RESEARCH ARTICLE

Platelet counts in patients with rheumatoid arthritis at the Kenyatta National Hospital- Nairobi, Kenya

Mbuthia BM[1], Oyoo GO[1], Kitonyi GW[2]

[1]*Department of Clinical Medicine and Therapeutics*
[2] *Department of Human Pathology, Unit of Haematology and Blood Transfusion School of Medicine, University of Nairobi, P O Box 19676 00202, Nairobi, Kenya*

Corresponding author:
Dr. BM Mbuthia. Email: muthonibg@yahoo.com

Abstract

***Background*:** Rheumatoid arthritis (RA) is a disease associated with significant morbidity and mortality. Thrombocytosis is one of the haematological manifestations of rheumatoid arthritis that occurs in active disease. Platelet counts may vary depending on disease activity and the variation has been shown to correlate with clinical and laboratory indices of disease activity in RA. Occasionally patients with RA may have drug induced thrombocytopenia.

Objectives: To determine the relationship between platelet counts and clinical disease activity in patients with RA at Kenyatta National Hospital (KNH).

Design: A cross-sectional descriptive study.

***Setting*:** Rheumatoid arthritis patients attending the KNH Rheumatology Outpatient Clinic (ROPC).

Methods: Patients presenting to the clinic were screened and those meeting the inclusion criteria recruited into the study. Consecutive sampling technique was done. *A* targeted history was obtained, following which a physical exam was done on the recruited patients. The patients' platelet counts were measured using Abbot Cell Dyn 1300. The patents' erythrocyte sedimentation rate (ESR)) was measured with the Wintrobe's method. The patients' clinical disease activity using the DAS 28 score was recorded.

Results: One hundred and four patients were recruited over the 6 months period between November 2010 and April 2011. Females were 90(86.5%) and 14(13.5%) were males giving a male to female ratio of 1:6.4. The mean age of the patients was 48 years. Regarding medication use, 75% of the patients were on disease modifying anti-rheumatic drugs (DMARDs), 72.1% on non-steroidal analgesics (NSAIDs) and 46.2% on steroids. The mean platelet count was 313.2 ±SD94 x 10^9/L with a range of 152 -611 x 10^9/L. Only 15 (14.4%) had thrombocytosis (>400x 10^9/L). No case of thrombocytopenia was recorded. Ninety two had active disease (88.5%) while 10(11.5%) were in remission. Among those with active disease,10(9.6%) had mild disease, 51(49%) moderate disease and 31(29.6%) high disease activity. The DAS28 score was not significantly different between those who had thrombocytosis and those who had normal platelet counts (p=0.413). However, HB, MCV and MCH were significantly lower in those with thrombocytosis at P values of 0.02, 0.002, 0.03 respectively. No correlation was found between platelet counts and clinical disease activity (DAS28).

Conclusion: While thrombocytosis was found in 14.4% of patients with RA, this study demonstrated that no relationship exists between platelet counts and disease activity in patients with rheumatoid arthritis seen at KNH.

Introduction

Rheumatoid arthritis is a chronic systemic inflammatory disorder characterised by deforming symmetrical polyarthritis often leading to joint destruction, deformity and loss of function. Extra-articular features and systemic symptoms can commonly occur and may antedate the onset of joint symptoms[1] **.** Chronic pain, disability and excess mortality are common sequelae. High standardised mortality rates have been observed in the RA population compared with the general population[2,3]**.** Thrombocytosis is among one of the haematological manifestations of rheumatoid arthritis (RA). Various studies have demonstrated thrombocytosis in RA with prevalence ranges from 16% to 51% in different studies[4-7].

Several possible mechanisms are thought to cause the increased platelet count. These include, decreased platelet survival, increased erythropoeitin levels, inflammatory cytokines, increased

thrombopoietin levels and analgesic-induced occult gastrointestinal bleeding [8-16]. Thrombocytosis has been shown to have consistent correlation with disease activity in different studies. Thrombocytosis is associated with more active disease and extra-articular manifestations are more common [4,5]. Elevated platelet counts are also associated with more joint damage[15]. Platelet counts have also been shown to positively correlate with acute phase reactants such as ESR, CRP in RA [5,18].

