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TREATMENT OF IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP): RESULTS OF A MULTICENTRIC PROTOCOL

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The results of a multicentric therapy protocol for Idiopathic Thrombocytopenic Purpura (ITP) in previously untreated patients are reported. Two different regimens of prednisone (A_1: 0.5 mg/kg/day; A_2: 1.5 mg/kg/day) were used in the induction phase. One hundred thirty patients (69 adults, 61 children), with a follow-up of almost 6 months, were evaluated. In adults good response to induction therapy was obtained in 30% of patients on schedule A_1 and in 34% on schedule A_2. In children good response was reached in 62% of cases with schedule A_1 and in 81% with schedule A_2. The difference in response to induction therapy in children vs. adults is statistically significant ($p<0.001$), whether the two schedules are considered separately or evaluated together. Antiplatelet, anti-nuclear, anti-smooth muscle, anti-mitochondrial, anti-viral and anti-toxoplasma antibodies were also determined at onset of the disease and after induction treatment.

Thirty-two patients (25 adults and 7 children) with refractory ITP were splenectomized, twenty-six of whom had been previously studied for platelet survival and kinetics.

KEY WORDS: Idiopathic thrombocytopenic purpura, therapy, splenectomy, antinuclear antibodies.

INTRODUCTION

It is well-established that Idiopathic Thrombocytopenic Purpura (ITP) is an acquired disorder caused by the production of antiplatelet antibodies [16]. Recently, some authors [10 11] defined this disease « Autoimmune Thrombocytopenic Purpura » or ATP.

Therapy has usually been based on the administration of immunosuppressive drugs, namely steroids, followed by splenectomy in resistant patients [1 9 13].

The aim of our study is to evaluate the response to two different regimens of steroid therapy in previously untreated thrombocytopenic patients.

MATERIALS AND METHODS

Patients

Our protocol included patients aged from 2 to 65, from 14 participating centers, affected by primary thrombocytopenia, not previously treated with steroids and with platelet counts less than 60×10^9/L. Diagnostic tests performed are shown in Table I.

TABLE I.
Screening tests for diagnosis of ITP.

- Platelet Count
- RBC, WBC count - Hb, Ht, MCV, MCHC, MCH
- Marrow Aspirate
- PT, PTT, Bleeding Time
- ESR
- Antiplatelet, Antinuclear, Anti DNA, Anti smooth muscle, Anti mitochondrial, Antiviral, Antitoxoplasma antibodies
- Biochemical tests
- Coombs' direct and indirect test
- Chest X-rays
- Search for infectious foci

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Other participating Centers: *Ospedale S. Maria - Terni* (Fausto Grignani), *Divisione di Ematologia - Ospedale Latina* (Antonio Bagnato), *Divisione Medica - Ospedale di Salò* (Carlo Almici), *III Divisione Medicina - Ospedale Provinciale di Bergamo* - (Benedetto Comotti), *Centro Emofilia e Malattie Emorragiche - Trombosi - Milano* (Daniela Mari), *Ospedale S. Elpidio a Mare - Ascoli Piceno* (Armando Morini), *Istituto di Semeiotica Medica - Perugia* (Giuseppe Giorgio Nenci), *Ospedale S. Gennaro - Napoli* (Ettore Volpe), *Ospedale Regionale - Bolzano* (Paolo Coser), *II Divisione Medica - Ospedale di Monza* (Eraldo Lanzi), *Cattedra di Ematologia - Policlinico S. Orsola - Bologna* (Luigi Gugliotta) - *Ospedale Bambin Gesù - Roma* (Domenico Rosati, Crescenzo Miano) - *Ospedale Generale Provinciale di Sora* (Filippo Pericoli Ridolfini).

Protocol

All patients were randomized for therapy according to the following schedules:

A1: prednisone (PDN) 0.5 mg/kg/day;

A2: PDN 1.5 mg/kg/day.

