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Platelet ultrastructural morphometry for diagnosis of partial δ-storage pool disease in patients with mild platelet dysfunction and/or thrombocytopenia of unknown origin. A Study of 24 cases

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ABSTRACT

Background and Objectives. **Exact diagnosis is sometimes difficult in patients presenting with a slight bleeding diathesis, prolonged bleeding times, non-specific aggregometric abnormalities, and/or mild thrombocytopenia. The objective of this study was to evaluate the use of platelet ultrastructural morphometry in detecting a partial δ-storage pool disease in such patients.**

Design and Methods. **Platelets from 52 patients and 15 controls were fixed immediately in glutaraldehyde in White's saline without anticoagulant and processed for transmission electron microscopy. Using computer-assisted morphometry, the size and shape of the platelets were measured, as were the size and number per platelet of the dense- and α-granules. Ultrastructural morphology of the above and other intraplatelet structures was observed.**

Results. **Twenty-four cases were diagnosed as having a partial δ-storage pool disease. Mean platelet area (2.28 μm²) and maximum diameter (2.58 μm) were significantly greater in patients than in control subjects (1.64 μm² and 2.25 μm, respectively) but discoid shape was preserved. Mean dense-granule number was decreased, both per platelet and per μm² of platelet area (patients 0.22 and 0.09; controls 0.42 and 0.24). Seven patients also had a marked decrease in α-granules, resulting in a significantly lower mean number of granules per μm² (patients 2.43; controls 3.15). Additionally, the patients' platelets had significant increases in both lipid droplets and surface-connected canalicular system.**

Interpretation and Conclusions. **A partial dense-granule deficiency, sometimes associated with partial α-granule deficiency, should be borne in mind faced with patients who have a slight bleeding diathesis, non-specific platelet dysfunction tests and/or mild thrombocytopenia of unknown origin. Platelet ultrastructural morphometry is useful in diagnosing this condition.**

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Disorders of platelet secretion result from abnormalities in the platelet secretory mechanism or from deficiencies of platelet specific granules. These disorders are a relatively frequent cause of mild to moderate bleeding diatheses, accompanied by a normal or decreased platelet count and often a prolonged bleeding time.[1,2] Dense-granule deficiency or δ-storage pool disease is a heterogeneous disorder with deficiency of dense-granules as a common feature. The platelet dense- or δ-granules are very electron-dense organelles, approximately 200 to 300 nm in diameter, which serve as intracellular storage sites for calcium, ADP, ATP, pyrophosphate, and serotonin. The contents of these granules are extruded during platelet secretion, coinciding with the second wave of platelet aggregation, and they play an important role in the propagation of the primary platelet response.

Dense-granule deficiency has been observed as a constituent trait in a variety of hereditary syndromes in which the bleeding tendency is associated with other clinical features, and also as a separate entity in which the platelet disorder is the sole abnormality.[2-4] In the recessively inherited Hermansky-Pudlak syndrome, the most typical of associated diseases, patients also present oculocutaneous albinism and accumulation of ceroid-like material in macrophagic cells of bone marrow and other tissues. Chediak-Higashi, Wiskott-Aldrich, and thrombocytopenia with absent radii are other congenital syndromes in which platelet dense-granule deficiency is also a constituent feature.

The isolated hereditary dense-granule deficiency, which is transmitted as an autosomal dominant trait, is commonly referred to as δ-storage pool disease. The variable degree of granule deficiency results in a wide range of clinical and laboratory manifestations.[3] When moderate involvement occurs, clinical bleeding is not significant and diagnosis can be missed, underestimating the number of subjects suffering from δ-storage pool disease.[5,6] Some individ-

uals with inherited dense-granule deficiency also have a partial reduction in α-granules. In these cases, the term of αδ-storage pool disease is applied.[3]

Moreover, some acquired clinical conditions also produce platelet specific granule deficiencies such as end-stage renal or hepatic diseases, myeloid leukemias, myelodysplasia, and myeloproliferative syndromes.[2,4]

The diagnosis of δ-storage pool disease is based on a clinical mild to moderate bleeding diathesis characteristic of platelet secretion defects, manifested by easy bruising, mucous bleeding, menorrhagia and excessive post-operative or obstetric bleeding. Platelet count and platelet morphology are generally normal, and bleeding time is usually, though not always, prolonged. When studied in an aggregometer, the platelets of these patients are commonly found to lack a second wave of aggregation when stimulated by ADP or epinephrine and to show decreased aggregation stimulated by low doses of collagen.[2,7] While this pattern is typical of dense-granule deficiency, examples have been reported with normal aggregation studies.[8]

