in the pre-treatment assessment, hence the use of ESR is more readily available and inexpensive compared to CRP" .

In this present study, complete blood count was studied in order to determine and compare their pre-treatment and treatment CBC results for prognostic values during the courses of chemotherapy to prevent the risk of unpleasant and life threatening side effects such as anaemia, fatigue, infections and bleeding. Also to prevent disruption of delivery of the treatment, due to none efficient monitoring of the CBC which can result in change to the planned dose and time.

NLR, PLR, LMR IN COLORECTAL CANCERS

"Colorectal cancer (CRC) is one of the most common cancers and one of the leading causes of cancer death worldwide. About 1.36 million were diagnosed with CRC and 0.7 million died of it in 2012"[85]. "Although the therapeutic strategies have been developed in recent decades, the 5-year overall survival (OS) of CRC is unsatisfactory because of local recurrence or metastasis" [86]. "Many factors can predict the prognosis of CRC, for instance tumor stage, cell differentiation grade, vascular invasion, and neural invasion. However, some patients with good prognostic factors still have poor prognosis" [87]. "Thus, there is an urgent need to find other new biomarker to predict the prognosis of CRC and help choose the optimal therapeutic strategies" [88]. "Published studies have demonstrated that several systemic inflammatory factors can be used to predict the prognosis for CRC patients, such as platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio" [89].

"NLR is the ratio of the absolute neutrophil count to the absolute lymphocyte count, and therefore the association between a high NLR and a poor prognosis, as revealed in the present study, is possibly indicative of the tumor-promoting activity associated with neutrophilia in the tumor environment. Neutrophilia promotes tumor growth and metastasis by remodeling the extracellular matrix and releasing reactive oxygen species, nitric oxide and arginase, which suppress the T cell response and increases the rate of mutagenesis" [90]. "Additionally, neutrophilia suppresses lymphocyte activity, therefore counteracting the antitumor immune response" [91]. "Among these laboratory markers, NLR is one of the most commonly used biomarkers. It was reported that tumor affected the hematopoietic progenitor cell of the host and thus myeloid lineage populations (including neutrophils) could expand" [92]. "These myeloid-derived suppressor cells (MDSCs) suppress host immune cells through various pathways and could diminish lymphocytes. NLR could be used to estimate the relative balance of myeloid and lymphocytic lineages, thus reflecting host immunity in cancer patients" [93]. "The prognostic impact of NLR was thoroughly investigated in different types of diseases including gastric cancer, hepatocellular cancer, pancreatic cancer, head and neck cancer, esophageal cancer, breast cancer, and thyroid cancer" [94].

"In accordance with these results, previous studies included meta-analysis demonstrated that NLR can predict survival outcome, or can also predict tumor regression grade, such as pathologic complete response (pCR), or good tumor response before and during treatment in patients with colorectal cancer" [95]. "An elevated NLR has been associated with a poor survival rate in breast [90], esophageal [96] and gastric cancers [97] and CRC" [98]. "Reported work done in 2012, demonstrated that patients with an elevated NLR (>3) in colorectal cancer appeared to possess larger tumors and a more advanced tumor stage, and patients with stages I–III CRC possessed a poorer 5-year disease-free survival rate" [98] . "An

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elevated NLR is always accompanied by lymphocytopenia, which is caused by systemic inflammation and leads to the release of a number of inhibitory immunological mediators, particularly IL-10 and transforming growth factor-β. These inhibitory immunological mediators may exert an immunosuppressive effect with an impaired lymphocyte function" [99]. "In 2013, another work demonstrated that an elevated NLR were significant predictors of a poorer OS rate and progression-free survival time following pre-treatment and first-line chemotherapy in patients with metastatic CRC" [100].

"Nevertheless, there are also a number of studies that have shown that NLR is not relevant in patients with colorectal cancer. Work done in 2016, reported that there were no differences in survival outcomes or pCR rate according to NLR cut-off point (2.0, 2.5, 4, and 5) in 175 patients who underwent treatment with surgery" [101]. "Another work also reported that NLR measured at diagnosis did not predict OS or disease-free survival (DFS) in patients with locally advanced colorectal cancer who underwent treatment" [102]. "Also in 2017, another work reported that NLR measured before commencement of treatment could not discriminate recurrence-free survival ($p = 0.07$) among 984 patients who underwent treatment" [103]. "The long course of treatment for locally advanced colorectal cancer patients usually takes 11–15 weeks from treatment commencement to the date of definite surgery. As has been recently suggested, neutrophil and lymphocyte counts are not constant over time during pretreatment for colorectal cancer" [104]. "So, the NLR will vary in value. Most previous studies evaluating the impact of NLR on survival or tumor response for colorectal cancer measured NLR at a specific time, mainly before initiating treatment, or used the NLR value assessed after surgery" [105]. "In contrast, the prognostic significance of change in NLR during treatment for colorectal cancer has not been widely assessed" [106] .The basis of this discrepancy among studies is not clearly understood.

"As a new factor of systemic inflammatory, lymphocyte-to-monocyte ratio (LMR) has been drawing increasing attention lately. A decreased LMR could generate a favorable immune microenvironment that promotes cancer development. In other words, a decreased LMR could be associated with poor prognosis in cancer patients" [107]. "Previous literatures have proved that an elevated pretreatment LMR is associated with survival benefit in hematologic malignancies. As a new factor of systemic inflammatory, LMR has been proved to be a predictor for haematologic malignancies and also for solid cancers" [107]. "Works done in 2015 showed that LMR was a prognostic factor for CRC patients in subgroup analysis, which included 4 studies" [108]. "However, several other studies published later, and the results of those studies remain controversial" [109]. "Eleven studies published between 2014 and 2016 with a total of 9045 patients were enrolled in this meta-analysis. Their findings indicated that a low LMR predicted a worse OS (HR 1.57, 95% CI 1.30–1.90, $P<.001$) and increased hazard ratio (HR 1.25, 95% CI 1.13–1.39, $P<.001$) for patients with CRC. Their up-to-date meta-analysis shows that a low LMR is associated with poor survival in patients with CRC, although the publication bias is existed" [110]. "The actual mechanisms of the prognostic impact of LMR in CRC are unclear. It has been suggested that cross-talk exists between the inflammatory response and tumor progression" [111]. "Furthermore, low lymphocyte counts are thought to be responsible for an insufficient immunological response, which leads to inferior survival in multiple solid cancers" [112]. "On the other hand, monocytes are also involved in tumor progression and metastasis. Tumor-associated macrophages (TMAs), which develop from circulating monocytes in the local tissues. TAMs can accelerate angiogenesis, invasion, migration, and tumor growth" [113]. "The peripheral blood absolute monocyte count is considered to reflect the formation and/or presence

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of TAMs. Thus, a high monocyte count reflects an elevated tumor burden of cancer patients" [114]. "Given this background, the LMR reflects both the immune status of the host and the degree of tumor progression. A low LMR combined with the effects of low lymphocyte count and high monocyte count reflects insufficient antitumor immunity and a high tumor burden. Thus, LMR might be a stronger predictor of prognosis in patients with CRC" [115]. "A total of nine studies comprising 8626 patients with CRC were included in the meta-analysis. The pooled analysis demonstrated that low LMR was significantly associated with decreased OS (HR: 0.63, 95% CI: 0.56–0.70, $P < 0.001$) and DFS/RFS (HR: 0.76, 95% CI: 0.68–0.84, $P < 0.001$). The negative prognostic impact of low LMR on OS was observed in patients with different ethnicity, treatment methods, cut-off values, and across disease stages" [116].

