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Application of a diagnostic algorithm for inherited thrombocytopenias to 46 consecutive patients

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A B S T R A C T

Background and Objectives. The Italian *Gruppo di Studio delle Piastrine* recently developed a diagnostic algorithm to assist clinicians in the diagnosis of inherited thrombocytopenias. This algorithm is based on the simplest possible diagnostic investigations and can also be used in centers that are not highly specialized. The aim of the present study was to validate this diagnostic algorithm by applying it to a case series of genetic thrombocytopenias.

Design and Methods. The diagnostic algorithm was applied retrospectively to 46 consecutive patients observed during the last five years at a single institution. Twenty-eight were affected by defined illnesses or their variants, while 18 had a disorder that did not fit the criteria for any known genetic thrombocytopenia. The study was based on the evaluation of clinical records and laboratory tests.

Results. The diagnostic algorithm recognized: 4 homozygous and 4 heterozygous Bernard-Soulier syndromes, 11 *MYH9*-related diseases, one von Willebrand's disease type 2B, one gray platelet syndrome and one X-linked thrombocytopenia with thalassemia. Moreover, it identified 4 patients with the clinical and laboratory features of heterozygous Bernard-Soulier syndrome not caused by mutations in the coding region of the GPIbα, GPIbβ, GPIX or GPV genes, and two patients with the clinical phenotype of *MYH9*-related disease but without *MYH9* mutations. Since the diagnostic flow chart did not allow prompt recognition of two subjects with *MYH9*-related disease, we introduced a small change to the previously proposed flow chart to obviate this defect.

Interpretation and Conclusion. The diagnostic algorithm correctly diagnosed 26 of 28 patients with known disorders or phenotypic variants of known disorders. By a simple modification of the investigation sequence, its sensitivity reached 100%. The algorithm also identified 18 patients with *new*, as yet uncharacterized forms of genetic thrombocytopenia.

Key words: inherited thrombocytopenias, diagnostic algorithm, platelets.

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Once considered exceptionally rare, inherited thrombocytopenias are increasingly recognized as a spectrum of clinical disorders ranging from severe diseases discovered early in infancy to mild conditions incidentally identified in adults. As for many other rare diseases, knowledge about inherited thrombocytopenias is not very widespread and the diagnosis is usually made only in a few specialized centers. As a consequence, several patients are not properly classified or receive a definite diagnosis only after a long delay. A definite diagnosis for patients with hereditary thrombocytopenias is required in order to determine the prognosis and the best therapeutic approach. Moreover, it defines the risk of transmitting the disorder to progeny, and in many cases allows antenatal diagnosis. The Italian *Gruppo di Studio delle Piastrine* has recently proposed an algorithm to facilitate the diagnosis of these disorders.[1] This algorithm is based on a first phase of clinical investigations and simple laboratory tests to put forward diagnostic hypotheses also in non-specialized centers, and is followed by a second phase of more complex studies. To validate this diagnostic algorithm, we applied it retrospectively to 46 consecutive patients who had been studied at a single institution during the last 5 years. For a detailed discussion of the clinical and biological features of genetic thrombocytopenias affecting our patients we refer the reader to recent reviews on this topic.[2,3]

Design and Methods

Diagnostic algorithm

The diagnostic algorithm was recently described in detail elsewhere.[1] In brief, it is composed of a first phase requiring simple

investigations, and a second phase based on more specialized studies. In the first phase, patients are categorized according to the presence (syndromic forms) or absence (non-syndromic forms) of clinical features other than those deriving from the platelet defects. In syndromic forms, history and physical examination lead to a diagnostic suspicion. For non-syndromic thrombocytopenias, platelet size directs the sequence of investigations required to generate a diagnostic hypothesis and to confirm it. In patients with small platelets, X-linked thrombocytopenia is suspected. In subjects with normal-sized platelet, a diagnosis of congenital amegakaryocytic thrombocytopenia (CAMT) or thrombocytopenia 2 (THC2) is considered. Specific investigations need to be carried out in patients with macrothrombocytopenia. These investigations include morphologic examination of May-Grünwald-Giemsa (MGG)-stained peripheral blood smears, evaluation of ristocetin induced *in vitro* platelet agglutination, and search for spontaneous *in vitro* platelet aggregation. Based on the findings of the screening phase, the diagnostic algorithm indicates the specialized investigations for the final diagnosis.

