Kanda *et al.*
Surgical and Experimental Pathology (2022) 5:9
https://doi.org/10.1186/s42047-022-00108-8

Surgical and Experimental Pathology

RESEARCH Open Access

Impact of reticulin stain in clinical outcome of Immune Thrombocytopenic Purpura (ITP): a pathologist perspective

Rafael Guimarães Kanda[1], Lígia Niero-Melo[2], Maria Aparecida Custódio Domingues[1] and Cristiano Claudino Oliveira[3*]

Abstract

Background: This study evaluated histopathological characteristics of bone marrow (BM) of patients with immune thrombocytopenic purpura (ITP) and sought to find possible associations among them and clinical aspects.

Method: A retrospective study was carried out with 120 patients using BM clot and BM biopsy samples, including morphological (cytological and histological) re-evaluation, reticulin and hemosiderin analysis, and clinical outcome review of medical records. Immunohistochemistry (CD34 and CD117) was applied in a group of patients with increased reticulin, with the objective of exclusion Myelodysplastic syndrome cases

Results: Megakaryocytic hyperplasia was observed in 109 (90.8%) patients and increased reticulin was diagnosed in nine patients, five of them with a clinically unfavorable outcome ($p = 0.042$). The increase in reticulin graduation was associated with a higher risk of an unfavorable outcome.

Conclusion: Increased reticulin degree in BM of patients with ITP is associated with an unfavorable outcome in this study. It is rarely explored in the literature and may provide information that contributes to understanding the patient's outcomes.

Keywords: Bone Marrow, Purpura, Thrombocytopenic, Idiopathic, Reticulin, Histology

Introduction

Immune thrombocytopenic purpura (ITP) is an autoimmune entity characterized by thrombocytopenia induced by the action of platelet autoantibodies, sometimes accompanied by a decrease in megakaryocytopoiesis. Biopsy/aspiration of bone marrow is recommended for selected cases, such as the elderly or those with atypical symptomatology, that raise neoplastic differentials between the diagnostic possibilities. Bone marrow (BM) morphology in ITP is not specific, and the real impact of the biopsy information and the exclusion of differential diagnoses is controversial (Bussel and Madhavi 2014; Bussel et al. 2009).

This study aimed to establish the relationship between histopathological findings of the bone marrow with clinical and outcome parameters of patients with ITP, with emphasis on reticulin staining.

Methods

Patients

A retrospective study comprised 120 Brazilian patients who underwent histopathological evaluation of BM. All patients had aspirated bone marrow clots (BMC) and 22 also had the bone marrow biopsy (BMB). All 120 patients had clinical and anatomopathological diagnoses

*Correspondence: Cristiano_c_oliveira@hotmail.com
[3] Department of Pathology, AC Camargo Cancer Center, Rua Professor Antonio Prudente, 211, Liberdade, São Paulo 01509-010, Brazil
Full list of author information is available at the end of the article

consistent with ITP and the biopsies were performed at the time of diagnosis.

To be included in the study, the patient needed to have a histopathological evaluation of the BM (by clot and/or biopsy) at the time of diagnosis, with a pathological and clinical diagnosis of the disease. Patients whose diagnoses were not confirmed or who did not have enough material for new histological sections without harming the patient were excluded. The study was conducted from 1998 to 2016 at the Hospital das Clínicas, Botucatu School of Medicine, São Paulo State University (FMB UNESP).

Bone marrow samples

BMC processing technical report is available in literature (Calpin et al. 1998). The BM histological slides of the 120 patients were reviewed in the standardized histochemical of hematoxylin and eosin (H&E), Perls, and reticulin stains. BMB samples were considered representative when they exhibited at least five intertrabecular spaces. BMC samples were considered representative when they exhibited at least five bone spicules. Regarding the H&E slides, the microscopic report included the global cellularity and each series description. Megakaryocyte hyperplasia was considered when three or more cells were found in a high-magnification field evaluated on a hotspot (Calpin et al. 1998).

The Perls histochemical technique for hemosiderin research was scored on a semi-quantitative system, ranging from zero to six (Cantadori et al. 2019). The reticulin staining was evaluated using a four-score system, following guidelines from the World Health Organization (WHO) (Cantoni et al. 2018; Cines et al. 2014). MF-2 and MF-3 are representative of pathological stages. Patients with increased reticulin underwent a complementary immunohistochemical (IHC) evaluation to determine myelodysplastic syndrome (MDS), an important differential diagnosis in these cases. MDS usually has megakaryocytic hyperplasia with dysplasia, associated or not to increased reticulin, which may be a feature in MDS]. Therefore, IHC studies were performed with CD34 and CD117 antibodies, aiming to detect immature cells in ectopic localization above 1% of all cellularity, which would favor the diagnosis of MDS over ITP. MDS diagnosis was made with immunophenotype, morphology, clinical and molecular criteria, in line with WHO recommendations (Cantoni et al. 2018).

Clinic aspects and complementary laboratorial data

Clinical and laboratory data of the medical records were collected, namely age, gender, initial signs and symptoms, hemogram with emphasis on the platelet counts, treatment, and outcomes (remission, unresponsiveness to treatment, remission with subsequent relapses, and death). Unfavorable outcomes included unresponsiveness to treatment, relapses, or death.

