Original Research Article

The use of peripheral blood cells as an assessment of inflammation in prostate cancer in patients attending in surgery department at ESUT teaching hospital, Parklane, Enugu, Nigeria

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ABSTRACT

Background: Full blood count (FBC) is a prerequisite investigation requested from all prostate cancer (PCa) patients pre and post treatment, poor parameter influences the outcome of cancers.

Methods: Total subjects consisted of 84 male subjects between the ages 41 to >80 years. Longitudinal study was conducted. Controls and test samples were collected at diagnosis and at different stages of the treatment. Demographic information was obtained using a questionnaire. The data was analyzed using IBM statistical package for social sciences (SPSS) PC, version 20.0; SPSS Inc., Chicago, III., USA; the receiver operating characteristic curve (ROC) curve was obtained via neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and platelet-to-lymphocyte ratio (PLR) ratios cut-off determinations. Cox proportional-hazards regression analyses the prognostic factors (duration, ratios) and overall survival (diagnosis to death or last follow-up). A paired sample t-test compared test of significance in pre/post treatment results. The analysis of variance (ANOVA) and Tukey HSD post-hoc, test susceptibility within age groups was done.

Results: Increased NLR and LMR were significantly associated with increased hazard ratio (HR) and OS at p<0.05 while PLR, no significant difference at P>0.05 in PCa. In complete blood count (CBC) and erythrocytic sedimentation rate (ESR), control and treatment period, all red blood cell (RBC) parameters showed a significant decrease at p<0.05 in treatment results compared to the pre-treatment results while total platelet (TPLT), total white blood cells (TWBC), NC, LC, ESR showed significant increase at p<0.05 in treatment results compared to pre-treatment results. Age group 41-50 years showed more susceptibility than other age groups with significant decrease at p<0.05 in NC, LC and increased MC.

Conclusions: This study supports CBC and ESR biomarkers as a prognostic tool in early detection, treatment and monitoring of disease progression in these subjects.

Keywords: Prostate, Inflammation, CBC

INTRODUCTION

Prostate cancer (PCa) is the development of cancer in the prostate gland.¹ Most prostate cancers are slow growing; however, some grow relatively quickly and become hyperplastic.² Prostate cancer is diagnosed by biopsy medical imaging and screening is by PSA.³⁴ In Nigeria, 2% of men develop prostate cancer, and 64% of them are dead after 2 years due to metastasis.⁵ Anemia in men with advanced prostate cancer may be caused by several factors,
including androgen deprivation, nutritional decline, inflammatory cytokine production, bone marrow infiltration, treatment-related toxicity, and the chronic inflammatory state.\(^6\)

Leucocytosis, even within the normal range, has been associated with solid cancer incidence and mortality rate.\(^7\) Neutrophils in cancer are multifactorial and reflect a state of host inflammation, a hallmark of cancer. Neutrophils participate in different stages of the oncogenic process.\(^8\) Neutrophilia is associated with worse outcomes in many solid cancers, both in early and advanced stage of cancer due to increased production of granulocyte colony-stimulating factor (G-CSF) which skews the neutrophil retention/release balance in bone marrow.\(^9\) However during treatment, neutropenia are seen in these subjects showing to be beneficial to the survival of the subjects which reflects adequate toxicity of the drug being achieved by killing tumour cells.\(^10\) Lymphocytes are key effectors of antitumor immune responses, due to release cytokines and stimulate natural killer T (NKT) cells causing elimination and equilibrium.\(^11\) The higher the lymphocyte count, the better the overall survival, the lower the platelet-lymphocyte ratio, the better the overall survival.\(^12\) Many hypotheses were reported on monocyte relationship with lymphocyte ratio, the better the overall survival.\(^13\) Many studies have demonstrated that elevated NLR, PLR and lower LMR were found to be significantly associated with worse OS.\(^14\)