Patients

The diagnostic algorithm was applied to 46 consecutive, unrelated patients who were studied over the last five years for an inherited thrombocytopenia of unknown origin at the Center for Thrombosis and Hemostasis of the IRCCS Policlinico San Matteo, Pavia, Italy. The genetic origin of thrombocytopenia was assigned when either the disease segregated within the family or after exclusion of all the causes of non-genetic thrombocytopenias in subjects with a low platelet count since birth. Complete clinical records, including medical history, physical examination, results of *in vitro* platelet aggregation and morphologic examination of peripheral blood films were available for all patients. When the results of other investigations required by the diagnostic algorithm were not available, the patients were recalled again and the examinations were performed. For those cases previously reported in the literature, we refer the readers to the corresponding references for a detailed description.

Methods

Evaluation of platelet count and size. Platelet count and volume were ascertained by automatic examination of blood samples. Since automated cell counters enumerate and size particles ranging within a specified volume window (e.g., 2-20 fL for platelets), platelet count and volume may be underestimated in patients with large platelets. Moreover, cell counters do not calculate mean platelet volume in the presence of low platelet numbers or severe platelet anisocytosis. For these reasons, we also counted platelets with a hemocytometer by phase microscopy, and determined the percentages of platelets with a diameter < 4 μm, from 4 to 8 μm, and larger than 8 μm on MGG-stained blood films by microscope observation. Normal value ranges were calculated in 50 healthy donors. Five hundred platelets were examined for each affected or control subject.

Morphologic and immuno-morphologic examination of peripheral blood smears

In addition to a correct evaluation of platelet size, MGG-stained peripheral blood smears were examined in order to identify: (a) platelets lacking in azurophilic granules that are typical of gray platelet syndrome (GPS); (b) red blood cell anisopoikilocytosis and microcytosis (not deriving from sideropenia), which are present in X-linked thrombocytopenias caused by mutations of GATA-1; (c) the light-blue inclusions in the cytoplasm of polymorphonuclear leukocytes (Döhle-like bodies) that are often detected in *MYH9*-related disease (*MYH9*-RD). In patients with suspected *MYH9*-RD, immunocytochemical studies on peripheral blood films with antibodies against the heavy chain of non-muscle myosin IIA (NMMHC-IIA) were performed in order to confirm the diagnostic hypothesis. In fact, whereas NMMHC-IIA is homogeneously distributed within granulocyte cytoplasm in controls, it always presents a spotty distribution in *MYH9*-RD.[4]

In vitro platelet aggregation

In vitro platelet agglutination induced by ristocetin (3, 1.5 and 0.5 mg/mL)(Sigma Chemical Co, St. Louis, MO, USA) was evaluated in citrated platelet-rich plasma (PRP) using Born's method, as previously reported.[5] The search for spontaneous platelet aggregation was done by stirring a PRP sample in the aggregometer for 10 minutes without agonists. When spontaneous *in vitro* platelet aggregation and/or enhanced platelet response to ristocetin were observed, mixing experiments were performed in order to distinguish between platelet-type von Willebrand's disease (PTvWD) and von Willebrand's disease type 2B (vWD2B).[2]

Flow cytometry

Surface expression of platelet glycoproteins (GP) was investigated in PRP by flow cytometry with an Epics XL flow cytometer (Coulter Corporation, Miami, FL, USA) as previously reported.[5] The following monoclonal antibodies (mAbs) were used: SZ21 (Immunotech) that recognizes GPIIIa (CD61); P2 (Immunotech) recognizing GPIIb in the intact complex with GPIIIa (CD41); MB45 (CLB, Amsterdam, the Netherlands) and SZ2 (Immunotech) against GPIbα (CD42b); FMC25 (kindly provided by Zola H, Adelaide, Australia) and SZ1 (Immunotech) that recognize GPIX (CD42a); SW16 (CLB) against GPV (CD42d); and 11E4B7.6 (Immunotech) against glycophorin A (CD235a). MO_2 (Coulter Corporation) was used as a neg-

Table 1. Diagnosis, age at diagnosis, platelet count and size in the series of patients to whom the diagnostic algorithm was applied.

