A Retrospective Analysis of Newly Diagnosed ITP Patients to Analyze Response to First and Further Line Treatments.

A RESEARCH THESIS SUBMITTED TO THE DIVISION OF HEMATOLOGY/DEPARTMENT OF INTERNAL MEDICINE, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY FOR PARTIAL FULFILLMENT FOR THE REQUIREMENTS OF SUB SPECIALITY IN CLINICAL HEMATOLOGY.

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Definitions and abbreviations

A. **Phases (Stages) of ITP** (International Working Group 2010):

1. Newly diagnosed ITP: 0-3 months (< 3 months) from diagnosis
2. Persistent ITP: After 3 to 12 months from diagnosis. Included are patients not reaching spontaneous remission or not maintaining complete response off therapy
3. Chronic ITP: lasting for more than 12 months
4. Severe ITP: Presence of bleeding symptoms at presentation sufficient to mandate treatment,

or occurrence of new bleeding symptoms requiring additional therapeutic interventions with a different platelet enhancing agent or an increased dose 5. Refractory ITP: Presence of severe ITP after splenectomy

B. Definitions of Response to Treatment in ITP (International Working Group 2010):

1. Complete response (CR): A platelet count of > 100, 000/µL measured on 2 occasions > 7 days apart and the absence of bleeding and response maintained during steroid tapering, or a documentation of CR by physician.

2. Response (R): A platelet count of > 30, 000/µL and the absence of bleeding and response maintained during steroid tapering, or a documentation of R by physician.

3. No response (NR): A platelet count of < 30, 000/µL or a less than 2-fold increase in platelet count from baseline or the presence of bleeding after 3 weeks on PDN or after 14 days on HD-DEX, physician documentation of no response.

4. Loss of CR: A platelet count of < 100, 000/µL measured on 2 occasions more than a day apart and/or the presence of bleeding, or such documentation by a physician.

5. Loss of response: A platelet count of < 30, 0000/µL or a less than 2-fold increase in platelet count from baseline or the presence of bleeding. Platelet count must be measured on 2 occasions more than a day apart, or such documentation by a physician.

6. Initial responses (CR or R): platelet count assessed by day 10 for HD-DXM treated patients (40) and day 28 for PDN treated patients.

7. Sustained response (SR)/ Sustained CR: platelet count maintained >/= 30 3109/L/>/= 100k/dl with an absence of bleeding symptoms or no requirement for additional ITP-modifying treatment of 6 consecutive months following achievement of initial response.(40)

C. Abbreviations

AAU: Addis Ababa University
CR: Complete response
CS : Corticosteroids
GC: Glucocorticoid
HBV: Hepatitis B virus
HCV: Hepatitis C virus
HIV: Human immunodeficiency virus B virus
HD-DEX: high dose dexamethasone
HRC: The Hematology referral clinic at TASH
ITP: primary immune thrombocytopenia (unless otherwise specified)
LPD: lymphoproliferative disorder
OR: Overall response
PDN: Prednisolone
PR: Partial response
rhTPO: Recombinant thrombopoietin
TASH: Tikur Anbessa Specialized Hospital
TPO – RA :thrombopoietin receptor agonist

Acknowledgment

My sincere acknowledgments go to Dr Amha Gebremedhin for his helpful comments.

Declaration

I, the under signed, declared that this is my original work & has never been presented in this university before, and that all the resources and materials used for the research have been fully acknowledged.

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Abstract

Background

Primary immune thrombocytopenia (ITP) is a relatively uncommon hematologic disorder with an estimated incidence of 3 to 6 patients /100,000 per year in the West. Initial response rates to corticosteroids average at 70%. In our country data on patient characteristics and outcome of therapy are scarce.

Objective

The primary objective of this study is to determine the rate of initial response to corticosteroids in newly diagnosed ITP patients.

Secondary objectives include duration of response, associations between independent/patient variables and response, duration of PDN therapy and outcomes with further line treatments.

Method: This is a retrospective, single-center study conducted at Tikur Anbessa Specialized Hospital, a tertiary care hospital located in Addis Ababa.

All newly diagnosed ITP patients for whom adequate information regarding their outcome after 1st line therapy was available were included in this study**. Data obtained thru a standardized questioner was analyzed by IBM-IPSS version 20 statistical software.**

Result: A total of 48 study participants with the age of 18 & above were included in the study. Females constitute 85% of the participants. 63% of the patients are less than 35 years old. The mean and range of age are 34 Yrs and 17 Yrs – 52 Yrs.
62 % had an initial response to steroids (52% complete response). The rate of SR at 6 months was 68%, a statistically significant difference from previous reports (P-value < 0.0001< , with a 95% C.I. = 0.4544 - 0.7856).
Rituximab and prednisolone were the most frequently used 2nd line drugs. 6 patients were splenectomized. Patients with a response to 1st line prednisolone had a marginally significant better outcome with 2nd /3rd line rituximab.

Conclusion: Initial response rate to steroids was in line with previous studies done elsewhere. The SR rate was 68% that is significantly more than that reported in previous studies of 1st line prednisolone therapy in newly diagnosed ITP patients.

1. Introduction

1.1. Background

Immune thrombocytopenia (ITP) is a non-malignant hematological disorder characterized by low platelet counts in peripheral blood with or without bleeding manifestations . It is a common reason for seeking consultation in hematology clinics.

