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Original Article

Pathology Section

Erythrocytes and Platelets: A Critical Analysis of their Ontogenic Relationship through Automated Parameters

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ABSTRACT

Introduction: Erythrocytes and platelets share an intimate development history, due to which special interest is centered on their relationship. Mature Red Blood Cells (RBCs) and platelets show a similar physiological rhythm, and are concurrently involved in numerous pathologic states.

Aim: To identify subtle relationships between various RBC and platelet parameters with an objective to analyse if a linear correlation co-exists between and among them in physiological/pathological states.

Materials and Methods: A prospective analysis of 1250 EDTA blood samples was conducted. The five RBC parameters (RBC count, HCT, MCV, RDW, Hb) and four platelet parameters (platelet count, PCT, MPV, PDW) obtained from each case were statistically analysed for linear dependence.

Results: A statistically significant direct linear relationship was found between RDW with platelet count and PCT. A statistically significant inverse linear relationship was noticed between the following parameters: i) HCT with platelet count and PCT; ii) MCV with platelet count and PCT; iii) Haemoglobin and platelet count.

Conclusion: A linear correlation, either direct or inverse, was seen among various parallel RBC and platelet parameters.

Keywords: Automation, Ontogeny, Platelet parameters, Red blood cell parameters

INTRODUCTION

The ontogeny of the human haematopoietic cells has been an area of constant research and immense interest. The three haematopoietic blood elements as we know today: the Red Blood Cells (RBC), White Blood Cells (WBC) and platelets are derived from a single Haematopoietic Stem Cell (HSC) [1]. One of the subsequent progenitors, the common myeloid progenitor in turn divides into Megakaryocyte/Erythroid Progenitor (MEP) and Granulocyte/Monocyte Progenitor (GMP). MEP gets further differentiated to form committed precursors, which give rise to RBC's and platelets [1].

Mature RBCs and platelets show a similar physiological rhythm and are concurrently involved in numerous pathologic states. Some of the features that are similar to both RBCs and platelets are: i) both erythrocytes and platelets develop from a common progenitor cell (MEP) [1]; ii) In the peripheral blood, both erythrocytes and platelets are in an anucleated form. Nucleated forms are present in the bone marrow and are seen in the peripheral blood only in diseased states, for example normoblasts in anaemia and micromegakaryocytes in myelodysplastic syndrome [2]; iii) Both the cells have an immature peripheral blood stage called as reticulocyte for RBCs and reticulated platelets for platelets [3]; iv) Erythropoietin, a cytokine growth factor has a significant effect on both the series of cells [4]; v) All the available haematology analysers determine the erythrocytes and platelet volume using the same aperture and the same dilution [5].

With remarkable advances in technology and advent of fully automated analysers, it has been easy to analyse various parameters of RBCs and platelets, such as RBC count, Haematocrit (HCT), Mean Corpuscular Volume (MCV), Red Cell Distribution Width (RDW), Platelet count, Plateletcrit (PCT), MPV and Platelet Distribution Width (PDW). Among the various advantages the fully automated multichannel haematological analysers offer, one of them is that, most of the parameters obtained through them have no manual equivalents. These parameters are often under-reported because of the lack of validated knowledge of their clinical utility.

This study was undertaken to identify linear co relationships between and among various RBC parameters (RBC count, HCT, MCV, RDW, Hb) and platelet parameters (Platelet count, PCT, MPV, PDW) in any given state – physiological or pathological and to determine their predictive value in certain pathological conditions.

MATERIALS AND METHODS

A prospective analysis of 1250 EDTA anticoagulated blood samples were conducted over duration of two months at the Department of Pathology, ESIC Medical College and PGIMSR, Bangalore, India. Random non-biased sampling technique was employed to select cases from patients attending hospital and for whom the treating physician advised a haematological investigation. The confidentiality of the patient details and ethical standards were maintained through out the study and only the relevant data was recorded in a proforma. This study was not subject to ethical clearance as only the automated analyser derived data was used for the study purpose. Since clinical indications and demographic data were not used for the study, the patients' confidentiality was not compromised.

Fully automated multichannel haematology analyser, SYSMEX XT 2000i was used for the study. All cases irrespective of age, sex and indication sent for complete haemogram were included in the study. Clinical and demographic details were not included to avoid bias and promote homogeneity and true random sampling in the population. A fixed number of samples were chosen from the daily samples by random selection.