	No. of patients	Mean age in yr.	Mean platelets $\times 10^9$/L (range)		Mean % of platelets by diameter size (range)		
			Cell counter	Microscopy	<4 μm	4-8μm	> 8 μm
Homozygous BSS	4	29.5 (6-52)	20 (5-32)	57.3 (51-69)	64 (45-83)	30 (15.5-43)	5.1 (1.5-12)
Heterozygous BSS	4	46.7 (20-60)	41.2 (30-54)	81.5 (60-104)	71.1 (60-82.4)	25.8 (16-36.5)	3.0 (1.6-4)
Heterozygous BSS-like	4	35.6 (22-45)	94.3 (54-119)	123.6 (65-176)	87.1 (79-91)	11 (7-18)	1.5 (0.6-3)
*MYH9*RD	11	30.6 (5-48)	11.1 (0-39)	61.8 (18-124)	41.3 (21-71)	33.8 (20.5-54)	22.6 (2.5-38.5)
*MYH9*RD-like	2	18 (15-21)	64 (28-100)	78 (46-110)	73.5 (60.1-87)	24.6 (12-37.2)	1.7 (1.1-2.3)
vWD2B	1	9	1	32	65.5	28	6.5
GPS	1	65	25	68	70	25	5
XLTT	1	10	75	126	88.7	11	0.3
Unknown disorder							
large platelets	16	31 (16-56)	75 (20-133)	105 (37-145)	77.3 (54-91)	18.9 (7-34)	2.9 (1-12)
normal-sized platelets	2	47 (33-61)	59 (42-77)	56.5 (39-74)	96.2 (95.5-97)	2.8 (2.5-3.1)	0.5 (0-1)
Normal range			150-400	150-400	87-100	0-10	0-1

BSS: Bernard-Soulier syndrome; MYH9RD: myosin 9-related disease; vWD2B: von Willebrand's disease type 2B; GPS: gray platelet syndrome; XLTT: X-linked thrombocytopenia with thalassemia.

ative control. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG (GAM-FITC) was purchased from Coulter Corporation. SDS-PAGE of platelets was performed as previously described.[6]

Globin chain synthesis

In vitro globin chain synthesis was evaluated according to Weatherall *et al.* as previously reported.[7]

Mutation screening

Molecular analysis of the genes encoding NMMHC-IIA, GATA-1 and GPIbα was performed as described elsewhere.[5,7,8] To screen GPIβ, GPIX and GPV for mutations, polymerase chain reaction (PCR) amplifications were carried out under standard conditions using specific oligonucleotides available on request (*savoia@tigem.it*). PCR products were directly sequenced using dye terminator chemistry (Big Dye Terminator Cycle Sequencing kit, Applied Biosystems) following the instructions in the user's manual. The electrophoresis of the cycle-sequencing products was carried out in an ABI 377 automatic sequencer (Applied Biosystems) and data analyzed using specific ABI sequencing analysis software (Applied Biosystems).

Results

Diagnostic algorithm: phase 1

The screening phase of the diagnostic algorithm, which includes medical history, physical examination, microscopy evaluation of MGG-stained peripheral blood films and *in vitro* platelet aggregation, allowed us to raise a diagnostic suspicion in 18 of 46 patients through the following steps.

History and physical examination identified 4 patients with syndromic thrombocytopenias. In addition to a bleeding diathesis ranging from mild to severe, all of them had the additional features of nephritis associated with cataract and/or hearing loss, suggesting MYH9-RD. Examination of MGG-stained blood films supported the diagnostic suspicion of *MYH9*-RD raised by history and examination in two patients who had a large number of giant platelets (platelets larger than 8 μm) and Döhle-like bodies in leukocytes. In contrast, the diagnosis of *MYH9*-RD remained doubtful in the other two patients because they had neither Döhle-like bodies nor giant platelets, but only mild platelet macrocytosis. Evaluation of blood smears in patients with non-syndromic thrombocytopenias suggested a *MYH9*-RD in 7 additional patients with Döhle-like bodies and giant platelets. Moreover, the finding of giant and *pale* platelets lacking azurophilic granules prompted the diagnostic suspicion of GPS in one case. Finally, the observation of large platelets plus red cell anisopoikilocytosis with microcytosis in one male patient with a possible X-linked disorder suggested a possible diagnosis of X-linked thrombocytopenia with thalassemia (XLTT).

