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Thrombocytosis and megakaryocyte changes associated with PRCA

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Abstract:

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Acquired pure red cell aplasia (PRCA) is a rare hematological disorder that results from failure of erythropoiesis [1, 2] and can be distinguished from other bone marrow failure disorders by reticulocytopenia and normal granulopoiesis and megakaryopoiesis[3, 4]. PRCA is often idiopathic and

likely due to cytotoxic T-cell mediated destruction of early erythroid precursors. Indeed, it can be associated with conditions such as T-cell Large Granular Lymphocytic (T-LGL) Leukemia, B-cell dyscrasia, thymoma, immunodeficiency, and infections[5-8].

Thrombocytosis refers to the abnormal elevation in platelet count of >450,000/µl. This condition can be related to a primary process, usually associated with a myeloproliferative neoplasm known as essential thrombocythemia, but more commonly presents as an epiphenomenon of other causes that include iron deficiency, chronic inflammatory conditions, and asplenia[9, 10]. Thrombocytosis has not been associated with PRCA. Herein, we report a cohort of PRCA patients with thrombocytosis that have not been defined in the literature.

A retrospective analysis was conducted on patient records diagnosed with PRCA at the University of Texas Southwestern and Cleveland Clinic Foundation between 2000 and 2022. The primary objective was to investigate the presence of thrombocytosis and/or megakaryocyte changes in these PRCA cases. Clinical, laboratory, and molecular data were abstracted, adhering to the guidelines established by the Declaration of Helsinki and the respective participating institutions.

Among a total of 90 patients, comprehensive analysis of 27 cases were identified as having acquired PRCA with thrombocytosis and/or megakaryocyte changes noted on bone marrow biopsies (Table 1) examined.To assess treatment efficacy, hemoglobin and platelet count before and after resolution of PRCA were compared. A Shapiro-Wilk test was used to assess normality. For normally distributed data, a paired t-test was performed. For non-normally distributed data, a Wilcoxon matched-pairs signed rank test was performed. For all relevant comparisons, a p-value < 0.05 was used to set statistical significance.

In a cohort of 90 PRCA patients, we identified 27 individuals with concurrent thrombocytosis and/or megakaryocyte changes (Table 1).

Of the 27 patients, the mean hemoglobin count at diagnosis was 7.6 +/- 0.7 g/dL, and the mean platelet count at diagnosis was 389,000 ± 80,400 /µL. 51.9% were female. 20 demonstrated megakaryocyte hyperplasia, 4 demonstrated hyper-lobation, and 1 displayed megakaryocyte dysplasia. Three patients tested positive for parvovirus on polymerase chain reaction , one patient had Chronic Lymphocytic Leukemia , and ten patients had LGL leukemia.

To delve deeper into the potential mechanisms linking PRCA and thrombocytosis, we then examined clinical variables in patients exhibiting thrombocytosis (n=7) following clinical response of their PRCA. This was defined as the achievement of a hemoglobin level > 9 g/dL with red blood cell transfusion independency. We excluded four patients due to unresolved hemoglobin levels or insufficient chart information. Bone marrow biopsies at the time of resolution were not available for any of the examined patients, as this procedure is generally not clinically indicated at this timepoint.

Among the subset of 7 patients with PRCA resolution, the mean hemoglobin count at diagnosis was 8.5 ± 1.4 g/dL, and the mean platelet count was 576,300 ± 71,800 platelets/µL. Post-resolution of PRCA, the mean hemoglobin count increased to 11.3 ± 1.3 g/dL, and the mean platelet count decreased to 431,600 ± 78,600 platelets/µL. Statistical analysis revealed significant differences in mean hemoglobin and mean platelet count before and after resolution, with p-values of 0.0225 and 0.0120, respectively. Interestingly, two of seven patients still exhibited thrombocytosis even after resolution of their PRCA.

Next, the mean EPO levels of those patients with thrombocytosis were examined. Of the 7 patients who had EPO levels drawn at the time of diagnosis, all had levels well over the upper limit of normal (26 mU/mL), 2476.5 +/- 2784.7 (range 72.4-10815) mU/mL.

In this study, we provide a detailed characterization of a cohort of patients with PRCA exhibiting notable alterations in megakaryocytes. Our cohort is heterogenous, and the etiology of each patient's PRCA is different. Therefore, there may not be one mechanism by which PRCA is linked to thrombocytosis. However, we theorize that there are two potential mechanisms that may explain the phenomenon in some cases.

We first hypothesize that the scarcity of erythroid production may redirect hematopoietic precursors towards the megakaryocytic lineage. The process of hematopoiesis, encompassing both erythroid and megakaryocyte lineages arising from a bipotential MEP, is well-documented[11-15]. We suggest that the interruption of erythroid differentiation at a critical stage may funnel differentiation towards the megakaryocytic lineage (Figure 1, I). Our findings lend support to this hypothesis, as the initial thrombocytosis observed prior to treatment resolves upon PRCA resolution with treatment.

