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Low COAT platelets are frequent in patients with bleeding disorders of unknown cause (BDUC) and can be enhanced by DDAVP

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To the Editor,

A large proportion of patients investigated for a mild bleeding tendency remain without a meaningful "diagnostic label" even after comprehensive and repeated laboratory testing [1, 2]. They are currently classified as having a "bleeding disorder of unknown cause" (BDUC) [3] and manifest a predominantly mucocutaneous bleeding diathesis, similarly to patients diagnosed with mild bleeding disorders. Thus, BDUC patients represent a diagnostic and treatment challenge [2-4].

Accurate quantification of the bleeding phenotype by a validated bleeding assessment tool (BAT) and the clinical gestalt is critical for determining the extent and depth of stepwise laboratory investigations [3]. We propose that in selected cases, these should include evaluation of procoagulant COAT-platelets by flow cytometry analysis (FCA) [5]. These platelets appear upon combined activation by COllagen And Thrombin, are coated by α-granule proteins retained on their surface by a serotonin- and transglutaminase dependent mechanism [6], and are highly efficient in sustaining thrombin generation [7]. They are also named "coated" platelets [8] and represent a phenotype of procoagulant platelets [9].

It is increasingly recognized that procoagulant platelets constitute a pathophysiological relevant component of the hemostatic response [10, 11]. They are generated under static conditions by the combined action of collagen and thrombin, and by collagen alone under high shear [9]. The procoagulant activity develops, after a transient activation of the fibrinogen receptor [6, 12, 13], in those platelets that are capable of increasing their intracellular free calcium into the micromolar range [13, 14], depolarize mitochondria [15], and express negatively charged phospholipids on their surface [7]. These platelets are characterized by a down-regulated fibrinogen receptor [6, 12, 13], a ballooned appearance [16], and are highly procoagulant [7].

According to our experience, 20-25% of patients with a high bleeding score but a negative standard laboratory work-up (thus satisfying the definition of BDUC [3]), have an impaired ability to generate procoagulant COAT-platelets. In our first cohort (01.2007–12.2011), we observed low levels of COAT-platelets (<20%) in 24% (n=16) of 67 BDUC patients with a clinically significant bleeding diathesis and normal or non-diagnostic light transmission aggregometry (LTA) [5]. Ad hoc analysis of a second cohort (01.2012–03.2017), revealed low COAT-platelets (<20%) in 19% (n=10) out of 53 BDUC patients [17].

Here, we present original data from our third cohort (01.2015 – 09.2019). Among 123 adult patients with an unexplained elevated ISTH-BAT score or bleeding gestalt investigated for a suspected platelet disorder after exclusion of a plasmatic coagulation defect, 37 (30%) had normal (n=23) or non-diagnostic (n=14) LTA and lumiaggregometry (LA), according to established criteria [18-20]. Platelets from these BDUC patients [3] were further characterized by FCA. Confirming our previous results, we observed a decreased ability to generate COAT-platelets (<25%) in 27% (n=10): in 4 out of 14 with non-diagnostic LTA and LA, and in 6 out of 23 with a normal workup. Overall, in our three cohorts we found 36 patients with decreased procoagulant COAT platelets among 157 patients provisionally classified as having BDUC, suggesting a prevalence of 23% (95%CI 20-27%).

We believe that it is clinically relevant to identify patients with a decreased ability to generate procoagulant COAT-platelets because of at least four reasons. First, it is reassuring for the patient and physician to know the reason for an increased bleeding tendency. Second, these patients represent up to 25% of those otherwise diagnosed with BDUC [5, 17]. Third, COAT-platelets are clinically relevant; e.g., Prodan and Dale showed that patients with low procoagulant platelets had increased mortality after a hemorrhagic stroke [21] and an early hemorrhagic transformation after an ischemic stroke [22]. Fourth, it is possible to modulate the individual amount of COAT-platelets [23].

In patients with primary platelet function defects (PFD), we have demonstrated that desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) is able to selectively increase procoagulant COAT-platelets [23]. This observation has been replicated in patients in whom DDAVP was given because of increased bleeding during cardiac surgery [24] and is confirmed by our current experience. In the period from January 2015 to August 2021, we have electively tested DDAVP in 53 adult patients, including primary PFD, von Willebrand disease, or BDUC, finding a relative increase of ≥5% COAT-platelets in 83% (n=44) of them, as observed in our previous cohort [23]. Currently, we define DDAVP-response more stringently as a relative COAT-platelet increase of ≥10% at one or more time points after DDAVP. This was the case in 74% (n=39) of all patients and 85% (n=17) of 20 with low COAT-platelets at baseline previously considered as BDUC. Table 1 highlights that these patients with low procoagulant COAT-platelets had a clinically significant bleeding diathesis as expressed by their ISTH-BAT score. Among the 17 patients who responded, COAT-platelets progressively increased up to 6 hours after DDAVP administration.