In a study done 2014 evaluating the prognostic significance of the pre-treatment LMR in patients with CRC also establish that a decreased LMR envisaged for shorter disease free survival and OS, and indicated that patients with decreased LMR may not profit from any adjuvant treatment[114]. However, in similar studies done in 2014 and 2015, no statistically significant relationship between LMR and OS were found [117] [118]. Since there have been several published studies assessing the prognostic role of LMR in CRC in the past 2 years, and the results of those studies remains controversial, even when an up-to-date meta-analysis was used to investigate the association between the LMR and the survival in CRC [119]. PLR and LMR, two representative indices of SIR, have been found to impact survival in a variety of solid malignancies including CRC [120]. As the collection of circulating inflammatory markers, including PLR and LMR, is simple, noninvasive, and easily accessible. Circulating levels of inflammatory markers have been investigated as applicable and cost-effective prognostic predictors in cancer patients [121]. Although the underlying mechanisms of altered PLR and LMR in CRC development remains unknown, numerous studies have investigated their value as prognostic factors and markers for predicting response to therapy. However, the results of these studies are conflicting [122].

Many studies have been reported that platelet-lymphocyte ratio (PLR) may be associated with the prognosis of colorectal cancer (CRC), but the results are inconsistent. Current opinion on the prognostic role of the PLR in CRC is inconsistent and inconclusive [115]. In PLR, a consensus on the prognostic value of the platelet to lymphocyte ratio (PLR) in CRC was not reached. However, works done in 2012 and 2015 reported that PLR was significantly associated with prognosis in CRC patients [123] ,[124], whereas another works in 2014 and 2016 indicated that elevated PLR did not predict poor prognosis [125],[107]. Recently in 2018, another work demonstrated that PLR was not associated with survival and recurrence in patients undergoing laparoscopic curative resection for colorectal cancer with or without treatment [126]. In addition, no studies have evaluated the potential use of PLR as an additional tool in the current tumor staging system, and the optimal cut-off value of PLR for predicting prognosis in CRC remains unknown. Erythrocyte sedimentation rate (ESR) is the most widely used laboratory test for evaluating the inflammatory status in clinical practice, including infection, autoimmune and malignant diseases [80].

Cancer development is influenced by many different host cell types. It has become clear that many cancer present infiltrating neutrophils. The exact role for these cancer-associated neutrophils (TANs) has yet to be completely elucidated. Neutrophil function in cancer has long been a matter of debate as these cells possess a range of tumor promoting as well as tumor limiting properties. Neutrophils are new players in cancer and have a potential role as biomarkers of disease outcome or as therapeutic targets. Also, the immune system and especially

lymphocytes play a key role in the development and progression of carcinoma. There has been no stipulated percentage about the use of CBC and especially pretreatment NLR/LMR/PLR score that can be considered valuable prognostic indicator in patients with this solid cancer to the best of my knowledge.

MATERIALS AND METHODS

STUDY SITE

The study was conducted in ESUTH Teaching Hospital Parklane GRA Enugu, Enugu State. Enugu was created on 27th August 1991. Enugu State is one of the five states in the South Eastern geopolitical zone of Nigeria and was the administrative capital of the former East Central State. It has an area of 8727.1 square kilometers. It is bounded by Anambra State on the west, Imo and Abia States on the south, Kogi state on the north and Ebonyi and Benue States on the east. The state has a projected population of over 3.5 million people. The major municipal cities are the capital, Enugu and Nsukka.

STUDY DESIGN

This was a longitudinal study. The control samples were collected from sex and age matched apparently healthy individuals. The pre-treatment samples were collected at diagnosis and the treatment samples collected at different stages [stage 1(localized); 11 (tumour); 111(lymph node); 1V (metastasis)] of the treatment. The controls, pre-treatment and treatment samples collected were compared and changes reported. The treatment samples were collected from seven days to at least a day before the next treatment. The study lasted for 14 months (August 2018 to December 2019) and a total of 6 subjects were lost to death.

STUDY POPULATION

This study comprised of fifty (50) colorectal cancer subjects with their control subjects. The control samples were from students and staff of College of Medicine, Enugu State University of Science and Technology (ESUCOM). The subjects used in this study were only adults between the ages of 21 years to 80 years. They were both males and females. There was no ethnicity differentiation. Questionnaire were used to obtain other demographic characteristics, clinical/provisional diagnosis, their life styles, types of solid cancers and the staging of these solid cancers. About 10% of the subjects have family history of the disease.

CRITERIA

Exclusion criteria:

Subjects suffering from other types of health problems like liver cirrhosis, active bleeding, intestinal obstructions, uncontrolled diabetes, uncontrolled high blood pressure, non-solid cancers examples lymphomas-leukemia syndrome, myelomas, mixed cancers like adenosquamous carcinomas and mixed mesodermal tumors were excluded from the study. Also subjects with the presence of any haematological system diseases such as pure red cell aplasia, immune haemolytic anaemia, microangiopathic haemolytic anaemia and polcythaemia. Subjects below the age of 21years. These subjects were excluded based on the clinical diagnosis already made by the clinician's report. Same exclusion criteria were used for control subjects.

Inclusion criteria:

All subjects suffering from all colorectal cancer, which has been diagnosed by the attending clinician who were at different stages of the illness according to solid cancer staging (ACS, 2020).

DATA COLLECTION

Subjects' data including demographics (ages, sexes, ethnicity, and body mass index, level of education occupation) and clinicopathological features (cancer location, size, histological type, and stages) were all obtained using questionnaires. The cancer staging was performed according to the 7th edition of the Union for International Cancer Control- American Joint Committee on Cancer Association on cancer classifications (ACS, 2020). Blood sampling were performed to measure erythrocyte sedimentation rate (ESR), total and differential leucocytes counts, platelet counts for the calculation of absolute neutrophil/ absolute lymphocyte ratio (NLR), platelet / absolute lymphocyte ratio (PLR), absolute lymphocyte / absolute monocyte ratio (LMR). These ratios are defined as the total number of absolute neutrophils, platelets, absolute monocytes divided by the total number of absolute lymphocytes.

SAMPLE PROCESSING

Sequestrated sample of a total four milliliters of blood were collected by venipuncture at the antecubital vein from all the subjects at different stages (pre-treatment and treatment) following standard protocol. The treatment samples were all collected from stage 1(localized); 11 (tumour); 111(lymph node); 1V (metastasis) stages. The blood samples were collected in dipotassium ethylenediaminetetraacetic acid (K_2EDTA) containers commercially prepared and processed immediately. Samples were taken from same environment. Stored blood samples were not used in this research work. The complete blood count (CBC) and ESR were done as soon as possible or at least within thirty minutes to one hour from the time of collection. The sample collections and processing were done at the different stages of cancer in this research work and results analyzed.