A similar phenomenon has been reported in the context of iron-deficient anemia leading to secondary thrombocytosis, albeit the precise underlying mechanism remains elusive[16]. One proposed mechanism posits that once the hematopoietic growth factors and cytokines required for erythrocyte development become available, differentiation of the MEP cell veers away from the megakaryocyte lineage and reverts towards the erythroid lineage. This skewing may be attributed to heightened MKL1 expression[14], transcription factors determining the megakaryocyte lineage [18], or an increase in thrombopoietin, stem cell factor, stromal-derived factor 1, or cytokines known to exert thrombopoietic effects[18]. Additionally, one study found that low iron in the bone marrow environment can bias MEP differentiation towards the megakaryocyte lineage via a reduction in ERK signaling[19]. It is plausible that one or more of these mechanisms may account for the PRCA-related thrombocytosis we have observed, although further research is warranted for a comprehensive understanding.

In our cohort, many patients for whom EPO levels were measured at the time of PRCA diagnosis exhibited levels well above the normal range. Therefore, we hypothesize that EPO may play a role in the development of thrombocytosis (Figure 1, II). EPO signaling has been shown to have a synergistic effect with TPO on thrombopoiesis[20]. A study involving TPO-knockout mice demonstrated that EPO exerts a direct and TPO-independent influence on late-stage thrombopoiesis, resulting in increased production of large platelets [21]. Furthermore, several human studies in healthy volunteers, uremic patients, and chronic liver disease patients have reported significant short-term increases in platelet count following EPO injections[22-25]. Thus, it is conceivable that, in certain PRCA patients, synergistic signaling between EPO and TPO may be occurring upstream at the level of the bipotential MEP cell, leading to increased megakaryocyte production and thrombocytosis.

The implications of thrombocytosis and megakaryocyte alterations in the context of PRCA remain enigmatic, necessitating comprehensive datasets to unravel their role in the pathophysiology of the disease and their potential as prognostic markers for treatment response. It is important to acknowledge the limitations of this study, including a modest sample size secondary to the rarity of the disorder, and the absence of post-treatment bone marrow biopsy results, which would have provided visual confirmation of resolved megakaryocyte abnormalities.

Our findings in aggregate suggest that perhaps the definition of PRCA be expanded to include those with megakaryote changes or thrombocytosis. Further research is warranted to establish a definitive correlation and assess whether the course and response of PRCA to standard immunosuppressive

treatment differ in cases with and without thrombocytosis. Future investigations should also encompass an examination of megakaryocyte hyperplasia and other abnormalities, such as hyper- and hypo-lobation, associated with PRCA.

This study was approved by the institutional review boards of both the University of Texas Southwestern Medical Center and Cleveland Clinic Foundation. Clinical, laboratory, and molecular data were meticulously abstracted, adhering to the guidelines established by the Declaration of Helsinki and the respective participating institutions.

Author Contributions

J.A., G.R.W and T.B. generated and conceived the study design. J.A., G.R.W, G.R., M.M., Y.O., W.C., and T.B. contributed to the table and manuscript; C.G., H.A., and J.P.M. reviewed the clinical data, took part in patients' selection and helped with writing the manuscript; J.A., G.R.W, G.R., M.M., Y.O., H.A., M.O., W.C., C.G., J.P.W., and T.B. reviewed clinical data and contribute to writing of this manuscript. All authors participated in data interpretation and critical review of the final paper and submission. All authors have read and agreed to the published version of the manuscript.

Disclosure of conflicts of interest

The authors declare no conflicts of interest.