Increased/premature removal of platelets from peripheral blood and inhibition/destruction of megakaryocyte by autoantibodies and CD8+ CTC leading to decreased platelet production (1,2) are the final outcomes of the autoimmunity of ITP and in action in all patients diagnosed with primary ITP. Initial responses to CSs are as high as 100 % because they address/control this mechanisms common to all primary ITP patients. Unfortunately the majority of initial responders will relapse because CSs do not address well the underlying mechanisms (leading to increased production of autoantibodies and autoreactive T cells), mechanisms not shared by all patients & probably more specific for an individual patient or a given population.

Now ITP is viewed as an autoimmune disorder with a complex immunopathogenesis where nearly any arm of the immune system has been demonstrated to play a role in the initiation/perpetuation of the autoimmunity (which most likely is evolving/gets complicated through time until an effective therapy is provided) with a possibility that patients could have a unique network of immune dysregulation with variable contribution from many immunologic players (3).

Studies using passive murine models of ITP suggest that anti- platelet antibodies initiate thrombocytopenia via both an Fc-dependent and Fc-independent pathways based on the type(s) of the anti – platelet autoantibody(ies) (10).

All these may partially explain the wide and complex variation of treatment outcome among ITP patients with the available therapies.

Another important aspect of ITP (and operating in all patients) that contributes to the low platelet production/count is that serum levels of TPO, the major regulator of platelet production, are abnormally normal in ITP (aplastic anemia patients have a 10 fold higher TPO level to the same level of thrombocytopenia (5). This is a secondary effect of premature destruction of platelets which minimizes the role of the Hepatic Ashwell-Morell Receptor Pathway , the regulator of TPO production by hepatocytes in response to (the physiologic) removal of deacetylated/aged platelets from the circulation (4,39,40), and increased TPO clearance. Inappropriately normal circulating TPO levels in people with ITP fail to effectively stimulate platelet production.

The abnormally normal serum TPO level in ITP is probably central in the pathogenesis of (clinical) ITP. **If the serum TPO levels could be increased proportional to the decrease in PB platelet count** (as in aplastic anemia), the otherwise normal bone marrow, which has a tremendous capacity to increase

platelet production up on demand, would increase/normalize the PB platelet count and, **probably, there would be no a clinical entity called ITP**. This view is supported by the satisfactory and sustained response to TPO – RA across the spectrum of ITP. Conversely, **this view suggests an impossibility of failure to TPO –RA therapy in ITP.**

The literature suggests polymorphisms in genes associated with the activation of T cells in ITP. The observed polymorphisms included cytokines associated with the activation and survival of CD4+ T cells (11,12), hypomethylation of genes associated with the proinflammatory response (12) , CD72 gene polymorphisms (11,13,14,15) and other, all of which have been suggested to contribute to the immunopathophysiology of ITP.

Observations made in patients treated TPO-RAs suggest that the low platelet count by itself may contribute in perpetuating the autoimmunity: Treg numbers recover when ITP patients are in remission even if patients are only treated with TPO mimetics, suggesting that platelets, perhaps through their role as transforming growth factor-β reservoirs, are themselves immunomodulatory (16,17).This is an indirect effect of TPOR agonists ; PDN requirements decline with longer durations of treatment (35). Theoretically there seems a possibility that patients could be tapered off treatment with TPOR agonists (with a possibility of relapse).

1.2 Statement of The Problem

The diagnosis of ITP is established by the exclusion of secondary causes of thrombocytopenia in patients presenting with low platelet counts (<100,000/micL) with or without bleeding.

Corticoteroids are the first-line therapy with a good but widely variable overall response rate.

Based on seven randomized controlled trials published over the past 12 years, the range of initial platelet response rates (platelet count ≥30 × 109/L over a period of at least 7 days) was10% to 100% with high-dose dexamethasone and 43.3% to 100% with prednisolone (6-9). Overall, initial response rates with corticosteroids across trials were ≈70%.

However, 30 – 80% of ITP patient become refractory to steroids either primarily or latter and require treatment with 2nd or further lines of therapies.

Options for steroid refractory patients include Splenectomy, rituximab, TPOR agonists, azathioprine, MMF and others. TPOR agonists are the latest introductions in the management of ITP and have revolutionized the management of ITP, especially patients with refractory ITP, defined as ITP requiring treatment after Splenectomy, which had been frustrating both to patients and their treating physicians.

Splenectomy, rituximab, and recently TPOR agonists are currently recommended by leading international groups as options for the treatment of ITP patients who failed steroids.

However, several constrains exist against the application of those recommendations in resource limited settings (best exemplified by our setting): availability of drugs, affordability by the individual patient,

limited ability to manage post-operative management of Splenectomy and the local epidemiology of infectious diseases such as malaria.

1.3 Significance of the Study

Given the wide range of initial response rates to GCs mentioned above, which is plausible considering the complex pathogenesis of ITP including possible genetic involvement as outlined above, there is a need to explore the effectiveness of the locally available therapeutic options in our setup including initial response rates, rates of sustained response, and any possible correlation between response and patient variables.

In Ethiopia, data on outcome of ITP patients treated with 1st line therapy barely exists. Also there is scarcity of data on management and outcome of ITP patients who failed 1st line therapy.

It should be emphasized that, even though much of the scientific knowledge (regarding the pathogenic mechanisms leading to ITP) generated so far are not matured enough to be translated to the ultimate goal of patient care, it is safe to say that they probably influence the initial response rates to the various therapeutic agents and the probability of maintaining responses not only on individual patient level but also on particular population level.

Therefor this study aims at evaluating the outcome ITP patients (treated at TASH hematology clinic) with 1st line CSs and their subsequent course (outcome with further line therapy). Efforts to detect correlations between treatment outcomes and pre-treatment patient variables have been made.