The effect of DDAVP on the procoagulant activity of platelets appears to be mediated by increased mobilization of intracellular free sodium (Na^+) and subsequently calcium (Ca^{2+}) [16, 23]. Upon activation, while all platelets initially increase their cytosolic Ca^{2+} [13, 25], only the subset that activates the reverse mode of the sodium/calcium exchanger [26], which extrudes Na^+ facilitating additional Ca^{2+} influx, will be able to depolarize mitochondria, further increasing cytosolic Ca^{2+} and eventually activating the transmembrane protein TMEM16F [27]. This culminates in the transfer of phosphatidylserine and phosphatidylethanolamine from the inner layer of the cell membrane to the outer one [27].

The mechanism modulating platelet procoagulant activity appears to be different from the one underlying DDAVP-induced increase of von Willebrand factor (VWF) and factor VIII (FVIII), which depends on cyclic AMP and protein kinase A [28]. This is nicely supported by following clinical observation. A 48-year-old woman was referred to our consultation to investigate a clinically significant bleeding diathesis, with an increased ISTH-BAT bleeding score (8 points). Laboratory evaluation showed normal results for VWF, coagulation factors, and fibrinolysis. Repeated LTA/LA were consistent with a signaling defect of the thromboxane A2 receptor. FCA [5] showed an impaired secretion of α-granules and a decreased percentage of procoagulant COAT-platelets (17-23%; reference range: 25-55%, Adler et al. manuscript submitted).

We performed an elective DDAVP-test with the standard dose of 0.3 µg/kg intravenously. VWF, FVIII, and COAT-platelets increased by 46%, 51%, and 35% after 2 hours, respectively, by 41%, 36%, and 56% after 4 hours, and by 32%, 21%, and 137% after 6 hours, whereas sodium remained within normal limits. Nevertheless, the patient did not tolerate DDAVP, which had caused an intense headache for three days. Because of the clinically relevant bleeding diathesis, the decreased ability to generate procoagulant platelets, and the excellent response observed after the canonical dose [23], we tested DDAVP at half-dose (0.15 µg/kg) subcutaneously (which is better tolerated than intravenously). Unfortunately, the patient described the same symptoms. Interestingly, while VWF/FVIII remained unaltered, COAT-platelets increased again by 15%, 35% and 41% after 2, 4, and 6 hours, respectively. The discordant response of COAT-platelets and VWF/FVIII to low DDAVP is consistent with different mechanisms modulating the development of platelet procoagulant activity [27] and the secretion of VWF from Weibel-Palade bodies [28]. From a practical point of view, this observation may be relevant for patients with a platelet-dependent bleeding diathesis and already high-normal or increased VWF/FVIII values, with a relative contraindication to DDAVP. Further studies should prospectively verify this hypothesis.

In conclusion, we confirm that low procoagulant COAT-platelets are frequently found in BDUC patients and we show that DDAVP can enhance their generation. Based on this evidence, we suggest that patients with a clinically relevant bleeding diathesis (based on ISTH-BAT score and/or clinical gestalt) and a negative laboratory work-up (including LTA and evaluation of dense granule content/secretion), as indicated by Baker and O' Donnell [3] could be investigated by FCA in order to assess their ability to generate COAT-platelets [5]. Conceptually, this is relevant because procoagulant platelet activity is a known endpoint of platelet activation, differing from and complementing their ability to aggregate [9-11] – and not detected by conventional platelet function studies [5]. Clinically, because the proportion of individuals with a decreased ability to generate procoagulant COAT-platelets among BDUC patients is relevant [5, 17] and because platelet procoagulant activity can be selectively improved by DDAVP [23].

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Author Contributions

A.S. was in charge of patient care and wrote the manuscript. M.A. was in charge of patient care, performed research, and wrote the manuscript. A.A. performed research and wrote the manuscript. E.M-G. Performed research. M.N. performed research. D.B.C. performed research. F.G. was in charge of patient care. F.J.G. performed research. L.A. supervised clinical and laboratory work, wrote the manuscript. All authors contributed to and approved the final version of the manuscript.

Declaration of competing interest

The authors have no conflict of interest to declare.

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References

1 Quiroga T, Mezzano D. Is my patient a bleeder? A diagnostic framework for mild bleeding disorders. *Hematology Am Soc Hematol Educ Program*. 2012; **2012**: 466-74. 10.1182/asheducation-2012.1.466.

