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Ultrastructural computerized morphometry of platelets in chronic myelogenous leukemia making use of ultra-thin sections and freeze-fracture procedure. A preliminary approach

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Summary. Biochemical and morphological data have shown that the circulating platelets are deeply altered in patients with chronic mielogenous leukemia. In this report we describe the results of ultrastructural morphometry performed by means of a computerized device of the platelet shape (ultra-thin sectioned platelets) and of the platelet plasma-membrane (freeze-fractured platelets). Platelets appeared deeply modified: reduced mean platelet area and perimeter, increased surface density of the openings of the surface connected canalicular system, abnormal features of the platelet cytoplasm and some aspects of platelets joined together even if with heterogeneity in these findings did appear from patient to patient.

Key words: Morphometry - Platelets - Leukemia

Introduction

It is known that thrombotic and haemorragic events take frequently place in myeloproliferative disorders, possibly due to impaired platelet function.

Pflieger et al. (1983) in patients affected with myeloproliferative disorders have described abnormal platelet aggregation induced by adrenalin or by collagen in about one half of patients. The prostaglandin metabolism and the beta-thromboglobulin levels were increased respect to normal healthy men.

Morphologic abnormalities of the platelets were also described in myeloproliferative disorders like myelofibrosis with myeloid metaplasia as well as in so-called «preleukemia» conditions in which large and rounded platelets as well as platelets with agranular (or hypogranular) forms were described frequently (Maldonado et al., 1974; Maldonado, 1975).

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In this preliminary study we have performed ultrastructural computerized morphometry of platelets collected from a little group of patients affected with chronic myelogenous leukemia, after freeze-fracturing and ultra-thin section examination, making use of a methodological approach used by us until now to study platelets from patients affected with diseases linked to atherosclerosis (Weber et al., 1979, 1984; Bianciardi et al., 1985, 1986).

Materials and methods

To get replicas for freeze-fracturing examination, citrated blood plasma was centrifuged at 800 rpm for 15' to obtain Platelet Rich Plasma (PRP). The PRP was taken away with a plastic pipette and subjected to a short fixation in 0.1% glutaraldehyde followed by 1.5% glutaraldehyde (in phosphate buffer) pH 7.35 fixation for 90' (t = 4°C). After centrifugation (3500 rpm for 10') the surnatant was discarded again and the platelets, as a pellet, were transferred to special gold holders and dropped immediately into solid nitrogen.

After the initial step of freezing, the samples were placed in liquid nitrogen. Replicas of the platelets have been obtained by means of a Freeze-etching device, BAF 301 FN 178, equipped with electron beam guns and quartz crystal thin film monitor. The replicas were observed at ×15000 by means of a Siemens TEM Elmiskop 1A. For each sample we have examined about 80 platelets counting the number of the openings of the surface conected canalicular system (SCCS) per micron square.

For transmission electron microscopy of ultrathin sections, the glutaraldehyde-fixed PRP was post-fixed with osmic acid (1%), dehydrated with ethanol, then treated with 1-2 propylen-oxid and embedded in araldyte. Ultrathin sections were obtained by means of an «Ultrotome 2088»-LKB and examined, after staining with lead citrate and uranyl acetate, in a Siemens TEM Elmiskope 1A at ×5000.

Evaluations of the surface density of openings of

SCCS and of the morphological parameters of platelet shape (area and perimeter) were performed by means of a computerized measuring system and a graphic tablet (Videoplan, Kontron-Zeiss).

Patients

The examined leukemic patients were 1 female: M.G. (1), 79 years old with leukocytosis (78000/μl) and 3 males: P.A. (2), 38 years old, with leukopenia (1300μl), A.G. (3) and L.B. (4), (60, 47, aged respectively) with slight leukocytosis. All the subjects were submitted to intensive therapy.

To obtain control platelets blood was collected from 4 healthy volunteers in the same range of age.

Results

Patients with chronic myelogenous leukemia have shown very different features in the different cases.

In patient number 1, some hypogranular platelets and platelets with unusual shapes, appearing joined together (Figs. 1, 2), were observed.

In patient number 2, the platelets appeared abnormally small: ultra-thin sectioned platelets presented mean area and perimeter smaller than the ones usually found in healthy subjects of the same range of age (Figs. 3a, b, 4a, b). After freeze-fracture, platelets with an abnormal high surface density value of the openings of SCCS (Figs. 5, 6a, b) were observed.

The patients number 3 and 4 presented instead normal parameters of the platelets.