A review of the myelograms' descriptions and conclusions was performed. The myelogram slides were stained with May Grünwald-Giemsa, and the characteristics of platelet genesis and global cellularity were important references. Retrospective evaluation of myelograms occurred in 114 patients. Six patients had no available medical reports or slides. The myelograms' evaluation was focused on thrombocytogenesis, aiming to identify how many, shape, and nuclear changes in the development of megakaryocytes from megakaryoblasts.

Statistical analysis

The study data were statistically evaluated using SPSS 15.0 software to evaluate the morphological data and the associations between morphological variables and clinical and outcome parameters. For the analysis of these associations, the Chi-square test or Fisher's exact test. Clinical and pathological data were also applied to the logistic regression model. A ROC (receiver operating characteristic) curve was constructed to study platelet quantity and its association with the outcomes. The area under the curve, the p-value, and the platelet count with the best sensitivity and specificity ratio were determined with the final patient outcomes. For all tests, the p-value used was 5%.

Results

Patients and clinic aspects

The patients were predominantly female ($n=75$, 62.5%) with the median age of 29 years (ranging from 0 to 80 years old), 17 over 60 years old. Ecchymosis ($n=94$, 78.3%) and petechiae ($n=80$, 66.7%) were the most frequent hemorrhagic manifestations, followed by oral bleeding ($n=61$, 50.8%) and mucous membranes bleeding ($n=54$, 45.0%). Therapeutic treatments included pulse therapy with corticosteroids ($n=80$, 66.7%); ($n=2$, 1.7%) or with immunoglobulins ($n=17$, 14.2%) or with plasmapheresis and immunoglobulins ($n=2$, 1.7%); in addition, splenectomy was performed on 18 patients (15.1%).

Pathological aspects

The morphological pattern consisted of reduced or normal global cellularity in 78 samples (65.0%), with an inverted relation between granulocytic and erythroid series ($n=84$, 70.0%, Fig. 1A) and megakaryocytic hyperplasia ($n=109$, 90.8%, Fig. 1B). In the megakaryocytic sector, moderate to severe dysplasia ($n=75$, 62.5%), marked nuclear lobulation ($n=35$, 29.2%), tendency for clusters ($n=62$, 51.7%), and the presence of micromegakaryocytes ($n=46$, 38.3%) were the most frequent

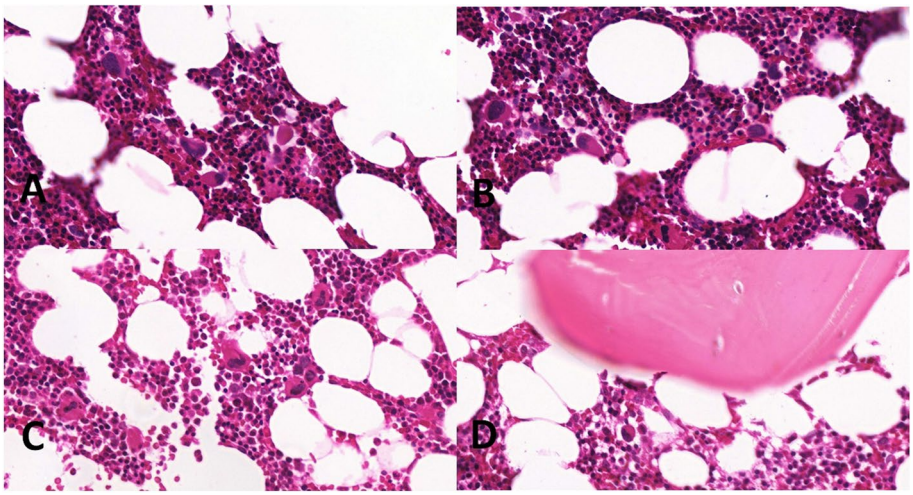

Fig. 1 Bone marrow biopsy from a patient with IPT. **A**, **B** and **C** - Megakaryocyte hyperplasia with dysplasia, **D** - Ectopic megakaryocytes. (A, B, C, D H&E, 400x)

characteristics (Fig. 1C and D). There was increased reticulin fiber in nine (7.5%) patients, all with MF2 score.

Review of myelogram data

The myelograms showed a predominantly hyperplastic pattern ($n=96/114$, 84.2%), with dysplasia ($n=104/114$, 91.2%), and mean of 28,670 platelets/mm^3] (standard deviation $= \pm 30.143/mm^3$).

Clinical outcomes

Among the 120 patients, 31 had unfavorable outcomes: three deaths and 28 relapses. For statistical analysis, the authors used the 31 patients with unfavorable outcomes as a group because the three individuals who died had relapses before the final complications. The increase in global cellularity ($n=16/31$, 51.6%, $p=0.022$) and moderate/severe dysplasia of the megakaryocytic series ($n=25/31$, 83.9%, $p=0.012$) had statistically significant associations with unfavorable outcomes (Tables 1 and 2).