This study was undertaken to describe the platelet counts in patients with RA and determine any relationship to clinical disease activity.

Materials and Methods

This was a cross-sectional descriptive study carried out at the Kenyatta National Referral and Teaching Hospital from November 2010 to April 2011. The study population were patients with RA on follow up at the KNH ROPC. The inclusion criteria were patients aged 18 years and above with rheumatoid arthritis attending the ROPC and those who gave informed consent. Patients excluded included those with acute febrile illnesses, bleeding disorders, haematological conditions, patients known to have malignancies and RA with mixed connective tissue disease. The main outcome variables were clinical disease activity and platelet count. Clinical disease activity as per DAS28 scores was classified as follows: Remission≤ 2.6, Mild 2.6-3.2, Moderate >3.2-5.1 and High ≥ 5.1. Platelet count was graded as follows: Thrombocytopenia <150 × 10^9/l , Normal platelet 150–400 × 10^9/l , Mild thrombocytosis 400-600 × 10^9/l , Moderate thrombocytosis 650-800 × 1010^9/l , Marked thrombocytosis >800 × 10^9/l .

In the ROPC, all patients on follow up for RA were screened for recruitment into the study. The files of patients who met the inclusion criteria were selected and consecutively sampled for study. Of the eligible patients, informed consent was obtained from them to participate in the study. Once consent was given, history was taken, physical examination performed and blood collected from them for laboratory investigation as outlined below. The principal investigator obtained socio- demographic data which included age, gender, marital status, place of residence, and occupation from both the patients and/or the patients' records. Disease history obtained included duration of illness, when first diagnosed, whether on any treatment, response to treatment and any current concurrent illness. Physical examination was carried out to check for features of active RA. All joints were examined for swelling and tenderness. The number of joints swollen and/ tender was recorded on the DAS28 score sheet. The patient was asked to assess his/her general well being using the Visual Analog Scale (VAS) and this too recorded in the DAS28 score sheet.

Three millilitres of venous blood was drawn aseptically from the forearm and collected in an EDTA bottle for a full blood count and ESR estimation in consenting patients. The blood was analyzed using Abbot Cell Dyn 1300 in the Department of Pathology, Haematology unit University of Nairobi. The ESR was also carried out by the Wintrobe's method. The ESR level was then recorded in the DAS score sheet. The total DAS score was then calculated using the DAS28 calculator .

All data was collected on the study proforma and entered into a computer data base MS Access. Statistical analysis was done using Statistical Package for Social Scientists (SPSS) version 17.0 software. Continuous variables such as age, DAS 28 scores platelet counts and ESR are summarized into means, median, and ranges. Comparison of means was done using Student's t test for normally distributed data and Mann Whitney U test for non-normal data. Platelet count was correlated to DAS28 score using the spearman Rho coefficients. Bivariate analysis was done using the Man Whitney test, Pearson's chi-square or Fisher exact test. Multivariate analysis done by linear regression was used to determine the relationship between platelet count and DAS28 adjusting for various factors. Comparisons were considered statistically significant at a P value ≤ 0.05. Ninety five percent confidence limits were used as a measure of certainty. Results are presented in form of charts, graphs and tables. The study was carried out upon approval by the local ethics board.

Results

In a period of 6 months, among the patients attending ROPC, 104 patients with RA were identified. These were screened and recruited into the study. Most of the patients were aged between 40 to 59 years at 53.9% with a mean age of 48 years ± 14 and a median age of 49 years (18-79 years) and a male to female ratio of 1: 6.4. Most of the patients had been diagnosed with RA over the last 1 to 5 years at 43.3% while in 25 (24%) the diagnosis had been made over the last one year. Majority of the patients were on DMARDs at 78 (75%) while on 48 (46.2%) were on steroids. The most commonly used DMARD was methotrexate in 72(69.2%) of the patients while only one patient was on leflunomide

The platelet counts in the study population varied between 152 to 611 x10^9/L with a mean of 313.2 ±SD 94.0 and a median of 294.5 x10^9/L. Eighty nine (85.6%) had normal platelet counts while 15 (14.4%) [95% CI: 8.9-22.4] had thrombocytosis. No case of moderate (more than 650 x10^9/L) or severe thrombocytosis (>800 x10^9/L) was recorded. Among those with thrombocytosis 12(80%) were female and 3(20%) were male. Platelet counts of the study population are shown in Table 1.

