

Platelets aggregation changes in patients with Rendu Osler Disease – Brief Report

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ABSTRACT

Background: Rendu Osler Disease is an autosomal dominant disease characterised by multiple telangiectatic lesions on the skin and mucosa. Pathogenesis of hemorrhagic diathesis in patients with Rendu Osler Disease is little understood. It may be due to both mechanical fragilities and haemostasis or aggregation platelet alterations.

Design and methods: We studied the platelet aggregation pattern for ADP, collagen, epinephrine and ristocetin in 4 patients with Rendu Osler Disease who were admitted in University Emergency Hospital Bucharest. All tests were performed on a Chrono-log aggregometer using optical method.

Results: The analysis of the graphic curves reveals a marked alteration of platelets' function in patients with Rendu Osler disease versus control, with a marked decrease in slope and amplitude. Regarding the lag phase's amplitude and duration, there were no modifications when compared to control. The analysis of the graphic curves for ristocetin and epinephrine reveals a severely altered response.

Blood coagulation tests, Quick Test, APTT and fibrinogen were within normal limits for all patients. The results were similar for blood glucose, renal tests and the lipid profile. Regarding the complete blood count, normal values were obtained excepting haemoglobin, which was constantly low in all patients, that being associated with low serum iron and morphologic abnormalities of the erythrocytes.

Conclusions: Patients with Rendu Osler Disease have severely altered platelet functions. Our results were similar with other reports, which indicated severe alterations of platelet aggregation.

Key words: platelet aggregation, hereditary hemorrhagic telangiectasia, Rendu Osler Disease

INTRODUCTION

Haemorrhagic teleangiectasia was described for the first time in 1864 by Sutton, later recognised and reported by Rendu, Osler and Weber; now it is known as the Osler-Weber-Rendu Syndrome. The prevalence is 1: 50000

but can increase up to 1: 16500, and its maximum frequency is around 40 years of age. It is an autosomal dominant disease characterised by multiple telangiectatic lesions on the skin and mucosa. Bleeding in hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease) results from fragile, easily bleeding mucosal

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telangiectases. All mucosal surfaces may be involved, with bleeding commonly originating from the nose and from the respiratory, gastrointestinal, and genitourinary tracts. Cutaneous haemorrhagic lesions grow in number with age. One third of the patients present a severe form that necessitates hospitalisation, transfusions, iron intake or surgical treatment.

Hypothesis: Haemorrhagic manifestations are very frequent in patients with Rendu Osler Disease, due to an increase in the mechanical fragility of telangiectatic vessels. However, there have been described other various changes of haemostasis. Platelets functional alterations have been reported by some studies but infirmed by others (1,2,3).

Objective: To highlight qualitative platelets alterations in patients with Rendu Osler Disease by using the technique of platelets aggregation.

Methods: Platelets aggregation was investigated in 4 patients with severe Rendu Osler Disease. The tests were performed in the laboratory of Haematology Clinics University Emergency Hospital Bucharest. Informed consent was taken for all patients and healthy volunteers. Patients had a history of frequent anterior epistaxis, about 1 episode/month, without other mucosal bleedings, with the onset of symptoms around the age of 40 and the necessity of receiving erythrocyte mass substitution. Genetic transmission was investigated in all patients because there were more cases with other family members having the disease. Two of the patients were brothers.

The blood samples were taken in the morning after fasting, in those periods when patients didn't associate severe anaemia. To obtain the platelet rich plasma (PRP), the blood was centrifuged for 15 minutes at 800G. The aggregation reagents used were ADP, collagen, epinephrine and ristocetin, all tests being performed on a Chrono-log aggregometer using the optical method.

The recorded variables for this study are:

- Independent variable – membership of the Rendu Osler diagnosed patients group.
- Dependent variables – amplitude, slope of aggregation graphic curves for different reagents, amplitude and duration of the lag phase for graphic curves obtained when using ADP or collagen reagents.