Complementary studies in δ-storage pool disease include analysis of dense-granule contents (adenine nucleotides, serotonin, calcium), measurement of adenine nucleotide secretion,[3,7,9] fluorescence microscopy or flow cytometry of mepacrine-treated platelets,[10,11] and platelet ultrastructure. Taking advantage of the intrinsic opacity of dense-granules, the examination by electron microscopy of whole mounted platelets directly on coated grids results in a very rapid, exact technique for quantification of these structures.[12] On the other hand, electron microscopic observation of platelet ultrathin sections from platelets previously embedded in plastic resins is a more laborious technique than that of examining whole mounted platelets, but gives a more complete vision of these cells.[3,4,13,14] It allows not only quantification of the number of dense-granules but also evaluation of their morphology, and analysis of the general platelet traits, the number and morphology of other intraplatelet structures, especially α-granules. However, platelet granules are present in variable amounts in different platelets, even within normal individuals. This irregularity is increased in platelet electron microscopic images due to the different planes produced by cell sectioning. Therefore, when a defect of platelet granules is not very marked, morphometric evaluation is essential in order to evaluate the degree of granule reduction correctly.

By applying morphometric measurements to electron micrographs of resting platelets, we were able to identify 24 cases of partial δ-storage pool disease among 52 individuals, the majority of them with non-severe functional platelet disorders and/or moderate thrombocytopenia of unknown origin.

Design and Methods

Patients

A total of 52 individuals were included. Forty-three were patients with a moderate bleeding diathesis and/or slight thrombocytopenia of unknown origin whose previous laboratory tests had demonstrated prolonged bleeding times or inconclusive aggregation defects; standard functional tests were normal in a few cases. The remaining 9 individuals were close family members with no previously known platelet pathology.

A careful clinical evaluation was carried out in all patients including a detailed estimation of bleeding and a complete family history was taken. Blood platelet counts were performed by phase contrast microscopy; a number $\geq 150\times10^9$/L was considered to be normal. Platelets were also observed by high-power light microscopy to assess their size, shape and granule morphology. Appropriate coagulation and von Willebrand tests were also carried out to discard other non-platelet primary hemostatic defects.

Bleeding time, measured by the Ivy method, was considered normal up to 9 minutes. Aggregometry was analyzed in platelet-rich plasma (PRP) samples adjusted to 150×10^9/L platelets, using the following substances as agonists: ADP (0.5, 1, 2, and 5 μmol/L), epinephrine (10 μmol/L), arachidonic acid (1 μmol/L), collagen (2 and 5 mg/L), and ristocetin (0.5 and 1 g/L). PRP from a normal individual, also adjusted to 150×10^9/L platelets, was analyzed in parallel for each agonist and concentration.

Platelet processing for transmission electron microscopy

Platelet electron microscopy was used to examine the platelets from all patients and from a group of 15 normal individuals used as controls. In order to obtain resting platelets, peripheral venous blood was collected directly in 9 volumes of 1.25% glutaraldehyde in White's buffer saline,[15] previously heated to 37ºC, without any anticoagulant, and left to fix for 20 minutes before centrifuging at 120 g to obtain the PRP.[14] PRP was allowed to settle for a further 30 minutes. The sample was then washed three times with White's buffer and a platelet pellet was obtained by centrifugation at 1,700 g. The postfixation was carried out with 1% osmium tetroxide in White's saline for 90 minutes at 4º C. All steps up to postfixation, were carried out at room temperature using plastic materials. Specimens were then dehydrated in a series of graded alcohols and embedded in Epon 812 following standard methods. The ultrathin sections were collected on uncoated 300-mesh copper grids and stained with uranyl acetate and lead citrate before being examined in a transmission electron microscopy at 80 Kv accelerating voltage.