Recent evidence indicated that increased NLR is associated with poor life expectancy in patients with prostate cancer (PCa). Similarly, with PLR can be used to predict the prognosis of patients with prostate cancer, but is still rarely used worldwide.\(^15\) Twenty-five studies with 26 datasets evaluated the association between pretreatment NLR and OS in PCa patients, significant differences at P<0.001 were present. Between pretreatment PLR and OS in PCa patients; no significant differences were present among these studies.\(^16\) One study with 2 datasets analyzed the pretreatment LMR for predicting the OS of PCa, no significant difference was present among studies (P=0.239). A work reported that NLR and PLR may be ineffective biomarkers for predicting PCa prognosis.\(^17\) So this work determined the CBC and ESR in PCa subjects at their pre/during-treatment period, calculated the NLR, LMR and PLR these subjects as prognostic biomarkers and determined age group susceptibility.

This study investigated the use of CBC and ESR as an assessment of inflammation in PCa subjects.

**METHODS**

The study was conducted in ESUTH teaching hospital, Parklane, G. R. A. Enugu, Enugu State. All subjects gave a verbal consent and they study were approved by the ethics committee of Enugu state university of science and technology teaching hospital, Park Lane G. R. A. Enugu-North local government area. The pre-treatment samples were collected at diagnosis and the treatment samples at stages of the treatment. The base line control samples were compared with other subsequent samples collected from same subjects at various stages of the treatment and changes reported.

This study comprised of 84 subjects between the ages of 41 years and 80 years with no ethnicity differentiation. Questionnaires used obtained other demographic characteristics. Follow up the subjects included.

In recent years, a number of studies have focused on hematological parameters, which can reflect the status of immune responses in cancer patients.\(^18\) Previous studies have explored the prognostic role of platelet-to-lymphocyte ratio (PLR) in patients with PCa, although the results are controversial. Neutrophil-to-lymphocyte ratio (NLR) from routine complete blood count (CBC) in the peripheral blood has been reported to be an independent prognostic factor in cancers.\(^19\) Pretreatment inflammatory factors, differential WBC as well as the ratios between them such as NLR, PLR and lymphocyte-to-monocyte ratio (LMR) have been suggested as potential prognostic predictors for patients with prostate cancer (PCa).\(^20\) Several studies have demonstrated that elevated NLR, PLR and lower LMR were found to be significantly associated with worse OS.\(^21\)
Exclusion criteria

Subjects suffering from other types of health problems like liver cirrhosis, active bleeding, intestinal obstructions, diabetes, hyper blood pressure, non-solid cancers were excluded.

Data collection

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. Blood sampling were performed to measure ESR by Westegren method. CBC were done using a haematological analyzer “Be-5300 – Mindray” Japan. These ratios were the total number of neutrophils, platelets, monocytes divided by the total number of lymphocytes.

Statistical analysis

The mean and standard deviation (mean value±SD) of the data were tabulated for each group. Data was analyzed using IBM statistical package for the social sciences (SPSS) PC. Version 20.0; SPSS Inc., Chicago, III., USA; the ROC curve: NLR, LMR and PLR ratios cut-off determinations. Cox proportional-hazards regression analyses the prognostic factors (duration, ratios) and overall survival (diagnosis to death or last follow-up). A paired sample t-test compared test of significance in pre/post-treatment results. The analysis of variance (ANOVA) and Tukey HSD post-hoc, test susceptibility within age groups.

RESULTS

A total of 84 male subjects with age range mean±SD of 66.3±10.7 were studied. Educational qualifications included primary, 6 (7.0%); secondary, 58 (69%) and tertiary, 20 (24%). Occupations included civil servants, 36 (43%); business, 48 (57%) and students, 0 (0%). Duration (months) mean±SD included diagnosis to death or last follow-up and grouped into three categories. The total number and percentage was reported for PCa.

ROC curve calculated using Youden index for area under curve (AUC) 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 84 subjects were classified into two groups: high and low NLR, LMR, and PLR with their respectively percentage.

