Sebastian Syndrome: Report of the First Case in a South American family

The Sebastian syndrome (SS) is a MYH9-related disorders, which are an extremely infrequent group of four autosomal dominant illnesses. SS consist of giant platelets, leukocyte inclusions and thrombocytopenia. To our knowledge, there are no case reports of this syndrome in South America. The propositus was a 35-year-old Argentine woman with a history of purpuric lesions in her lower limbs and thrombocytopenia. Idiopathic thrombocytopenia purpura (ITP) was previously diagnosed, but she did not respond to treatment with steroids. Family history failed to provide any evidence of hearing loss, easy bruising, nephritis, renal failure or cataracts. The patient and 11 members of her family were evaluated. The diagnosis of SS was established by demonstrating giant platelets, thrombocytopenia and leukocyte inclusions in peripheral smear in two relatives and by peripheral smear and electronic microscopy in the propositus. MYH9-related disorders should be suspected whenever a patient has a low platelet count or a bleeding diathesis of unknown origin. In these cases, the history, carefully peripheral smear exam, immunocytochemistry and electronic microscopy will be of great help. Differentiation ITP with SS is important to avoid unnecessary diagnostic studies and treatments.

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Introduction.

The MYH9-related disorders are an extremely infrequent group of four autosomal dominant illnesses.[1] May-Hegglin anomaly is characterised by giant platelets, thrombocytopenia and spindle-shaped inclusion bodies in granulocytes, which consist of 7-10 nm parallel-lying filaments.[2] The Fechtner syndrome is a variant of the Alport syndrome, with inclusion bodies consisting of dispersed filaments, clusters of ribosomes and a few segments of rough and smooth endoplasmic reticulum.[2] Epstein syndrome is associated with Alport syndrome but without leukocyte inclusions.[3] The Sebastian syndrome (SS), described in 1990 by Greinacher *et al.* [4] shows giant platelets, thrombocytopenia, leukocyte inclusions similar to those in the Fechtner syndrome, mild bleeding tendency, however lacking the additional anomalies seen in Alport syndrome. These syndromes arise from a similar defect. The mutation responsible is in MYH9 gen, on chromosome 22, which encodes the heavy chain of nonmuscle myosin IIA (NMMHC-IIA).[1,5-7] The MYH9 gene is expressed in platelets and upregulated during granulocyte differentiation.[6]

Immunofluorescence studies in patients with MYH9-related disorders revealed that NMMHC-IIA distribution in neutrophils appeared to mimic the characteristic inclusions (Döhle-like bodies).[8] The basic defect of platelets is related to a profound abnormality of the cytoskeleton.[1] Renal failure pathogenesis is being clarified and it is likely to be related to anomalies in the podocyte cytoskeleton.[1] The pathogenesis of hearing loss and cataracts remains obscure.[1]

Cases of SS have been reported in Germany, Japan, Saudi Arabia, Spain, Italy and United States.[2,4,9-12] We have not found any reports of SS in South America.

Case report.

The propositus was a 35-year-old Argentine woman with a history of purpuric lesions in her lower limbs that appeared with intermittence. The platelet count varied from $19x10^9$/L to $23x10^9$/L and phase microscopy count was bigger than the automated count in previous studies. Idiopathic thrombocytopenia purpura (ITP) was previously diagnosed, but she did not respond to treatment with steroids. She had three vaginal deliveries without excessive bleeding and had no other bleeding manifestations except the skin lesions. She never received either platelet or red blood cell transfusions. Family history failed to provide any evidence of hearing loss, easy bruising, nephritis, renal failure or cataracts.

Material and methods. The patient and 11 members of her family (Figure 1.) were evaluated with complete physical exam including ophthalmologic and audiologic examination, serum creatinine and urine sediment. A complete blood count (CBC), a platelet count on a Cell-Dyn 1600 (Abbott, California, USA) and by phase microscopy with ethylenediamine tetraacetic acid (EDTA) and sodium citrate as anticoagulants to rule out EDTA agglutination were performed. Moreover, peripheral smear exams stained with May Grünwald-Giemsa (MGG) stain and leukocyte alkaline phosphatase were performed.