Table 1: Platelet counts in study population

Platelet counts	No. (%)
Median	294 x10^9/L
Mean	313.2±94 x10^9/L
Range	152-611 x10^9/L
Normal platelet counts (150- 400 x 10^9/L	89 (85.6%)
Thrombocytosis (mild) Above 400 x 10^9/L	15 (14.4%)

Comparison of various parameters between those with thrombocytosis and those with normal platelet counts was done. Comparison between those with normal versus elevated platelet counts showed that the median DAS28 score was slightly lower in those with normal platelet scores at 4.2 versus 4.6 in those with thrombocytosis but insignificant (p 0.413). Differences in the median age and erythrocyte sedimentation rate (ESR) levels were also insignificant and P values of 0.715 and 0.185 respectively. Significant differences in the median of the haemoglobin levels, RBC indices and WBC counts were found between the two groups. Lower HB, MCV and MCH were associated with elevated platelet counts while higher WBC was associated with thrombocytosis (Table 2).

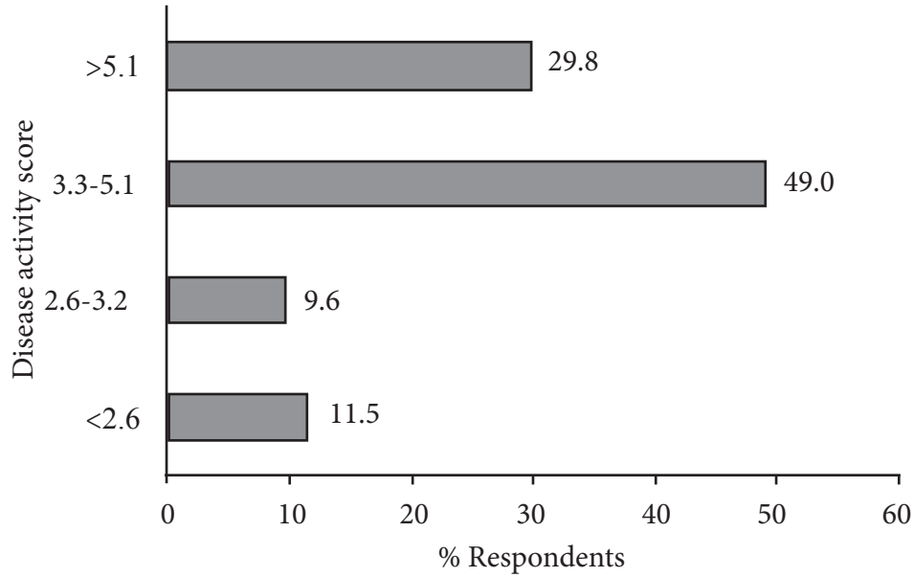
The significant values were then subjected to a multivariate analysis by linear regression as shown in Table 5. While haemoglobin lost significance MCV remained significant demonstrating that the MCV is independently associated with platelet levels as is WBCs.

Table 2: Comparison of various parameters in those with normal vs high platelet counts

Laboratory parameters	Platelet count (>400x10^9/L)		Platelets count (150- 400x 10^9/L)		
	No.	Median (Range)	No.	Median (Range)	P value
DAS28 Score	15	4.6 (2.2-7.1)	89	4.2 (1.7-8.4)	0.413
Age (years)	15	46.0 (25.0 - 76.0)	89	50.0 (18.0 - 79.0)	0.715
ESR(mm/hr)	15	40.0 (6.0-60.0)	89	35.0 (2.0-63.0)	0.185
HB(g/dl)	15	11.5 (4.4-13.9)	89	12.8 (7.4-16.3)	0.020
MCV(fl)	15	72.0 (56.0-87.0)	89	83.0 (55.0-101.0)	0.002
MCH(pg)	15	22.8 (15.6-28.9)	89	26.8 (16.0-34.0)	0.001
WBC x10^9/L	15	7.6 (4.8-13.6)	89	6.1 (3.0-12.2)	0.030