A total of 71%, 67% and 55% of PSA subject had low NLR, LMR and PLR respectively while total of 29%, 33% and 45% had high NLR, LMR and PLR respectively. The coefficient (B) NLR (2.08) and LMR (1.06) have a positive value. HR for NLR is 8.03 (95% CI: 3.76-17.17; p=0.000001) and LMR is 1.06 (95% CI: 1.03-1.08; p=0.000001) meaning that high NLR and lower LMR ratios are associated with increased HR and decrease or shortened survival time in the subjects. A unit increase in NLR and decrease LMR by 1.0 increases HR of the ratio values by 8.03 (NLR) and 1.06 (LMR) folds. Also a unit increase in NLR and decrease LMR decreases the survival time in the subjects by 17.17 and 1.08 months respectively. But in PLR, HR equals 1.0, meaning that there no effect between high PLR and low PLR in these subjects even with the high significant p value. The graphs summarize the result (Figure 1-3), increase NLR and decrease LMR, increase HR and decreases survival time.

Table 1: Demographic table of the prostate cancers.

| Characteristics       | Total number (n) | Percentage (%) |
|-----------------------|------------------|----------------|
| Gender (males)        | 84               | 100            |
| Level of education    |                  |                |
| Primary               | 6                | 7              |
| Secondary             | 58               | 69             |
| Tertiary              | 20               | 24             |
| Occupation            |                  |                |
| Civil servants        | 36               | 43             |
| Business              | 48               | 57             |
| Students              | 0                | 0              |
| Age (years) mean±SD   | 66.3±10.7        |                |
| Age groups (in years) |                  |                |
| 41-50                 | 6                | 7.1            |
| 51-60                 | 22               | 26.2           |
| 61-70                 | 29               | 34.5           |
| 71-80                 | 19               | 22.6           |
| >80                   | 8                | 9.6            |
| Duration (months) mean±SD | 37.8 ±10.9    |                |
| 11-30                 | 24               | 28.6           |
| 31-50                 | 55               | 65.5           |
| 51-70                 | 5                | 5.9            |

Table 2: High and low optimal cut-off values in the prostate cancers with their total number and percentages respectively.

| Parameter | NLR | LMR | PLR |
|-----------|-----|-----|-----|
| Optimal cut-off | 1.95 | 8.55 | 4901.5 |
| Sensitivity  | 0.897 | 0.755 | 1.000 |
| Specificity  | 0.211 | 0.263 | 0.000 |
| AUC         | 0.848 | 0.791 | 1.000 |
| P value     | 0.0001 | 0.0001 | 0.000 |
| High (N%)   | 24 (29) | 38 (45) | 38 (45) |
| Low (N%)    | 60 (71) | 46 (55) | 46 (55) |

Significant difference at P<0.05 were observed in the pretreatment results of all RBC parameters assayed, total platelet count, total leucocyte count, neutrophil count, lymphocyte count and ESR compared with the treatment test results. Monocyte and eosinophil count showed no significant different at P>0.05.
Table 3: The prognostic purposes of NLR, LMR and PLR in prostate cancer.

| Covariates (%)           | Coefficient (B) | Standard error | P value | Exp (B) (hazard ratio) | 95% CI for exp (B) |
|--------------------------|-----------------|----------------|---------|------------------------|--------------------|
| NLR [0<1.95 (71%); 1>1.95 (29%)] | 2.08            | 0.39           | 0.000001 | 8.03                   | 3.76 to 17.17     |
| LMR [0<8.55 (67%); 1>8.55 (33%)] | 0.06            | 0.01           | 0.00001  | 1.06                   | 1.03 to 1.08      |
| PLR [0<4901.5 (55%); 1>4901.5 (45%)] | 0.00            | 0.00           | 0.00001  | 1.00                   | 1.00 to 1.00      |