Platelet count was performed weekly during the first month. Induction treatment was stopped after two positive controls or continued for no longer than one month in non responder patients.

Steroid administration was continued in the case of persistent or relapsed thrombocytopenia at dosages ranging between 0.05 mg/kg/day and 0.2 mg/kg/day, according to the severity of the hemorrhagic syndrome.

For refractory patients, platelet survival and kinetics were studied by the ^{51}Cr labeling technique, 6-8 months from the onset of the disease.

Splenectomy was performed according to the site of platelet sequestration and/or clinical evaluation. For patients who failed to respond to splenectomy, steroids administration was resumed when necessary (0.05-0.2 mg/kg/day).

Results of therapy were evaluated excluding patients with a follow-up of less than 6 months.

Response categories after induction therapy, successive evolution and splenectomy, are as follows:

Good, if platelet count $\geqslant 150 \times 10^9$/L;

Fair, if platelet count $\geq 60 < 150 \times 10^9$/L;

Poor, if platelet count $< 60 \times 10^9$/L (Tab. II).

Platelet count: was performed electronically (Technicon Hemalog H6000) or by phase contrast microscopy. Normal values ranged from 150 to 400 $\times 10^9$/L.

Antiplatelet antibodies: serum platelet bindable IgG (S-PBIgG) were evaluated by antiglobulin consumption assay [4,12]. Normal sera contain less than 100 ng Ig/10^7 platelets.

TABLE II.
Protocol.

Induction therapy { A 1: prednisone 0.5 mg/kg/day
A 2: prednisone 1.5 mg/kg/day

— if positive result stop therapy

— if persistence of thrombocytopenia or relapse after 1 month of therapy → maintenance.

Maintenance therapy: prednisone 0.05-0.2 mg/kg/day

— if persistence of thrombocytopenia after 6-8 months from the onset of the disease

↓

Platelet survival and kinetics (^{51}Cr)

↓

Splenectomy

Response definitions:

Good if platelet count $\geqslant 150 \times 10^9$/L

Fair if platelet count $\geqslant 60 < 150 \times 10^9$/L

Poor if platelet count $< 60 \times 10^9$/L

Antinuclear (ANA), anti-DNA, anti-smooth muscle (SMA), anti-mitochondrial (AMA) antibodies: ANA determinations were performed by indirect immunofluorescence on cryostat sections of rat liver. Antibodies to ds-DNA and to ss-DNA were evaluated by radioimmune assay (RIA) using ^{125}I as label.

SMA and AMA studies were performed by the indirect immunofluorescence test on sections from composite blocks of rat organs [22].

Intensity of fluorescence was arbitrarily graded from 0 to +++. In a control group of 250 normal subjects, the positivity rates of ANA, SMA, AMA were 2%, 4.4% and 0.4%, respectively.

Anti viral antibodies: virological investigation was meant to determine serum levels of Epstein Barr virus (EBV), rubeola and cytomegalovirus specific antibodies.

Anti toxoplasma antibodies were also evaluated.

The specific anti EBV antibodies test was completed with the determination of anti viro-capsidic antigen (VCA), anti nuclear antigen (EBNA) and anti early antigen (EA) antibodies by the anti IgG and anti Complement (C_3) indirect immunofluorescence test [8].

To determine complement fixing antibodies for cytomegalovirus and toxoplasma a standard microtechnique was employed [6]. The titration of rubeola antibodies was performed using the hemoagglutinoinhibition test according to the microtechnique described by Sever [20].

^{51}Cr platelet kinetics: the technique used was similar to that previously reported by Gugliotta et al.[5]. In all cases homologous platelets from ABO-Rh compatible, HBsAg negative donors, were used.

The platelet sequestration site was determined by the use of directional scintillation counters (1.5 × 1.5 in NaI crystals) and expressed as splenic, spleno-hepatic, hepatic and diffuse [18].