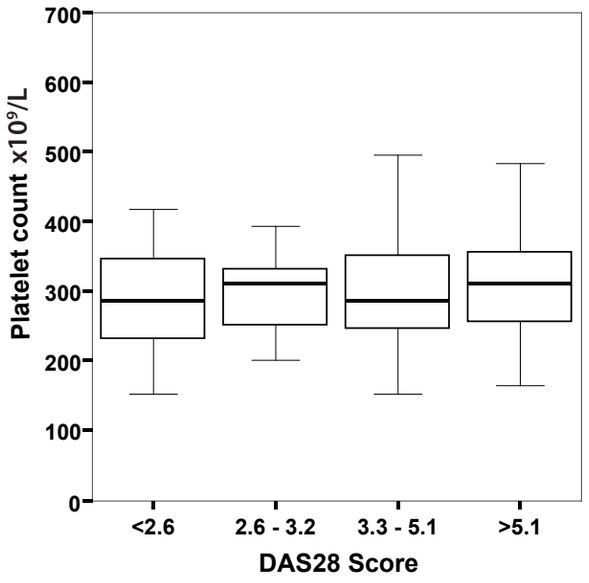
Regarding the clinical disease activity, the mean DAS28 score was 4.5 ± 1.5 with a median of 4.3 and a range of 1.7-8.4. Majority of the patients had moderate disease activity at 52 (49 %) while only 12 (11.5) % had their disease in remission. Thirty (29.8%) had high disease activity and 10 (9.6%) mild disease as shown in Figure 1.

Figure 1: Distribution of clinical disease activity.



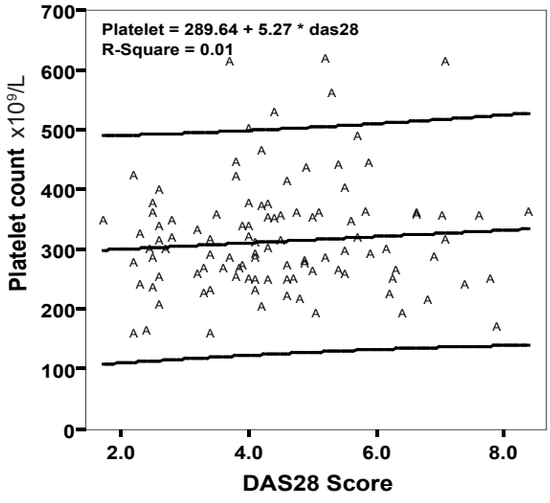
In terms of platelet counts for the various levels of disease activity,the median platelet count for those in remission was 285x 10^9/L, mild disease 310x10^9/L, 285x10^9/L for moderate disease and 311x10^9/L for those with high disease activity. This is best illustrated in Figure 3.

Figure 2: Boxplot of various DAS scores and respective platelet counts



Correlation between platelet counts and DAS28 scores revealed a correlation coefficient of r=0.084 which is statistically insignificant at p=0.394 as shown in the scatter plot below (Figure 3).

Figure 3: Correlation between plaletet counts and DAS28 scores



r = 0.084, p=0.394

Discussion

The mean platelet count in the study population was 313.2 ±94 x10⁹/L , a median of 294 x10⁹/L and a range of 152-611x 10⁹/L. These figures are much higher than platelet counts in normal healthy adults. In 1981 Mukibi *et al*[19] found a mean platelet count of 200 xl0⁹/L in healthy Kenyan adults. A more recent study by Rajab *et al*[20] on haematological parameters in healthy Kenyan blood donors found a mean platelet count of 241.2 ±86.6 x109/L and median of 235.1 x109/L. Bain[21] found even lower platelet counts of a mean of l83 xl09/L in black females and 207 x109/L in black males in a study comparing ethnic and gender differences in healthy adults though in a different set up. It can therefore be inferred from these previous studies that patients with RA have higher platelet counts. Our results are comparable to a Turkish study by Yacizi *et al* [22]who found mean platelets of 307±99 x109/L in patients with active RA compared to a mean of 258 ±58 x109/L in healthy controls .

It is worth noting that despite the significant use of DMARDs, no case of thrombocytopenia was recorded. The prevalence of thrombocytopenia in various studies ranges from 0.8 to 3.1% in patients using DMARDs[23,24]. Buhroo *et al*[24] in a study in India found only 2 out of 245 patients (0.8 %) had thrombocytopenia. This is close to our study that recorded no case of thrombocytopenia The consistent concurrent use of folate use in our patients may account for this finding as folate has been shown to reduce the adverse effects of methotrexate[25].