Coagulation studies [bleeding time (Modified Ivy method), prothrombin time, partial thromboplastin time and fibrinogen] and platelet aggregation studies (Lumi-aggregometer; Chrono-Log Corporation, Hawertown, Pennsylvania) with adenosine diphosphate (ADP) 2,5

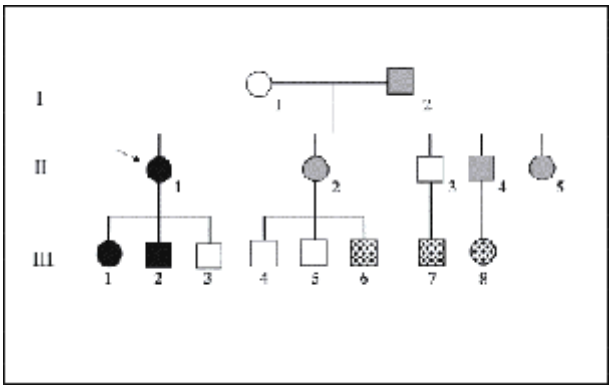

Figure 1. Familiar lineage. Circles represent females and squares represent males. Open symbols denote unaffected family members, black symbols denote affected family members with giant platelets and thrombocytopenia and grey symbols denote affected family members with anisocytosis and normal platelet count. Plucking symbols denote family members not analysed. Arrow denote the propositus.

mM, epinephrine 100 mM, collagen 2 mcg/mL, ristocetin 15 mg/mL and ristocetin-induced agglutination of normal platelets (von Willebrand factor ristocetin cofactor) were performed to the propositus and four relatives (I-2, II-2, III-1 and III-2).

Platelets and leukocytes ultrathin sections of the patient were stained with uranyl acetate and lead citrate, and observed under an Elmiskop 1A electron microscope (Siemens, Berlin, Germany).

Results. Alterations in the CBC were found in seven of the 12 members studied. Three of them had giant platelets and thrombocytopenia (II-1, III-1 and III-2) and four anisocytosis with normal platelet count (I-2, II-2, II-4 and II-5). In the seven members, the phase microscopy count was bigger than the automated count (mean: 38,1%) and in members with giant platelets and thrombocytopenia the mean difference was 73,4%. Leukocyte inclusions in neutrophils, monocytes and eosinophils were observed in the peripheral smear of four members (I-2, II-1, III-1 and III-2) (Figure 2. and Table 1.).

Leukocyte alkaline phosphatase was normal in all members. A decrease of the basal line and an extension of the curve that exceeded 30 fl were observed in the platelet histograms of the members that presented platelet macrocytosis and thrombocytopenia. Platelets had spherical shape with an average diameter of 14 μm.

Electronic microscopy showed inclusions in neutrophils consisting of dispersed filaments, clusters of ribosomes and a few segments of rough and smooth endoplasmic reticulum not bounded by a membrane, similar to those in the Fechtner syndrome (Figure 3. and 4.). Platelet ultrastructural morphology revealed the presence of large but otherwise normal platelets.

Coagulation studies were normal in all members except in III-1, who presented a long bleeding time (Table 2). Platelet aggregation studies showed two types of aggregation: type 1, with a mild decreased response with all inductors and type 2 with mild decreased response to ADP and epinephrine and normal with the rest (Table 2).

In the family members evaluated, ophthalmologic and audiologic examination were normal as were serum creatinine and urine sediment.

Table 1. Platelet features

Patient	Platelet count (x 10⁹ /L) Counter	Microscopy	Difference (% of CC)	Morphology	WC with inclusion bodies (%)
I-2	207	220	6,3	anisocytosis	12
II-1	23	42	82,6	giant platelets	83
II-2	154	184	19,5	anisocytosis	0
II-4	228	239	4,8	anisocytosis	0
II-5	190	220	15,8	anisocytosis	0
III-1	28	47	67,8	giant platelets	78
III-2	48	68	70	giant platelets	90

The rest of the relatives evaluated presented normal count and morphology in platelets without leukocyte inclusions. **CC**: counter count. **WC**: white cells.

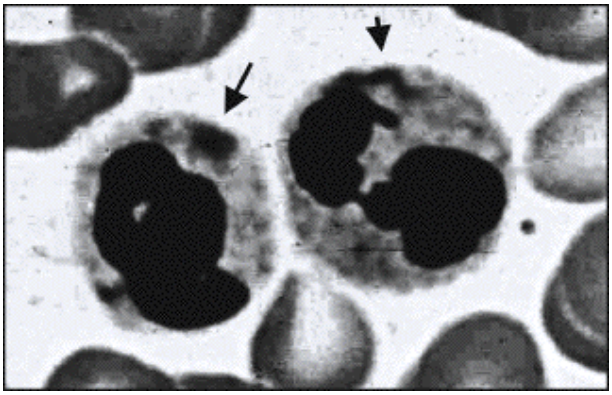

Figure 2. Peripheral smear photomicrograph with May Grünwald-Giemsa stain of propositus demonstrating leukocytes with inclusions (arrows) (magnification, x 1 000)

Discussion.

To our knowledge, there are no case reports of SS in South America. We report the first South American case of SS in an Argentine family.