2. Methods

2.1.Study design

This was a retrospective, single-center study conducted at Tikur Anbessa Specialized Hospital. The study was approved by the Institutional Review Board .

2.2. Outcomes Measures

2.2.1. Primary end point

The primary end point of the study is to determine initial response rates to 1st line CS therapy in newly diagnosed ITP patients.

2.2.2. Secondary End Points

Secondary outcomes include rate of sustained response to 1st line therapy, duration of initial CS therapy, pattern of 2nd & further line agents used and their outcome, testing associations between independent variables and treatment outcomes, and comparison between treatment outcome groups.

2.3.Patients

2.3.1.Inclusion & Exclusion Criteria

Patients were required to meet the diagnostic criteria set by the IWG. Other inclusion criteria include Age >/= 18 years, and a documentation on response to initial 1st line therapy (CR, R, or no response).

Exclusion criteria:

1. Evans syndrome
2. Any LPD
3. Any autoimmune disorder
4. HIV positive, uncontrolled
5. HCV positive, uncontrolled
6. Uncontrolled HBV infection
7. Recent vaccination or exposure to a drug suspected to cause thrombocytopenia

2.4. Sampling & Data Collection Methods

First a list of Medical registrations numbers (MRN) of 246 ITP patients from four registration books (2016-2022) at the hematology clinic of TASH was made.

From the list, MRNs at regular intervals were selected and the corresponding patient file was revised if it fulfills the inclusion criteria and passes the exclusion criteria until the required sample size (48) was attained.

A structured questioner was used to collect data on the dependent & independent variables felt necessary for the study. All the data were collected by the investigator (to avoid the very likely abstractor associated errors).

Medical treatment details were accessed thru the outpatient clinic files thru ICare, an electronic data source.

2.5. Statistical Analysis

2.5.1.Sample size

Sample size was calculated based on the assumption that 80% of ITP patients would have an initial response to GCs. A standard score of 1.65 for a 90% confidence interval was used to get the corresponding sample size of 43.

2.5.2.Data Analysis

Data were entered in to the IBM-SPSS version 20 software which was used to generate the descriptive statistics of the various variables (both dependent & independent) felt required for the study including baseline characteristics of study subjects, response to the 1st line therapy, duration of response, and responses to the other therapies used for the individual cases. Correlative analyses between various patient (and subgroup) characteristics (e.g. platelet counts, presence of bleeding, age, sex) and different outcomes of treatment were made using the same software and Minitab Statistical Software 2022 version.

3.RESULTS

3.1. Patient Characteristics

Table 1 summarizes the baseline characteristics of all study participants.

85 % of the study participants were female.

63% of all the patients were less than 35 years old and only 10% are older than 50 years (Fig.2). The mean age was 34 years with SD of 10.4.

Table 1. Baseline Characteristics of All Patients

Characteristics		
	Including Patients With Missing Data	Excluding Patients with missing Data
Age		No missing data
Mean (Range)	34 (17 – 55)	
Distribution no. (%)		
18 – 35 Yrs	30 (62.5)	
36 – 50 Yrs	13 (27.1)	
>50 Yrs	5 (10.4)	
Sex: no. (%)		No missing data
Male	7 (14.6)	
Female	41 (85.4)	
Address		
Total no.(%)	48 (100)	41 (100)
Addis Ababa no.(%)	28 (58.3)	28 (68.3)
Oromia no.(%)	6 (12.5)	6 (14.6)
Amhara no.(%)	3 (6.3)	3 (7.3)
Others no.(%)	4 (8.3)	4 (9.75)
Missing Data no. (%)	7 (14.6)	
Platelet Count:		
Mean (Range)		
</= 10,1000/µL no. (%)	10 (20.1)	10 (43)
>10,1000/µL no.(%)	13 (27)	13 (57)
Total no. (&)	48 (100)	23 (100)
Bleeding Symptoms		
Present no. (%)	18 (37.5)	18 (78.3)

Absent no. (%)		5 (21.7)
Total no. (%)	48 (100)	23 (100)
Duration of ITP		
<3 months no,(%)	39 (81)	39 (93)
3 – 12 months no.(%)	3 (6)	3 (7)
>12 months no.(%)	0 (0)	0 (0)
Total no.(%)	48 (100)	
Comorbidity		
Present no. (%)	14 (29.2)	
Absent no.(%)	34 (70,8)	

Data on presence or absence of bleeding at presentation was missing in 25 cases. 5 patients had no bleeding. Vaginal bleeding was the most frequent symptom .

Baseline platelet counts were missing in 3 of the 18 patients who had bleeding symptoms (Table 2).

Table 2: Description Of Baseline Platelet Count For The 15 patients Who had bleeding symptom at presentation

Baseline Platelet Count For The 15 Bleeding Patients						
	N	Range	Minimum	Maximum	Mean	Std. Deviation
Platelet Count	15	53000.00	6000.00	59000.00	19333.3333	16043.09672
Valid N (listwise)	15					

Table 3: Description Of Baseline Platelet Count For the whole group

Baseline Platelet Count, The Whole Group					
	N	Minimum	Maximum	Mean	Std. Deviation
Platelet count at Dx absolute	24	2000.00000	59000.00000	18666.6666667	15110.22304864
Valid N (listwise)	24				

For the whole group, data on platelet counts were available only for 24 patients making it difficult draw any conclusion on relation between bleeding and level of thrombocytopenia (Table 3). The mean platelet count for the 15 bleeding patients was around 19,000 (table 2).