Platelet ultrastructural morphometry and morphology

One high quality cut covering an entire mesh of the grid was generally sufficient to obtain six to eight photos of different areas, at ≈5,500 magnification, with a total of at least 150 different platelet profiles. When there were fewer platelets, additional photos were taken from other embedding blocks, not in consecutive cuts, to ensure that platelet profiles belonged to platelets not contained in the first cut. A cross-grating replica (Agar S-106) was photographed before and after the takes to perfect the exact magnification of the negatives. Two prints were made for each negative: one at ≈11,500 final magnification to analyze

platelets and another at ≈22,000 to analyze intraplatelet structures. The final magnification of the prints was carefully calculated by means of a comparative measurement with the negative.

Two-dimensional morphometry was performed on the platelet electron micrographs using computer-assisted image analysis in a MIP system (Microm, Barcelona, Spain) connected to an AVC-D1 black-and-white CCD video camera (Sony Corporation). Platelet images were captured by the video camera and transferred to the computer where they were digitalized and analyzed with MIP version 1.6 software (Microm, Barcelona, Spain). Based on the identification of platelets and their organelles according to previous morphological descriptions for transmission electron microscopy,[3,12] the following parameters were measured:

- *Platelet population*: mean area, mean maximum diameter and mean circular deviation index (CDI) according to the formula: CDI = $(4\Pi \times \text{area}) / \text{perimeter}^2$. CDI was used to evaluate discoid or ellipsoid shape: the more rounded the shape the higher the CDI, and the more discoid the shape the lower the CDI;
- *Intraplatelet particle structures*: mean diameter, mean number per platelet and mean number per μm^2 of platelet area for dense-granules, α-granules, mitochondria, and lipid droplets;
- *Other intraplatelet structures*: mean area and mean percentage of total area with respect to the platelet area for surface-connected canalicular system, and total area with respect to the platelet area for glycogen.

Apart from morphometric measurements, we evaluated morphological traits of the platelets and the organelles measured above. Moreover, dense-granules were classified into 4 different types according to Weiss *et al*.[13]: type 1) solid core occupying more than 50 % of the granule; type 2) solid core occupying less than 50 % of the granule; type 3) fragmented core; 4) empty granule, no visible core (Figure 1). Dense tubular system, membranous complexes, microtubules, and other ultrastructural characteristics were evaluated only by morphology.

The results were expressed as mean±standard deviation. The one-way analysis of variance was used to compare means between results obtained in patients and controls. p-values of ≤ 0.05 were considered to indicate statistical significance.

Results

A partial decrease of dense-granules was demonstrated in 20 of the 43 patients and in 4 of the 9 previously considered normal family members studied. Nine were males and 15 females, with ages ranging from 6 to 64 years old (Table 1). Sixteen cases were grouped in 6 families and another 2 (#11 and 14), analyzed individually, also presented familial platelet involvement. Most patients were studied because of a moderate bleeding diathesis and/or slight thrombocytopenia but one of them (#17), who was studied as a family member, had no clinical bleeding or thrombocytopenia. Previous platelet functional tests had been inconclusive or, in some cases, normal. In a previously reported case (#11), a type IV Ehlers-Danlos syndrome was also observed, a condition sometimes known to be associated with δ-storage pool disease.[16] No traits suggestive of Hermansky-Pudlack or other congenital syndromes with known constituent dense-granule deficiency were detected. No patients suffered from renal or hepatic diseases or neoplastic myelopoietic pathology, nor had they been treated with drugs which may affect platelets. The analysis of familial presentation suggested a pattern of autosomal dominant inheritance in the majority of the patients (Table 1). Some cases presented familial particularities, such as the child with Ehlers-Danlos syndrome whose mother had died of the same disease several years earlier, having never been studied for functional or ultrastructural platelet defects. Ultrastructural analysis of the platelets of the father of case #14 demonstrated partial deficiency of α-granules, with preservation of dense-granules.

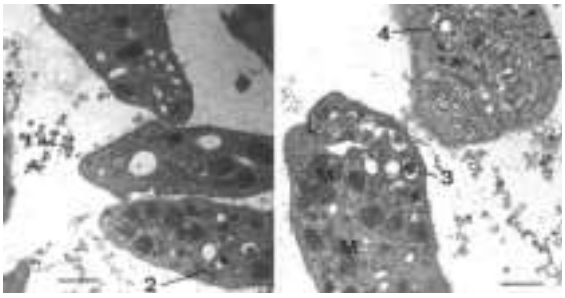

Figure 1. A. Several platelets presenting two type 1 dense-granules (1), and one type 2 (2); b. A platelet showing a type 3 dense-granule (3), and another showing type 4 (4). A lipid droplet (L) and some enlarged mitochondria (M) can also be seen. The dense tubular system (arrowheads) is well developed; bars = 0.5 µm.