Incomplete platelet parameters, spurious thrombocytopenia and cases with flags for suspected haematological malignancy were excluded from the study. Hence, out of 1250 samples, 1214 samples were selected for the study.

The following RBC parameters – RBC count, HCT, MCV, RDW and haemoglobin and platelet parameters – platelet count, PCT, MPV, PDW were noted. All the present haematological parameters were obtained through the Sysmex autoanalyser and validated as our haematology laboratory undergoes regular external quality control checks as a part of the National Quality Control Programme.

The five RBC and four platelet parameters obtained from each case were statistically analysed for linear dependence using the Pearson Correlation coefficient matrix. Statplus statistical analysis software for Mac was used for computation. High, medium and low correlation was considered when the r value (Pearson Coefficent value) was in the range of - 0.5 to – 1.0 or 0.5 to 1.0; -0.3 to -0.5 or 0.3 to 0.5; -0.1 to -0.3 or 0.1 to 0.3 respectively. Comparable parameters demonstrating direct or inverse relationships were also analysed for statistical significance. A p-value of < 0.05 was taken to be statistically significant.

RESULTS

Pearsons correlation coefficient value for various RBC and platelet parameters in a consolidated form are presented in [Table/Fig-1].

Upon computation of relationship of five RBC parameters with four platelet parameters, the following observations were noted [Table/Fig-2].

A statistically significant direct linear relationship was found between RDW with platlet count and PCT. A statistically significant inverse linear relationship was noticed between the following parameters: i)

Correlation Coefficients Matrix										
Sample size	1214	Critical value (5%) RBC count	1.96389 Hb	RDW	MCV	HCT	PLT Count	PDW	MPV	PCT
RBC count	Pearson Correlation Coefficient	1.								
	R Standard Error									
	t									
	p-value									
	H0 (5%)									
Hb	Pearson Correlation Coefficient	0.74263	1.							
	R Standard Error	0.00074								
	t	27.27544								
	p-value	0.E+0								
	H0 (5%)	rejected								
RDW	Pearson Correlation Coefficient	-0.33549	-0.58537	1.						
	R Standard Error	0.00147	0.00109							
	t	-8.75966	-17.75873							
	p-value	0.E+0	0.E+0							
	H0 (5%)	rejected	rejected							
MCV	Pearson Correlation Coefficient	-0.34584	0.28673	-0.26338	1.					
	R Standard Error	0.00146	0.00152	0.00154						
	t	-9.06602	7.36177	-6.71552						
	p-value	0.E+0	5.95524E-13	4.32552E-11						
	H0 (5%)	rejected	rejected	rejected						
HCT	Pearson Correlation Coefficient	0.81427	0.95578	-0.50877	0.22275	1.				
	R Standard Error	0.00056	0.00014	0.00123	0.00157					
	t	34.50284	79.94266	-14.53611	5.62022					
	p-value	0.E+0	0.E+0	0.E+0	0.					
	H0 (5%)	rejected	rejected	rejected	rejected					
Platlet Count	Pearson Correlation Coefficient	-0.01286	-0.16697	0.14049	-0.16319	-0.10458	1.			
	R Standard Error	0.00165	0.00161	0.00162	0.00161	0.00163				
	t	-0.31634	-4.16539	3.49026	-4.06861	-2.58646				
	p-value	0.75185	0.00004	0.00052	0.00005	0.00993				
	H0 (5%)	accepted	rejected	rejected	rejected	rejected				
PDW	Pearson Correlation Coefficient	0.05008	0.03708	0.01941	-0.03729	0.01969	-0.37022	1.		
	R Standard Error	0.00165	0.00165	0.00165	0.00165	0.00165	0.00143			
	t	1.23339	0.91262	0.47761	-0.91783	0.48451	-9.80289			
	p-value	0.21791	0.36181	0.6331	0.35907	0.6282	0.E+0			
	H0 (5%)	accepted	accepted	accepted	accepted	accepted	rejected			
MPV	Pearson Correlation Coefficient	0.02304	0.03765	-0.04958	0.00116	0.01519	-0.36493	0.89257	1.	
	R Standard Error	0.00165	0.00165	0.00165	0.00165	0.00165	0.00143	0.00034		
	t	0.56677	0.92676	-1.22097	0.02844	0.37366	-9.64109	48.68905		
	p-value	0.57108	0.35442	0.22257	0.97732	0.70879	0.E+0	0.E+0		
	H0 (5%)	accepted	accepted	accepted	accepted	accepted	rejected	rejected		
PCT	Pearson Correlation Coefficient	0.00558	-0.20275	0.22453	-0.22602	-0.12585	0.7729	-0.33356	-0.30466	1.
	R Standard Error	0.00165	0.00158	0.00157	0.00157	0.00163	0.00067	0.00147	0.0015	
	t	0.13723	-5.09281	5.66728	-5.70717	-3.12029	29.96106	-8.70282	-7.86771	
	p-value	0.89089	0.	0.	0.	0.00189	0.E+0	0.E+0	1.66533E-14	
	H0 (5%)	accepted	rejected	rejected	rejected	rejected	rejected	rejected	rejected	