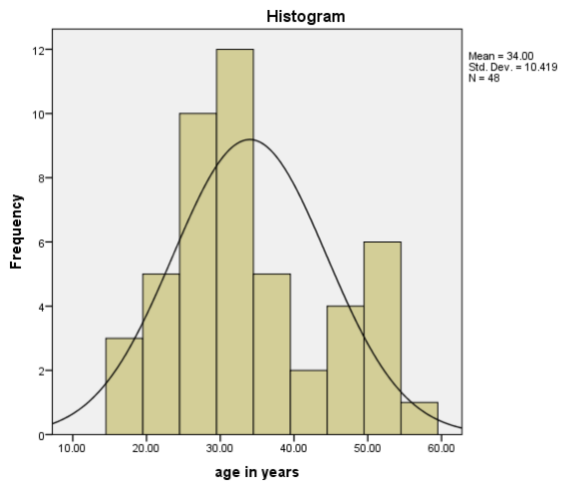


Figure 2. A histogram of age distribution of the study subjects.

Data regarding both HCV and HBV status were available in 75% of the patients. All were negative for HCV. Three patients were positive for HbsAg, all under hepathology care: 2 are in chronic inactive phase, one patient is receiving active therapy and the viral load was undetectable by the time ITP therapy was initiated. All the three patients were considered eligible to be included in the study.

Data regarding HIV status were available only in 21% of patients; all were negative for the screening test.

Bone marrow aspiration was done for seven patients and all were described as 'suggestive of ITP'; no abnormal findings were reported**.**

3.2. Response Rates for 1st Line Steroid Therapy

3.2.1. Initial Response

All the studied patients received prednisolone as the 1st line therapy: three patients received dexamethasone and one patient received methylprednisolone as part of the 1st line therapy in addition to prednisolone**.**

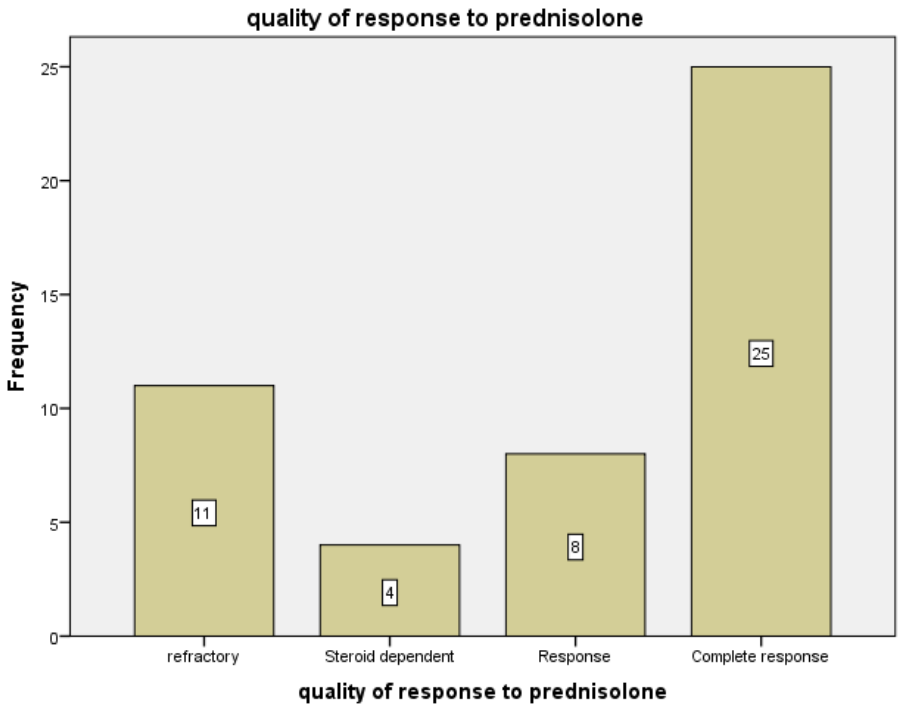


Figure 3. A histogram showing frequencies of the different outcomes of 1st line therapy

Data on initial response to 1st line steroid therapy were available for all patients. 68.7% (33/48) patients had initial response (Figure 3). This is not Significantly different from the hypothesized population proportion of 80% (P= 0.07).

The CR rate (52%, 25/45) was higher than the average CR rate reported in several trials of 1st line PDN (30%), which was statistically significant (P- value 0.002, significance level 0.05).

11 patients showed no increase in platelet count at any time while receiving 1st line prednisolone (primary steroid refractory) while four patients showed increase in platelet count (3 above 100,000/micL) but became steroid dependent and were said to fail 1st line steroid therapy**.**

3.2.2.Long-term Outcomes

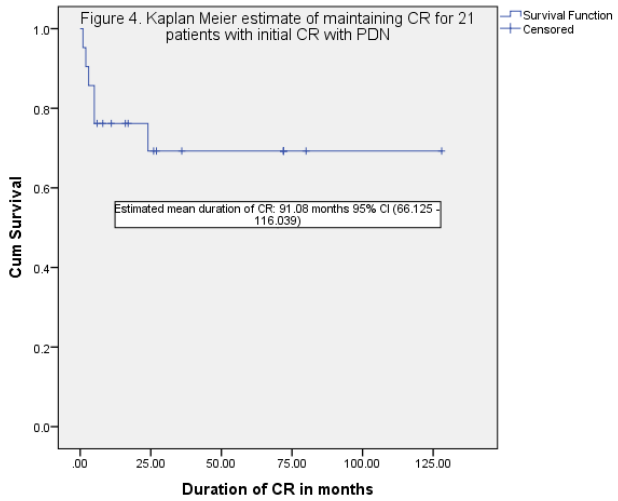
Data on duration of initial response were available for 22 patients: 3 in the response group and 19 in the CR group.