Regarding reticulin (Fig. 2), none of the patients had MF3 reticulin. Among the nine patients with MF2 reticulin, 05 (55.6%, $p=0.042$) had poor clinical outcomes. Reticulin represented a factor significantly associated with unfavorable outcome of ITP. Increased reticulin (grade MF2) in the histopathological context of ITP indicated a 3.889 times higher chance of association with unfavorable outcome (OR: 3.889; 95% CI: 0.974–15.527; $p=0.055$). In this study, we observed that MF1 reticulin increases 2.627 times the chance of association with unfavorable outcomes (OR: 2.627; 95% CI: 1.073–6.433, $p=0.034$) and that MF2 reticulin increases the chance of association with unfavorable outcomes by 6.375 times (OR: 6.375; 95% CI: 1.452–27.984; $p=0.014$), both concerning individuals with absent reticulin. From these data, an increased reticulin observed in progressively higher patterns indicates the possibility of unfavorable outcomes. The other clinical and pathological data were applied to the logistic regression model, however the results observed were not statistically significant.

All nine patients of our research with increased reticulin underwent immunophenotypic evaluation with CD34 and CD117. The results disfavored MDS in these patients. Clinical records also did not indicate MDS.

Regarding the platelet counts, unfavorable outcomes were found for 39.3% ($n=11$) of patients with less than 33,500 platelets/mm^3 ($p<0.001$), the best evaluation point for sensitivity and specificity in the ROC curve.

Concerning the clinical aspects, systemic arterial hypertension ($n=14/31$, 45.2%, $p=0.001$), dyslipidemia ($n=6/31$, 19.4%, $p=0.033$) and viral hepatitis, ($n=6/31$, 19.4%, $p=0.009$) represent important comorbidities related to unfavorable outcomes.

Evaluation was performed for the morphological and clinical characteristics according to the age group, separating those under 18 years (pediatric group) from those over 18 years old (adult group). Among the 42 patients with increased cellularity, 36 (85.7%, $p<0.001$) were adults (Tables 3 and 4). Higher rates of moderate/severe megakaryocytic dysplasia were found among adults ($n=51$, 68.9%, $p=0.050$) compared to the pediatric group ($n=24$, 52.2%). In addition, there was a significant

Table 1 Association between morphological parameters and evolution in 120 patients with ITP

	Favorable outcome ($n=89$)		Unfavorable outcome ($n=31$)		p-value*
	n	%	n	%	
Global hypercellularity	26	29.2	16	51.6	**0.022**
Erythrocyte series					
Increased in number	71	79.8	22	71.0	0.220
Delayed maturation	6	6.7	1	3.2	0.419
Megaloblasts	26	29.2	12	38.1	0.224
Granulocytic series					
Reduced/Normal	69	77.5	19	61.3	0.066
Delayed maturation	6	6.7	6	19.4	0.053
Eosinophilia	7	7.9	5	16.1	0.164
Lymphoplasmacytic series					
Reduced/Normal in number	54	60.7	17	54.8	0.359
Lymphocytosis	18	20.2	9	29.0	0.220
Lymphoid aggregate	4	4.5	3	2.5	0.257
Plasmacytosis	28	31.5	9	29.0	0.495
Delayed maturation	0	0.0	2	6.5	0.065
Megakaryocytic series					
Increased in number	81	91.0	28	90.3	0.578
Lobulated nucleus	28	31.5	7	22.6	0.242
Micromegakaryocytes	34	38.2	12	38.7	0.562
Grouping	45	50.6	17	54.8	0.421
Nuclear fragmentation	33	37.1	11	35.5	0.527
Hyperchromasia	19	21.3	7	22.6	0.534
Moderate/intense dysplasia	50	56.2	25	80.6	**0.012**
Stroma					
Cytotoxicity	37	41.6	11	35.5	0.353
Edema	35	39.3	16	51.6	0.163
Congestion	35	39.3	15	48.4	0.251
Reticulin Grade 2/3	4	4.5	5	16.1	**0.049**
Positive hemosiderin	17	19.1	7	22.6	0.428
Myelogram					
Compromised platelet production	80	93.0	24	85.7	0.205
Platelet count less than 33,500 platelets/mm^3	66	78.6	11	39.3	**< 0.001**

* Statistical tests used: Chi-square and Fisher exact

difference in the hemosiderin deposits between the age groups, almost three times greater in adults ($n=20$, 27%, $p=0.011$). There was no statistically significant difference related to reticulin in these age groups. In the pediatric group, the history of infection ($n=21$, 45.7%, $p<0.001$) preceding the hematological condition and the presence of lymphopenia ($n=11$, 23.9%, $p=0.050$) were significant. The adult group had significance for systemic medication ($p<0.001$), systemic arterial hypertension ($p<0.001$), diabetes mellitus ($p=0.018$), dyslipidemia ($p=0.004$), obesity ($p=0.050$), smoking ($p=0.001$) and viral hepatitis ($p=0.011$), epidemiologically classic conditions of this range.