Most patients had a bleeding diathesis, as a single manifestation in 13 and combined with thrombocytopenia in 8. Easy bruising, mucous bleeding (epistaxis, menorrhagia, gum bleeding), excessive post-operative or post-partum blood loss, and/or excessive bleeding after superficial cuts or tooth extractions were the most common bleeding symptoms reported (Table 1). Two cases showed thrombocytopenia without bleeding, and an additional case, a father of 2 affected children (case#17), was free of clinical manifestations and thrombocytopenia.

Using light microscopy, platelets appeared normal in size and shape in most patients. However, macrocytic platelets were seen in 7 cases, either alone (cases#1, 4, 5) or accompanied by a number of giant platelets (cases#2, 10, 17, 23). In approximately half the patients, some platelets exhibited slight hypogranularity, especially in the cases in which ultrastructural morphometry showed associated α-granule deficiency. In these cases, a few completely degranulated platelets (gray platelets) were also seen. The presence of vacuolated platelets in some patients, mainly in those without α-granule reduction, was another finding of interest.

Bleeding time was prolonged in 11 cases, mainly

Table 1. Patients with partial δ-storage pool disease. Clinical characteristics, blood platelet counts and functional platelet studies

N	Family		Age/Sex	Clinical beeding			Platelets	Bleeding	Aggregometry. Impaired response to:				
	Id	Rel		Easy bruising	MB	STO	x 10^9/L	time (min)	ADP	Epi	AA	Col	Rist
1	A	P	30 F	++			180	15		+			
2	A	Br	26 M		+		170	17		+			
3	A	Mo	63 F	+++	+		100	22	++	++	±		±
4	B		19 F	+		+	110	13.5		+			±
5	C		26 F	++	+	+	130	21		+			
6	D	P	32 F	+	+	+	350	7.5		±			
7	D	Mo	61 F		++	±	400	5		+			
8	E	P	31 F	++	±	++	85	13	+	+	+++	±	
9	E	Br	17 M	+		+	110	10	±	±	+	+	
10	E	Br	30 M	+	±	++	90	24	±	+			
11	F		6 F	+	+	+	450	7			±		±
12	G		64 F		++		300	17		±	±	±	±
13	H		20 M		+	+++	200	11.5		±			
14	I		17 M	±	±		130	8					
15	J	P	8 F	+	+		400	9	+	+		+	
16	J	Br	6 M	±	±	±	475	8.5					
17	J	Fa	37 M				180	6.5				±	
18	J	Mo	36 F	±	++	++	330	9				+	
19	K		10 F	++	±		450	6	±				
20	L	P	42 F	++		+	275	8	±			±	
21	L	So	25 M		±	+	230	7					
22	M	P	8 F				95	7					
23	M	Si	8 F				80	7.30					
24	N		31 M		±		110	11					

Id = Family identification; Rel = Familial relationship: P = propositus; Fa = father; Mo = mother; Br = brother; Si = sister; So = son ; MB = Mucous bleeding: epistaxis, menorrhagia, gingivorrhagia; STO = surgical, traumatic or obstetric bleeding ; Epi = epinephrine; AA = arachidonic acid: Col = collagen; Rist = ristocetin.

Table 2. Patients with partial δ-storage pool disease and controls. Platelet and dense-granule ultrastructural morphometry and morphology.

	Controls (n=15)		Patients (n=24)		p
	mean	SD	mean	SD	
Platelets					
Area (μm²)	1.64	0.29	2.28	0.73	0.018
Maximum diameter (μm)	2.25	0.20	2.58	0.33	0.009
Circular deviation index (CDI)	0.62	0.03	0.60	0.02	0.078
Dense-granules					
Diameter (nm)	222	27	235	20	0.107
Number/platelet	0.42	0.13	0.22	0.09	0.005
Number/μm²	0.24	0.05	0.09	0.05	<0.001
Dense-granule classification (%)					
Type 1	41.6	13.2	30.9	16.9	0.136
Type 2	29.9	4.6	29.9	12.2	0.998
Type 3	19.9	10.6	30.3	18.8	0.180
Type 4	8.7	4.4	9.2	7.6	0.876

SD = Standard deviation

corresponding to those with thrombocytopenia associated with clinical bleeding (Table 1). Aggregometry was variably affected in 18 cases, generally using epinephrine, ADP and/or collagen as agonists, whereas in 6 patients it failed to demonstrate any defect.