Among the 8 patients with initial (partial) response, the duration of response was found in 3 patients (one 7 months, the others below 2 months).Data was missing for the 5 patients.

Sustained Response for 1st line PDN at 6 months was 48% (16/33), significantly different from 30%, the average reported for 1st line PDN (P-value 0.034, at 0.05 significance level).

Figure 4 shows the Kaplan Meier curve for probability of maintaining CR among patients who attain CR after 1st line PDN and with available data on duration of CR.

14 cases has ongoing CR; Censored



3.3.Duration of Initial PDN Therapy

59% of patients received PDN during initial therapy for a maximum of 4 weeks before tapering started.

The minimum and maximum duration were 2 weeks and 16 weeks, respectively.

The mean treatment duration was 6 weeks.

3.4. Correlation Analysis

3.4.1.Comorbidity or additional diagnosis & Initial Response to Prednisolone

29% (14/48) of the patient had comorbidities (Table 4)**.** No correlation detected when all the comorbidities were taken together.

Table 4: Frequency of Comorbities							
Comorbidity/additional diagnosis	IDA	Polycythemia secondary to?	HBV infection	Diabetes	Hypertension	Thyrotoxicosis	Stroke
Frequency	**4**	**3**	**3**	**1**	**1**	**1**	**1**

3.4.2. Additional Hematologic diagnosis & Initial Response to Prednisolone

14.6% (7/48) of the whole group has hematologic comorbidity (Table 4). These patients with other hematologic disorders constitute 40% (6/15) of patients who failed 1st line PDN treatment.

There was a statistically significant correlation between presence of hematologic comorbidity (both IDA & polycythemia) and initial response to PDN (Pearson correlation index -0.412, at a significance level of 0.01). See table 5.

Table 5 **Pearson Correlation Index Between Presence of additional hematologic**

abnormality & Initial Response To Prednisolone			
		Presence of additional Hematologic diagnosis	Response To PDN
Presence of additional Hematologic diagnosis	Pearson Correlation	1	-.412**
	Sig. (2-tailed)		.004
	N	48	48
Response To PDN	Pearson Correlation	-.412**	1
	Sig. (2-tailed)	.004	
	N	48	48
**. Correlation is significant at the 0.01 level (2-tailed).			

3.5.Outcomes of Second & Further Line Therapies

	Name of The 2nd Line Drug Used	Frequency	%	
1	Rituximab	9	32%	
2	Prednisolone	9	32%	
3	Splenectomy	5	18%	
4	Azathioprine	5	18%	
Total		28	100%	
Table 6				

22, 19, and 4 patients received 2nd , 3rd , and 4th lines of therapy. One patient received 5th and 6th lines of treatment.

Rituximab & PDN were used in 9 patients each as 2nd line.

3.5.1.Rituximab Therapy

14 patients were treated with rituximab: 9, 4, & 1 patient(s) as a second, third & 5th line therapy.

Data on response were available for 12 patients:

Table 6: Summary of Rituximab Response				
	Response to Rituximab	Time to Response	Duration of Response (Months)	Current Status Of Rituximab Response
1	Failure			
2	CR	0.75	3	
3	R	0.75	12	
4	R	6		
5	R	2	14	
6	CR	0.5	8	Ongoing response
7	R			
8	Ongoing Rx			
9	CR		9	Ongoing response
10	CR			
11	CR		9	Ongoing response
12	CR	4	9	
13	Unknown			
14	Failure			

83.3% (10/12) patients have responded to rituximab (6 CR, 4 R).

Time to response was available for 6 patients: Mean 2.3 months, range 5.5 months (0.5, 6).

Duration of response was available for 11 patients: Mean 7.1 months, range 14 months (0,14).

Table 6 summarizes the outcome of rituximab treated patients.

Comparison of response rates between 2nd line & 3rd line Rituximab.

- **2nd Line Rituximab,** 9 patients

Overall Response= 87.5% (37.5% CR, 50% R)

- **3rd Line Rituximab**, 5 patients

OR = 60% (40% CR, 20% R)

A pattern relating response to rituximab and type of response to previous PDN was observed.

Table 7: Relation between response to Retuximab and response to preceding PDN

Case ID No	Response to previous Prednisolone	Response to Rituximab
1	R	CR
2	R	CR
3	CR/D	CR
4	CR/D	CR
5	CR/D	R
6	Failure	Failure
7	Failure	CR
8	Failure	R
9	R	R
10	Failure	Failure

To analyze possible association using Pearson correlation , first R or CR was assigned 1 and failure assigned 2 (Table 7). Patients with platelet counts above 100,000 /micL but became steroid dependent (CR/D) were included with the R/CR group. Data were then entered in to the IBM/SPSS V.20 software. (Table 8)

Table 8: Pearson Correlation between Previous PDN response & Rituximab Outcome

Correlations

		R2	R1
R2	Pearson Correlation	1	.612
	Sig. (2-tailed)		.060
	N	10	10
R1	Pearson Correlation	.612	1
	Sig. (2-tailed)	.060	
	N	10	10

Considering the small sample size, the P-value of 0.060 and the Pearson correlation 0.612 indicate a positive correlation between R/CR/CRd and response to later rituximab therapy, a correlation demonstrated in a large long term study (21).

3.5.2.Use of Prednisolone for a 2nd & 3rd time

Prednisolone was used for a 2nd /3rd time after relapse in 8 (17%) of the patients studied. The treatment course of these cases is summarized in table 9.