[Table/Fig-1]: Pearsons correlation coefficient matrix – consolidated for all RBC and platelet parameters.

RBC Parameters	Platelet parameters							
	Platelet count		PCT		MPV		PDW	
	r value	Statistical significance	r value	Statistical significance	r value	Statistical significance	r value	Statistical significance
RBC Count	-0.01 (NC)	Not Significant	0.005 (PC)	Not Significant	0.02 (PC)	Not Significant	0.05 (PC)	Not Significant
RDW	0.14 (PC)	Significant	0.22 (PC)	Significant	-0.04 (NC)	Not Significant	0.01 (PC)	Not Significant
HCT	-0.10 (NC)	Significant	-0.12 (NC)	Significant	0.01 (PC)	Not Significant	0.01 (PC)	Not Significant
MCV	-0.16 (NC)	Significant	-0.22 (NC)	Significant	0.001 (PC)	Not Significant	-0.03 (NC)	Not Significant
Hb	-0.16 (NC)	Significant	-0.20 (NC)	Significant	0.03 (PC)	Not Significant	0.03 (PC)	Not Significant

[Table/Fig-2]: Relationship of five different RBC parameters with four different platelet parameters.
NC – Negative correlation; PC – Positive correlation.

HCT with platlet count and PCT; ii) MCV with platlet count and PCT; iii) Hb and platlet count. No association of statistical significance was seen between total erythrocyte count and any other platelet indices.

The observations derived from the present study also shows that among RBC parameters, a direct linear significant relationship between RBC count and Hb; and also between RBC count and HCT was seen. An inverse linear significant relationship between RBC count and MCV; and RBC count and RDW was also seen.

Among the platelet parameters, a direct linear significant relationship between platelet count and PCT, and an inverse linear significant relationship between platelet count and MPV; platelet count and PDW was seen. These well known observations validate the present study.

DISCUSSION

This study was based on the premise of the following two observations; one, undeniable ontogenic relationship between erythrocytes and megakaryocytes which is reflected in their maturation stages and two, RBCs and platelets are analysed in the same detector using hydrodynamic focusing and yield similar parallel comparable parameters.

Erythropoiesis and thrombopoiesis originate from a common bipotent haematopoietic stem cell–Colony Forming Unit –Erythrocyte megakaryocyte (CFU-EMk). This cell divides into divergent pathways and leads to the specific cell lines – the RBCs and the platelets [1]. During the maturation stages of an erythron, the normoblasts lose their nuclei, enter the peripheral circulation to form an immature RBC, known as reticulocyte and eventually develops into a mature RBC. Nucleated forms, named normoblasts are found only in the bone marrow and are seen in the peripheral circulation only in disease states, as in bone marrow damage, stress and potentially serious underlying disorders [6]. Reticulocytes give an estimate of erythropoiesis.

On the other hand, platelets are anucleate cytoplasmic fragments, which are derived from megakaryocytes – a nucleated cell. Platelets, like RBCs also have an immature stage in the circulation, known as reticulated platelet. Newly released platelets are called as reticulated platelets that retain residual RNA, analogous to red cell reticulocytes. Reticulated platelet counts give an estimate of thrombopoiesis and can be used in distinguishing platelet destruction syndromes from hypoplastic platelet production [1]. Nucleated forms of platelets–micromegakaryocytes are seen in the peripheral blood only in disease conditions such as myelodysplastic syndromes and myeloproliferative disorders [2,3].