Finally, *in vitro* platelet function studies generated diagnostic hypotheses in 5 cases. An absent or severely reduced ristocetin-induced platelet agglutination strongly suggested the diagnosis of homozygous Bernard-Souli-

er syndrome (BSS) in four patients with giant platelets, while the association of spontaneous *in vitro* platelet aggregation with platelet agglutination after 0.5 mg/mL ristocetin (a dose of ristocetin that does not evoke a response in normal platelets) indicated the possibility of PTvWD or vWD2B in one patient.

In summary, phase I of the diagnostic algorithm allowed us to suspect the following diagnoses: 11 *MYH9*-RD (n=1), homozygous BSS (n=4), XLTT (n=1), GPS (n=1) and PTvWD or vWD2B (n=1). However, the majority (28 out of 46) of the patients remained without any potential diagnostic suspicion. Based on microscope evaluation, 26 had a platelet macrocytosis, which ranged from mild to severe, while only two had platelets of normal size.

Diagnostic algorithm: phase 2

Patients with a diagnostic hypothesis generated by the screening phase

Patients with a suspected diagnosis generated by phase I underwent additional specific investigations in order to confirm the diagnostic hypothesis.

Immunocytochemical study of NMMHC-IIA localization within granulocytes detected spots of NMMHC-IIA aggregates in all the 9 patients presenting with Döhle-like bodies and giant platelets. The diagnosis of *MYH9*-RD was confirmed by the identification of *MYH9* mutations in all of them (4 patients have already been reported).[4] In contrast, the two patients (one has been described previously)[4] (Medicine) with autosomal dominant macrothrombocytopenia and nephritis plus cataract and/or hearing loss, but without giant platelets and Döhle-like bodies had normal NMMHC-IIA distribution within granulocytes and no *MYH9* mutations, thus indicating genetic heterogeneity of subjects with this clinical phenotype.

Platelet flow cytometry showed severely reduced or absent binding of monoclonal antibodies against components of the GPIb/IX/V complex in the 4 subjects with defective *in vitro* platelet agglutination induced by ristocetin, thus confirming the diagnostic suspicion of homozygous BSS.

SDS-PAGE of platelets demonstrated severe deficiency of α-granule proteins in the patient with pale platelets on MGG blood films, thus confirming the diagnosis of GPS.

Analysis of globin chain synthesis and morphologic bone marrow examination further supported the diagnostic suspicion of XLTT by demonstrating an unbalanced α/non-αglobin chain ratio and a severe dysmegakaryocytopoiesis, respectively. The diagnosis was definitely confirmed by identification of the R216Q mutation in *GATA-1* (this case has been recently published).[7]

The study of *in vitro* platelet function in the patient with possible PTvWD or vWD2B showed that the patient's plasma was able to enhance the response of normal platelets to ristocetin, while washed platelets from the patient agglutinated normally after ristocetin addition when resuspended in normal plasma. On this basis, a diagnosis of vWD2B was made.

Patients without a diagnostic hypothesis generated by the screening phase

Phase 1 of the diagnostic algorithm did not raise a diagnostic suspicion for 28 patients.

In 10 of the 26 subjects with platelet macrocytosis, flow cytometry showed that binding of monoclonal antibodies against components of GPIIb/IIIa complex was higher than normal, as expected for large-sized platelets. The levels of the GPIb/IX/V components were instead low or normal. However, in all cases the ratio between GPIb/IX/V and GPIIb/IIIa was near 50% of that in controls, thus suggesting a diagnosis of heterozygous BSS. The Ala156Val mutation of GPIbα (also known as Bolzano mutation) was identified in 4 cases (two of them have been published),[5] while no mutation in the coding region of the GPIbα, GPIb, GPIX or GPV genes has been found in other 4 subjects (preliminary data of these patients have been reported).[5] In the remaining two patients the diagnostic suspicion of heterozygous BSS was denied by *immunocytochemistry* for NMMHC-IIA, which identified the spotty distribution of the protein typical of *MYH9*-RD in neutrophils, although no recognizable Döhle-like bodies were present on MGG-stained blood films. One of these patients has been described previously,[4] and it was suggested that the discrepancy derived from the very small size of NMMHC-IIA clumps, which made them unrecognizable as Döhle-like inclusions on MGG smears. In both patients the presence of the R702H mutation of *MYH9* confirmed the diagnosis of *MYH9*-RD.