Thrombocytosis was found in only 15(14.4%) of the study population. Studies elsewhere have recorded higher prevalences of thrombocytosis. Hutchingsons *et al* [5] and Selroos[4] found prevalences of 51% and 33% respectively. Notably these studies were done in the 70s and 80s when use of DMARDs was not widespread. A higher prevalence of thrombocytosis in Caucasians could also be attributable to the fact that Caucasians have been shown to have higher levels of platelets compared to Africans in several studies[20,21]. Therefore a relatively lower baseline platelet count to start with will result to fewer cases of thrombocytosis when using the same cutoff as the Western studies despite a similar increase in platelet counts. A case control study in future may bring out these differences. A more recent Saudi Arabia study found a prevalence of 16%[6].This is comparable to the findings of this study. No similar study has been done in Africa to which the findings can be compared.

Most of the patients had active disease at 88.5% (DAS28 scores >2.6). The majority of these had moderate disease activity (49%) while 29.7% had high disease activity and only 9.6% had mild disease activity. This is probably because a significant number of the patients (24%) were diagnosed during the study period and had previously not been on treatment and therefore had high disease activity. Another possible explanation is that more aggressive treatment maybe needed for these patients such as anti-TNF antagonist or use of biological agents of which none of the patients was on. The study did not assess compliance to treatment which could also affect the levels of disease activity seen in the study population.

While comparing different parameters in those with thrombocytosis versus normal PCs, no significant difference was found in the median DAS28 scores between the groups i.e 4.6 versus 4.2 (p=0.413). This precludes any meaningful relationship between platelet counts and disease activity in this study. The Hb, MCV

and MCH were however noted to be significantly lower in the group with thrombocytosis at p of 0.20,0.002 and 0.001 respectively. This is similar to what other studies have reported. Hutchingsons *et al*[5] found higher platelet counts in those with lower Hb mean of 12.47(50.10) in platelets <450 x10^9/L and 11.27± 0.77 in >450 x10^9/L**.** The lower Hb was noted to be mainly microcytic hypochromic. This was confirmed by the multivariate analysis that confimed MCV to be an independent contributor to platelet counts. The microcytic anaemia is usually due to either iron deficiency anaemia(IDA) or less commonly anaemia of chronic disease (ACD). IDA is usually associated with a reactive thrombocytosis. ACD may also be associated with a reactive thrombocytosis secondary to chronic inflammation[11]. This can explain the association between thrombocytosis and low MCV. Further studies are needed to define the exact cause(s) of the low MCV/MCH in our study population.

Correlation between disease activity and platelet count revealed no significant correlation at p=0.394. These findings are in contrast to earlier studies by Hutchingsons *et al*[5] and Farr *et al*[9] who both found a positive correlation. However they used different measures of disease activity with Hutchingsons *et al*[5] including a presence of extraarticular manifestations, morning stiffnes and grip strength which are not assessed in the DAS 28 score. Farr *et al*[9] used the total articular index i.e summation of pain on movement, stiffness, swelling, heat and tenderness of each joint. Notably, the other studies had recorded higher levels of thrombocytosis to start with**.**

Our findings are more comparable to Yacizi's *et al*[22] who used the DAS score in assessment of disease activity**.** The study demonstrated a fall in mean platelet counts after a period of treatment but the platelet counts did not correlate with disease activity however.

In conclusion, patients with RA have relatively increased platelet counts compared to the general population in our black patients. While thrombocytosis was found in 14.4% of the patients with RA, no relationship was found between platelet counts and disease activity in patients with RA in this study.