Statistical analysis: was performed with the « Chi square homogeneity test ».

RESULTS

One hundred sixty-eight patients, 98 adults and 70 children, were included in this protocol. The results of therapy were evaluated in 130 patients: 69 adults [17 males (25%) and 52 females (75%)] and 61 children [36 males (59%) and 25 females (41%)].

Median age was 27 years (range 13-65) for adults and 6 years (range 2-12) for children. Limit of pediatric age was considered to be 12 years.

Thirty-three patients with a follow up of less than 6 months, and 5 patients who showed evidence of connective tissue disease were excluded from the evaluation (Tab. III).

Response to therapy

— Adults (69)

37 patients were randomized in schedule A1 and 32 in A2.

TABLE III.
Distribution of age and sex.

Adults: 69 Median Age 27 (range 13-65)	M : 17 (25%)	F : 52 (75%)
Children: 61 Median Age 6 (range 2-12)	M : 36 (59%)	F : 25 (41%)

Distribution of sex among adults and children is statistically different ($p<0.001$).

A1

Good response to induction therapy was obtained in 11 patients, fair in 13, and poor in 13 (Tab. IV).

A2

Good response to induction therapy was obtained in 11 patients, fair in 10, and poor in 11 (Tab. IV).

TABLE IV.
Response to therapy in adults.

	Response to induct. ther.	Evolution			Maintenance therapy
		Good	Fair	Poor	
A1	Good 11 (30%)	4	3	4	7
	Fair 13 (35%)	3	5	5	10
	Poor 13 (35%)	1	3	9	10
Total	37	8 (22%)	11 (30%)	18 (48%)	27 (73%)
A2	Good 11 (34%)	5	3	3	6
	Fair 10 (32%)	1	4	5	8
	Poor 11 (34%)	1	4	6	9
Total	32	7 (22%)	11 (34%)	14 (44%)	23 (72%)

Comparison of response to induction therapy A 1 vs. A 2: $p>0.5$.

Data concerning successive evolution and maintenance therapy for both groups are summarized in Table IV.

— Children (61)

29 patients were randomized in schedule A1 and 32 in A2.

A1

Good response to induction therapy was obtained in 18 patients, fair in 9, and poor in 2 (Tab. V).

TABLE V.
Response to therapy in children.

	Response to induct. ther.	Evolution			Maintenance therapy
		Good	Fair	Poor	
A 1	Good 18 (62%)	18	—	—	1
	Fair 9 (31%)	2	6	1	8
	Poor 2 (7%)	—	—	2	2
Total	29	20 (69%)	6 (21%)	3 (10%)	11 (38%)
A 2	Good 26 (81%)	23	1	2	3
	Fair 2 (6%)	2	—	—	2
	Poor 4 (13%)	—	2	2	4
Total	32	25 (78%)	3 (9%)	4 (13%)	9 (28%)

Comparison of response to induction therapy A 1 vs. A 2: $p<0.05$.

A2

Good response to induction therapy was obtained in 26 patients, fair in 2, and poor in 4 (Tab. V).

Data concerning successive evolution and maintenance therapy for both groups are summarized in Table V.

Comparison of response after induction therapy between adults and children is reported in Table VI.

TABLE VI.
Comparison of response to induction therapy: adults vs. children.

	Response			Total
	Good	Fair	Poor	
Adults	22 (32%)	23 (33%)	24 (35%)	69
Children	44 (72%)	11 (18%)	6 (10%)	61

Comparison of three types of response to induction therapy, adults vs. children, is statistically different: $p<0.001$.

Platelet survival and splenectomy

Of 49 adults and 11 children available for platelet survival and kinetics study, 28 and 9, respectively, were studied.

Nineteen splenic, 4 spleno-hepatic, 1 hepatic and 4 diffuse sequestrations were found in adults.

All children showed splenic sequestration.