Clinical expression of MYH9-related disorders may vary widely, even in member of the same family, which probably contributes to underestimating the frequency of these anomalies.[1,12] In symptomatic patients, bleeding first occurs during infancy and its severity does not change during life.[1] Mucosa and skin involvement, as in the case reported, is present in 40% of SS cases.[12] Despite this, cases of massive surgical, gastrointestinal and cerebral bleedings have been reported.[2,4,12] The only effective measures for preventing or treating bleeding events in this patients are platelet transfusions and desmopressin.[1,13]

ITP is a common cause of thrombocytopenia and can be associated with giant platelets.[3] Differentiation of SS is important to avoid unnecessary diagnostic studies and the morbimortality of immunosupressor (steroids, gammaglobulins, etc.) or surgical (splenectomy) treatments of the ITP.[11]

Table 2. Coagulation and aggregation studies

Patient	PT	PTT	Fibrinogen	vWF:RCof (%)	BT (h:m)	Aggregation
I-2	9	42	249	84	2 min 45 sec	Type 1
II-1	10,4	41	278	92	4 min 45 sec	Type 2
II-2	10,1	42	284	82	2 min 30 sec	Type 1
II-4	10,1	41	249	78	3 min 15 sec	Type 1
III-2	9,4	45	231	68	3 min	Type 2

PT: prothrombin time (normal value: 9,4-12 sec).
PTT: partial thromboplastin time (normal value: 35-45 sec).
Fibrinogen: normal value: 160-380 mg/dl.
vWF:RCof: von Willebrand factor ristocetin cofactor (normal value: 50-150%).
BT: bleeding time (normal value: 1 min 30 sec - 4 min 30 sec).
Type 1: mild decreased response with all inductors.
Type 2: mild decreased response to ADP and epinephrine and normal with the rest.

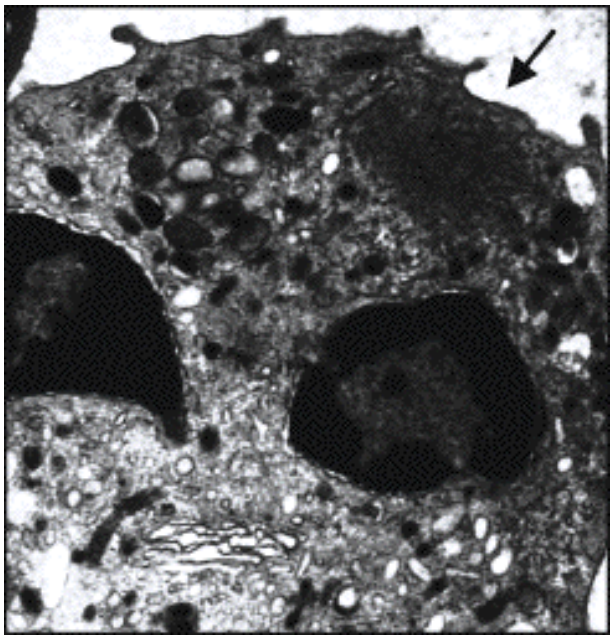

Figure 3. Thin section electron photomicrograph of a neutrophil revealing an inclusion (arrow) typical of those found in granulocytes of patients with SS (magnification, x 14 000).

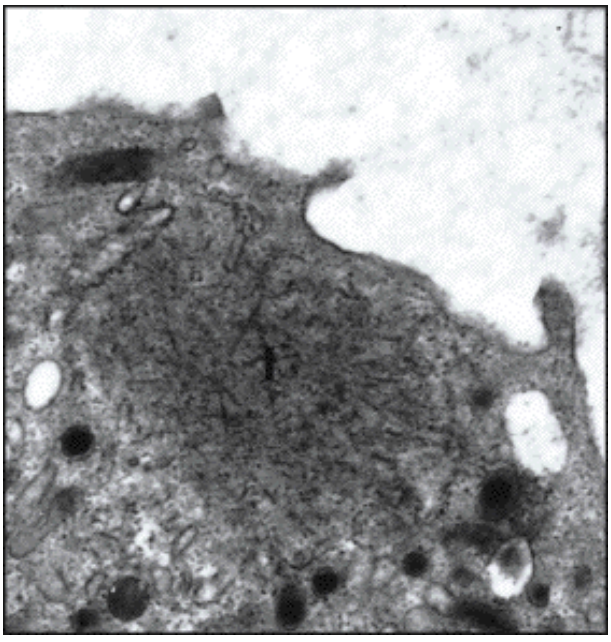

Figure 4. Thin section electron photomicrograph with higher magnification of the inclusion (I) not enclosed by membrane in the same neutrophil (magnification, x 52 000).