In Table 1 are summarized the morphometric results obtained with freeze-fracture in the platelets of leukemic patients.

Table 1. Platelet mean area and perimeter (from ultra-thin sections) and surface density of the openings of the surface connected canalicular system (from freeze-fracturing) in patients affected with chronic myelogenous leukemia

PATIENTS AFFECTED WITH CHRONIC MYELOGENOUS LEUKEMIA			
	Pt/μm^2	Area (μm^2)	Perimeter (μm)
1 M.G.	2.2	1.38	4.66
2 P.A.	3.1	0.72	3.65
3 A.G.	2.4	1	4.19
4 L.G.	2.6	1.42	4.86

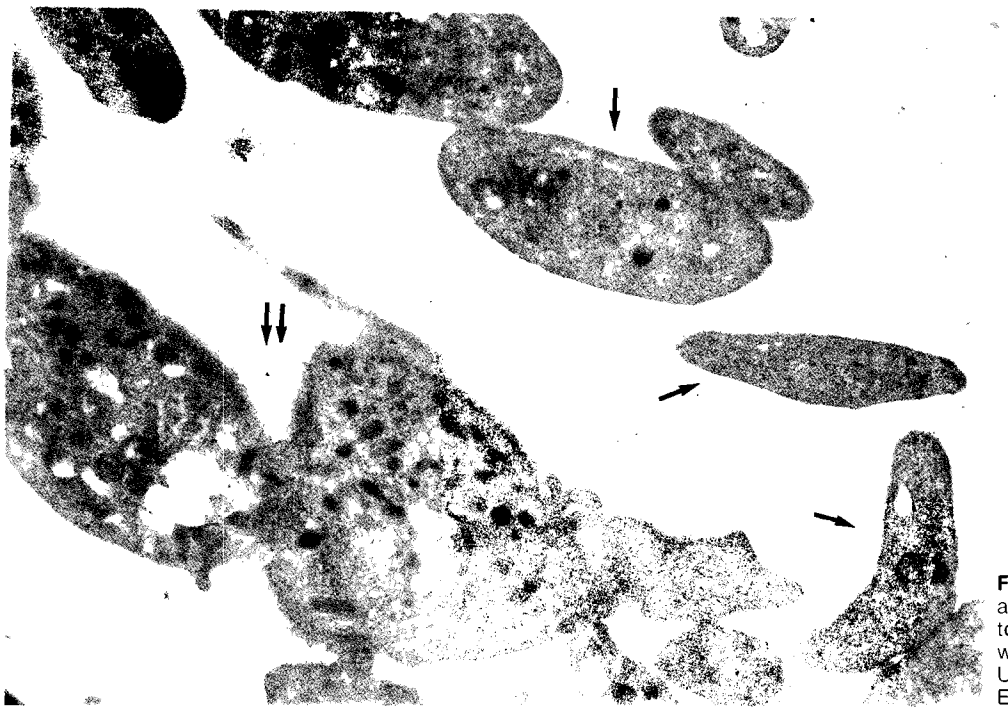


Fig. 1. «Hypogranular» platelets (↑) and platelets that appear joined together (↑↑) in a patient (1) affected with chronic myelogenous leukemia. Ultra-thin section, Transmission Electron Microscopy. ×7,000