The sample group followed in this study is small and therefore a nonparametric test was chosen. The small number of patients does not permit to obtain statistical results sure enough to be extended in the general population.

The descriptive statistical analysis was performed by representing the whole distribution with graphic columns for each dependent variable and comparing the results with the mean value of dependent variable's amplitude obtained from the control group represented by 30 healthy volunteers (students, doctors, nurses) who did not receive any medication capable to alter the platelets function (aspirin, nonsteroidal anti-inflammatory drugs). Hypothesis testing was performed by applying the Mann-Whitney U test.

RESULTS

We attempted to find whether there are platelet aggregation abnormalities at various stimuli.

The analysis of the graphic curves obtained in studied patients reveals a marked decrease in amplitude for all reagents.

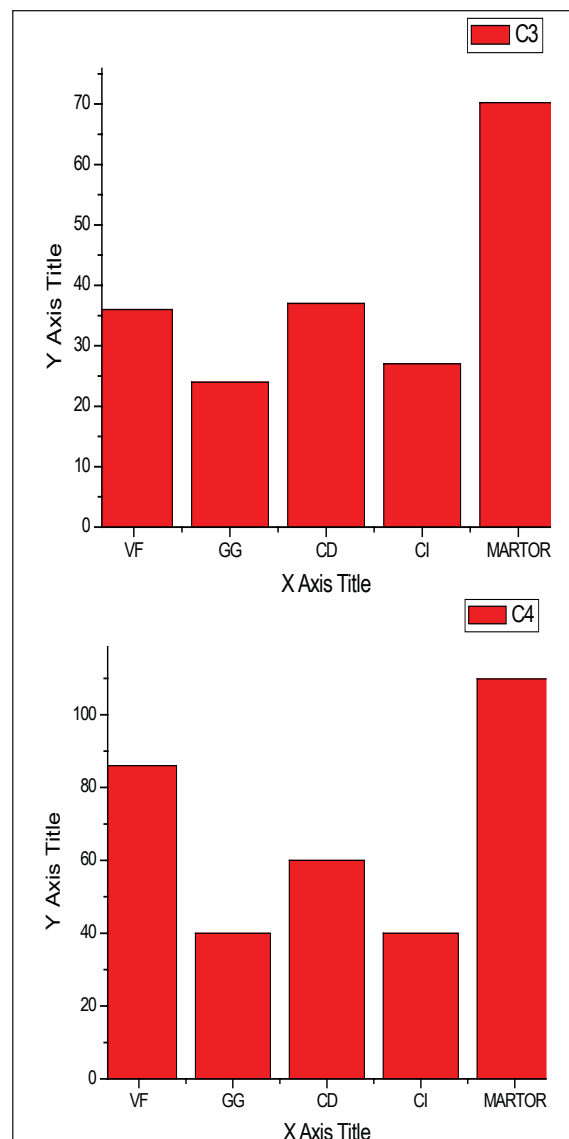


FIGURE 1. Graphic curve's amplitude and slope for ADP 10ug/ml

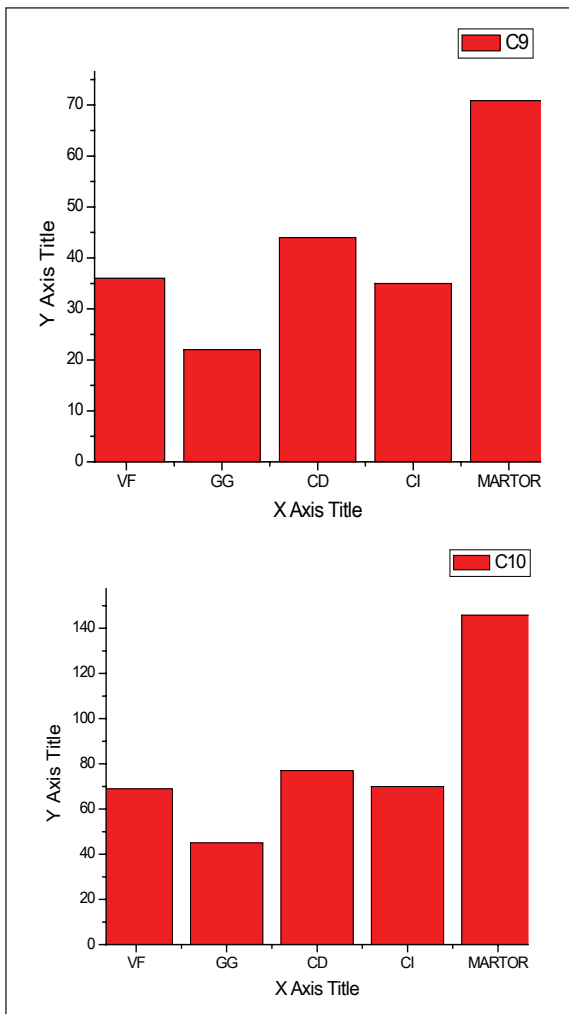


FIGURE 2. Graphic curve's amplitude and slope for collagen

In one patient we observed deaggregation with ADP. The presence of a second ADP stimulation graphic curve was noticed only in one patient, corresponding to the age group between 50 and 60 years old. Two patients in the age group over 70 years old presented a severe alteration of platelets aggregation with ADP – the absence of the second phase of aggregation. The analysis of the graphic curves reveals a marked alteration of platelets function in patients with Rendu Osler Disease versus control, with a marked decrease in slope and amplitude. Regarding the lag phase's amplitude and duration there were no modifications when compared to control. Platelets retain their capacity to change shape at the moment they become activated.

The analysis of the graphic curves for ristocetin and epinephrine reveals a severe altered response, with a marked decrease in amplitude, one patient having no response at all. However, three cases showed the presence