Table 9: Summary of Patients treated with PDN as a 1st ,2nd % 3rd line Therapy

Summary Of Patients Treated With 2nd/3rd Line Prednisolone[a]

	Age	Sex	Response to 1st line prednisolone	FirstDur	Response to 2nd line prednisolone	SecD	Response to 3rd line prednisolone	ThirdD	Total duration in therapy	Current status of ITP	Steroid toxicity identified
1	35.00	F	Complet response	5 months	Response	24 months+	NA	NA		CR	None
2	35.00	F	Complete R	22	Complete R	33	Complete R	44	6 years	CR	None
3	30.00	F	Complete R	22	Complete R	33	Complete R	44	8 years	CR	None
4	27.00	F	Response	24 months	Complete R	33	Complete R	44		PR	None
5	25.00	M	55		66		Response	44		PR	None
6	27.00	F	Complete R	24 months	Complete R	24 months+	NA	NA		CR	None
7	23.00	F	Complete R	5 months	Complete R	28 months+	NA	NA		CR	None
8	24.00	F	Complete R	2 months	66	33	NA			Unknown	None
Total N	8	8	7	8	6	8	8	4	8	8	8

a. Limited to first 10 cases.

Note: 22 , and 33 represent missing data on response duration after 1st & 2nd line PDN, respectively.

With the available data the average duration of response after 1st & 2nd line PDN were 12 months & 76 months respectively.

No toxicity associated with steroid use was documented for all patients.

3.5.3. SPLENECTOMY

Splenectomy was done in 6 patients: 2nd line in 5 patients and 3rd line in 1 patient. (Table 10)

Three patients were primarily refractory to initial therapy with PDN.

Two are in CR, two refractory, one patient (outcome undetermined).

Two CR, 2 refractory, outcome undetermined for 1 patient (operated a month ago), unknown for 1 patient.

CR rate for splenectomy was 50%.

Table 10: Summary of the treatment course of patients Treated with Splenectomy

SUMMARY OF SPLENECTOMIZED CASES[a]

	Gender	Order of splenectomy	Name of 1st line drug	Response to 1st line	Duration of response to prednisolone	Response to splenectomy	Received further therapy	Name of comorbidity	Current status of ITP	Age in years
1	Male	2nd line	**Prednisolone**	CR/D	22.00	R	Pred,Azath, Ritux,	Polycythemia 2ndyto?	CR, Azathiop + low dose Pred	46.00
2	Male	2nd line	Prednisolone	No response	.00	R	LD pred,Azath	Polycythemia Asthma	Refractory	19.00
3	Female	2nd line	Prednisolone	No response	.00	R	Ritux,Azath, MMF	None	Refractory	17.00
4	Female	2nd line	Prednisolone	No response	.00	CR		None	Continued CR	23.00
5	Female	2nd line	Prednisolone	44.00	22.00	No response	Ritux,Azath	None	Refractory	31.00
6	Male		Prednisolone	44.00	22.00	.				50.00
Total	6	6	6	**4**	6	5	6	6	6	6

a. CR/D: Normalized platelet count but steroid dependent

3.5.4.Azathioprine

10 patients (3 as a second line, 5 as a 4th line, 2 as a 3rd line) were treated with azathioprine. Table 11 summarizes the outcome of patients treated with azathioprine.

The overall response rate was 60%.

Table 11. Summary of Azathioprine treated patients							
	Age	Sex	Number of prior treatment lines	Response	Time to response, days	Duration of response, months	Prior splenectomy
	46	M	3	Failure			Yes
	31	M	3	Failure			Yes
	31	F	3	Failure			Yes
	30	F	2	R			No
	46	M	3	CR	112	24, ongoing CR	Yes
	29	F	2	R			No
	31	F	1	R	33	6	NA
	51	F	1	R	42	13	NA
	44	F	1	R	43	9	NA
	31	F	3	Failure			Yes

Table 12. Correlations among response to azathioprine, line of azathioprine and splenectomy				
		Post splenectomy	Response to azathioprine	Line of azathioprine therapy
Post splenectomy	Pearson Correlation	1	**-.816****	-.918**
	Sig. (2-tailed)		.004	.000
	N	10	10	10
Response to azathioprine	Pearson Correlation	-.816**	1	.749*
	Sig. (2-tailed)	.004		.013
	N	10	10	10
Line of azathioprine therapy	Pearson Correlation	**-.918****	**.749***	1
	Sig. (2-tailed)	.000	.013	
	N	10	10	10
**. Correlation is significant at the 0.01 level (2-tailed).				
*. Correlation is significant at the 0.05 level (2-tailed).				

Determinant of outcome with azathioprine therapy:

Tables 12 & 13 present the correlations among response to azathioprine, line of azathioprine therapy and status of splenectomy. There were significant correlations between treatment outcome and status of splenectomy and line/timing of azathioprine therapy.

Table 13. Summary of the correlations; response to azathioprine, line of azathioprine and splenectomy

Pearson correlation 1. Outcome of azathioprine Rx 2. Splenectomy 3. Line of azath. therapy	**Line of azathioprine therapy & outcome of azathioprine treatment**	**Splenectomy & outcome of azathioprine treatment**	**Splenectomy & Line of azathioprine therapy**
Correlation	74.9%	81.6%	91.8%
Significance level	0.05	0.01	0.01

One patient attained CR (as a 4th line). 5 patients attained R. The OR rate is 60%.

Four patients failed therapy (all after Splenectomy, as a 4th line).

Time to response: mean 57.5 days, median 42.5 days, SD 36.6

Duration of response; Mean 13 months, SD 7.87,Median 11

The mean of prior lines of therapy is 2.2, SD = 0.91, median = 2.5

50% of the patients had splenectomy prior to azathioprine & 80% of them failed azathioprine.