DETERMINATION OF HAEMATOLOGICAL PARAMETERS

Haematological parameters such as; haematocrit (HCT), haemoglobin concentration (Hb), total white cell count (TWCC), differential white cell count, total platelets count, MCHC, MCV, MCH were immediately analyzed on samples collected in EDTA tubes by a haematological analyzer "Be-5300 – Mindray" Japan. Determination of erythrocyte sedimentation rate was done using Westegren method.

STATISTICAL ANALYSIS

Sample size was calculated using Graphpad Prism of Statmate Software version 2.0. A sample size of 50 has 90% power to detect a difference between means of 0.33 with significant difference level (alpha) of 0.05 (two-tailed). The mean and standard deviation (mean value ± SD) of the data were tabulated for each group. The data was analyzed with IBM Statistical Package for Social Sciences (SPSS PC. version 20.0; SPSS Inc., Chicago, III., USA). The ROC curve was used to determine the different cut-offs in the ratios (NLR, LMR and PLR). Cox proportional-hazards regression analysis was used to evaluate the prognostic factors (ages, duration, ratios and their cancer diagnosis). Overall survival (OS) was defined as the duration from diagnosis to death or last follow-up. The ANOVA and Tukey HSD post- hoc test were used to compare the results obtained within controls, pre-treatment and treatment result; age groups and the age group susceptibility.

RESULTS

Demographic table of the six solid cancers and control subjects.

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A total of sixteen colorectal cancer subjects both male and female were enrolled in this work. Six different solid cancers were studied. Colorectal cancer (CRC), 50(100%) [Males (34)68% and females (16)32%]. The age ranges of the subject were between 21 and 70 years. The individual age range mean±SD were in colorectal cancer (52.0±21.0); Educational qualifications were: primary, 0(0.0%); secondary, 8(16%) and tertiary, 42(84%). There occupations were: civil servants, 35(70%); business, 2(4%) and students, 13(26%). The duration was calculated from the onset of diagnosis to the end of this research and it was calculated in months. A total number of four cancer subjects were lost to death. The duration on the cancer were reported as (28.3 ±14.8). The total number and percentage was reported for colorectal cancers. The staging was done according to the spread of the disease, which was from localized to metastasis stage. Average treatment intervals in weeks (mean±SD) were in CRC (5.0±3.2). The controls of fifty subjects used in this work were apparently healthy individuals who were sex and aged matched.

TABLE 1: DEMOGRAPHIC TABLE OF THE COLORECTAL CANCERS

	Total number		Percentage	
	Test Subjects	Control	Test Subjects	Control
Gender (Females)	50	50	100	100
Level of Education				
Primary	0	0	0	0
Secondary	8	8	16	0
Tertiary	42	42	84	100
Occupation				
Civil Servants	35	40	70	80
Business	2	0	4	0
Students	13	10	26	20
Age(years)Mean±SD	**52.0±21.0**			
Age Groups				
21-30	2	10	4.0	20.0
31-40	20	20	40.0	40.0
41-50	15	18	30.0	36.0
51-60	5	2	10.0	4.0
61-70	8	0	16.0	0.0
Duration (months)mean±SD	**28.3 ±14.8**			
11-30	22	0	44.0	0
31-50	20	0	40.0	0
51-70	8	0	16.0	0

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High and Low optimal cut-off values in the Colorectal cancers with their total number and percentages respectively.

Receiver Operating Characteristic (ROC) curve calculated using Youden index for AUC (area under the curve) were constructed between death events and censors. The optimal cut-off values of pretreatment NLR, LMR, and PLR were calculated using ROC curve. According to these optimal cut-off values, the 50 subjects were classified into two groups: high and low NLR, LMR, and PLR with their respectively percentage.

TABLE 2: HIGH AND LOW OPTIMAL CUT-OFF VALUES IN COLORECTAL CANCERS WITH THEIR TOTAL NUMBER AND PERCENTAGES RESPECTIVELY USING ROC ANALYSIS.

	NLR	LMR	PLR
Optimal cut-off	2.35	5.00	2175.0
Sensitivity	100	0.97	1.000
specificity	0.00	0.450	0.000
AUC	1.00	0.983	0.980
p-value	0.02	0.02	0.50
High (N %)	26(52%)	22(44%)	16(32%)
Low (N %)	24(48%)	28(56%)	34(68%)

The prognostic purposes of NLR, LMR and PLR in Colorectal cancer

A total of 24(48%) of Colorectal Ca subjects had low NLR (<2.35) while 26(52%) had high NLR (>2.45). In LMR, 28(56%) had low ratio (5.00) and 22(44%) had high ratio (>5.00). 34(68%) had low PLR (<2175.0) while 16(32%) had high PLR (>2175.0). In CRC, the coefficient (B) NLR (1.00) and LMR (0.22) have a positive value. HR for NLR is 2.65 [95%CI: 1.15-6.10; p=0.02] and LMR is 5.65 [95%CI: 1.04-1.50; p=0.02], meaning that high NLR and lower LMR ratios are associated with increased HR and decrease or shortened overall survival(OS) time in the subjects. A unit increase in NLR and decrease LMR by 1.0 increases HR of the ratio values by 2.65(NLR) and 1.24(LMR) folds. Also a unit increase in NLR and decrease LMR decreases the OS time in the subjects by 6.10 and 1.50 months respectively. But in PLR, there was no significant difference at p>0.05 meaning that there was no evidence of risk of death observed in either group (high PLR and low PLR).

TABLE 3: THE PROGNOSTIC PURPOSES OF NLR, LMR AND PLR IN COLORECTAL CANCER

Covariates (mean/N)	Coefficient (β)	Standard error	P-value	Exp(B) (Hazard ratio)	95% CI for Exp(B)	
					Lower	Upper
NLR(2.58) [:0 <2.58(24); 1>2.5(26)	1.00	0.43	0.02*	2.65	1.15	6.10
LMR (5.65) [0:<5.65(28);1>5.65(22)]	0.22	0.10	0.02*	1.24	1.04	1.50
PLR (138.8) (0:<138.8(34);1>138.8(16)	0.10	0.20	0.50	1.11	0.81	1.51

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P<0.05*-signifies a significant difference.