Discussion

One differential of this study is the use of BMC in the diagnostic routine (Fig. 3A, B). This technique is not widespread within the Departments and Laboratories of Pathological Anatomy. The evaluation of the BMC follows the same protocol of evaluation of the bone marrow biopsy. The representativeness of the material is given by the amount of hematopoietic tissue represented in

Table 2 Association between clinical parameters and evolution in 120 patients with ITP

	Favorable outcome (*n* = 89)		Unfavorable outcome (*n* = 31)		*p*-value*
	n	%	n	%	
Petechiae	60	67.4	20	64.5	0.466
Ecchymosis	71	79.8	23	74.2	0.339
Hematoma	19	21.3	6	19.4	0.518
Epistaxis	29	32.6	13	41.9	0.234
Purpura	21	23.6	6	19.4	0.415
Oral bleeding	45	50.6	16	51.6	0.543
Mucosal bleeding	41	46.1	13	41.9	0.427
Internal bleeding[a]	12	13.5	4	12.9	0.609
Anemia	18	20.2	6	19.4	0.572
Leukopenia	1	1.1	0	0.0	0.742
Lymphopenia	16	18.0	3	9.7	0.214
Thrombocytopenia	83	93.3	29	93.5	0.660
Infection	23	25.8	4	12.9	0.105
Splenomegaly	4	4.5	1	3.2	0.615
Non-hematological neoplasia	1	1.1	2	6.5	0.165
Chemotherapy/radiation therapy	0	0.0	1	3.2	0.258
Use of systemic medication	23	25.8	9	29.0	0.449
Systemic arterial hypertension	13	14.6	14	45.2	**0.001**
Diabetes mellitus	5	5.6	3	9.7	0.340
Dyslipidemia	5	5.6	6	19.4	**0.033**
Obesity	5	5.6	5	16.1	0.079
Smoking	13	11.2	3	9.7	0.555
Past autoimmune disease	4	4.5	1	3.2	0.615
Hypothyroidism	3	3.4	3	9.7	0.178
Viral hepatitis	3	3.4	6	19.4	**0.009**
H. pylori gastritis	6	6.7	2	6.4	0.622

* Statistical tests used: Chi-square and Fisher exact. [a] Hemorrhages of the gastrointestinal tract, respiratory tract, or central nervous system

clusters dispersed among the erythrocytes. These clusters are called spicules, and at least five are needed to guarantee their representativeness. The entire evaluation sequence is maintained, including specific histochemical stains (Calpin et al. 1998).

The histopathological evaluation of BM, both by BMC and BMB, is an important ancillary resource but not essential for ITP diagnosis. Its usefulness is to unveil differential diagnoses. However, the BM evaluation may contribute to the diagnosis, follow-up, and therapeutic management of patients with ITP, even after another differential had been ruled out (Cuker et al. 2016; Ettrup et al. 2010; Fattizzo et al. 2019; Fattizzo et al. 2019).

ITP has its diagnostic bases in the clinical manifestations and laboratory finding of thrombocytopenia (platelet count less than 100×10/L). In defining the diagnosis, it is essential to exclude other causes of hemorrhagic manifestations, which leads to the reasoning of ITP as a diagnosis of exclusion. Patients need to be investigated for hereditary causes of thrombocytopenia, viral infections (hepatitis B virus, for example) and *Helicobacter pylori* infection. Bone marrow biopsy is a necessary resource in order to exclude hematologic malignancies, such as leukemia and MDS. In addition, biopsy is often ordered for patients with relapsed ITP, splenomegaly, and unresponsive to treatment. In our study, these biopsies were performed at diagnosis, especially to exclude clinical differences whose laboratory tests were not completely elucidative. Considering that the bone marrow approach is an invasive procedure, it is certain that the pathologist uses, as much as possible, the analysis of the hematopoietic tissue to provide useful information to the hematologist who will monitor the patient and define his treatment. It is in this context that our research worked, aiming to identify histopathological and histochemical factors that can infer aspects of the evolution of these patients (Gale et al. 1963).

Regarding the differential with MDS, our data showed that patients with increased reticulin and ITP did not have MDS, which could explain the poor clinical evolution. MDS involves diagnostic criteria. Reticulin is not a necessary criterion for diagnosis. However, it is known that these patients with MDS may evolve with increased reticulin fibers on bone marrow biopsy. In the case of IPT, bone marrow biopsy plays an important role in excluding differential diagnoses. Given the relationship observed between unfavorable clinical evolution and increased reticulin fibers, we could think about whether the diagnosis of IPT would be correct or whether, eventually, the patient would have an MDS. Therefore, we chose to carry out this complementary immunohistochemical study. CD34 and CD117 collaborate in the visualization and quantification of immature cells, which in normal bone marrow should be around 1–2% (Cantoni et al. 2018).

Mahabir et al. (2013) (Fattizzo et al. 2019)studied patients with ITP by showing the BM sections to three hematopathologists randomly. Singly, their morphology showed to be non-diagnostic in most cases, rendering the sensitivity and specificity 24% and 90%, respectively (Fattizzo et al. 2019). The histopathological variability found in the ITP cases precludes the creation of specific diagnostic criteria. In most cases, the pathologist provides information on whether the observed histological characteristics are present in this data. Thus, the diagnosis of ITP is based on the anatomoclinical correlation (Cuker et al. 2016; Ettrup et al. 2010; Fattizzo et al. 2019; Fattizzo et al. 2019; Gale et al. 1963; Ghanima et al. 2011).