Results of morphometric measurements on ultrastructural images of patients' platelets showed that mean platelet area and maximum diameter were significantly increased with respect to those of the controls, whereas discoid or elliptic shape was preserved as demonstrated by a not increased CDI (Table 2). The number of dense-granules was significantly decreased, both per platelet and per μm² of platelet area (Table 2) (Figure 2). Morphologic evaluation showed no statistically significant difference in dense-granule types with respect to normal individuals. However, from the individual values obtained, 11 patients showed a reduction in type 1 dense-granules, which was very marked in 6 (#6, 7, 11, 13, 19, and 22). This reduction was associated with a relative increase in other types of granules, particularly those of the fragmented core (type 3). In some of these cases, fragments of the dense-granule electron-

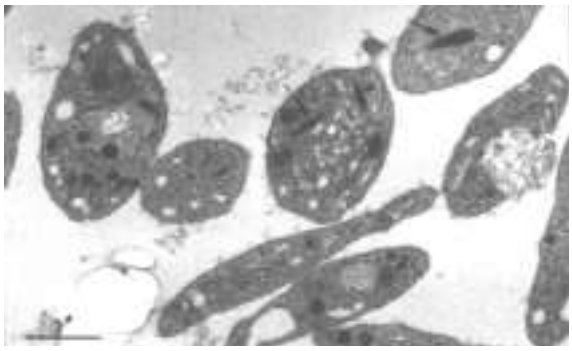

Figure 2. Platelets from a patient with partial d-storage pool disease with associated partial α-granule deficiency showing enlarged size, preserved discoid shape and decreased dense- and α-granules. Some α-granules show an elongated shape (arrows); bar = 1 µm.

Figure 3. A platelet showing distended channels of its surface-connected canalicular system; bar = 0.5 µm.

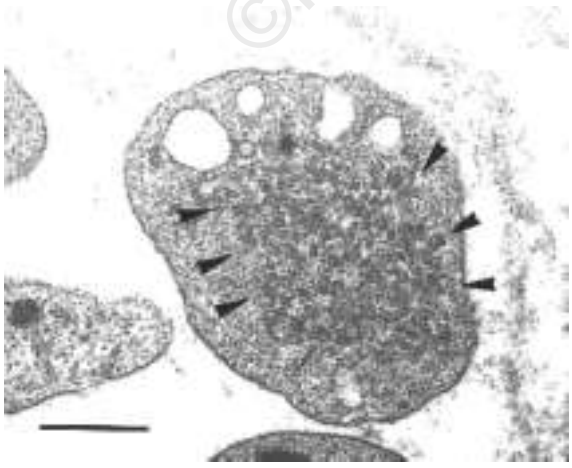

Figure 4. A platelet showing a dense tubular system complex (arrowheads); bar = 0.5 µm.

Table 3. Patients with partial δ-storage pool disease. Ultrastructural morphometry of α-granules and other intraplatelet structures.

	Controls (n=15)		*Patients (n=24)*		*p*
	mean	*SD*	*mean*	*SD*	
α-granules					
Diameter (nm)	181	27	180	22	0.911
Number/platelet	5.02	0.56	5.48	1.67	0.464
Number/µm²	3.15	0.72	2.43	0.47	0.002
Mitochondria					
Diameter (nm)	215	35	280	26	<0.001
Number/platelet	0.41	0.21	0.50	0.22	0.277
Number/µm²	0.26	0.08	0.22	0.08	0.206
Lipid droplets					
Diameter (nm)	315	76	330	23	0.480
Number/platelet	0.023	0.028	0.181	0.211	0.035
Number/µm²	0.036	0.044	0.095	0.095	0.086
Glycogen					
Total area/platelet area (%)	1.82	1.01	2.43	1.20	0.228
Surface-connected canalicular system					
Individual element area (µm²)	0.014	0.002	0.017	0.005	0.145
Total area/platelet area (%)	5.31	0.95	8.50	2.71	0.005

SD = Standard deviation.

dense content could be seen free in the platelet cytoplasm or in the extracellular space.