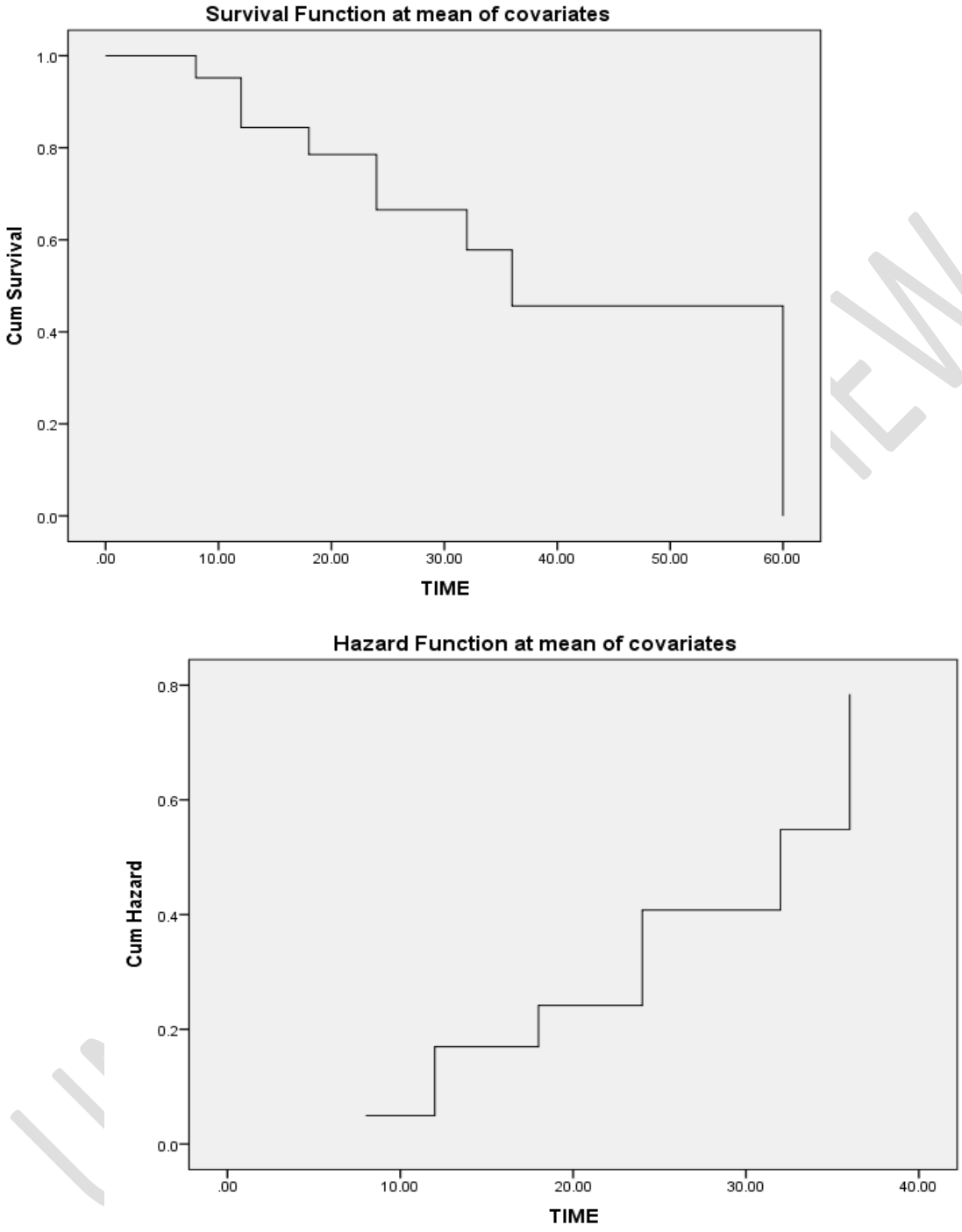

FIGURE 1: THE PROGNOSTIC PURPOSES IN CRC SHOWING NLR SURVIVAL AND HAZARD FUNCTION

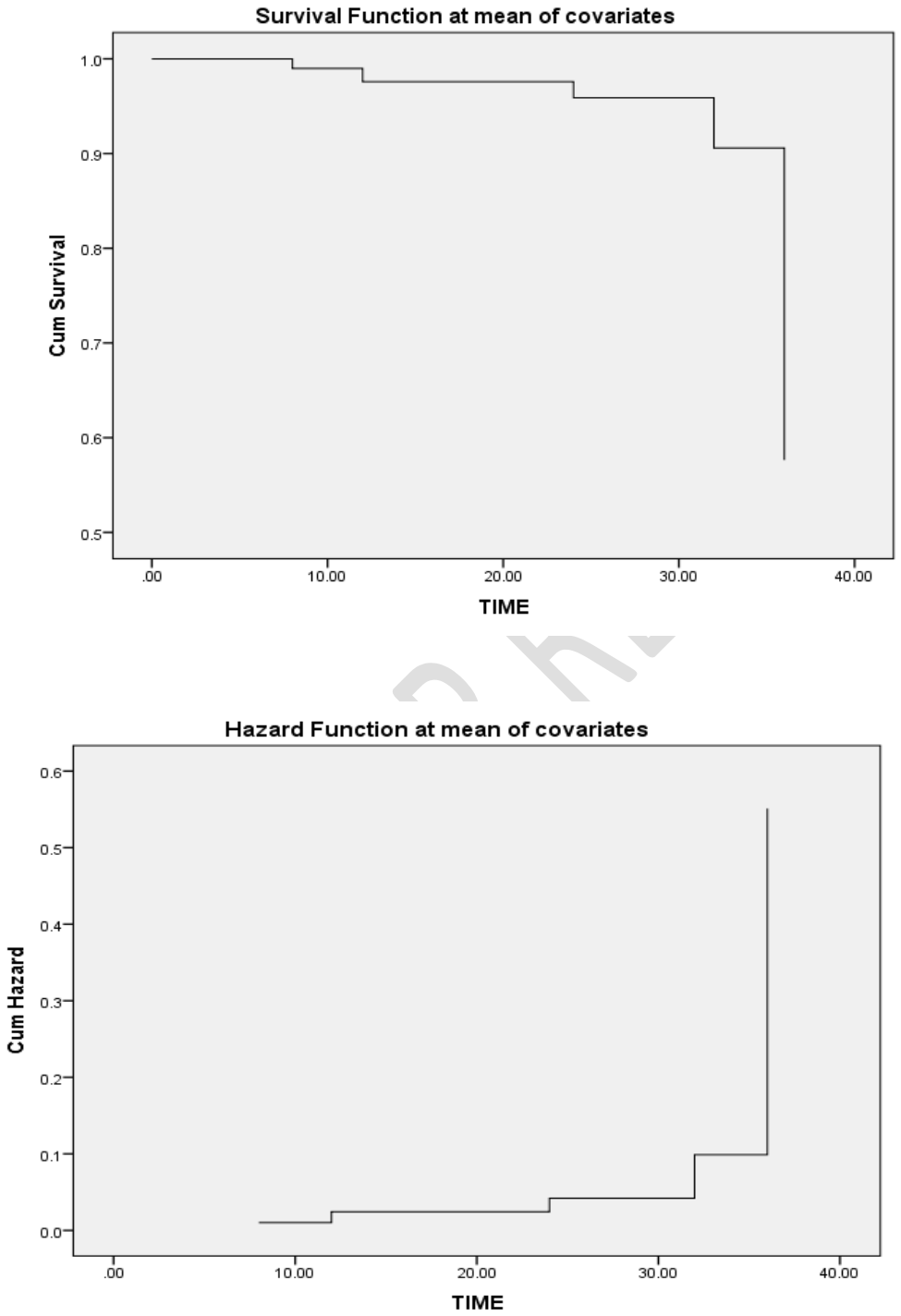

FIGURE 2: THE PROGNOSTIC PURPOSES IN CRC SHOWING LMR SURVIVAL AND HAZARD FUNCTION

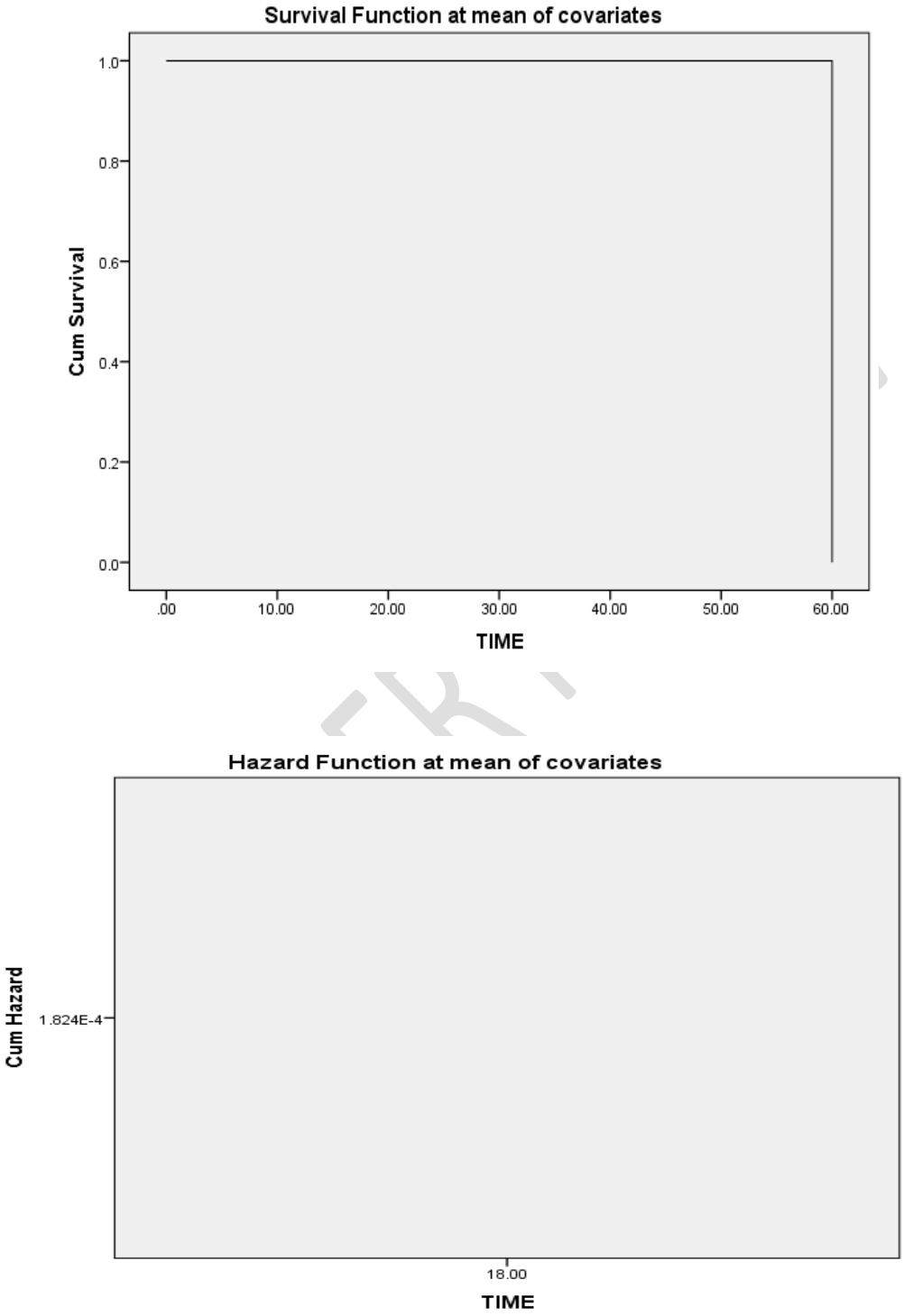

FIGURE 3: THE PROGNOSTIC PURPOSES IN CRC SHOWING PLR SURVIVAL AND HAZARD FUNCTION

Comparing the CBC and ESR results in controls, pre- treatment and treatment in Colorectal Cancer (CRC) subjects using ANOVA Turkey HSD Post- Hoc

Analysis of Variance (ANOVA) was used to calculate the difference between and within the controls, pre-treatment and treatment CBC and ESR result in CRC subject. A significant difference at $P \leq 0.05$ between and within the RBC, TPLT and some WBC parameters were observed. A Turkey post-hoc of the mean±SD was carried out on the RBC parameters, ESR TPLT, MPV, TWBC, ANC, ALC, AMC and AEC within and between the control, pre-treatment and treatment results. In TRBC, a significant increase at p=0.003 with control (4.7±0.4) and pre-treatment (4.22±0.3); also a significant decrease at p=0.02 between pre-treatment (4.22±0.3) and treatment (4.64±0.5) was seen. In HB, a significant increase control (13.6±0.6) at p=0.001 and pre-treatment (9.36±1.9); control (13.6±0.6) and treatment (10.75±1.5); pre-treatment (9.36±1.9) and treatment (10.75±1.5) at p=0.02 was observed. In HCT, a significant increase control (41.1±2.1) and pre-treatment (29.00±5.5) at p=0.0001; control (41.1±2.1) and treatment (33.00±4.6); significant decrease pre-treatment (29.00±5.5) and treatment (33.00±4.6) at p=0.03 was observed. In MCHC, a significant increase control (33.5±1.3) and pre-treatment (30.06±1.6); control (33.5±1.3) and treatment (31.00±1.3) at p=0.0001 was observed. In MCV, a significant increase control (91.4±7.0) and pre-treatment (64.04±8.0) at p=0.0001; control (91.4±7.0) and treatment (70.30±6.5) at