A significant difference at P≤0.05 within and between the age groups NC [f (82)=5.7577, p=0.0004]; LC [f (82)=5.7770, p=0.0004]; MC [f (82)=3.3441, p=0.0140]; TPLT [f (82)=2.6265, p=0.0402]. A Turkey HSD post-hoc of the mean±SD was carried out on NC, LC, MC, and TPLT within and between the different age groups. In TPLT, age groups 71-80years (195368.4±60386), a significant difference at p=0.00001 with age group >80 (195395.0±46810.0) and in age group 41-50 (259500.0±121227.0), at a significant difference at p=0.0189 was seen. In NC age group 41-50 (71.3±6.7) showed a significant difference at p=0.002 to age groups 51-60 (56.1±6.0) with 71-80 (61.2±5.9) at p=0.002 and at >80 (58.3±12.3) at p=0.009. In LC, age group 41-50 (25.7±7.2) a significant difference at p=0.002 to 51-60 (40.8±5.2), with 61-70 (40.0±7.0) at p=0.003; at 71-80 (37.8±5.5) at p=0.0046 and at >80 (40.4±13.4) at p=0.0025. In MC, age group 41-50 (3.2±2.6) had a significant difference at p=0.0206 with age group 71-80 (0.6±1.0).

Figure 1: NLR survival function.

Figure 2: LMR survival function.

Figure 3: PLR survival function.

Table 4: Pre-treatment and treatment CBC and ESR results in prostate cancer subjects.

| Parameters | Pre-treatment | Treatment | P≤0.05 |
|------------|---------------|-----------|---------|
| TRBC       | 4.23±0.7      | 3.84±0.6  | 0.0001* |
| HB         | 11.66±1.6     | 10.52±1.4 | 0.0001* |
| PCV        | 34.81±1.5     | 31.48±1.7 | 0.0001* |
| MCHC       | 31.69±1.5     | 28.81±1.7 | 0.0001* |
| MCV        | 82.33±7.3     | 77.82±6.0 | 0.0001* |
| MCH        | 26.24±2.7     | 23.54±2.5 | 0.0001* |
| PLT        | 190881.0±66299.0 | 210310.0±65092.7 | 0.0001* |
| TWBC       | 6.85±5.4      | 5.69±2.3  | 0.043*  |
| Neutrophils| 59.30±7.3     | 61.87±7.6 | 0.0001* |

Continued.
**DISCUSSION**

**Prostate cancer ratios**

The prognostic roles of NLR, LMR and PLR in subjects with PCa have been explored, but the results were controversial. Some works demonstrated that elevated NLR, PLR and lower LMR were found to be significantly associated with worse OS while other works contradict this. These work observed that high NLR (>1.95) and low LMR (<8.55) were associated with significant difference increased HR and shortened OS in these subjects using duration as a constant while PLR had no significant difference on HR and survival in PCa subjects.
(Table 3 and Figure 1-3). Most works done reported no significant difference in pre-treatment LMR for predicting OS in PCa subjects. The association between PLR and OS in PCa subjects evaluated reported no significant effect on predicting OS in these subjects. All these discrepancies could be attributed to their small sample size and their methods of analysis. Many hypotheses were reported on monocyte relationship with PCa but the results were inconclusive. Based on these hypothesis monocyte counts have been believed as a clinical prognostic factor for PCa and some other solid cancers. Higher monocyte count leads to lower LMR and correlates with poor OS while high LMR predicts more favourable outcome. Study reported that elevated NLR, PLR and decreased LMR were found to be significantly associated with worse OS in PCa. This work is consistent with works done by only. So these ratios though inexpensive, readily available can be used to predict progression, recurrence, inform treatment decisions and potential treatment outcomes in these subjects.

**Prostate cancer pre-treatment and treatment**

In this study, classical cases of pre-treatment anaemia in these subjects were seen in PCa values when compared with their treatment results at p<0.05 (Table 4). This change could be as result of production of inflammatory cytokines that impedes erythropoiesis hence leading to insufficient differentiation and proliferation of erythroid precursors leading to anaemia. Also these cytokines can be produced by the cancer cells themselves which then induces iron sequestration, thereby decreasing RBC production. This is work is consistence with works done by.