4. DISCUSSION

The OR rate observed in this study (68.7%) is probably an underestimation of the real rate in our community for two reasons:

- First, patients with a good response to 1st line therapy are not well represented in this study.
- Second, most of the patients referred to TASH and included in this study had already failed PDN.

The 68% rate of SR was superior to the 41% for 1st line PDN reported in a meta-analysis study of 1st line ITP treatments (26) (P- value 0.0013, 95% CI= 10.6463 – 40.8417%, using statistical methods for comparing two proportions , 0.68 & 0.41 from two populations ,n1=33,n2=148).

A possible reason for this difference could be the very nature of ITP: complex, not completely understood pathogenesis with a high probability of involvement of genetic polymorphism in various genes involved in the regulation of T cell activity (3, 11 -14).

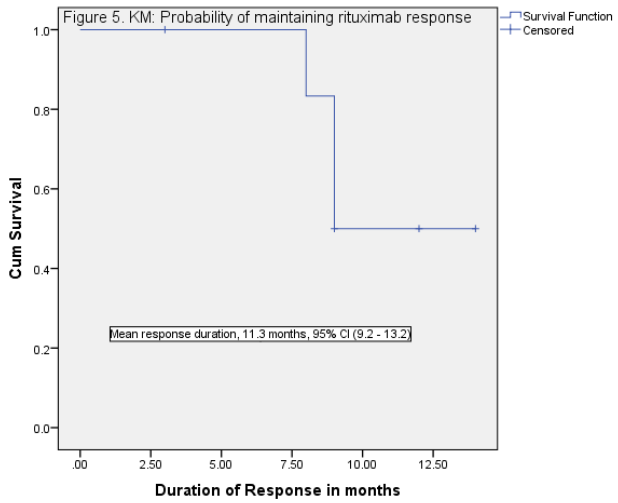
The observed duration of PDN therapy at initial therapy is in line with the current international recommendations (maximum of 4 weeks in 60%). In a Spanish registry study (484 ITP patients), the duration of 1st -line corticosteroid treatment was >6 weeks in 59.5% of patients (18).

Table 14 compares outcomes with rituximab in this study and a French prospective registry study of 248 ITP patients treated with rituximab (21,31).

Table 14. Comparison of A French rituximab Study (21) & This Study

Parameter for comparison	This Study (N= 14, Mean age	The French Study (N= 248, Mean age	Statistical Significance	REMARKS

	= 31 years)	51 years)	between the differences	
Proportion of 2nd line Rituximab	64%	90%	Significant difference z-stat=-2.54 P-value = 0.011 (one-tailed)	Generally, complexity of the autoimmunity increases with duration of ITP (3) Peripheral intolerance → central intolerance (33)
Overall Response Rate	84%	61%	Significant: P-value= 0.012	
Time to Response in months (mean)	2.1	2		
Estimated duration of response, in months (median)	12	25		**Contradicted by a finding of a recent publication on sequence of rituximab & splenectomy (32)**
Proportion of splenectomy	21.4% (3/14)	10 %	Not significant z-stat=1.02 P-value = 0.308	



The outcome of rituximab in this study was significantly inferior to the French study (median duration of response, months: 7.1 Vs 25, P = 0.000015, Chi- square analysis).

Two out of three patients who received post splenectomy rituximab had no response, and the mean duration of response is 3 months while for the seven 2nd line rituximab patients it is 7.8 months. A Mayo clinic study analyzed the sequence of splenectomy & rituximab in 218 ITP patients who failed CSs reported that patients who received **rituximab after splenectomy** seem to derive **superior** benefit **than** do those who are treated with **rituximab with an intact spleen**. **The 2-year FFR** for 2nd -line rituximab Vs post-splenectomy rituximab was **29.0%** (95% CI, 20.7%- 40.6%) **Vs 73.4%** (95% CI, 57.2%-94.2%) (**P<.001**) (32).

Table 14: Possible determinants of rituximab outcome Comparison between this & the French study	This Study	The French Study	Statistical significance
Duration of ITP * < 1 year; %	**33.3**	**41**	Not significant
Transient CR to PDN %	**57**	**16**	Significant P = 0.002
Splenectomy %	**21.4**	**10**	Not significant P = 0.3
Estimated duration of response, in months (median)	**12**	**25**	Significant
* At the start of rituximab therapy			

Possible reasons for the significantly inferior rituximab outcome in this study:

1. Timing & regularity of rituximab therapy in our setup (Rituximab is not always the 1st drug after splenectomy, 5th line in one of the 3 patients, delayed initiation of rituximab, interruption of doses.)

2. Population difference (e.g. The rate of SR after initial response to PDN was significantly superior to other studies done elsewhere)

The outcome of rituximab in our setup should be studied further separately.

PDN was used for a 2nd and 3rd time for ITP in 19% of the studied patients. All these patients are currently in CR after an average of 34 months since the diagnosis of ITP was made. Given the limited access to 2nd & further line therapy, this approach might be considered for selected cases.

Two studies, a Spanish registry study of 484 ITP patients (18) and a Korean registry study of 3382 ITP patients (19), have reported the use of PDN as a further line treatment.

Regarding azathioprine the OR rate was 100% (3/3) for 2nd line azathioprine and 20% (1/5) for post splenectomy azathioprine, suggesting either a better outcome with earlier use of azathioprine or a worse outcome with post splenectomy azathioprine, or both.