p=0.001; significant decrease pre-treatment (64.04±8.0) and treatment (70.30±6.5) at p=0.02 were observed. In RDW, a significant decrease control (12±0.8) and pre-treatment (15±2.0); control (12±0.8) and treatment (16.7±2.5) at p=0.0001; pre-treatment (15±2.0) and treatment (16.7±2.5) at p=0.05 were observed. In TPLT, a significant decrease control (189.188±19.563) and pre-treatment (302.563±163.773); control (189.188±19.563) and treatment (311.000.0±16.156) at p=0.0001 was observed. In MPV, a significant increase control (11±0.8) and pre-treatment (10±1.4) at p=0.04; significant increase control (11±0.8) and treatment (9.0±1.2) at p=0.0001; pre-treatment (10±1.4) and treatment (9.0±1.2) at p=0.005 were observed. In TWBC, a significant decrease control (3.2±0.7) and pre-treatment (6.34±3.7) at p=0.001; control (3.2±0.7) and treatment (5.67±1.7) at p=0.02 was observed. In ANC, a significant decrease control (3.2±0.5) and pre-treatment (5.6±1.8) at p=0.0001; control (3.2±0.5) and treatment (4.4±0.5) at p=0.03; significant increase pre-treatment (5.6±1.8) and treatment (4.4±0.5) at p=0.03 was observed. In ALC, a significant decrease control (3.0±0.3) and pre-treatment (2.5±0.9) at p=0.01; significant increase control (3.0±0.3) and treatment (1.9±0.7) at p=0.0001; pre- treatment (2.5±0.9) and treatment (1.9±0.7) at p= 0.01 was observed. In AMC, a significant increase the control (0.20±0.09) and pre-treatment (0.03±0.05 at p=0.0001); control (0.20±0.09) and treatment (0.01±0.1) at p=0.0001. In AEC, a significant decrease control (0.15±0.04) and pre-treatment (0.01±0.03) at p=0.0001; control (0.15±0.04) and treatment (0.05±0.08) at p=0.0001. In ESR, a significant decrease control (10.4±8.1) and pre-treatment (82.14±29.0), p=0.0001; control (10.4±8.1) and treatment (62.94±25.0), p=0.0001; pre-treatment (82.14±29.0) and treatment (62.94±25.0) observed a significant difference at p=0.05.