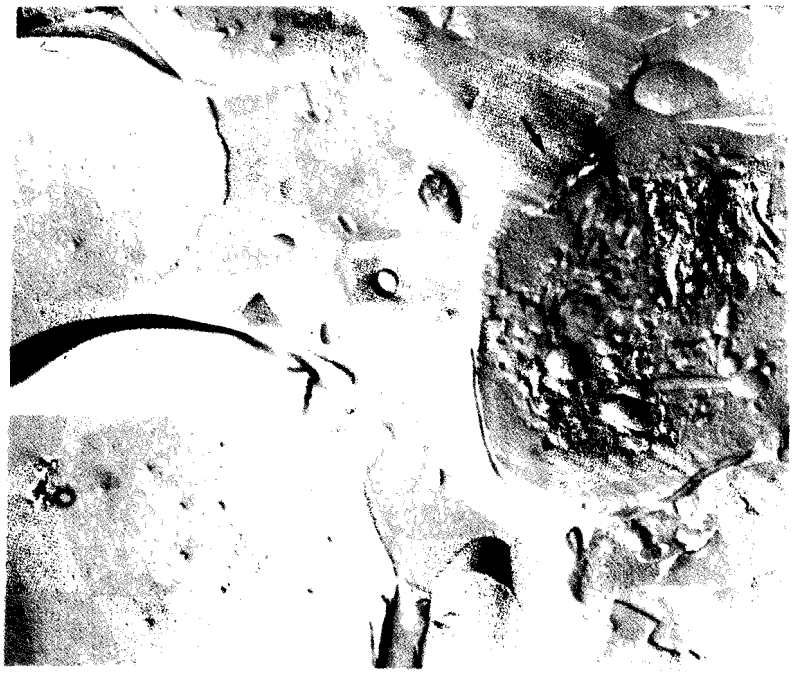


Fig. 2. A «hypogranular» platelet in a patient (1) affected with chronic myelogenous leukemia. Freeze-etching technique. × 20,000

Fig. 5. Platelet plasma-membrane of a patient (2) affected with chronic myelogenous leukemia. The plasma-membrane presents high number of openings of the surface connected canalicular system (**1**). Freeze-etching technique, × 20,000

Fig. 3a.

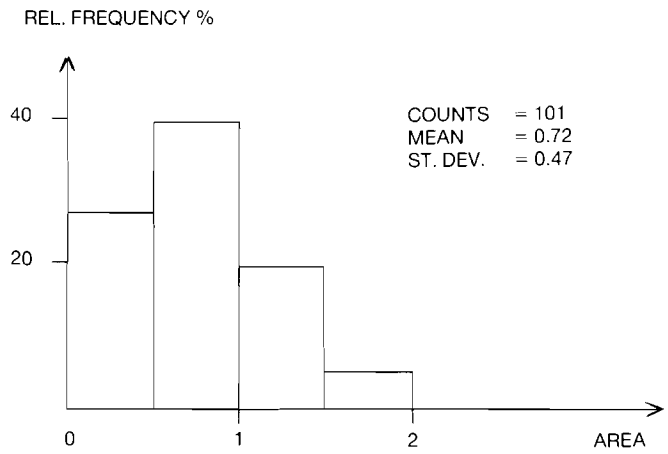


Fig. 3b.

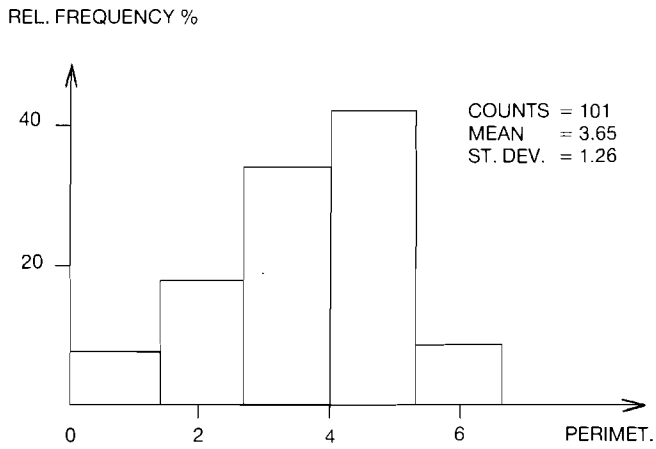


Fig. 4a.

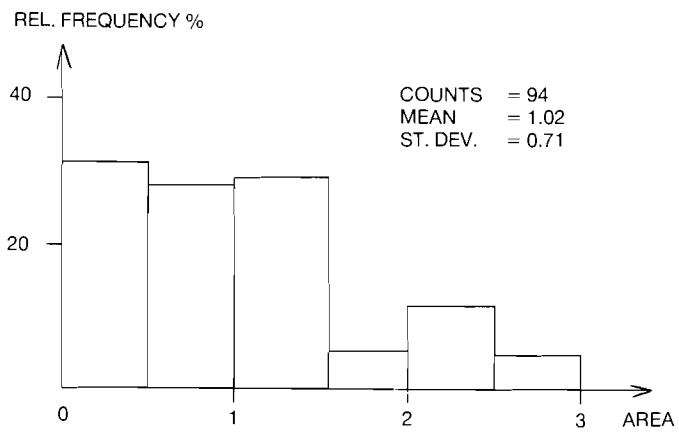
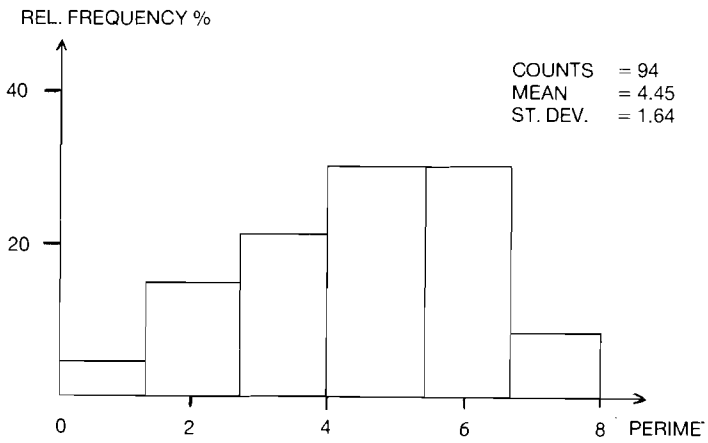