Splenectomy was performed in 25 adults, 20 of whom had been previously studied for platelet survival. Results of splenectomy were evaluated in patients with a follow-up of almost two months.

Of 16 patients with splenic sequestration, 13 (81.25%) showed good response to splenectomy, 3 poor response (18.75%).

Two patients had spleno-hepatic sequestration and showed good response to splenectomy.

Diffuse sequestration was noted in 2 patients: after splenectomy one had a good response and the other a fair one.

Of 5 patients who underwent splenectomy without prior platelet survival study, 3 had a good response, one a poor response, and one was not evaluable.

Seven splenectomies were performed in children, in one case without prior platelet survival study. All 6 previously studied cases had splenic sequestration; good response was obtained in 5 and poor response in one. The patient without prior study had a poor response to splenectomy (Tab. VII).

Of the 19 adult patients who had good response to splenectomy, 13 had failed induction therapy, 6 had had an initial good response to steroids which did not persist in successive evolution. Patients who failed splenectomy had not been responsive to induction therapy. In all children who underwent splenectomy induction therapy had been unsuccessful.

Platelet antibodies

Determination of S-PBIgG was performed in 78 patients (35 adults and 43 children) at the onset of the disease. Positivity was found in 43 cases (55%): 21 adults (60%) and 22 children (51%).

A second determination was performed after induction therapy in 38 patients (24 adults and 14 children).

TABLE VII.
Splenectomies.

	N. cases	^{51}Cr study	Sequestr.	Result			No. ^{51}Cr study	Result		
				G	F	P		G	F	P
Adults	25	20	S 16	13	—	3	5*	3	—	1
			SH 2	2	—	—				
			D 2	1	1	—				
Children	7	6	S 6	5	—	1	1	—	—	1

S: Splenic; SH: Spleno-hepatic; D: Diffuse; G: Good; F: Fair; P: Poor.
* One case was not evaluable.

Positivity was found in 24 cases (63%): 15 adults (63%) and 9 children (64%) (Tab. VIII).

TABLE VIII.
Antiplatelet antibodies (Dixon's Indirect Test).

	I determination		II determination	
	N. cases	Positivity	N. cases	Positivity
Adults	35	21 (60%)	24	15 (63%)
Children	43	22 (51%)	14	9 (64%)
Total	78	43 (55%)	38	24 (63%)

Positivity: S-PBIgG > 100 ng. IgG/10^7 platelets.

ANA, aDNA, SMA, AMA studies

Determination of ANA, aDNA, SMA, AMA was performed in 44 adult patients at the onset of the disease. Presence of ANA antibodies was noted in 11 cases (25%). Ten of these were women aged from 16 to 47 (median age 28), and nine were of childbearing age.

No aDNA antibodies were noted.

Determination of SMA was positive in 9 cases (20%); AMA were found in 1 patient (2.3%).

In 8 of 11 patients, presence of ANA was also confirmed in further determinations with evidence of a-DNA antibodies in 3 cases. These subjects were not considered in the evaluation of response to therapy.

Determination of ANA, aDNA and SMA, AMA was performed in 51 adults and 48 children, respectively, at the beginning of the disease. Only one of the 51 cases tested for ANA was positive (1.9%). No positivity for ANA or aDNA was found in further controls.

SMA were noted in 10 patients (21%).

AMA were negative in all determinations (Tab. IX).

Anti viral and toxoplasma antibodies

Anti viral and toxoplasma antibodies were evaluated at the beginning of disease (Fig. 1) and after induction therapy, without any significant modification of the titer (data not shown).

DISCUSSION

The aim of our investigation was to compare clinical response to two different schedules of prednisone in the induction phase of therapy.

TABLE IX.
Antinuclear, anti-DNA, anti-smooth muscle, anti-mitochondrial antibodies.