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TABLE 4: COMPARING THE CBC AND ESR RESULTS IN CONTROLS, PRE-TREATMENT AND TREATMENT IN COLORECTAL CANCER (CRC) SUBJECTS USING ANOVA WITH TURKEY POST HOC

Parameter	Control (mean±SD) (N=50)	Pre-treatment (mean±SD) (N=50)	Treatment (mean±SD) (N=44)	f-value	p-value	A vs B	A vs C	B vsC
TRBC($\times10^{12}$l)	4.7±0.4	4.22±0.3	4.64±0.5	6.7	0.003*	0.003*	0.8	0.02*
HB(g/dl)	13.6±0.6	9.36±1.9	10.75±1.5	36.1	0.0001*	0.0001*	0.0001*	0.02*
HCT (%)	41.1±2.1	29.00±5.5	33.00±4.6	32.7	0.0001*	0.0001*	0.0001*	0.03*
MCHC(g/dl)	33.5±1.3	30.06±1.6	31.00±1.3	24.1	0.0001*	0.0001*	0.0001*	0.08
MCV(fl)	91.4±7.0	64.04±8.0	70.30±6.5	62.2	0.0001*	0.0001*	0.001*	0.02*
MCH(pg/cell)	33.0±2.5	-	-	NA	NA	NA	NA	NA
RDW (%)	12±0.8	15±2.0	16.7±2.5	23.9	0.0001*	0.0001*	0.0001*	0.05*
PLT($\times10^{9}$l)	189.188 ±19.563	302.563 ±163.773	311.000 ±16.156	243.6	0.0001*	0.0001*	0.0001*	0.4
MPV (%)	11±0.8	10±1.4	9.0±1.2	10.8	0.0002*	0.04*	0.0001*	0.005*
TWBC($\times10^{9}$l)	3.2±0.7	6.34±3.7	5.67±1.7	7.6	0.001*	0.002*	0.02*	0.7
ANC($\times10^{3}$l)	3.2±0.5	5.6±1.8	4.4±0.5	17.1	0.0001*	0.0001*	0.03*	0.03
ALC($\times10^{3}$l)	3.0±0.3	2.5±0.9	1.9±0.7	18.6	0.0001*	0.0001*	0.05*	0.01
AMC($\times10^{3}$l)	0.20±0.09	0.03±0.05	0.01±0.1	25.0	0.0001*	0.0001*	0.0001*	0.80
AEC($\times10^{3}$l)	0.15±0.04	0.01±0.03	0.05±0.08	31.3	0.0001*	0.0001*	0.0001*	0.12
ABC($\times10^{3}$l)	-	-	-	-	-	-	-	-
ESR(mm/hr)	10.4±8.1	82.14±29.0	62.94±25.0	43.2	0.0001*	0.0001*	0.0001*	0.05*

P<0.05*-signifies a significant difference. A (control), B (pre- treatment), C (treatment).
NA-Not Available

Table 5a and 5b: showing CBC and ESR results of different age groups in colorectal cancer

There were no significant differences at P>0.05 observed in all the CBC and ESR parameters measured within and between the different age groups.

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TABLE 5a: RED BLOOD CELL PARAMETERS RESULTS OF DIFFERENT AGE GROUPS IN CRC USING ANOVA

Age groups (years/N)	TRBC ×1012l	HB g/dl	HCT %	MCHC g/dl	MCV fl	MCH pg	RDW %	ESR mm/hr
21-30 (n=2)	4.2± 0.5	9.0± 1.8	27.3±5.1	30.3± 2.9	62.7± 5.5	22.0± 1.0	21.3 ±1.3	60.0 ±22.9
31-40 (n=20)	4.1± 0.3	8.6± 2.0	26.0± 5.7	29.0± 1.5	59.0± 3.5	20.0± 1.5	17.5 ±5.7	86.3 ±41.2
41-50 (n=15)	4.2± 0.3	9.0± 1.0	27.0± 3.0	30.0± 0.0	71.3± 14.1	22.0± 1.2	20.0 ±3.8	77.0 ±36.2
51-60 (n=5)	4.3± 0.5	9.9± 3.0	29.7± 8.9	30.0± 2.7	61.0± 6.2	24.5± 3.1	21.0 ±3.5	74.7 ±41.1
61=70 (n=8)	4.3± 0.2	9.9± 2.3	32.0± 4.8	31.0± 0.6	69.0± 18.0	23.5± 3.9	17.2 ±6.0	68.0 ±38.0
F (p) value	0.18 (0.9)	0.25 (0.9)	0.63 (0.7)	0.53 (0.7)	0.64 (0.7)	1.04 (0.4)	0.60 (0.7)	0.22 (0.9)

TABLE 5b: PLATELET AND WHITE BLOOD CELL PARAMETERS RESULTS AT DIFFERENT AGE GROUPS IN COLORECTAL CANCER(CRC) USING ANOVA

Age groups (years/N)	PLT $\times 10^9$l	MPV %	TWBC 10^9l	ANC 10^9l	ALC 10^9l	AMC 10^9l	AEC 10^9l	ABC 10^9l
21-30 (n=8)	450.000± 204.404	8.5 ±1.3	5.2± 0.4	9.9. ±5.7	2.9 ±1.6	0.07 ±0.06	0.01 ±0.02	0
31-40 (n=18)	246.667± 100.161	10.7 ±1.5	10.4± 6.0	4.3 ±0.6	1.7 ±0.1	0.03 ±0.06	0.00 ±0.0	0
41-50 (n=8)	396.667± 191.398	8.9 ±1.6	7.2± 5.1	6.4 ±5.5	2.3 ±0.8	0.03 ±0.06	0.03 ±0.06	0

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51-60 (n=8)	222.333± 118.277	12.8 ±5.1	4.7± 1.2	4.0 ±1.2	1.7 ±0.6	0.0 ±0.0	0.00 ±0.0	0
61=70 (n=8)	223.500± 131.637	14.3 ±5.4	4.9± 0.6	3.9 ±0.3	2.0 ±0.5	0.03 ±0.05	0.0 ±0.0	0
F (p) value	1.56 (0.3)	1.6 (0.2)	1.57 (0.3)	0.4 (0,8)	1.1 (0.4)	0.7 (0.6)	0.7 (0.6)	0

DISCUSSION

Full blood count is a prerequisite investigation requested from all cancer patients before surgery, use of chemotherapy and/or radiotherapy. Poor parameters adversely correlated with prognosis in several solid cancers especially in CRC [27].