2 Mezzano D, Quiroga T. Diagnostic challenges of inherited mild bleeding disorders: a bait for poorly explored clinical and basic research. *J Thromb Haemost*. 2019; **17**: 257-70. 10.1111/jth.14363.

3 Baker RI, O'Donnell JS. How I treat bleeding disorder of unknown cause. *Blood*. 2021; **138**: 1795-804. 10.1182/blood.2020010038.

4 Thomas W, Downes K, Desborough MJR. Bleeding of unknown cause and unclassified bleeding disorders; diagnosis, pathophysiology and management. *Haemophilia*. 2020; **26**: 946-57. 10.1111/hae.14174.

5 Daskalakis M, Colucci G, Keller P, Rochat S, Silzle T, Biasiutti FD, Barizzi G, Alberio L. Decreased generation of procoagulant platelets detected by flow cytometric analysis in patients with bleeding diathesis. *Cytometry Part B, Clinical cytometry*. 2014; **86**: 397-409. 10.1002/cyto.b.21157.

6 Dale GL, Friese P, Batar P, Hamilton SF, Reed GL, Jackson KW, Clemetson KJ, Alberio L. Stimulated platelets use serotonin to enhance their retention of procoagulant proteins on the cell surface. *Nature*. 2002; **415**: 175-9.

7 Alberio L, Safa O, Clemetson KJ, Esmon CT, Dale GL. Surface expression and functional characterization of alpha-granule factor V in human platelets: effects of ionophore A23187, thrombin, collagen, and convulxin. *Blood*. 2000; **95**: 1694-702.

8 Dale GL. Coated-platelets: an emerging component of the procoagulant response. *J Thromb Haemost*. 2005; **3**: 2185-92. 10.1111/j.1538-7836.2005.01274.x.

9 Agbani EO, Poole AW. Procoagulant platelets: generation, function, and therapeutic targeting in thrombosis. *Blood*. 2017; **130**: 2171-9. 10.1182/blood-2017-05-787259.

10 Heemskerk JW, Mattheij NJ, Cosemans JM. Platelet-based coagulation: different populations, different functions. *J Thromb Haemost*. 2013; **11**: 2-16. 10.1111/jth.12045.

11 Mazepa M, Hoffman M, Monroe D. Superactivated platelets: thrombus regulators, thrombin generators, and potential clinical targets. *Arterioscler Thromb Vasc Biol*. 2013; **33**: 1747-52. 10.1161/ATVBAHA.113.301790.

12 Kulkarni S, Jackson SP. Platelet factor XIII and calpain negatively regulate integrin alphaIIbbeta3 adhesive function and thrombus growth. *J Biol Chem*. 2004; **279**: 30697-706. 10.1074/jbc.M403559200

13 Alberio L, Ravanat C, Hechler B, Mangin PH, Lanza F, Gachet C. Delayed-onset of procoagulant signalling revealed by kinetic analysis of COAT platelet formation. *Thromb Haemost*. 2017; **117**: 1101-14. 10.1160/TH16-09-0711.

14 Abbasian N, Millington-Burgess SL, Chabra S, Malcor JD, Harper MT. Supramaximal calcium signaling triggers procoagulant platelet formation. *Blood Adv*. 2020; **4**: 154-64. 10.1182/bloodadvances.2019000182.

15 Remenyi G, Szasz R, Friese P, Dale GL. Role of mitochondrial permeability transition pore in coated-platelet formation. *Arterioscler Thromb Vasc Biol*. 2005; **25**: 467-71.

16 Agbani EO, van den Bosch MT, Brown E, Williams CM, Mattheij NJ, Cosemans JM, Collins PW, Heemskerk JW, Hers I, Poole AW. Coordinated Membrane Ballooning and Procoagulant Spreading in Human Platelets. *Circulation*. 2015; **132**: 1414-24. 10.1161/CIRCULATIONAHA.114.015036.

17 Adler M, Kaufmann J, Alberio L, Nagler M. Diagnostic utility of the ISTH bleeding assessment tool in patients with suspected platelet function disorders. *J Thromb Haemost*. 2019; **17**: 1104-12. 10.1111/jth.14454.

18 Dawood BB, Lowe GC, Lordkipanidzé M, Bem D, Daly ME, Makris M, Mumford A, Wilde JT, Watson SP. Evaluation of participants with suspected heritable platelet function disorders including recommendation and validation of a streamlined agonist panel. *Blood*. 2012; **120**: 5041-9. 10.1182/blood-2012-07-444281.