References

1. Mitchell DM, Spitz PW, Young DY *et al*. Survival, prognosis, and causes of death in rheumatoid arthritis. *Arthritis Rheum.* 1986; **29**:706–714.
2. Sokka T, Mottonen T, Hannonen P. Mortality in early 'sawtooth' treated RA patients during the first 8-14 years. *Scand J Rheumatol.* 1999; **28**:282-287.
3. Gabriel SE, Crowson CS, O'Fallon WM. Mortality in RA: Have we made an impact in four decades? *J Rheumatol.* 1999; **26**:2529-2533.
4. Selroos O. Thrombocytosis in rheumatoid arthritis. *Scand J Rheumat.* 1972; **1**(3) 136-140.
5. Hutchingsons RM, Davis P, Jayson M. Thrombocytosis in rheumatoid arthritis. *Ann Rheum Dis.* 1976; **35**:138-142.
6. Dixon JS, Martin MF, Wright VA. Comparison of platelet count and acute phase proteins in measurement of disease activity in RA. *Rheumatology*. 1983; **22**(4):233-238.
7. Aisha AG, Suzan MA. Extra-articular manifestations of rheumatoid arthritis: A hospital-based study. *Ann Saudi Med.* 2009: **29** (3);189-193.
8. Garg SK, Amorosi EL, Karpatkin S. Use of megathrombocyte as an index of megakarocyte number. *New Eng J Med.* 1971; **284**:11.
9. Farr M, Scott T, Constable TJ. *et al* .Thrombocytosis of active RA. *Ann Rheum Dis.* 1983; **42**:545-549.
10. Farr M, Wainwright A, Salmon, M., *et al.* Platelets in the synovial fluid of patients with rheumatoid arthritis. *Rheumatol Int*. 1984; **4**(1):13-17.
11. Beguin Y. Erythropoeitin and platelet production. *Hematologica.* 1999; **84**(6):541-547.
12. Ertenil I, Kiras Z, Ozturk MA. *et al*. Pathological thrombopoeisis of RA. *Rheumatol Int*. 2003; **23**(2):49-60.
13. Haznedaroğlu IC, Ertenil I, Ozcebe OI. *et al*. Megakaryocyte-related interleukins in reactive thrombocytosis versus autonomous thrombocythemia. *Acta Haematol.* 1996; **95**(2):107-111.
14. Hollen CW, Henthorn J, Koziol JA. *et al.* Serum IL-6 levels in patients with thrombocytosis. *Leukemia Lymphoma*.1998; **8**(3):235-241.
15. Ceresa IF, Patrizia N, Chiara A. *et al*. Thrombopeitin is not uniquely responsible for thrombocytosis in inflammatory disorders. *Platelets.*2000; **18**(8):579-582.
16. Kiraz S, Ertenli I, Ozturk MA. *et al.* Bloodstream thrombopoeitin in rheumatoid arthritis with thrombocytosis. *Clinical Rheumatol.* 2002; **21**(6):453-456.
17. Milovanovic M, Nilsson E. Järemo P. Relationships between platelets and inflammatory markers in rheumatoid arthritis. *Clinica Chimica Acta*. 2004; **343**: 237-240.
18. Aiping LZ. Correlations among cartilage erosions, IgA levels, red bloodcell and platelet counts in 436 RA patients with path analysis. *Bioinformat Biomed Engineering.*2009; **6**:1-3.
19. Mukibi JM, Okelo GA, Kanja C. Platelet in normal Kenyan adults. *East Afr Med J.* 1981; **58**:136-139.
20. Rajab JA, Muchina WP, Orinda DA *et al*. Blood donor haematology parameters in two regions of Kenya. *East Afr Med J.* 2005; **82**: 123-127.
21. Bain JB. Ethnic and sex differences in the total and differential white cell count and platelet count. *J Clin Path.* 1996; **49**:664-666.
22. Yacizi S, Yacizi M., Erer B. The platelet indices in patients with rheumatoid arthritis. *Platelets*. 2010; **21**(2):122-125.
23. Franck H, Rau R. Herborn G. Thrombocytopenia in patients with RA on long term treatment with low dose methotrexate. *Clin Rheumatol.* 1997; **16**(4): 429-430.
24. Buhroo AM, Baba AN. Adverse effects of low dose methotrexate in patients with rheumatoid arthritis. *Indian J Physical Med Rehab.*2006; **17**(2): 21-25.
25. Hornung N, Ellingsen T, Stengaard-Pedersen K. *et al.* Folate, homocysteine, and cobalamin status in patients with rheumatoid arthritis treated with methotrexate, and the effect of low dose folic acid supplement. *J Rheumatol.* 2004;**31**(12):2374.