The relationship between NLR, LMR and PLR and prognostic significance in patients with different solid cancers have been reported by many studies, however inconsistent results have been presented so far. No reference values have been established in Nigeria and Africa to the best of my knowledge. We made an attempt to determine different cut-off values in CRC using the pre-treatment CBC results. And in addition, determine the NLR, LMR and PLR ratio with their respective percentage in each group using longitudinal approach. Previous studies determined these ratios in retrospective studies. The duration of the disease was used to determine the overall survival using the different ratio cut-off values.

In this study, the treatment red cell parameters (TRBC, Hb, PCV, MCV) results were significantly lower than the pre- treatment and control result. The treatment MCHC and MCH were also associated with decreased value when compared with the control subjects but their pre-treatment values but had no significantly difference. The RDW were significantly increased in the treatment results compared with the pre- treatment and control results. These results showed a classical case inflammation and anaemia in these subjects which can be caused by malnutrition, cytotoxicity of the chemotherapy drug. RDW reflects impaired erythropoiesis and abnormal red blood cell survival but it correlates also with inflammation, under nutrition and impaired renal function, with inadequate production of erythropoietin (EPO) [69]

Quite recently, red cell distribution width (RDW), have also been shown to associate with survival of solid tumors [68]. Growing evidence indicated that high RDW is associated with systemic inflammation and elevated RDW harbored the potential to predict poor survival in a variety of human cancers, consisting of breast cancer, lung cancer, prostate cancer, endometrial cancer, colon, esophageal cancer and upper tract urothelial carcinoma [69] [70]. Work done in 2005 reported anaemia in solid cancer patients. They suggested that this anaemia which is mostly of iron deficiency may be due to the malignancy itself or a direct consequence of the treatment due to the decreased value in red blood cell indices. They also reported that this anaemia in these cancers may be evident at initial diagnosis and develops due to activation of immune system which appears to be the driving force of global diminution of erythropoiesis [50]. A work done in 2006, hypothesed that this immune system once activated stimulates the production of inflammatory cytokines that impedes erythropoiesis hence leading to insufficient

differentiation and proliferation of erythroid precursors leading to anaemia [51]. In 2017 some works reported that these inflammatory cytokines also impairs iron metabolism which results in reduced serum iron levels and iron retention within the reticuloendothelial system (RES)[52]. Another researchers in their work reported that, these cytokines can be produced by the cancer cells themselves which then induces iron sequestration, thereby decreasing RBC production and over expression of these inflammatory cytokines causes shortened RBC survival [53]. Reported works [55], [54] [56] all observed that chronic blood loss at common sites can exacerbate anaemia from bone marrow invasion by these solid cancers causing myelophthsis resulting from bone marrow replacement causing pancytopenia. This is work is consistence with works done by [51], [55], [54], [56], [52], [53].

In this present work, there is significant decrease of the treatment TWBC compared to the pre-treatment and control TWBC even though the TWBC are within the normal range. This change could be attributed to effect of chemotherapy which exerts cytotoxic destructive effect during treatment on the bone marrow. Studies by [57], [58], [59], reported leucocytosis in solid cancer subjects. Several studies by [60],[61], had attempted to identify the association between TWBC and solid cancer risk, but no consistent evidence has been found most reports were done on neutrophils/ lymphocytes ratios. This work reported leucopenia which is not consistent with other published works done by [57], [58], [59].

In differential TWBC, most data were available for the ratio of NLR. The role of neutrophils in cancer is multifactorial and not fully understood. Some works reported that neutrophils participate in different stages of the oncogenic process including tumor initiation, growth, proliferation or metastatic spreading [62]. The various roles of neutrophils in cancer development and progression by several researchers have recently explored the role of neutrophils and other markers of host inflammation on clinical outcomes. In their work reported also that an elevated absolute neutrophil count is an adverse prognostic factor incorporated in a contemporary prognostic score for metastatic carcinoma treated with targeted therapy [63]. A lot of controversies in neutrophil counts have been involved in neutrophil count report. Reported works done in 2011 stated that the expression of neutrophils in the tumour had been linked with detrimental outcome in some cancer examples include: head and neck cancer, esophageal cancer whereas in other cancers, it has been associated with better survival [64]. However, another work reported that neutrophils assessment can be used as indirect measurement of the tumor inflammation outcome [65].

In this work, significant increases were observed in absolute neutrophil count (even though the values fell within the normal range) in their pre- treatment samples results compared with their treatment and control results. In 2017 works reported that peripheral bloods neutrophils are increased in subjects with solid cancer before treatment. They suggested that this is because tumors at initial stage produces granulocyte colony – stimulating factor (G-CSF) which skews the neutrophil retention or release balance in bone marrow, leading to this increase in circulating neutrophil[66]. However during treatment, reported by [67], reduce neutrophil count are seen in these subjects showing to be beneficial to the survival of the subjects and this may of course just be a reflection of adequate toxicity of the drug being achieved as it kills tumour cells. The direct effect of toxicity during therapy on neutrophils should be closely monitored in order to prevent the occurrence of neutropenia. This work is however consistent with works done by [67], [66].

Recently, increasing attention has been paid to the assessment of MPV in cancer patients. The observation of decreased platelet size in cancer patients has been explained by an

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increased cancer-associated platelet activation and exhaustion [68]. In this context, a low MPV may reflect degranulated "exhausted" platelets that have already secreted their potentially tumor growth-promoting cytokines, and thus are associated with a worse outcome in cancer patients [103]. Some works done in 2014, examined the effect of chemotherapy treatment on MPV levels in colorectal cancer patients. Pre-treatment MPV findings were similar in the whole study group, but decreases during treatment. They indicated that changes in MPV could be due to the effect of chemotherapy on the formation of blood platelets and cyclic drug administration. Hence no thrombocytosis or thrombocytopenia was observed [74]. All the literatures about PLT were of western countries origin none has been reported in Nigeria to the best of my knowledge. From the result of this work, it was observed that the solid cancers rarely affect the platelets as most of the subjects during treatment had no bleeding tendencies. So the result obtained in this study is not consistent or in agreement with other past researchers.