In the remaining 16 macrothrombocytopenic patients without a diagnosis, flow cytometry did not identify glycophorin A on the platelet surface, thus excluding macrothrombocytopenia with platelet expression of glycophorin. In half of them an autosomal dominant transmission of the disorder was identified, and therefore a diagnosis of *Mediterranean macrothrombocytopenia* should be assigned. Since Mediterranean macrothrombocytopenia is a vague diagnosis deriving from the exclusion of all autosomal dominant disorders with platelet macrocytosis, and likely to be genetically heterogeneous, we prefer to refer to these 8 patients as affected by an *undefined* macrothrombocytopenic disorder.

In the two patients with normal platelet size, bone marrow examination excluded congenital amegakaryocytic thrombocytopenia, while the hypothesis of a plausible implication of the *THC2* gene was not tested because the two families were not large enough to establish a linkage on chromosome 10p12.1, where the gene is localized.[9]

Additional investigations

All patients without a diagnosis based on the investigations indicated by the diagnostic algorithm underwent additional testing. In all of them flow cytometry for platelet GP, *in vitro* study of platelet aggregation induced by ADP, collagen, epinephrine and ristocetin, immunocytochemistry for NMMHC-IIA and complete blood counts were performed independently of the algorithm's requirements. No specific alteration was detected, leaving these patients without a diagnosis because they did not fulfill the criteria for any of the known inherited platelet disorders.

Discussion

The diagnosis of inherited thrombocytopenias is commonly considered difficult and physicians often do not deal with a patient's disorder, especially when the platelet count is not severely reduced and/or a bleeding diathesis is absent or mild. Our experience confirms this situation, in that 40 of the 46 patients recently investigated at our institution for a diagnostic purpose were adults. The long delay in reaching a definite diagnosis was not without consequences, since 7 subjects underwent undue splenectomy before our observation (three with *MYH9*-RD, three with homozygous BSS, and one with heterozygous BSS).[10] In contrast, a correct diagnosis defines prognosis and translates into improvement of the patients' care. For instance, stem cell transplantation can cure patients with poor prognosis disorders,[11,12] and desmopressin infusion can transiently reduce the bleeding tendency in specific illnesses.[13,14] Since patients might remain without a diagnosis despite accurate investigation, a systematic diagnostic approach to inherited thrombocytopenias also serves the purpose of classifying affected individuals within homogenous classes of *new* disorders, which are worthy of scientific research. The main aim of our study was to validate the diagnostic algorithm recently proposed by the Italian *Gruppo di studio delle piastrine*[1] and define potential limitations or improvements on a cohort of 46 unrelated patients. Based on the investigation sequence indicated by the diagnostic flow chart, we were able to classify 22 of 46 patients as having well-defined forms of genetic thrombocytopenias and 6 patients as having *new* variants of known disorders. Moreover, 8 subjects with autosomal dominant macrothrombocytopenia could be classified as having Mediterranean macrothrombocytopenia but, as already discussed, this diagnosis is a wastebasket, and we decided to include these cases within the category of *undefined* macrothrombocytopenias. On this basis, 18 subjects remained without a definite diagnosis in spite of additional investigations other than those required by the diagnostic algorithm, thus indicating that they were affected by *new* forms of inherited thrombocytopenias.

Although the first screening phase (history and examination, morphologic evaluation of MGG-stained peripheral blood film, *in vitro* platelet response to stirring, ristocetin-induced *in vitro* platelet agglutination) is quite simple and can be performed in non-specialized institutions, it was considerably specific in formulating diagnostic hypotheses, since 16 out of 18 suspected diagnoses were subsequently confirmed by specialized tests. Moreover, a definite diagnosis or a correct diagnostic suspicion was obtained in 20 of 22 patients with *classical* forms of inherited thrombocytopenias by a few, simple investigations: examinations of the screening phase, immunocytochemistry for NMMHC-IIA, and flow cytometry for platelet GPIb/IX/V and GPIIb/IIIa. Since the instruments required for these investigations (light microscope, platelet aggregometer and flow cytometer) are available in many clinical institutions, the proposed algorithm could assist a large number of non-specialized physicians in the diagnostic approach to patients with genetic thrombocytopenias. When required, the diagnostic hypothesis could be subsequently confirmed in centers with specialized experience in the suspected disorder, and patients with *new* illnesses referred to research institutions.