Bussel et al. (2009) (Ghanima et al. 2011), Jubelier & Harpold (1999) (Godeau 2014), Westernman & Grigg (1999) (Hasserjian 2017), and Calpin et al. (1998)

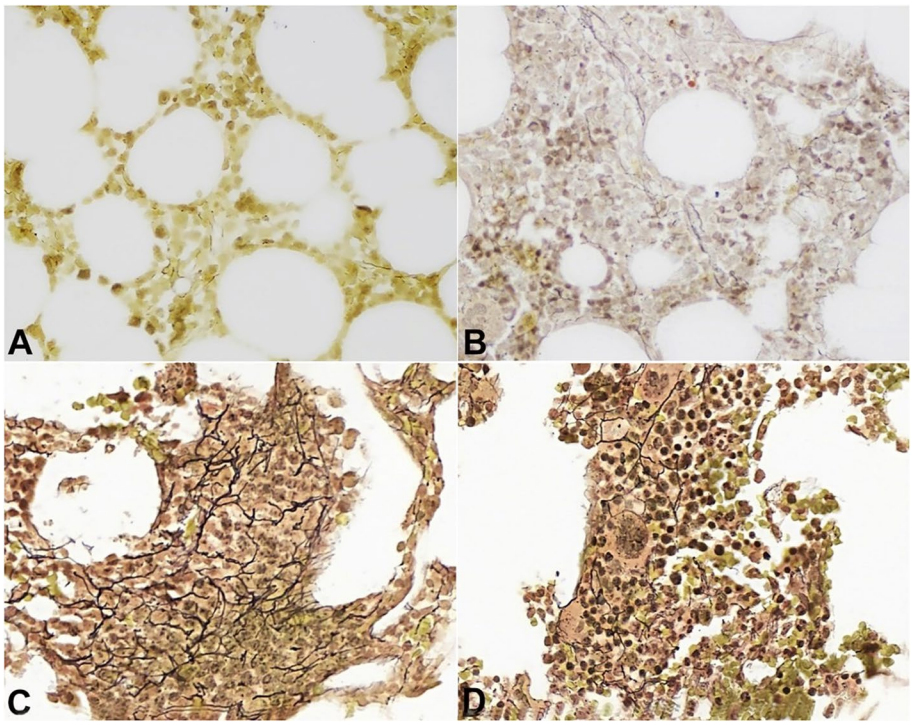

Fig. 2 **A** - Grade zero (Reticulin, 400x), **B** - Grade 1/MF1 (Reticulin, 400x), **C** - Grade 2/MF2 (Reticulin, 400x), **D** - Reticulin fibers involving the megakaryocyte (Reticulin, 400x)

Table 3 Association between BM morphological parameters and age group in 120 patients with ITP

	Younger than 18 years (*n* = 46)		18 years or older (*n* = 74)		*p*-value*
	n	%	n	%	
Global hyperplasia	6	13.0	36	48.6	**< 0.001**
Megaloblasts	11	23.9	27	36.5	0.107
Eosinophilia	4	8.7	8	10.8	0.483
Lymphocytosis	12	26.1	15	20.3	0.300
Lymphoid aggregate	1	2.2	6	8.1	0.174
Plasmacytosis	12	26.1	25	33.8	0.248
Megakaryocytic series					
Hypercellularity	39	84.8	70	94.6	0.070
Lobulated nucleus	13	28.3	22	29.7	0.516
Micromegakaryocytes	13	28.3	33	44.6	0.054
Grouping	22	47.8	40	54.1	0.317
Nuclear fragmentation	16	34.8	28	37.8	0.455
Nuclear hyperchromasia	11	23.9	15	20.3	0.400
Moderate/severe dysplasia	24	52.2	51	68.9	**0.050**
Reticulin Grade 2/3	1	2.2	8	10.8	0.077
Positive hemosiderin	4	8.7	20	27.0	**0.011**

* Statistical tests used: Chi-square and Fisher exact

(Jubelirer and Harpold 1999) studied pediatric and adult patients. They concluded that BMB did not provide additional information for the diagnosis in typical cases, since very rare diagnoses of malignancy were identified. Notwithstanding, these researchers did not study the associations between morphological findings and clinical/epidemiological parameters, nor did they mention the use of reticulin staining.

Our study is groundbreaking because we used the BMC data and reticulin staining. We also investigated the relationships between these variables and the clinical outcome of the patients. The retrieval of as much information as possible from the anatomopathological examination, especially from the BM, renders the possibility of additional relations between new variables, improving the histopathology study of these patients (Godeau 2014; Hasserjian 2017; Jubelirer and Harpold 1999; Kashiwagi et al. 2020; Kuter et al. 2007).

A Danish study (Mahabir et al. 2013), comprising 75 patients with ITP and their respective BM samples, found increased reticulin in less than 2% of patients. In the study, the increased reticulin was associated with a platelet count below 30,000 cells/mm^3, over 75 years old, female, and hepatosplenomegaly (Mahabir et al.