Seven patients (#3, 5, 8, 9, 10, 14, 19) were considered to show an associated deficiency of α-granules because the number of granules per µm² of platelet area was lower than 2.08 (the minimum value found in normal individuals, equivalent to 1.5 standard deviations below the normal mean). Low numbers of α-granules in these cases resulted in a significantly low mean α-granule per µm² of platelet area in the total group of patients (Table 3).

However, the mean number of α-granules per platelet was not affected in the patients. Irregular shaped α-granules, mainly elongated, are often seen (Figure 2). Other morphometric anomalies observed consisted of significant increases in mean diameter of mitochondria, number of lipid droplets and total area of surface-connected canalicular system per platelet area (Table 3) (Figures 1b and 3). The dense tubular system was generally more developed than normal, and occasional tubular complexes were observed (Figures 1b and 4). Specific anomalies of microtubules and glycogen were not seen.

Discussion

Functional studies are often definitive for diagnosis of δ-storage pool disease when there are very low or absent residual granules. However, in cases of partial granule deficiency, they are, at most, only sug-

gestive.[5,7] Platelet ultrastructural morphometry performed with special technical conditions has demonstrated to be a useful diagnostic tool in these cases.[3,17] Moreover, when diagnosis of δ-storage pool disease is suspected, morphometric measurements of platelet images obtained by electron microscopy may confirm a dense-granule anomaly and can also quantify the defect. Furthermore, this technique can detect other non-suspected associated platelet deficiencies such as those in α-granules.

The patients' general characteristics, including familial presentation and clinical bleeding corresponded to those previously described by others.[2-4] Prolonged bleeding times and variable abnormalities in aggregometry in the majority but not in all patients, also coincided with previous reports on δ-storage pool disease.[5,8] Additionally, 10 of our patients showed moderate thrombocytopenia, 6 with an associated partial α-granule deficiency. Unlike α-storage pool disease or gray platelet syndrome, which are associated with constituent thrombocytopenia, platelet counts are generally described as being normal in δ-storage pool disease.[2,4] It should be pointed out that thrombocytopenia, either associated with bleeding tendency or as an isolated feature, was the main reason for referring the patient to our center in most cases.

Since the aim of the study was to analyze structural anomalies, it was essential to work with resting platelets with no acquired structural modifications. Other technical requisites were to ensure optimal preservation and identification of dense-granules and to obtain good reproducibility of the results. It is well known that blood anticoagulants generally modify platelet structure to a variable degree giving less discoid, more rounded, cells.[18] Cooling blood samples below 37ºC or placing them in contact with glass also disturbs discoid platelet shape as a sign of early activation.[19] A number of pseudopodia and some degree of granule centralization can also be seen, indicating a more advanced stage of platelet activation. When these changes are produced, platelet size and shape, and the number and morphology of granules suffer variable modifications that do not correspond to real platelet structure.

Collected blood poured directly into a large volume of buffered saline glutaraldehyde solution was used both to obtain immediate fixation and to make the use of anticoagulant unnecessary. Thus, it was ensured that resting platelets with minimal acquired structural modifications was obtained. Moreover, the use of White's buffer in fixation, washing and postfixation, led to the best identification of dense-granules, as has been previously described.[19]

Using a computer-assisted system, we previously demonstrated that the analysis of a minimum of 150 different platelets is essential to obtain reproducible results.[14,17] In 1979, Weiss *et al*.[3] applied ultrastructural morphometry to quantify platelet granules. Precisely for this reason, they were able to determine different types of dense-granule deficiencies, including those with associated reduction of α-granules. Although our quantitative results were not identical to those obtained by these authors, similar differences between controls and patients were observed. Some technical differences, in obtaining and fixing the blood platelets, staining their dense granules or morphometric sampling could explain the disparate results. We also analyzed other parameters which gave interesting results: mean diameter of organelles, area of platelets, maximum diameter, and CDI.

Using ultrastructural morphometric analysis, patients' platelets were shown to have increased mean areas and mean maximum diameters, whereas their CDI was normal or tended to be lower than normal, indicating a very good preserved discoid shape (Table 2). All these data suggest that the increased size of patients' platelets is a constituent feature and is not due to platelet rounding caused by activation.

