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RESEARCH LETTER

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

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, similar 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] and 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 workup (thus satisfying the definition of BDUC[3]), have an impaired ability to generate procoagulant COAT platelets. In our first cohort (January 2007–December 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 nondiagnostic light transmission aggregometry (LTA).[5] Ad hoc analysis of a second cohort (January 2012–March 2017), revealed low COAT platelets (<20%) in 19% (n = 10) of 53 BDUC patients.[17]

Here, we present original data from our third cohort (January 2015–September 2019). Among 123 adult patients with an unexplained elevated Bleeding assessment tool of the International Society on Thrombosis and Haemostasis (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 nondiagnostic (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 four of 14 with nondiagnostic LTA and LA, and in six 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% confidence interval 20–27).

We believe that it is clinically relevant to identify patients with a decreased ability to generate procoagulant COAT platelets for 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, 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

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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 platelet function defects, 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 h 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, although 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 for 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 h, respectively; by 41%, 36%, and 56% after 4 h; and by 32%, 21%, and 137% after 6 h, whereas sodium remained within normal limits. Nevertheless, the patient did not tolerate DDAVP, which had caused an intense headache for 3 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, although VWF/FVIII remained unaltered, COAT platelets increased again by 15%, 35%, and 41% after 2, 4, and 6 h, 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 workup (including LTA and evaluation of dense granule content/secretion), as indicated by Baker and O'Donnell[3] could be investigated by FCA 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

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

Note: Responders, relative increase of COAT platelets of at least 10% at one or more time points after DDAVP.

Abbreviations: COAT, collagen and thrombin activated platelets; DDAVP, 1-deamino-8-D-arginine vasopressin (= desmopressin); IQR, interquartile range (25th–75th percentiles); ISTH-BAT, Bleeding assessment tool of the International Society on Thrombosis and Haemostasis.

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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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Amandine Segot was in charge of patient care and wrote the manuscript. Marcel Adler was in charge of patient care, performed research, and wrote the manuscript. Alessandro Aliotta performed research and wrote the manuscript. Elena Matthey-Guirao performed research. Michael Nagler performed research. Debora Bertaggia Calderara performed research. Francesco Grandoni was in charge of patient care. Francisco J. Gomez performed research. Lorenzo Alberio supervised clinical and laboratory work, wrote the manuscript. All authors contributed to and approved the final version of the manuscript.

INFORMED CONSENT

This investigation was conducted according to the guidelines of the competent ethical board [Commission cantonale (VD) d'éthique de la recherche sur l'être humain, CER-VD] upon its approval (protocol number CER-VD 2020-00995. Specific patient informed consent for the study was waived, according to the Federal Act on Research involving Human Beings on Human Research (Human Research Act, HRA). However, our centre is using a general research consent for reuse of patient's data. Patients were excluded in case of documented refusal of this general research consent.

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