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Tables

Table 1: Cohort of patients with PRCA

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	Megakaryocyte changes only	*Thrombocytosis only*	*Both*	Megakaryocyte changes and/or thrombocytosis	*Neither (PRCA in the absence of thrombocytosis or megakaryocyte changes)*	**Total**
Number of patients	16	4	7	27	63	90
Mean age at diagnosis	57.6 +/- 8.3 (33.8-82)	68 +/- 16.1 (45.3-84.3)	53.5 +/- 11.2 (28-73.9)	58.1 +/- 6.2 (28-84.3)	56.7 +/- 5.1 (0-85)	57.1 +/- 4 (0-85)
Patients with BM Megakaryocyte changes						
• Dysplasia	1	0	0	1	0	1
• Hyper-lobation	3	0	1	4	0	4
• Hyperplasia	13	0	7	20	0	20
• Total with any changes	16	0	7	23	0	23
Mean hemoglobin at diagnosis, g/dL	7.6 +/- 0.9 (3.5-9.6)	6.4 +/- 1.3 (4.5-7.4)	8.3 +/- 1.6 (5.1-12.4)	7.6 +/- 0.7 (3.5-12.4)	7.7 +/- 0.6 (2.6-11.7)	7.6 +/- 0.5 (2.6-12.4)
Mean platelet count, 100,000 platelets/µL	232.7 +/- 70.3 (9-440)	550.8 +/- 96.6 (476-695)	564.7 +/- 59.2 (472-693)	389 +/- 80.4 (9-695)	254.2 +/- 26.7 (45-432)	299.1 +/- 35.2 (9-695)
Mean ferritin at diagnosis, µg/L	2309.4 +/- 1750.2 (648.4-6579)	2254.9 +/- 2350.7 (434.1-5505)	705 +/- 310.1 (315-1569)	1635.9 +/- 861.5 (315-6579)	2021 +/- 583.6 (50.1-7597)	1899.8 +/- 481 (50.1-7597)
Mean WBC at diagnosis, 1,000 WBC/µL	8.1 +/- 2.7 (3.9-18.8)	8.3 +/- 0.7 (7.8-9.4)	8 +/- 1.8 (3.3-10.3)	8.1 +/- 1.5 (3.3-18.8)	5.6 +/- 0.7 (2-12.6)	6.4 +/- 0.7 (2-18.8)
Female (% of total)	6 (37.5%)	2 (50%)	6 (85.7%)	14 (51.9%)	29 (46.0%)	43 (47.8%)
Mean bone marrow cellularity, %	55.3 +/- 10.9 (25-90)	53.8 +/- 26.9 (40-95)	73.6 +/- 8.2 (50-80)	60 +/- 8.2 (25-95)	39.6 +/- 5.4 (5-90)	1973.2 +/- 745.8 (46.6-11560)
Next generation sequencing findings	•STAT3 p.S614R c.1840A>C 8 •IDH1, SETBP1	•SF3B1 p.K666N 18.21% •BCOR p.1252_1253del 9%	• c.490G>T p.G164C 43.2%, c.1292C>T p.P431L 41.7%, c.6460G>A p.D2154N 41.4%, c.3974A>G p.K1325R 40.6%, c.588_589insACCCGC p.P196_P197insTR 13.0% •NF-kappaB2, JAK2, TYK 2 •c.490G>T p.G164C 43.2%, c.1292C>T p.P431L 41.7%, c.6460G>A p.D2154N 41.4%, c.3974A>G p.K1325R 40.6%, c.588_589insACCCGC p.P196_P197insTR 13.0%		•SPTB, SPTA1, EPB42 •TET2 p.N275lfs* 4.3% •RPS19, HFE C282Y, H63D •PB, ASXL1, U2AF1 •ASXL1, JAK2, STAT3, U2AF1 VUS •STAT3 p.D661Y VAF 12.7% • ASXL1 p.K912Q c.2734A>C VAF 50.3% and PTPN11 p.K131R c.392A>G 51.1% VAF •N6471 mutation in STAT3 gene	
Mean absolute reticulocyte count at diagnosis, reticulocytes/µL	2.924 +/- 3.162 (0.01-10)	0.013 +/- 0.004 (0.011-0.015)	3.685 +/- 3.322 (0.007-8.9)	2.84 +/- 1.987 (0.007-10)	1.45 +/- 0.884 (0-7.1)	1.894 +/- 0.881 (0-10)
Mean reticulocyte percent at diagnosis, %	0.684 +/- 0.647 (0.3-2)	0.543 +/- 0.283 (0.3-0.8)	0.263 +/- 0.084 (0.04-0.4)	0.459 +/- 0.231 (0.04-2)	0.5 +/- 0.191 (0.1-2.1)	0.487 +/- 0.149 (0.04-2.1)
Mean erythropoietin level, mU/mL	748.6 +/- 532.1 (46.6-1646)	2012 (n=1)	2553.9 +/- 3290 (72.4-10815)	1679 +/- 1542.6 (46.6-10815)	2105 +/- 845.3 (91-11560)	1973.2 +/- 745.8 (46.6-11560)

Parvovirus B19	0	2	1	3	3	6
Chronic Lymphocytic Leukemia	0	1	0	1	1	2
Large granular lymphocyte leukemia	4	2	4	10	15	25

Table 2: Subset of 7 patients who had resolution of transfusion dependency

VARIABLE	MEAN BEFORE RESOLUTION ± CI	MEAN AFTER RESOLUTION ± CI	P
Platelet count, 100,000 platelets/μL	576.3 ± 71.8	431.6 ± 78.6	0.0120
Hemoglobin, g/dl	8.5 ± 1.4	11.3 ± 1.3	0.0225

Figure legends

Figure 1: **Proposed mechanism for PRCA-related thrombocytosis**. Abbreviations: MPP- multipotent progenitor; CFU- colony-forming unit; BFU- burst-forming unit; GEMM- granulocyte-erythrocyte-monocyte-megakaryocyte. GM- granulocyte-monocyte; Eo- eosinophil; E- erythrocyte; MEG-megakaryocyte; MEP- megakaryocyte-erythrocyte progenitor. EPO- Erythropoietin. TPO-Thrombopoietin. **(I)** Proposed mechanism I: Decreased erythropoiesis shunts differentiation toward megakaryocyte lineage. **(II)** Proposed mechanism II: Increased EPO due to PRCA leads to synergistic signaling between EPO and TPO at the bipotential MEP, stimulates TPO receptors and resulting in increaseds megakaryocyte formation. Created with BioRender.com.