Thrombopoietin and erythropoeitin belong to the same haematopoietic growth factor subfamily, are majorly produced in the kidney and similarly act by activating the JAK/STAT pathway and Ras signal transduction on their respective precursors [7].

Erythropoietin and thrombopoietin share a high degree of amino acid sequence homology (first 155 amino acids are common). Based on this theory, thrombocytosis in children with iron deficiency anaemia was explained by Bilic E et al., [8]. GATA-1, a transcription factor is expressed in primitive and definite erythroid and megakaryocytic cells and expression of both lineages are dependent on the presence of an intact GATA site [9].

The other noteworthy similarities between RBCs and platelets are that both are anucleate and lack mitochondria, both show mass changes in hyperdestructive and proliferative conditions. All the available haematology analysers determine the erythrocytes and platelet volume using the same aperture and the same dilution [5].

Only a handful of studies have been conducted stating the correlation of RBC and platelet parameters [Table/Fig-3] [10-12]. Wiwanitkit V studied plateletcrit, mean platelet volume and platelet distribution width, and defined their expected values and correlated the same with parallel red cell indices like haematocrit, mean corpuscular volume and red cell distribution width in 215 volunteers [10]. The authors demonstrated a significant correlation between PDW and RDW, whereas no significant correlation between PCT and HCT, MPV and MCV were seen. Saouli Z et al., also performed a study similar to Wiwanitkit V in 303 volunteers [11]. The author's results were similar to the study done by Wiwanitkit V. Kodikoylu G et al., studied platelet parameters in women with iron deficiency anaemia in 87 cases and concluded that an inverse relationship between PDW and MCV was noted [12].

The direct linear relationship between RDW and platelet count/plateletcrit is observed in inflammatory conditions, for example in slow

Study	Parameters compared	Inference
Wiwanitkit V [10]	PCT, MPV, PDW with parallel red cell parameters (215)	• No significant correlation between PCT and HCT, MPV and MCV. • A significant correlation between PDW and RDW.
Saouli Z [11]	PCT, MPV, PDW with parallel red cell parameters. (303 cases)	• No significant correlation between PCT and HCT, MPV and MCV. • A significant correlation between PDW and RDW.
Kadikoylu G [12]	Iron metabolism and thrombopoiesis. (87 cases)	• Inverse relationship between PDW and MCV.
Present study	Platelet count, PCT, MPV, PDW with parallel red cell parameters and Hb (1214 cases)	• Statistically significant direct linear relationship between RDW and platlet count; PCT. • Significant inverse linear relationship between HCT and platlet count, PCT. MCV and platlet count, PCT. Hb and platlet count, PCT.

[Table/Fig-3]: Comparison of present study with different studies [10-12].

coronary flow phenomenon, a subclinical inflammatory condition, where both PCT and RDW are independent predictors [13]. Similarly, in inflammatory bowel disease, a significant relationship between RDW and platelet count has been documented [14]. Higher RDW has also been shown to concurrently occur with higher platelet counts in advanced cancer stages, particularly in lung cancer [15].

An inverse linear relationship between MCV and plateletcrit is seen in inflammatory bowel disease [14]. Another important clinical implication of this inverse relationship would be in voluntary plateletpheresis. The AABB demonstrated that higher donor platelet counts reflected a decrease in MCV, thus projecting a higher likelihood of donor iron deficiency in high plateletpheresis yields [16]. An inverse linear relationship between Hb and platelet count is documented in multiple sclerosis [17], inflammatory bowel disease [14] and various other clinical conditions [18].

LIMITATION

An important limitation of the study was limitation related to the use of automated analysers. Only average values were quantified. Morphological scatter may be analysed for better correlation. Precision/reproducibility is much lesser for platelets than for red blood cells. However, on the brighter note, one of the highlights of the study was that already known facts showed a linear correlation, which validates the study.

CONCLUSION

Increasingly, numerous studies are demonstrating a significant association between routine haematological parameters and disease conditions. While most of these studies document similar relationships between RBC and platelet parameters as in the present study, they fail to elucidate in detail the cause for these subtle yet coincidental relationships. The present study demonstrates the inherent ontogenic association at play in these correlations; however, further in-depth evaluation is required to validate these results.