Table 4 Association between clinical parameters and age group in 120 patients with ITP

	Younger than 18 years (*n* = 46)		18 years or older (*n* = 74)		*p*-value*
	n	%	n	%	
Petechiae	30	65,2	50	67,6	0,471
Ecchymosis	39	84,8	55	74,3	0,130
Hematoma	7	15,2	18	24,3	0,168
Epistaxis	15	32,6	27	36,5	0,408
Purpura	11	23,9	16	21,6	0,469
Oral bleeding	25	54,3	36	48,6	0,338
Mucosal bleeding	17	37,0	37	50,0	0,113
Internal bleeding[b]	3	6,5	13	17,6	0,069
Anemia	9	19,6	15	20,3	0,560
Leukopenia	0	0,0	1	1,4	0,617
Lymphopenia	11	23,9	8	10,8	**0,050**
Thrombocytopenia	44	95,7	68	91,9	0,345
Infection	21	45,7	6	8,1	**< 0,001**
Splenomegaly	3	6,5	2	2,7	0,286
Use of systemic medication	2	4,3	30	40,5	**< 0,001**
Systemic arterial hypertension	1	2,2	26	35,1	**< 0,001**
Diabetes mellitus	0	0,0	8	10,8	**0,018**
Dyslipidemia	0	0,0	11	14,9	**0,004**
Obesity	1	2,2	9	12,2	**0,050**
Smoking	0	0,0	13	17,6	**0,001**
Past autoimmune disease	0	0,0	5	6,8	0,085
Hypothyroidism	0	0,0	6	8,1	0,051
Viral hepatitis	0	0,0	9	12,2	**0,011**
H. pylori gastritis	1	2,1	7	9,4	0,800

* Statistical tests used: Chi-square and Fisher exact. [b] Hemorrhages of the gastrointestinal tract, respiratory tract or central nervous system

2013). However, no information was provided regarding the patients' outcome.

The study of the reticulin in the BM can improve the understanding of the disease and may, depending on future research, be indicated as a biological marker of the biological behavior or the responsiveness to treatment (Godeau 2014; Hasserjian 2017; Jubelirer and Harpold 1999; Kashiwagi et al. 2020; Kuter et al. 2007). In this study, we observed that the evaluation of reticulin in BM is a potentially useful tool for establishing prognosis, although analysis with more patients is necessary.

Every process of increase in reticulin fibers takes place in the context of transformation of the bone marrow microenvironment. These are contexts that can be exemplified: 1-Leukemia and lymphoma infiltrations; 2-Pulmonary hypertension; 3-HIV infection; 4-Visceral leishmaniasis; 5-Pre-treatment with bone marrow stimulating drugs or growth factors; 6-Pulmonary arterial hypertension; 7-Connective tissue diseases; 8-Paroxysmal nocturnal haemoglobinuria; 9-Tuberculosis and granulomatous diseases; 10-Sjögren's Syndrome; 11- Bone marrow necrosis and irradiation. As can be seen from the tables, patients do not specifically have the conditions listed. Therefore, we believe that our results have the possibility of bias greatly reduced (Neunert et al. 2019).

In the pediatric group, a history of prior infectious disease, mostly viral etiology, is present in 20% of the cases (Kuter et al. 2007; Mahabir et al. 2013; Nugent et al. 2009; Perricone et al. 2014; Provan et al. 2019; Rinaldi et al. 2014; Smock and Perkins 2014; Tang et al. 2017; Thiele 2017). This infection triggers the ITP by cross immune reactions, as was observed in our study. However, no statistically significant difference was noted in this regard. The*Helicobacter pylori* infection has also been implicated as having a high immune potential for triggering ITP. No association with other clinical and outcome parameters was present among the eight patients with ITP and *Helicobacter pylori*gastritis. In this sample, the reticuline framework showed no difference between adults and the pediatric group (Ghanima et al. 2011; Godeau 2014; Hasserjian 2017; Jubelirer and Harpold 1999).

Interestingly, hypercellularity was more commonly found among the adults than in the pediatric group, and an increase in reticulin was associated with worse clinical outcomes. Gathering the hypercellularity, increased reticulin, and dysplasia of the megakaryocytic series in the BM, we observed a similar morphological context to that

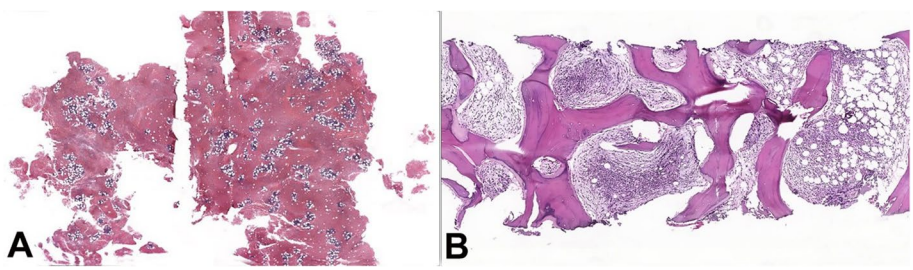

Fig. 3 **A** - Bone marrow clot, **B** - Bone marrow biopsy. (A and B H&E,10x)

observed in patients with myeloproliferative neoplasms and myelodysplastic syndromes. In the sample presented, the possibility of the myelodysplastic syndrome was excluded (Cantoni et al. 2018; Neunert et al. 2019; Nugent et al. 2009; Perricone et al. 2014; Provan et al. 2019; Rinaldi et al. 2014; Smock and Perkins 2014; Tang et al. 2017; Thiele 2017).

In a recent study, Fattizzo et al. (2019a, b) (Wei and Hou 2016) also reported ITP patients with a morphological presentation similar to myeloproliferative neoplasia. In their series, the patients had poor responsiveness to the thrombopoietin analogs. In our series, no patient received this type of treatment. Myeloproliferative neoplasia-like morphology pattern was, in general, described in patients after the use of thrombopoietin analogs.