19 Cattaneo M, Cerletti C, Harrison P, Hayward CP, Kenny D, Nugent D, Nurden P, Rao AK, Schmaier AH, Watson SP, Lussana F, Pugliano MT, Michelson AD. Recommendations for the Standardization of Light Transmission Aggregometry: A Consensus of the Working Party from the Platelet Physiology Subcommittee of SSC/ISTH. *J Thromb Haemost*. 2013. 10.1111/jth.12231.

20 Badin MS, Graf L, Iyer JK, Moffat KA, Seecharan JL, Hayward CP. Variability in platelet dense granule adenosine triphosphate release findings amongst patients tested multiple times as part of an assessment for a bleeding disorder. *Int J Lab Hematol*. 2016; **38**: 648-57. 10.1111/ijlh.12553.

21 Prodan CI, Stoner JA, Dale GL. Lower Coated-Platelet Levels Are Associated With Increased Mortality After Spontaneous Intracerebral Hemorrhage. *Stroke; a journal of cerebral circulation*. 2015; **46**: 1819-25. 10.1161/STROKEAHA.115.009068.

22 Prodan CI, Stoner JA, Cowan LD, Dale GL. Lower coated-platelet levels are associated with early hemorrhagic transformation in patients with non-lacunar brain infarction. *J Thromb Haemost*. 2010; **8**: 1185-90. 10.1111/j.1538-7836.2010.03851.x.

23 Colucci G, Stutz M, Rochat S, Conte T, Pavicic M, Reusser M, Giabbani E, Huynh A, Thurlemann C, Keller P, Alberio L. The effect of desmopressin on platelet function: a selective enhancement of

procoagulant COAT platelets in patients with primary platelet function defects. *Blood*. 2014; **123**: 1905-16. 10.1182/blood-2013-04-497123.

24 Swieringa F, Lance MD, Fuchs B, Feijge MA, Solecka BA, Verheijen LP, Hughes KR, van Oerle R, Deckmyn H, Kannicht C, Heemskerk JW, van der Meijden PE. Desmopressin treatment improves platelet function under flow in patients with postoperative bleeding. *J Thromb Haemost*. 2015; **13**: 1503-13. 10.1111/jth.13007.

25 Aliotta A, Bertaggia Calderara D, Alberio L. Flow Cytometric Monitoring of Dynamic Cytosolic Calcium, Sodium, and Potassium Fluxes Following Platelet Activation. *Cytometry A*. 2020; **97**: 933-44. 10.1002/cyto.a.24017.

26 Aliotta A, Bertaggia Calderara D, Zermatten MG, Alberio L. Sodium-Calcium Exchanger Reverse Mode Sustains Dichotomous Ion Fluxes Required for Procoagulant COAT Platelet Formation. *Thromb Haemost*. 2021; **121**: 309-21. 10.1055/s-0040-171670.

27 Aliotta A, Bertaggia Calderara D, Zermatten MG, Marchetti M, Alberio L. Thrombocytopathies: Not Just Aggregation Defects-The Clinical Relevance of Procoagulant Platelets. *J Clin Med*. 2021; **10**. 10.3390/jcm10050894.

28 Kaufmann JE, Vischer UM. Cellular mechanisms of the hemostatic effects of desmopressin (DDAVP). *J Thromb Haemost*. 2003; **1**: 682-9. 10.1046/j.1538-7836.2003.00190.x.

Table 1. The effect of DDAVP on COAT platelet generation in patients with low baseline values.

Patients					**ISTH-BAT**		**Procoagulant COAT platelets**		
	Number	Females	Age		Score			**Absolute value, %**	
	n (%)	n (%)	years	IQR	median	IQR		median	IQR
Total	20 (100)	14 (70)	30.9	23.6 – 49.6	6.5	5 – 9	**Baseline**	20.3	17.1 – 22.0
Responders	17 (85)	12 (71)	33.5	23.6 – 49.6	7	5 – 9	**Baseline**	18.1	17.0 – 21.2
							After DDAVP	**Relative increase, %**	
							hours	median	IQR
							+2	**14.7**	10.3 – 27.3
							+4	**24.8**	8.6 – 35.1
							+6	**38.4**	8.0 – 50.0

Legend:

COAT, Collagen and thrombin activated platelets

DDAVP, 1-deamino-8-D-arginine vasopressin (= desmopressin)

IQR, Interquartile range (25^{th} – 75^{th} percentiles)

ISTH-BAT, Bleeding assessment tool of the International Society on Thrombosis and Haemostasis

Responders, Relative increase of COAT platelets of at least 10% at one or more time points after DDAVP