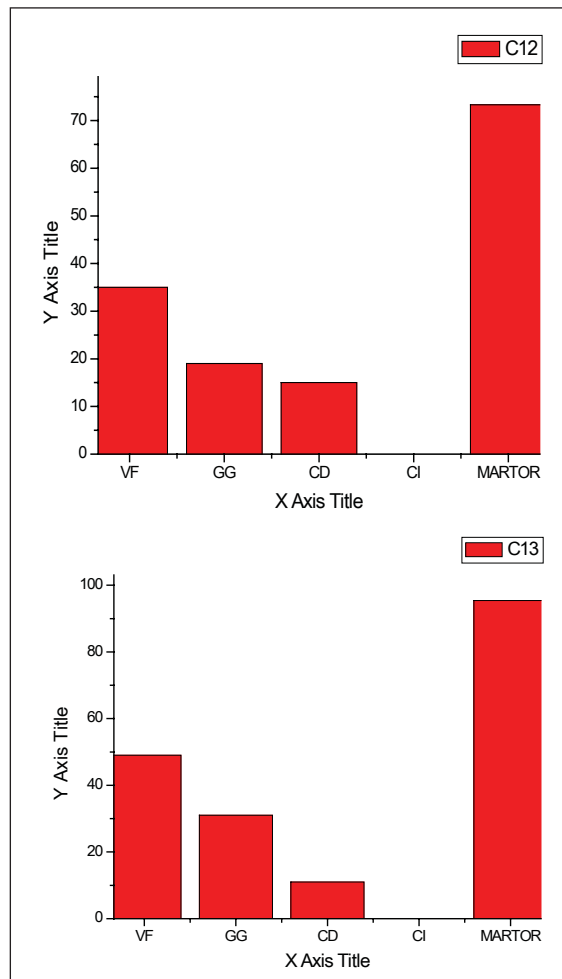


FIGURE 3. Graphic curve's amplitude and slope for epinephrine

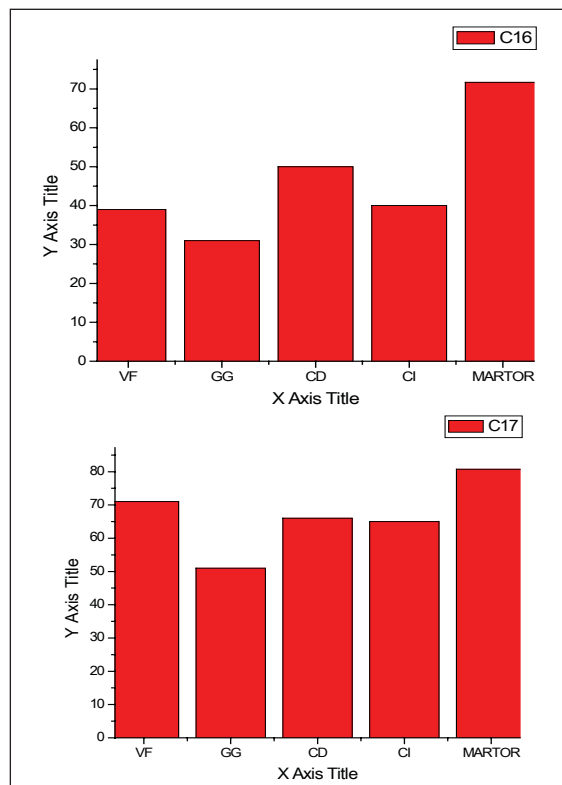


FIGURE 4. Graphic curve's amplitude and slope for ristocetin

of the second graphic curve which indicates the secretory capacity of these patient's platelets. The patient with no response for epinephrine had a severe form of the disease, needing frequent erythrocyte mass transfusion, presenting clinically numerous telangiectasias on the face, lips and tongue and frequent epistaxis. For ristocetin, the differences were less apparent.

Blood coagulation tests, Quick time, APTT and fibrinogen were between normal limits for all patients. The results were also normal for blood glucose, renal tests and the lipid profile. Regarding the complete blood count, normal values were obtained excepting haemoglobin which was constantly low in all patients; the anaemia was associated with impaired erythrocytes indices, reticulocytosis, microcytosis and low serum iron.

DISCUSSIONS

Rendu Osler Disease was described for the first time in 1864 by Sutton, later recognised and reported by Rendu, Osler and Weber, being known as the Osler-Weber-Rendu Syndrome. It is an autosomal dominant disease characterised by multiple telangiectatic lesions on the skin and mucosa, associating epistaxis and other haemorrhagic complications. Its prevalence is 1:50000, but it can increase up to 1:16500, and its maximum frequency is around 40 years of age (4).

Three defective genes responsible for the vascular malformations were identified. Thus the OWR1 locus, located on 9q33-34 chromosome was implicated. Later the endoglin gene was identified. Endoglin is a membrane glycoprotein expressed by endothelial cells of arterioles, venules and capillaries. This glycoprotein role is to bind TGF- β (tumor growth factor β). In other cases, it was identified a second locus, OWR2, located on 12q chromosome, corresponding to the ALK1 gene whose role is to synthesise another TGF- β receptor. These mutations are responsible for the alterations in angiogenesis (3,4,5,6).

The mechanisms by which these gene defects lead to the development of telangiectasias were not yet identified. Postcapillary venule enlargement appears to be the first detectable morphologic modification. Later, direct communications between arterioles and venules appear, with an infiltrate of mononuclear cells in perivascular region of telangiectasias.

The pathogenesis of haemorrhagic diathesis in Rendu Osler Disease patients is little understood. One possible explanation includes