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REFERENCES

[1] Chow A, Frenette PS. Origin and Development of Blood Cells. In:Greer JP, Arber DA, Glader B, List AF, Means RT Jr, Paraskevas F, et al. Wintrobe's Clinical Haematology. 13th ed. Philadelphia: Lippincott Williams and Wilkins; 2014. pp 65-82.
[2] Gracia-Manero G. The Myelodysplastic Syndromes. In: Greer JP, Arber DA, Glader B, List AF, Means RT Jr, Paraskevas F, et al. Wintrobe's Clinical Haematology. 13th ed. Philadelphia: Lippincott Williams and Wilkins; 2014. pp. 1673-87.
[3] Smock KJ, Perkins SL. Examination of Blood and Bone Marrow. In: Greer JP, Arber DA, Glader B, List AF, Means RT Jr, Paraskevas F, et al. Wintrobe's Clinical Haematology. 13th ed. Philadelphia:Lippincott Williams and Wilkins; 2014. pp. 1-18.
[4] Stohlawetz PJ, Dzirlo L, Hergovich N, Lackner E, Mensik C, Eichler HG, et al. Effects of erythropoietin on platelet reactivity and thrombopoiesis in humans. Blood. 2000;95(9):2983–89.
[5] Fujimoto K. Principles of measurement in haematology analysers manufactured by sysmex corporation. Sysmex J Int. 1999;9:31-44.
[6] Constantino B, Cogionis B. Nucleated RBCs–significance in the peripheral blood film. Lab Med. 2000;31(4):223-29.
[7] Munker M, Hiller E, Glass J, Paquette R. Modern Haematology 2nd ed. New Jersey: Humana Press; 2007.
[8] Bilic E, Bilic E. Amino acid sequence homology of thrombopoietin and erythropoietin may explain thrombocytosis in children with iron deficiency anaemia. J Pediatr Haematol Oncol. 2003;25(8):675–76.
[9] Ferreira R, Ohneda K, Yamamoto M, Philipsen S. GATA1 Function, a paradigm for transcription factors in haematopoiesis. Mol Cell Biol. 2005;25(4):1215-27.
[10] Wiwanitkit V. Plateletcit, mean platelet volume, platelet distribution width; its expected values and correlation with parallel red blood cell parameters. Clin Appl Thromb Hemost. 2004;10(2):175-78.
[11] Saouli Z, Kaiafa G, Girtovitis F, Kontoninas Z, Ntaios G, Charisopoulos G, et al. Correlation of red blood cell and platelets parameters. Blood. 2007;110(11):3764.
[12] Kodikoylu G, Yavasoglu I, Bolaman Z, Senturk T. Platelet parameters in women with iron deficiency anaemia. J Natl Med Assoc. 2006;98(3):398-402.
[13] Akpinar I, Sayin MR, Gursoy YC, Aktop Z, Karabag T, Kucuk E, et al. Plateletcrit and red cell distribution width are independent predictors of the slow coronary flow phenomenon. Journal of Cardiology. 2014;63(2):112-18.
[14] Arhan M, Onal IK, Tas A, Kurt M, Kalkan IH, Ozin Y, et al. The role of red cell distribution width as a marker in inflammatory bowel disease. Turk J Med Sci. 2011;41(2):227-34.
[15] Koma Y, Onishi A, Matsuoka H, Oda N, Yokota N, Matsumoto Y, et al. Increases red blood cell distribution width associated with cancer stage and prognosis in patients with lung cancer. PloS One. 2013;8(11):e80240.
[16] Vinsett EM. The Evaluation of Platelet Count as an Indicator of Iron Status in Voluntary Plateletpheresis Donors. AABB. Available from https://www.aabb.org/development/scholarships/Documents/15vinesett.pdf.
[17] Hon GM, Hassan MS, VanRensburg SJ, Erasmus RT, Matsha T. The haematologival profile of patients with mutliple sclerosis. Open Journal of Modern Neurology. 2012;2:36-44.
[18] Shah AR, Chaudhari SN, Shah MH. Role of platelet parameters in diagnosing various clinical conditions. National Journal of Medical Research. 2013;3(2):162-65.

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