Nevertheless, the previous algorithm should be partially modified because two patients with platelet macrocytosis, no recognizable Döhle-like bodies at MGG smears, and defective GPIb/IX/V complex were indeed affected by *MYH9*-RD, as confirmed by molecular analysis. In the diagnostic algorithm, flow cytometry was established as the initial screening for non-syndromic macrothrombocytopenias without abnormalities of platelet aggregation or peripheral blood films. Conversely, immunocytochemistry for *NMMHC-IIA* was recommended only in patients with normal GPIb/IX/V complex. Our study indicates that immunocytochemistry for NMMHC-IIA should precede flow cytometry, in that a defect of the GPIb/IX/V complex[15] and the absence of Döhle-like bodies[4] do not exclude the diagnosis of *MYH9*-RD. The diagnostic algorithm including this change is reported in Figure 1. In our case series it recognized all patients with *classical* forms of inherited thrombocytopenias (Figure 2).

Apart from testing the diagnostic algorithm, our review of a large series of consecutive patients with inherited thrombocytopenias deserves a few comments. First of all, the findings for 39% of patients did not fit with any well-defined platelet disorder. This is likely to be an overestimation of the real prevalence of *new* disorders in Italy, since our institute is specialized in platelet disorders, and it is therefore possible that

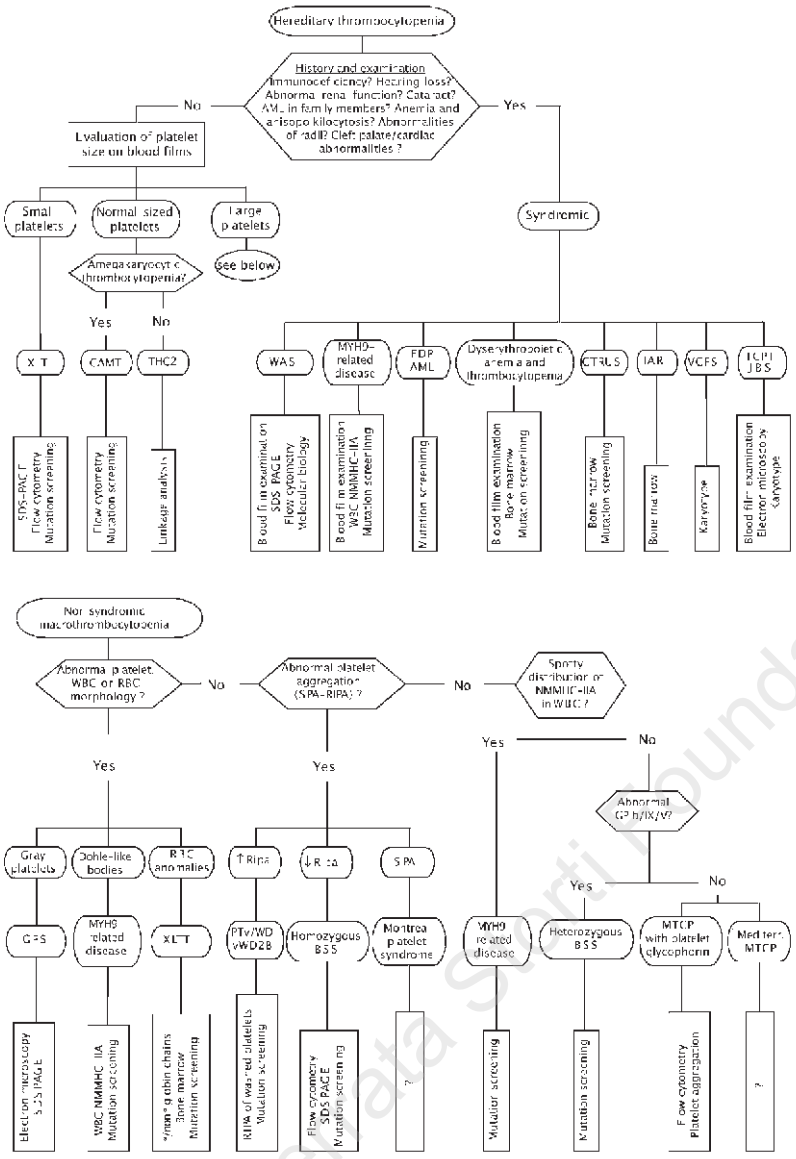

Figure 1. Diagnostic algorithm for hereditary thrombocytopenias prepared by the Italian *Gruppo di Studio delle Piastrine* and modified according to the results of the present study. The changes are included in the square. XLT: X-linked thrombocytopenia; CAMT: congenital amegakaryocytic thrombocytopenia; THC2: autosomal dominant thrombocytopenia 2; VCFS: velocardiofacial syndrome; TCPT: Paris-Trousseau type thrombocytopenia; JBS: Jacobsen's syndrome; CTRUS: amegakaryocytic thrombocytopenia with radio-ulnar synostosis; TAR: thrombocytopenia with absent radii; WAS: Wiskott-Aldrich syndrome; FPD/AML: familial platelet disorder and predisposition to acute myelogenous leukemia; GPS: gray platelet syndrome; XLTT: X-linked thrombocytopenia with thalassemia; BSS: Bernard-Soulier syndrome; PTvWD: platelet-type or pseudo von Willebrand's disease; MTCP: benign Mediterranean macrothrombocytopenia; RIPA: ristocetin-induced platelet aggregation; SPA: spontaneous platelet aggregation; NMMHC-IIA: non-muscle myosin heavy chain IIA.