In coagulation studies, bleeding time may become increased or in the upper limit. Patients are not functionally thrombocytopenic, probably because total platelet mass is preserved.[4] Platelet aggregation is normal, although diminished aggregation with some inductors has been reported in patients with SS.[2,4]

The presence of Döhle-like bodies in leukocyte cytoplasm is a hallmark of MYH9-related disorders.[1] On MGG stained peripheral blood smears they appear as faint, light blue, round or spindle shaped amorphous inclusions located at the cell periphery or in the inner cytoplasm.[1] Leukocyte inclusions are found mainly in mature neutrophils, although they have been reported in myeloid precursors, basophils and monocytes.[1,2,4] There are no reports in either erythroid precursors or lymphocytes.[12] Ultrastructural studies showed that Döhle-like bodies consist of ribosomes and microfilaments 7-10 nm in diameter. Two ultrastructural patterns have been described.1 Immunocytochemistry with antibodies against NMMHC-IIA is the simplest method to confirm the diagnosis of MYH9-related disorders and does not require specialized centers.1 It is more sensitive than MGG staining and cheaper than electron microscopy.[1]

The only constant feature of patients with MYH9-related disorders is platelet macrocytosis, most patients have thrombocytopenia but few have normal platelet count.1 In patients of this case with giant platelets and thrombocytopenia, the phase microscopy count was higher than in the automated count. This is explaining because automated counts measure big-size platelets similar to erythrocytes and underestimate the platelet count and volume.[11]

In conclusion, MYH9-related disorders should be suspected whenever a patient has a low platelet count or a bleeding diathesis of unknown origin. In these cases, the history, carefully peripheral smear exam, immunocytochemistry and electronic microscopy will be of great help. Differentiation ITP with SS is important to avoid unnecessary diagnostic studies and treatments.

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References

1. Balduini CI, Iolascon A, Savoia A. Inherited thrombocytopenias: from genes to therapy. Haematologica 2002; 87: 860-80.
2. Khalil S, Qari M. The Sebastian platelet syndrome. Report of the first native Saudi Arabian patient. Pathology 1995; 27: 197-98.
3. Young G, Luban N, White J. Sebastian Syndrome: case report and review of the literature. Am J Hematol 1999; 61: 62-65.
4. Greinacher A, Nieuwenhuis H, White J. Sebastian platelet syndrome: a new variant of hereditary macrothrombocytopenia with leukocyte inclusions. Blut 1990; 61: 282-8.
5. Toren A, Rozenfeld-Ganot G, Rocca B, Epstein C, Amariglio N, Laghi F et al. Autosomal-dominant giant platelet syndromes: a hint of the same genetic defect as in Fechtner syndrome owing to a similar genetic linkage to chromosome 22q11-13. Blood 2000; 96: 3447-51.
6. The May-Hegglin/Fechtner Syndrome Consortium. Mutations in MYH9 result in the May-Hegglin anomaly, and Fechtner and Sebastian syndromes. Nat Genet 2000; 26: 103-5.
7. Kelley M, Jawien W, Ortel T, Korczak J. Mutation of MYH9, encoding non-muscle myosin heavy chain A, in May-Hegglin anomaly. Nat Genet 2000; 26:106-8.
8. Kunishima S, Kojima T, Matsushita T, Tanaka T, Tsurusawa M, Furukawa Y et al. Mutations in the NMMHC-A gene cause

autosomal dominant macrothrombocytopenia with leukocyte inclusions (May-Hegglin anomaly/Sebastian syndrome). Blood 2001; 97: 1147-49.
9. Tsuda H, Yamasaki H, Miyayama H. Sebastian platelet syndrome: two Japanese families originally diagnosed with May-Hegglin anomaly. Int J Hematol 1999; 70: 290-93.
10. Tsurusawa M, Kawakami N, Sawada K, Kunishima S, Agata H, Fujimoto T. The first Japanese family with Sebastian platelet syndrome. Int J Hematol 1999; 69: 206-10.
11. Young G, Luban N, White J. Giant Platelet Disorders in African-American Children Misdiagnosed as Idiopathic Thrombocytopenic Purpura. J Ped Hematol Oncol 1999; 21: 231-36.
12. Aguilar Franco C, Lucia Cuesta J, Gomez Ara A, Navarro M. The Sebastian platelet syndrome: description of 2 cases and review of the literature. Sangre (Barc) 1998; 43: 262-4.
13. Noris P, Spedini P, Belletti S, Magrini U, Balduini C. Thrombocytopenia, giant platelets, and leukocyte inclusion bodies (May-Hegglin anomaly): clinical and laboratory findings. Am J Med 1998; 104: 355-60.