In another study by Fattizzo et al. (2019a, b) (Westerman and Grigg 1999), the use of these drugs in ITP and aplastic anemia patients showed positive results, mainly in individuals with milder disease and small lymphoid infiltrate in the BM. Even in a pathophysiological scenario of proliferation, the patients did not develop clonal diseases. The most important information of the two studies from Fattizzo et al. (2019a, b) (Rinaldi et al. 2014; Smock and Perkins 2014) is that BM evaluation before treatment with thrombopoietin analogs may provide predictive data of clinical outcome.

Conclusions

Our findings are relevant since patients with morphological and/or clinical characteristics suggesting a possible negative outcome could have started treatment with a more aggressive regimen. The evaluation of the BM, including clot samples, is an invasive procedure. Therefore, the histopathological evaluation should be explored to the maximum, providing data to help the clinicians. This study demonstrates the relevance of the histopathological examination of BM and places the increase of reticulin as a factor associated with the unfavorable outcome of ITP.

The present study shows,for the first time, association of increased reticulin fibers and unfavorable outcome in patients with IPT, without treatment. This finding is in line with the unfavorable prognostic value of increased bonemarrow reticulogenesis previously described in myelodysplastic syndrome. It also corroborates the predictive value of unfavorable response to thrombopoietin analogs for patients with IPT, which presented increased bone marrow reticulin fibers.

Our paper, similar to the literature (Jubelirer and Harpold 1999; Kashiwagi et al. 2020; Kuter et al. 2007; Nugent et al. 2009; Perricone et al. 2014; Provan et al. 2019; Rinaldi et al. 2014; Smock and Perkins 2014) highlights the evaluation of bone marrow in patients with ITP. In the current scenario of new treatments, morphological information could provide more than differential diagnoses, providing prognostic markers. Attention should be given to high cellularity, increased reticulin, megakaryocytic dysplasia, and lymphoid infiltrate. New and more extensive studies are needed to confirm some of the insights provided by our results. However, given the literature discussions, the role of BM evaluation in patients with ITP is undeniable, especially in adults and in candidates for treatment with thrombopoietin analogs.

Abbreviations

BM: Bone marrow; BMB: Bone marrow biopsy; BMC: Bone marrow clot; ITP: Immune thrombocytopenic purpura; ROC: Receiver operating characteristic.

Acknowledgements

Not applicable.

Authors' contributions

RGK was responsible for the study design, bibliographic review, data collection, interpretation of clinical and pathological data. CCO is the project advisor, being directly responsible for supervising the writing of the manuscript, text review, statistics, interpretation of clinical and pathological data. LNM, CCO and RGK were responsible for interpreting clinical data and referring to myelogram reports, in addition to writing the text, reviewing the text and interpreting the results. CCO, MACD and RGK carried out the pathological study, the review of the reports, the integration of data, actively participating in the writing, design and review of all stages of the project. The authors read and approved the final manuscript.

Funding

The scientific project received financial support from the Foundation of Research Support of the State of São Paulo (FAPESP), Brazil, number 2016/1903-5.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethics approval was obtained from the research ethics committee at São Paulo State University, Botucatu School of Medicine (FMB UNESP). Protocol number: 54591216.8.0000.5411.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interests.

Author details

[1]Department of Pathology, Botucatu School of Medicine, São Paulo State University (FMB UNESP), Botucatu, São Paulo, Brazil. [2]Department of Internal Medicine, Botucatu School of Medicine, São Paulo State University (FMB UNESP), Botucatu, São Paulo, Brazil. [3]Department of Pathology, AC Camargo Cancer Center, Rua Professor Antonio Prudente, 211, Liberdade, São Paulo 01509-010, Brazil.

Received: 21 October 2021 Accepted: 7 February 2022
Published online: 20 May 2022

References

Bussel JB, Madhavi L. Thrombopoietic agents: There is still much to learn. Presse Med. 2014;43:e69–78.