the mechanical fragility and rupture of telangiectatic vessels. There were also reported other various changes in haemostasis, platelets aggregation and adhesion and an abnormal synthesis of von Willebrand factor by endothelial cells (7,8,9,10,11). Platelet's functional alterations have been reported by some studies but infirmed by others. Disseminated *intravascular coagulation and fibrinolytic system activation were also reported. There were described prolonged bleeding time and APTT, an increase in capillary fragility, the decrease of vWf:Ag and VIII:C levels, the decrease of ristocetin cofactor and severe decrease of platelets adhesion and aggregation especially with ristocetin and collagen reagents (4,12,13). Referring to clinical manifestations, cutaneous haemorrhagic lesions grow in number with age. They range in size between 1-3 mm diameter, being apparently well delimited. They disappear on diascopy. The lesions are evident on the face, lips, tongue, nose alae, nail bed and hands. These are not clinically important because of the rare bleedings occurring at these sites. Epistaxis represents a symptom present in almost 90% of patients. It originates from telangiectatic lesions located on nasal septum and inferior vestibule. It frequently manifests before 35 years of age and is highly variable. One third of the patients present a severe form which necessitate hospitalisation, transfusions, iron intake or surgical treatment. Pulmonary arteriolar-venular malformations appear in 5-30% of patients. Recent genetic studies showed that mutations in chromosome 9q have a higher rate of pulmonary vascular malformations. These are multiple and they are located in the inferior pulmonary lobes. They may cause a significant right-left shunt, determining the patients to present with significant dyspnoea, cyanosis, physical asthenia, a decrease in effort tolerance and polyglobulia. A paradoxal embolus may appear, determining a cerebral transient ischemic attack, stroke, or cerebral abscesses. Pulmonary arteriolar-venular malformations may bleed, causing haemoptysis or haemothorax.*

20% of the patients may develop upper and lower digestive tract hemorrhages. Digestive tract hemorrhage (DTH) is rare before 50 years of age. About 40% of DTH are upper hemorrhages, only 10% are located in the large bowel and in 50% of cases the origin cannot be revealed (4).

Hepatic involvement is rare. The localisation at this site determines hepatomegaly and altered hepatic tests. A significant arteriolar-venular shunt may lead to right heart failure. Anicteric cholestasis and atypical cirrhosis were