Table 14 compares the outcome of azathioprine treatment in this study with a retrospective study in Taiwan that analyzed the outcome of azathioprine treatment in 79 patients, as a 2nd line therapy in all patients. (34).

Table 14. Comparison of Azathioprine Outcome

Parameters compared	This study; n=10, Median age 31 years Retrospective	The Taiwan study; n 57 Median age 51 years Retrospective	Statistical significance	
Overall Response	60%	71.4%	Not significant P = 0.541	
Time to response ;	Mean 57 days	Median 96 days		
Starting dose of azathioprine	50 mg	100 – 150 mg		
Number of previous lines of therapy: Median, range	2.5 (1-4)	1 , all as 2nd line		
Duration of response Median	11 months	72 months	Chi-sq-stat= 41.548, P-value < 0.00001	

Determinant of outcome with azathioprine therapy:

- Prior splenectomy Vs
- Line of azathioprine treatment

Pearson correlation 1. **Outcome of azathioprine Rx** 2. **Splenectomy** 3. **Line of azath. therapy**	**Line of azathioprine therapy & outcome of azathioprine treatment**	**Splenectomy & outcome of azathioprine treatment**	**Splenectomy & Line of azathioprine therapy**
Correlation	74.9%	81.6%	91.8%
Significance level	0.05	0.01	0.01

In an Indian study (2021, retrospective, 63 ITP patient);

OR was38%,CR 1.8%, azathioprine was used as a 4th or further line in 70% (close to this study), and

starting dose was 1mg/Kg/Day (similar to our setting)

The correlation between splenectomy and line of azathioprine is clearly confounding (so could be the correlation between splenectomy and outcome of azathioprine therapy). The interval between Dx of ITP and azathioprine therapy, & starting dose seem to matter most regarding treatment outcome.

In the Taiwan study, azathioprine was compared to splenectomy (also 2nd line, 38 patients); long term outcomes were comparable (Median response duration 72 months Vs 87 months).

The usual starting dose of azathioprine is ½ to 1/3 used in the Taiwan study. This and the fact that azathioprine was used much later from ITP diagnosis in this study than the Taiwan study could explain the much better outcome in the later.

Azathioprine as a 2nd line agent at a higher dose (than usually used in our setup) should be used as an option for CS refractory/relapsed patients.

Mycophenolate mofetil (MMF) a potent immunosuppressive agent, which selectively inhibits the proliferation of T- and B-lymphocytes, the production of antibodies, and the generation of cytotoxic T cells in response to immune stimuli (27).

Based on available evidences in the literature and availability and cost of MMF, and the fact that options for steroid refractory ITP patients are limited in our setup (cost and availability), the drug should be used more often as an option for CS refractory cases. It might also be used as an add-on agent when patients are judged most likely to fail 1st line treatment early in the course of therapy.

6 out of 7 patients with additional hematologic diagnosis (3 IDA, 3 polycythemia) have failed 1st line PDN. They constitute 39% (7/18) of patients who failed 1st line PDN.

Scenarios for co-occurrence of anemia & thrombocytopenia include

1. IDAT (unknown mechanism), defined as IDA, usually sever, plus thrombocytopenia which will completely normalize soon after initiation of iron therapy (30).

2. ITP with secondary IDA (bleeding)

3. Evan's syndrome (one of the exclusion criteria in this study)

4. Primary BM disease

In one study of 10 patients with IDAT (median PLT 30k), platelet counts increase to > 150k in all cases within 4 weeks of iron therapy (30).

ITP with secondary IDA (bleeding): outcomes with PDN therapy are not different from other primary ITP patients.

For Polycythemia + ITP: Only case reports (all JAK -2 positive PV + ITP) were found, all responded to CS similar to other ITP patients . This is probably coincidental.

Since these 6 patients had failure rates significantly worse than the other patients in this study and their response pattern does not fit to one of the 1st 3 scenarios mentioned above, they probably need a separate analysis after collecting more detailed data.

5. Limitations

- The retrospective nature of the study
- The limited sample
- Most referred patients had already failed PDN which probably made the initial response rates lower
- The 'copy-paste' way writing progress notes on electronic data…. When a treatment was started/completed

6. Conclusion

This study demonstrates that prednisolone as a 1st line therapy for newly diagnosed ITP patients is effective with initial response rates comparable with that observed in other studies.

The initial CR rate and sustained response rate are significantly better than reported in other studies of 1st line PDN which may indicate ITP patients in our setup do better with 1st line PDN.

For patients who primarily fail PDN, second line therapies other than rituximab might do better.

In selected cases (e.g. patients with fast and sustained response to 1st line PDN, no contraindications for GC, young, difficulty to access recommended therapies) further line treatments with CSs might be considered.

Patients with a presumed diagnosis of ITP having & coexisting anemia should be managed with special attention to response to therapy.

Azathioprine should be considered at an earlier line and at more intensive doses especially for patients with **poor response to PDN &/or if there is financial uncertainty for rituximab** , and **timely splenectomy** is not available & if patients are from malaria endemic areas.

Considering the following facts

1. Long term outcome of 2nd line rituximab is unsatisfactory
2. The financial issues in our setup
3. Mechanisms of action of rituximab are more similar to CSs than to agents directly targeting activated autoreactive T & B cells (37,38)

prospective studies using 2nd line agents like azathioprine & MMF, especially for steroid dependent patients, should be conducted in our setup.

MMF should be used more frequently as an agent for CS refractory ITP patients in our setup.

Conflict of Interest: None

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22 Safety and efficacy of azathioprine in immune thrombocytopenia

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38 **Rituximab in the treatment of immune thrombocytopenia: what is the role of this agent in 2019?**
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