Fig. 4b.



Figs. 3a, b-4a, b. Platelet areas and perimeters in a patient (2) affected with chronic myelogenous leukemia and in a healthy volunteer of the same range of age. The mean platelet area and perimeter are very low in the patient.

Fig. 6a.

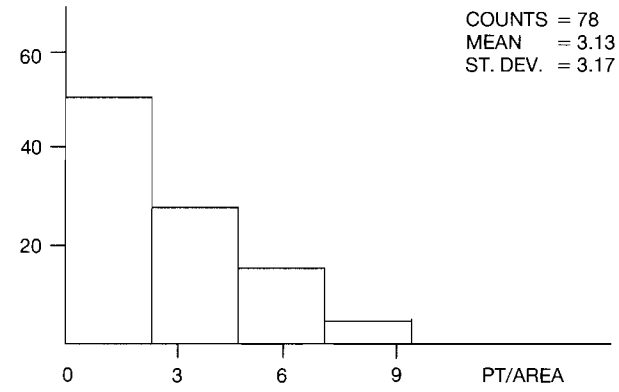


Fig. 6b.

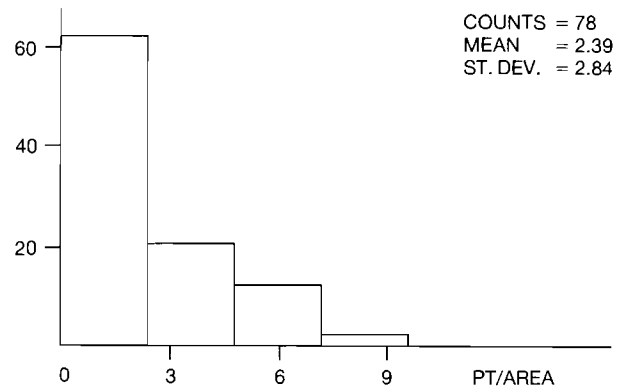


Fig. 6a, b. The surface density of the openings of the surface connected canalicular system is very high in platelets from a patient (2) affected with chronic myelogenous leukemia (mean value = 3.1), more than the one of a healthy volunteer of the same range of age (mean value = 2.4)

Discussion

Platelets collected from patients with chronic myelogenous leukemia, subjected to therapy, have shown highly heterogenous features: in some patients the platelets appeared abnormal in the cytoplasm («hypogranular» platelets rich in canalicular systems), or in their shapes (platelets joined together) or in their morphometric parameters (area and perimeter reduced).

Viceversa, in two patients no ultrastructural modifications of the platelets have appeared.

The aspect of platelets joined together observed in one patient might be interpreted as a defect in the platelet release from the megakariocyte: in patients with preleukemia or with chronic granulocytic leukemia, ultrastructural morphologic alterations of the megakariocytes have been described (Saarni et al., 1971; Maldonado et al., 1974).

The high heterogeneity of the platelet populations in the different patients is in a good agreement with the observations by Maldonado et al. (1974, 1975) that described agranular and hypogranular forms, as well as platelets with abnormal granules probably due to the dishemopoietic state in some but not in all patients affected with myeloproliferative disorders.

The abnormal ultrastructural aspects observed by us might constitute the basis for the platelet dysfunctions described by several authors in myeloproliferative disorders (Inceman and Tangun, 1972; Sultan and Caen, 1972) even if effects due to the intensive therapy cannot be excluded.

The computerized morphometric system has resulted a good methodological approach to evaluate the platelet modifications in this disease.

We are now going to collect platelets from further, possibly not-treated, patients with myeloproliferative disorders, to better characterize the morphological modifications also of the circulating platelets in these diseases together with some biochemical parameters of the platelet function (beta-thromboglobulin, fibrinopeptide A) in plasma.

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