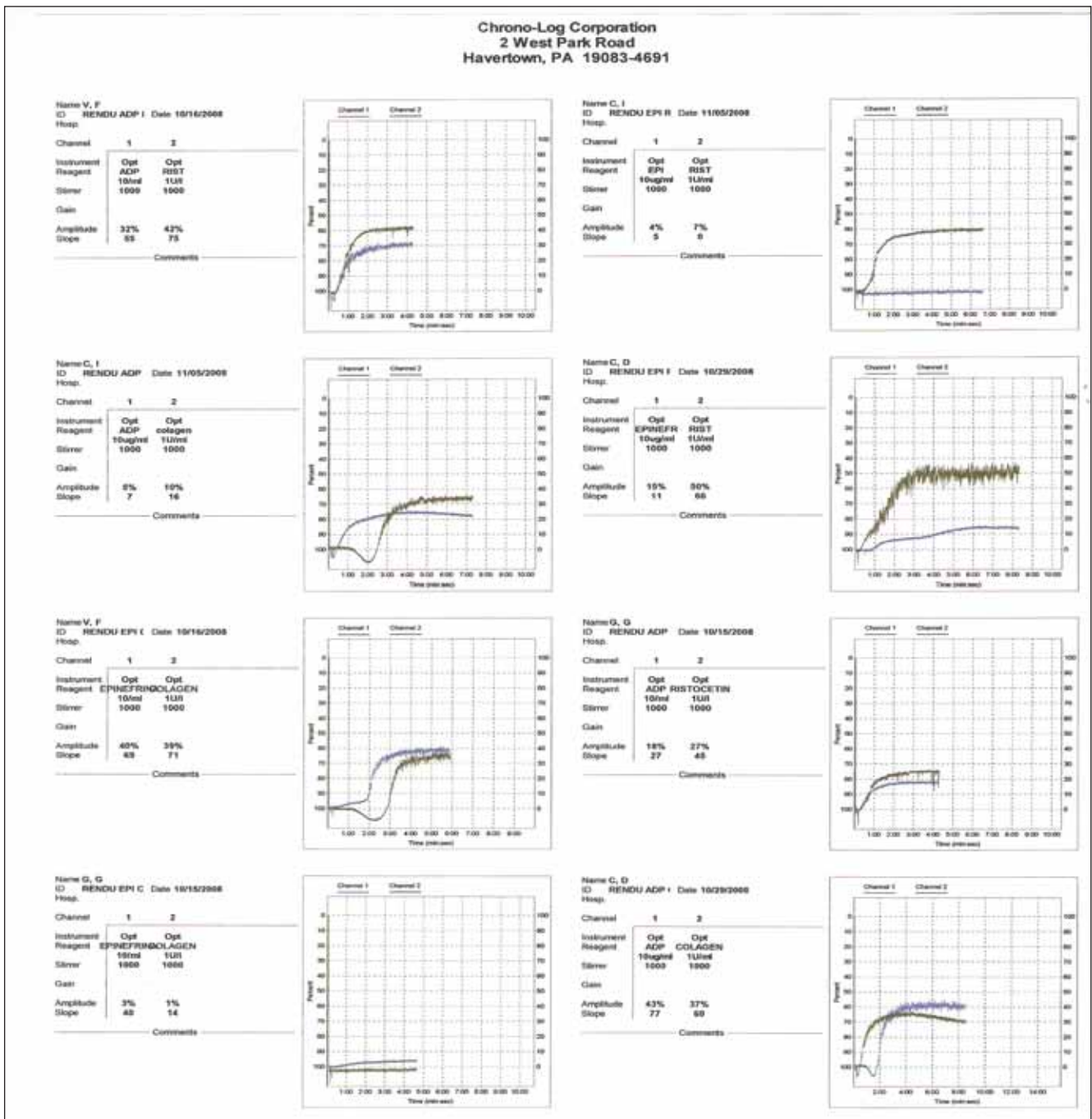


FIGURE 5. Comparative platelet aggregation slopes for ADP, epinephrine, collagen, ristocetin in 4 patients with Rendu-Osler disease

also reported. Patients with type 1 mutation have large telangiectasias at the level of small and large bowel mucosa, but those with type 2 mutation have more frequent vascular abnormalities in the liver (14, 15).

Neurologic symptoms are due to cerebral telangiectasias, aneurysms and cavernous haemangiomas, being able to determine haemorrhage and palsy (4, 5, 6).

Concerning clinical pictures for all patients who were studied, we observed more cutaneous hemorrhagic lesions. Patients who were admitted

in our study are 60-75 years old; two of them are over 70 years old. The medical history shows an increase of the number of cutaneous hemorrhagic lesions, which grow in number with age. This data was reported in literature too.

Three of patients had a reduced response for epinephrine, and for one patient this response was associated with a very low response for collagen.

For all these patients the hemorrhagic episode (medium or severe epistaxis - 2-3 times per month, upper and lower digestive tract hemorrhage) was

clinically obvious and these patients had to be admitted in the hospital and to receive substitutive treatment with iron and transfusions.

Comparative with reagents type and platelet aggregation response, we observed more alterations in the platelet aggregation pattern for collagen, epinephrine and ADP than for ristocetin.

The literature data described an association between Rendu Osler Disease and von Willebrand Disease (7,8,9,10,11,15). This association seems to be due to von Willebrand factor which is synthesized by the vascular endothelial cells and is a common pathogenic factor in Rendu Osler Disease.

Blood coagulation tests for all patients were normal and the platelet aggregation curve for ristocetin was present but with reduced amplitude. Von Willebrand Disease type IIB or IIC should be under debate. Multimers of Willebrand factor-ristocetin cofactor or antigen must be studied for correct diagnosis.

CONCLUSIONS

The platelet function is altered in patients with Rendu-Osler disease and the presence of

this alteration explains the severity of the haemorrhagic syndrome. The low number of patients included in our study does not allow an exact statistical analysis. Still, all the patients presented altered platelet aggregation patterns to various stimuli. Although without statistical value, it is important to underline the direct association between the severity of the altered aggregation patterns and the clinical severity of the disease (increased number of teleangiectatic lesions, more severe and frequent haemorrhagic syndrome). These results highlight the importance of extending the study to a larger number of patients.

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