	ANA		aDNA Positivity	SMA		AMA	
	N. cases	Positivity		N. cases	Positivity	N. cases	Positivity
Adults							
I Determination	44	11 (25%)*	—	44	9 (20%)	44	1 (2.3%)
II Determination	34	11 (32%)	3	34	6 (18%)	34	2 (6%)
Children							
I Determination	51	1 1.9%)	—	48	10 (21%)	48	—
II Determination	21	—	—	21	3 (14%)	21	—

* 10/11 female; age 16-47 (median age 28); 9/10 in childbearing age.

Diagnostic tests performed allowed us to identify primary thrombocytopenia end to exclude any secondary form. We were unable to demonstrate any relationship between onset of thrombocytopenia and viral or bacterial illness from available data [2 17 23].

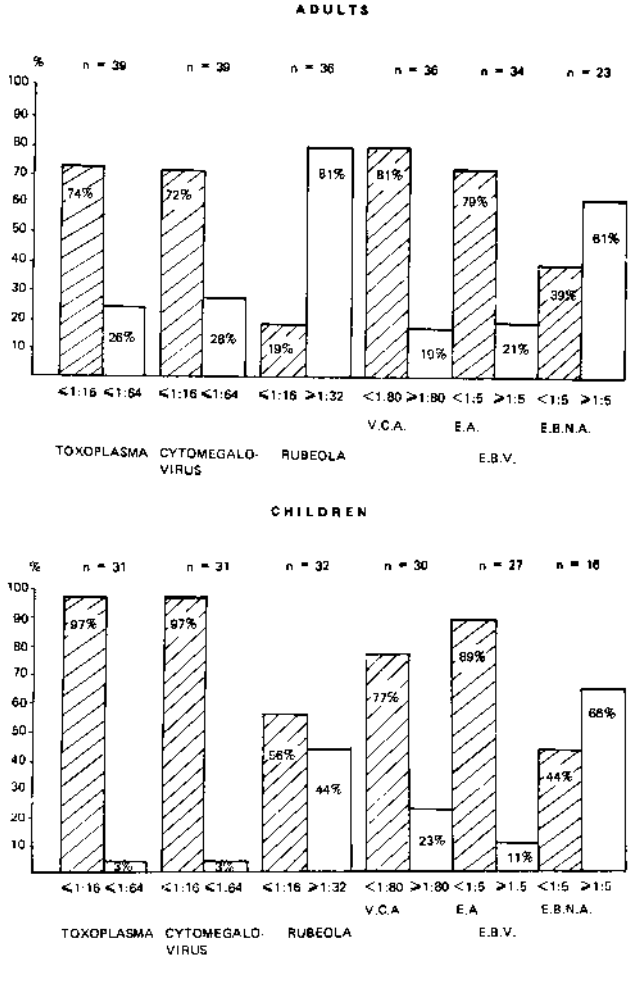

Fig. 1.
Antiviral and antitoxoplasma antibodies. — In abscissa antibody titers are reported. — In the columns percentages of patients with low ▨ or high □ titers are reported. — E.B.V.: Epstein-Barr virus; V.C.A.: virocapsidic antigen; E.A.: early antigen; E.B.N.A.: E.B.V. nuclear antigen.

We considered response to therapy in adults and children separately.

We did not see any significant difference between A1 and A2 in adults (30% vs 34% response rate after induction therapy). These results indicate that low or intermediate doses of corticosteroids induce the same response in adult patients affected by ITP. A future approach could be the use of high dose prednisone to obtain a higher rate of positive responses.

Evolution of ITP was not modified by maintenance therapy. Only 22% of patients achieved a lasting clinical remission, while the others developed a chronic disease that can be considered typical of adult age [3 19]. Low dose prednisone, however, is useful in a maintenance regimen to prevent and/or reduce hemorrhagic symptoms.

In children, on the contrary, higher doses of prednisone in induction phase gave better results than did lower doses, with statistical significance (81% vs 62%: $p = <0.05$). The problem of the usefulness of steroid therapy in children is still debated [14 21]. Our data indicate a rapid increase in platelet count in a large number of treated patients. Steroid therapy is required in those cases with a hemorrhagic syndrome, which is often present in children with acute forms of the disease.