In this work, there is pre-treatment leucopenia results compared to the treatment (within the normal range) (Table 4). This change could also be as a result of chemotherapy cytotoxic destruction effect on bone marrow resulting in these changes observed in this TWBC work. Studies had attempted to identify the association between TWBC and other solid cancer risk, but no consistent evidence has been found most reports were done on neutrophils/ lymphocytes ratios. This work is not consistent with other published works.

The role of neutrophils in cancer is multifatorial. It participates in different stages of the oncogenic process including tumor initiation, growth, proliferation or metastatic spreading. Neutrophilia is associated with worse outcomes in many solid cancers, both in early and advanced stage of cancer due to increased production of G-CSF which skews the neutrophil retention/release balance in bone marrow. However during treatment, neutropenia are seen in these subjects showing to be beneficial to the survival of the subjects which reflects adequate toxicity of the drug being achieved by killing tumour cells.

In this work, neutrophilia were observed (even though the values fell within the normal range) in their pre-treatment samples results compared with their treatment samples results (Table 4). This work is however consistent with works done by.

Lymphocytopenia was seen in PCa subjects in their pre-treatment sample result when compared to their treatment results at p<0.05 (Table 4). May be as a result cytotoxicity of these drugs could be caused by proliferative arrest in lymphocyte precursor or by direct induction of apoptosis in mature cells; mechanisms by which cytotoxic drugs include depletion of lymphocytes have not been defined. In this work, pre-treatment monocytopenia and reduced eosinophil count were observed when compared with the treatment sample results, however with no significant difference.

Thrombocytopenia is usually seen in pre-treatment PCa subjects; however on commencement of the treatment, thrombocytosis is seen. A study reported thrombocytosis in pre-treatment subjects while thrombocytopenia was reported by other studies. So many theories postulated the causes of this thrombocytopenia in these patients, as an increase in anti-platelet antibody induced by the cancer as well as the presence of immune – modulating oncogenic viruses; however the mechanism of this cancer ITP has not yet been elucidated. In this work, the pretreatment PLT was within normal range, no thrombocytosis or thrombocytopenia was observed when compared with the treatment results. All the literatures about PLT were of western countries origin none has been reported in Nigeria to the best of my knowledge. In this work, it was observed that the PCa 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.

This work observed a significant increase of treatment ESR to pre-treatment ESR test results in the PCa. The result coincides with the anaemia observed in these patients who may be caused by several factors including androgen deprivation, nutritional decline, bone marrow filtration, treatment – related toxicity and chronic inflammatory state. This work is in agreement with works done and reported by.

**Prostate cancer and ages**

In Table 5 of this work, age group 71–80 years showed thrombocytopenia when compared to other age groups. This signifies that men in age group 71–80 years have a tendency of having thrombocytopenia before treatment. This thrombocytopenia observed can actually change their treatment options. In same table, neutrophilia was observed age group 41–50 years. Increase changes in the neutrophil counts in any solid cancer have been associated not only with the presence of cancer but also stage and prognosis of the disease. Neutrophilia seen in advanced cancer and causes increased mortality. No literature has
correlated NC with a specific age group in prostate cancer to the best of my knowledge. Also literature were limited to an effect of prostate cancer on WBC and it components. In age group 41–50 years lymphocytopenia and monocytopenia was observed when compared to other age groups. In conclusion, the result obtained so far has shown that subjects in age group 41–50 years were more susceptible disposed in developing prostate cancer than other age group in this work. This work however has set a precedent in PCa and NL, LC count.

CONCLUSION

It is evident that components of CBC and ESR had provided valuable prognostic information in prostate cancer by predicting survival, assessment of diseases progression and response to treatment. Thus, these ratios may be considered for routine clinical use as reliable and low-cost biomarkers.

This works proved that pre-treatment ratios of NLR, LMR and PLR should be introduced in clinical practice as a routine laboratory for early detection, prognosis, easily reproducible and accessible.

Recommendations

Identification of adequate cut-off values in these ratios over a pre-treatment and treatment period of time could add more accurate information in the type of therapy for use in these patients.

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