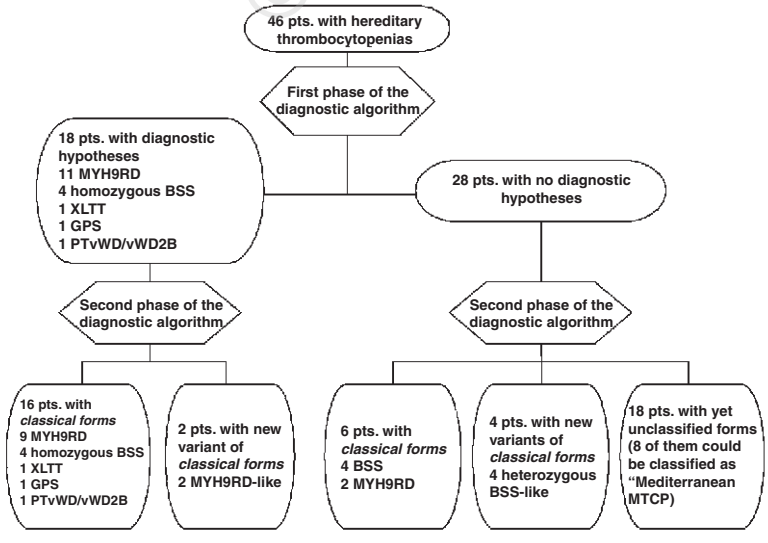

Figure 2. Results of the application of the diagnostic algorithm shown in Figure 1 to our case series. Eight patients with autosomal dominant macrothrombocytopenias could be classified as being affected by Mediterranean macrothrombocytopenia. However, this diagnosis is vague and we decided to include affected patients within the group of unclassified forms.

our case series was rich in patients with diagnostic difficulties. Nevertheless, the percentage of subjects with unknown diseases is impressive and confirms that many platelet disorders are still waiting to be characterized. Second, our study contributes to the identification of *new* inherited thrombocytopenias. The present case series includes two patients with autosomal dominant macrothrombocytopenia and glomerulonephritis plus cataracts and/or hearing loss, but without the *MYH9* mutations of *MYH9*-RD. A patient with a similar phenotype was described by Kunishima in Japan,[16] thus indicating that at least one disorder with the clinical features of *MYH9*-RD but a different, yet unknown, genetic defect occurs in different parts of the world. The present study also includes 4 previously published patients with suspected heterozygous BSS for whom preliminary experiments had suggested the absence of the more frequent BSS mutations.[5] Now the sequence of the coding region of the genes for GPIbα, GPIbβ, GPIX and GPV has been completed and no mutations have been found. Whether these patients carry alterations in intronic or regulatory sequences of the GPIb/IX/V subunits or in a new gene, is at present unknown. Finally, although our study has little epidemiological value due the enrollment bias discussed previously, in our experience BSS and *MYH9-RD* are the most frequent inherited thrombocytopenias in Italy.

In conclusion, we suggest that the diagnostic algorithm proposed by the Italian *Gruppo di Studio delle Piastrine* might assist non-specialized staff in the process of diagnosing inherited thrombocytopenias and provide criteria for selecting patients for the characterization of *new* disorders that we are, at present, unable to classify.