Evolution of thrombocytopenia shows a pattern of acute disease in about 75% of cases (both random groups). It is not always possible to assert whether acute thrombocytopenia in children is idiopathic or subsequent to drug assumption or to viral or bacterial illness [15].

In the remaining cases (25%) the disease acquires the characteristics of a chronic form, similar to that of adults. Onset, evolution and response to therapy of ITP present special features in children. Comparison of response to induction therapy in children vs adults is statistically different whether the two schedules are considered separately (A_1: $\chi^2 = 9.65820$; d.f. $= 2$: $p<0.01$ – A_2: $\chi^2 = 14.68186$; d.f. $= 2$: $p<0.001$) or if they are evaluated altogether ($\chi^2 = 20.01$; d.f. $= 2$: $p<0.001$).

Results of splenectomy are similar to those previously described [5].

Platelet kinetics and clinical observation may select patients eligible for splenectomy. This strategy is recommended for patients with splenic sequestration. In patients with hemorragic syndrome, low platelet count and/or need for high dose prednisone, splenectomy may be indicated even if platelet sequestration sites are spleno-hepatic or diffuse.

The usefulness of determining antiplatelet antibodies is well documented [4 12]. The difficulty of collecting blood samples forced us to

determine only S-PBIgG. We found a lower percentage of positivity than other Authors [7], and no significant difference appeared between adults and children either before or after induction therapy. Determination of S-PBI-gG after induction treatment was performed mainly in non responder patients. This probably explains why S-PBIgG levels appeared unmodified. On the other hand, inactive ITP should present elevated antiplatelet antibodies.

Problems arise regarding the significance of ANA present in some adults but absent in children. The presence of ANA was noted in 11 cases (25%), 3 of whom later developed evidence of connective tissue disease and were excluded from the protocol evaluation. In the other 8 subjects persistence of ANA was confirmed in further controls without other clinical or laboratory signs. The clinical course of the disease was chronic in 7 of them, acute in the other. The significance of these findings in adults is not well clarified. The association with females in childbearing age and absence in children is noteworthy. These data suggest that this group of patients needs a longer follow-up period.

TERAPIA DELLA PORPORA TROMBOCITOPENICA IDIOPATICA (ITP): RISULTATI DI UN PROTOCOLLO MULTICENTRICO

Si riferiscono i risultati di un protocollo terapeutico multicentrico che prevede l'uso del prednisone secondo due differenti dosaggi (schema A_1: 0.5 mg/kg/die; schema A_2: 1.5 mg/kg/die) nella terapia di induzione della porpora trombocitopenica idiopatica in pazienti non trattati precedentemente. Centotrenta pazienti (69 adulti, 61 bambini) sono valutabili per un « follow-up » superiore od eguale a 6 mesi.

Per i pazienti adulti, si è ottenuta normalizzazione del numero delle piastrine nel 30% dei casi, con lo schema A_1 e nel 34% con lo schema A_2.

Per quanto riguarda i bambini, si è avuta risposta positiva allo schema A_1 nel 62% dei soggetti e nell'81% allo schema A_2. Esiste una differenza statisticamente significativa ($p<0.001$) tra bambini e adulti nelle risposte alla terapia di attacco con entrambi gli schemi terapeutici, sia considerati separatamente che congiuntamente. Vengono altresì riportati i risultati dello studio degli anticorpi antipiastrine, antinucleo, antimuscolo liscio, anti mitocondri, antivirus ed antitoxoplasma eseguito all'esordio della malattia ed in successivi controlli. In 32 pazienti resistenti alla terapia steroidea e con sindrome emorragica è stato praticato intervento di splenectomia, di cui si riferiscono i risultati correlati allo studio della cinetica piastrinica.

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