Two main parameters demonstrated the statistically significant reduction of dense-granules in patients with respect to controls: the number of granules per platelet and the number of granules per μm^2 (Table 2). Statistical analysis of morphological types of dense-granules[13] failed to demonstrate significant differences between patients and controls. However, individual observation of all δ-storage disease cases showed very low numbers of type 1 granules (dense core $\geq 50\%$) in some of them (#6, 7, 11, 13, 19, and 22). This favored the number of less electron-dense types, particularly type 3 with a fragmented core. Moreover, in some platelets in these cases, fragments of electron-dense material were seen free in platelet cytoplasm and even in the extracellular space. This phenomenon was especially evident in the girl suffering from Ehlers-Danlos syndrome (#11) as was described in a previous report.[16] We were not able to suggest any explanation for consequence of this special defect of dense-granules, also described by other authors,[13] in the context of δ-storage pool disease.

In 8 cases a parallel study of dense-granules using electron microscopic observation of whole mounted platelets was performed (data not shown). It should be kept in mind that the total number of granules per platelet is counted using this method. This is not the case in the transmission electron microscopy method used in this study, in which we only observed the granules contained in a thin platelet section. However, an acceptable correlation between the two methods was obtained.

In 7 cases, in addition to δ-granule deficiency, a partial reduction of α-granules was also observed suggesting the diagnosis of αδ-storage pool disease (Table 3). However, as the number of α-granules per platelet section was not very low, the increased platelet size was sufficient to neutralize the granule reduction. On the other hand, the number of α-granules was significantly decreased with respect to the platelet area (in μm^2).

There was no clinical or biological evidence of any conditions or drugs known to affect platelets.[20] Moreover, as there was no ultrastructural evidence of platelet activation, a storage pool disease secondary to increased platelet release was also ruled out. On the other hand, familial presentation and the association of partial α-granule deficiency in some patients or their relatives (father of case #14) suggested a constitutional defect. As hereditary thrombocytopenias are often considered rare diseases, they are not always taken into account in diag-

nosis.[6] However, our results obtained using platelet ultrastructural morphometry should alert hematologists to the presence of δ-storage pool disease in patients with thrombocytopenia of unknown origin, either alone or associated with moderate bleeding.

The pathogenesis of δ-storage pool disease is currently not completely understood. Based on the different types of dense-granule pathology described – pure numeric decrease, reduced or fragmented core, presence of empty sacs instead of dense-granules – a granulogenesis defect has been suggested.[3] Moreover, associated α-granule reduction and abnormal morphology, observed in some cases, also support the hypothesis of a defect in platelet granule formation in megakaryocytes.

In conclusion, when δ-storage pool disease does not produce profound clinical or functional platelet abnormalities, it is often not identified. Quantification of platelet adenine nucleotides and serotonin, or fluorescence analysis of mepacrine-treated platelets can strongly suggest the disease. However, ultrastructural platelet study provides conclusive proof for diagnosis. Additionally, when the dense-granule deficiency is not complete, morphometry of resting platelet ultrastructure, under certain technical conditions, can give an accurate diagnosis of partial δ-storage pool disease. This technique not only allows quantification of the dense-granule defect, but may also be useful for detecting associated partial α-granule deficiencies allowing the diagnosis of αδ-storage pool disease. Platelet ultrastructural morphometry is thus useful in detecting partial δ- or αδ-storage pool disease in patients presenting with a slight bleeding diathesis, non-specific platelet dysfunction tests and/or mild thrombocytopenia of unknown origin.

Potential implications for clinical practice

- Application of platelet ultrastructural morphometry for diagnosis may be useful in demonstrating and quantifying a granule-dense deficiency in patients with non-catalogued platelet dysfunction. Moreover, in cases with previously suggested partial δ-storage pool disease this methodology can provide conclusive evidence of the structural defect.
- Unlike other laboratory tests, specifically for analysing dense-granule content and function, platelet ultrastructural morphometry may also demonstrate associated α-granule defects.
- In the case of mild thrombocytopenia of uncertain origin with no clear traits of thombocytopathy the possibility of a δ- or αδ-storage pool disease diagnosis should be kept in mind.

Contributions and Acknowledgments

NP-M was primarily responsible for the conception of the study and production of the article and evaluated the ultrastructural platelet morphometry and morphology. NP-M and IE clinically assessed of the patients. AH performed the platelet processing for electron microscopy and collaborated in the ultrastructural morphologic and morphometric evaluation. GE, FM-B and JM performed functional analyses, contributed by referring patients for the study, and participated in the discussion of the results.

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