ESR is the most widely used laboratory test for evaluating the inflammatory status in clinical practice, including infection, autoimmune and malignant diseases [80]. Reported works done in 2014 stated that an elevated ESR in cancer patients could be as a result of underlying disorder, the stage and duration of the disease, the regimen and intensity of the antitumor treatment [78]. In 2014, another researcher also reported on elevated ESR level as a prognostic factor adversely affecting survival in cancer patients [79]. This present work observed a significant increase of treatment ESR to pre- treatment ESR test and control results in all the in solid cancers. The result coincides with anaemia observed in these patients which may be caused by several factors including nutritional decline, bone marrow filtration, treatment – related toxicity and chronic inflammatory state. This work is in agreement with works done and reported by [80], [81], [84].

In this present work, NLR (2.58) and LMR (5.65) showed significant difference while PLR (138.8) showed no significant difference in CRC. This actually means that both NLR and LMR are associated with HR and survival prediction in these subjects. This work observed a significant difference in LMR (5.65) in its association with OS in CRC subjects. A decreased LMR showed an increased HR and decreased OS in these subjects. As a new factor of systemic inflammation, LMR has been drawing increasing attention lately. This result can be due to neutrophils, lymphocyte and platelet counts variation over time during diagnosis for CRC subjects. NLR is the ratio of the absolute neutrophil count to the absolute lymphocyte count, and therefore the association between a high NLR and a poor prognosis, as revealed in the present study, is possibly indicative of the tumor-promoting activity associated with neutrophilia in the tumor environment. Neutrophilia promotes tumor growth and metastasis by remodeling the extracellular matrix and releasing reactive oxygen species, nitric oxide and arginase, which suppress the T cell response and increases the rate of mutagenesis [90]. Additionally, neutrophilia suppresses lymphocyte activity, therefore counteracting the antitumor immune response [91]. The pre-treatment NLR assessment were also seen in works done by [105],[106], [101], [102],[103], all reported that NLR had no significant prognostic value for predicting survival in CRC even with various cut-off values and large sample sizes employed in their various works. However works done by [98], [100] reported that an elevated pre-treatment NLR has been associated with a poor survival rate in CRC. However, in 2016 work reported that decreased pre-treatment LMR is associated with poor prognosis in CRC while increased pre-treatment LMR is associated with good prognosis in haematological malignancies [107]. Works done by [117], [118] found no significant relationship between LMR and OS in CRC patients. Works done by [125], [115],[109] reported that increased or decreased PLR cannot be used as

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prognostic biomarker in predicting HR and OS in CRC patients or subjects and optimal cut-off values for PLR for predicting prognosis in CRC remains unknown. So the current reports on the prognostic role of PLR as a biomarker in CRC are inconsistent and inconclusive In this present work, LMR is not consistent with other reported works while NLR is consistent with works done by [98], [100]only . PLR were in conformity with other reported works on CRC. The discrepancy among these several works done on this CRC using NLR, LMR and PLR were not clearly understood

In this present work, there were no significant difference between and among the different age groups observing that no age group were spared in CRC. This disease cuts across all age groups but in the age group 31 – 40, their CBC and ESR results showed decreased value all through while their WBC parameters showed increased values. This showed that this particular age group is more susceptible to this disease than other age group. The reason(s) underlying this trend is unclear. Reported cases in 2019 stated that over 86 percent of those diagnosed with CRC under the age of 50 years are symptomatic, and the disease is being diagnosed at later stages, suggesting that the increased incidence is real and not representative of a shift in age at diagnosis attributable to earlier detection [33],[31]. In 2010, some works done reported increasing incidence between 20 – 30 years [32]. It was hypothesed that this may be as result of life style, smoking, high consumptions fried meats and fishers, sedentary television viewing in this particular age group [34]. .Also ingesting hides processed with a flame fuelled by firewood and spent engine oil may contain toxic organic compounds such as polyaromatic hydrocarbons (PAHs), dioxins, furan, and benzene which can potentiates the onset of CRC in young adults between the ages 30-40years [25].. This work has shown increased incidence of CRC in Enugu as compared with other works done from 1975 to 1980[43]. So an aggressive awareness programme should be mounted and screening started earlier than 50 years so that survival in these patients will be assured. This work is consistent with works done by [34] [32] [33].

CONCLUSION:

It is evident that components of CBC count in this study had provide valuable prognostic information in solid cancer that are not limited to survival predictions or assessment of diseases progression, but also were important tools when evaluating response to treatment. The findings of this work indicate that about 50% use of CBC especially the pre-treatment NLR/ LMR/PLR score can be considered a valuable prognostic indicator in patients with this solid cancer. A close relationship between NLR/LMR/PLR and cancer progression was also observed in subject with this solid cancer. Thus, these ratios may be considered for routine clinical use as reliable and low-cost biomarkers.

ETHICAL APPROVAL AND CONSENT

All subjects gave a written consent and the study was approved by the local Ethics Committee of Enugu State University of Science and Technology Teaching Hospital Park Lane G.R.A. Enugu-North Local Government Area. Questionnaires were used to extract some useful data required in this study. The subjects attending surgical out-patient (SOP) clinic at ESUT Teaching Hospital Parklane GRA Enugu State were used.

CONFLICT

There was no conflict of interest in this work

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APPENDIX

QUESTIONAIRE

SURNAME……..OTHER NAMES……..

AGE…….OCCUPATION……

STATE OF ORIGIN……..

(PLEASE TICK IN THE BOX BELOW)

SEX

MALE ☐

FEMALE ☐

LEVEL OF EDUCATION

PRIMARY ☐ SECONDARY ☐ TERTIARY ☐

BODY MASS INDEX

Weight ----------Height----------

Waist Circumference----------

Parity ----------Age of Menarche……..

Age of First Parity----------

Breast Feeding ☐ No Breast Feeding ☐

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Use of Contraceptives: yes ☐ No ☐

TYPE OF SOLID CANCER……..

Date of Onset----------

STAGES OF CANCER

Zero ☐

Stage 1(T) ☐

Stage 11(T) ☐

Stage 11 &111 (N) ☐

Stage 1V (M) ☐

TUMOR SIZE

T0 (<1mm) ☐

T1 (1mm-5mm) ☐

T2 (20mm-50mm) ☐

T3 (>50mm) ☐

T4 (metastasis) ☐

LIFE STYLE

Smoking ☐ Obesity ☐

Poor Diet ☐ Lack of Exercise ☐

Alcohol Intake ☐

PRE DISPOSING FACTORS

Hepatitis A, B, C, D, &E ☐

Human Papillomavirus infection (HPI) ☐

Genetic ☐

SCREENING TESTS

Self Examination ☐

Fecal Occult Blood ☐

Scanning ☐

Pap test ☐

PSA ☐

Urinalysis ☐

CONFIRMATION TESTS

Scanning ☐

Biopsy ☐

MRI ☐

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TREATMENT INTERVAL

Fortnightly (2wks) ☐

Three weeks ☐ Monthly ☐ Three month ☐

Six Monthly ☐ Yearly ☐

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