All the authors contributed to: design of the study, analysis and interpretation of data, drafting the article or revising it critically, final approval of the version to be published. PN, AP, MDP, IFC, NA, CA, CLB were responsible for generation and management of clinical and hematological data. FDB, MTDS, AS were involved in generation and analysis of genetic data. The authors reported no potential conflicts of interest.

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References

1. Balduini CL, Cattaneo M, Fabris F, Gresele P, Iolascon A, Pulcinelli FM, et al. Inherited thrombocytopenias: proposal of a diagnostic algorithm by the Italian "Gruppo di Studio delle Piastrine". Haematologica 2003;82:203-15.
2. Balduini CL, Iolascon A, Savoia A. Inherited thrombocytopenias: from genes to therapy. Haematologica 2002;87:860-80.
3. Balduini CL, Savoia A. Inherited thrombocytopenias: molecular mechanisms. Semin Thromb Haemost (in press).
4. Seri M, Pecci A, Di Bari F, Cusano R, Savino M, Panza E, et al. May-Hegglin anomaly, Sebastian, Fechtner, and Epstein syndromes are not distinct entities but a unique 'MYH9-related disease'. Medicine 2003;82:203-15.
5. Savoia A, Balduini CL, Savino M, Noris P, Del Vecchio M, Perrotta S, et al. Autosomal dominant macrothrombocytopenia in Italy is most frequently a type of heterozygous Bernard-Soulier syndrome. Blood 2001;97:1330-5.
6. Noris P, Arbustini E, Spedini P, Belletti S, Balduini CL. A new variant of Bernard-Soulier syndrome characterized by dysfunctional glycoprotein (GP) IB and severely reduced amounts of GPIX and GPV. Br J Haematol 1998;103:1004-13.
7. Balduini CL, Pecci A, Loffredo G, Izzo P, Noris P, Grosso M, et al. Effects of the R216Q mutation of GATA-1 on erythropoiesis and megakaryocytopoiesis. Thromb Haemost 2004;91:129-40.
8. The May-Hegglin/Fechtner Syndrome Consortium. Mutations in the nonmuscle myosin heavy chain IIA gene (MYH9) result in the diverse phenotypes of the May-Hegglin anomaly, Fechtner and Sebastian syndromes. Nat Genet 2000; 26:103-5.
9. Savoia A, Del Vecchio M, Totaro A, Perrotta S, Amendola G, Moretti A, et al. An autosomal dominant thrombocytopenia gene maps to chromosomal region 10p. Am J Hum Genet 1999;65:1401-5.
10. Balduini CL. Role of splenectomy in inherited thrombocytopenias. Blood 2004; 104: 1227.
11. Locatelli F, Rossi G, Balduini C. Hematopoietic stem-cell transplantation for the Bernard-Soulier syndrome. Ann Intern Med 2003;138:79.
12. Lackner A, Basu O, Bierings M, Lassay L, Scaefer UW, Revesz T, et al. Haematopoietic stem cell transplantation for amegakaryocytic thrombocytopenia. Br J Haematol 2000;109:773-5.
13. Noris P, Spedini P, Belletti S, Magrini U, Balduini CL. Thrombocytopenia, giant platelets, and leukocyte inclusion bodies (May-Hegglin anomaly): clinical and laboratory findings. Am J Med 1998; 104: 355-60.
14. Greinacher A, Potzsch B, Kiefel V, White JG, Muller-Burghaus G, Mueller-Eckhardt G. Evidence that DDAVP transiently improves hemostasis in Bernard-Soulier syndrome independent by von Willebrand factor. Ann Hematol 1993; 67:149-50.
15. DiPumpo M, Noris P, Pecci A, Savoia A, Seri M, Ceresa IF, Balduini CL. Defective expression of GPIb/IX/V complex in platelets from patients with May-Hegglin anomaly and Sebastian syndrome. Haematologica 2002; 87:943-7.
16. Kunishima S, Matsushita T, Kojima T, Sako M, Kimura F, Jo EK, et al. Immunofluorescence analysis of neutrophil nonmuscle myosin heavy chain-A in MYH9 disorders: association of subcellular localization with MYH9 mutations. Lab Invest 2003; 83:115-22.