Bussel JB, Kuter DJ, Pullarkat V, Lyons RM, Guo M, Nichol JL. Safety and efficacy of long-term treatment with romiplostim in thrombocytopenic patients with chronic IPT. Blood. 2009;113:2161–71.
Calpin C, Dick P, Poon A, Feldman W. Is bone marrow aspiration needed in acute childhood idiopathic thrombocytopenic purpura to rule out leukemia? Arch Pediatric Adolesc Med. 1998;152:345–7.
Cantadori LO, Gaiolla RD, Niero-Melo L, Oliveira CC. Bome marrow aspirate clot: a useful techinique In Diagnosis and Follow-Up of Hematological disorders. Case Rep Hematol. 2019;10:7590948.
Cantoni S, Carpenedo M, Mazzucconi MG, De Stefano V, Carrai V, Ruggeri M, et al. Alternate use of thrombopoietin receptor agonists in adult primary immune thrombocytopenia patients: A retrospective collaborative survey from Italian hematology centers. Am J Hematol. 2018;93(1):58–64.
Cines DB, Cuker A, Semple JW. Pathogenesis of immune thrombocytopenia. Presse med. 2014;43:e49–59.
Cuker A, Cines DB, Neunert CE. Controversies in the treatment of immune thrombocytopenia. Current opinion in hematology. 2016;23(5):479–85.
Ettrup MS, Jensen AØ, Engebjerg MC, Farkas DK, Nørgaard M, Cha S, et al. Bone marrow reticulin and collagen content in patients with adult chronic immune thrombocytopenic purpura: a Danish nationwide study. Am J Hematol. 2010;85(12):930–4.
Fattizzo B, Pasquale R, Carpenedo M, Cantoni S, Auteri G, Gramegna D, et al. Bone marrow characteristics predict outcome in a multicenter cohort of primary immune thrombocytopenia patients treated with thrombopoietin analogs. Haematologica. 2019a;104(10):e470–3.
Fattizzo B, Kulasekararaj AG, Hill A, Benson-Quarm N, Griffin M, Munir T, et al. Clinical and morphological predictors of outcome in older aplastic anemia patients treated with eltrombopag. Haematologica. 2019b;104(11):e494–6.
Gale E, Torrance J, Bothwell T. The quantiative estimation of total iron stores in human bone marrow. J Clin Invest. 1963;42(7):1076–82.
Ghanima W, Junker P, Hasselbalch HC, Boiocchi L, Geyer JT, Feng X, et al. Fibroproliferative activity in patients with immune thrombocytopenia (ITP) treated with thrombopoietic agents. Br J Haematol. 2011;155:248–55.
Godeau B. Immune thrombocytopenic purpura: Major progress in knowledge of the pathophysiology and the therapeutic strategy, but still a lot of issues. Presse Med. 2014;43:e47–8.
Hasserjian RP, Orazi A, Brunning RD, Germing U, Le Beau MM, Porwit A et al. Myelodysplastic syndromes: overview. In: Swerdlow S, Campo E, Harris NL, Jaffe ES, Pileri SA, Sein H et al. WHO classification of tumors of haematopoietic and lymphoid tissues. Revised 4th edition. Lyon, 2017, p.98.
Jubelirer SJ, Harpold R. The role of the bone marrow examination in the diagnosis of immune thrombocytopenic purpura: case series and literature review. Med J Aust. 1999;170(5):216–7.
Kashiwagi H, Kuwana M, Hato T, Takafuta T, Fujimura K, Kurata Y. Reference guide for management of adult immune thrombocytopenia in Japan: 2019 revision. Int J Hematol. 2020;111(3):329–51.
Kuter DJ, Bain B, Mufti G, Bagg A, Haserjian RP. Bone marrow fibrosis: pathophysiology and clinical significance of increased bone marrow stromal fibres. British Journal of Haematology. 2007;139:351–62.
Mahabir VK, Ross C, Popovic S, Sur ML, Bourgeois J, Lim W, et al. A blinded study of bone marrow examinations in patients with primary immune thrombocytopenia. Eur J Haematol. 2013;90(2):121–6.
Neunert C, Terrell DR, Arnold DM, Buchanan G, Cines DB, Cooper N, et al. American Society of Hematology 2019 guidelines for Immune thrombocytopenia. Blooc Adv. 2019;3(23):3829–66.
Nugent D, McMillan R, Nichol JL, Slichter SJ. Pathogenesis of chronic immune thrombocytopenia: increased platelet destruction and/or decreased platelet production. Br J Haematol. 2009;146(6):585–96.
Perricone C, Ceccarelli F, Nesher G, Borella E, Odeh Q, Conti F, et al. Immune thrombocytopenic purpura (ITP) associated with vaccinations: a review of reported cases. Immunol Res. 2014;60:226–35.
Provan D, Arnold DM, Bussel JB, Chong BH, Cooper N, Gernsheimer T. Updated international consensus report on the investigation and management of primary immune thrombocytopenia. Blood Adv. 2019;26(3):3780–817.
Rinaldi M, Perricone C, Ortega-Hernandez D, Perricone R, Shoenenfeld Y. Immune Thrombocytopaenic Purpura: an Autoimmune Cross-Link between Infections and Vaccines Lupus. 2014;23:554–67.
Smock KJ, Perkins SL. Thrombocytopenia: an update. Int Jor Lab Hem. 2014;36:269–78.
Tang Y-T, He P, Li Y-Z, Chen H-Z, Chang X-L, Xie Q-D, et al. Diagnostic value of platelet indices and bone marrow megakaryocytic parameters in immune thrombocytopenic purpura. Blood Coag Fibrinol. 2017;28(1):83–90.
Thiele J, Kvasnicka HM, Orazi A, Gianelli U, Barbui T, Barosi G et al. Primary myelofibrosis. In: Swerdlow S, Campo E, Harris NL, Jaffe ES, Pileri SA, Sein H et al. WHO classification of tumors of haematopoietic and lymphoid tissues. Revised 4th edition. Lyon, 2017, p.47.
Wei Y, Hou M. T cells in the pathogenesis of immune thrombocytopenia. Seminars in hematology. 2016;53(1):S13–5.
Westerman DA, Grigg AP. The diagnosis of idiopathic thrombocytopenic purpura in adults: does bone marrow biopsy have a place? Med J Aust. 1999;170(5):216–7.

Publisher's Note