Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(4): 4241-4247. **OPEN ACCESS**

Tumor Necrosis Factor-α (TNF-α) Influence in Pathogenesis of Childhood Primary Immune Thrombocytopenia

Mervat Abdallah Hesham[1], Asmaa Mohamed Husny Esh[2] and Ahmed Mokhtar Beshik[3]

[1]Department of Pediatric, Faculty of Medicine, Zagazig University, **Egypt**
[2]Department of Clinical Pathology, Faculty of Medicine, Zagazig University, **Egypt**
[3]Department of Pediatric, Zliten Teatching Hospital, **Libya**

***Correspondence:** Ahmedbeshik@gmail.com **Received 27-04-2020, Revised: 29-05-2020, Accepted: 09-06-2020 e-Published: 30-12-2020**

Immune thrombocytopenic purpura (ITP) is a benign, self-limiting disease in children complete recovery is usually expected within a few days or weeks, regardless of treatment. In approximately 30% of cases, the disease will follow a chronic course. Tumor Necrosis Factor alpha (TNF- α) is a multifunctional cytokine and is involved in the promotion of inflammatory responses. It also plays critical roles in the pathogenesis of autoimmune diseases.This study aimed to assess the level of TNF- α in children with primary ITP and its influence to pathogenesis of the disease. A case control study conducted on (21 ITP cases and 21 healthy controls) at inpatient and outpatient clinic of hematology and oncology unit of pediatric department at Zagazig university hospitals. The subjects of total 42 were divided into two equal groups (21 in each group): Group (A): children diagnosed with ITP (newly diagnosed, persistent and chronic).Group (B): healthy children with matched age and sex as a control group. Full history, clinical examination and laboratory investigation including CBC and serum TNF- α were recorded and its correlation was estimated.There was statistically significant difference between the chronic cases and control group, newly diagnosed and persistent cases regarding age where chronic cases had higher age than other groups .Concerning bleeding manifestation, purpuric rash was the commonest bleeding manifestation followed by ecchymosis, epistaxis, gingival bleeding, bruises and lastly sub-conjuctival hemorrhage and hematuria. There was statistically significant increase in platelets count at discharge and after 3 months among newly diagnosed, persistent and chronic cases (7.6±4.5 versus 286.7±105.9, 12.4±11.1 versus 128±103.6 and 16.7±15.4 versus 86.6±68.9) respectively. Also, there was no statistically significant difference between the ITP cases regarding platelets count initially and at discharge but after 3 months follow up, there was statistically significant higher platelets count among newly diagnosed thanpersistent and chronic cases. Immune thrombocytopenia (ITP) is one of the most common acquired bleeding disorders of childhood. The finding of significant differences in TNF alpha levels between ITP patients and healthy controls indicates that TNF alpha disturbances might be involved in the pathogenesis of ITP in pediatric patients.

Keywords: Immune thrombocytopenia, TNF-α, bleeding disorders and platelet destruction.

INTRODUCTION

Immune thrombocytopenia (ITP) is an acquired hematological disorder that is developed secondary to the production of auto-antibodies

against platelets leading to isolated thrombocytopenia (peripheral platelet count <100,000/ml) in the absence of other causes of thrombocytopenia such as drugs, infections, malignancy, or other autoimmune diseases (Blandizzi et al. 2014). A report on standardization of terminology, definition and outcome of ITP was published in Blood by International Working Group (IWG). The group found that the term purpura is not accurate, because many patients do not present with bleeding symptoms and even thrombocytopenia could be discovered incidentally during routine clinic visit (Kashiwagi et al. 2013). Only 5% or fewer children develop serious bleeding, most commonly from the nose or gastrointestinal tract. While intracranial hemorrhage is the most serious complication, it is rare, occurring in about 0.3% of cases and if treated promptly usually has a good outcome (Zufferey et al. 2017). The rationale for treating children with ITP is to increase platelet count to a safer level and prevent serious bleeding, mainly intracranial haemorrhage (ICH). Corticosteroids produce its effects by reducing the production of anti-platelet antibodies and by decreasing clearance of opsonized platelets and increase vascular stability in ITP. It is commonly used as 1–2 mg /kg /day for up to 2 weeks and discontinued by the third week (Chang et al. 2010). Tumor necrosis factor has been shown to be an endogenous pyrogen that causes fever. Chronic exposure to a low dose of TNF may cause cachexia, wasting syndrome, and depression (Talaat et al. 2014). Additionally, TNF is a key mediator of both acute and chronic systematic inflammatory reactions. TNF not only induces its own secretion, but it also stimulates the production of other inflammatory cytokines and chemokines (El Sissy et al. 2014). TNF-α is considered an inflammatory cytokine that is involved in the inflammatory response and protective immune response to infectious agents. It participates also in the pathogenesis of many autoimmune diseases e.g. ITP. ITP children were found to have helper t-1 cytokine pattern with increased TNF-α as in most autoimmune disorders (Tomiyama et al. 2017).Therefore, the present study aimed to assess the level of TNF- α in children with primary ITP and its influence to pathogenesis of the disease.

MATERIALS AND METHODS

A case control study carried out at Hematology and Oncology Unit, Pediatric Department, Zagazig University Hospital during a period from (November 2019 to April 2020). Approval of the study was obtained from pediatric department after approval from institutional review board - Zagazig University (IRB – ZU).

Study design:

The sample size was 42 using OPEW EPI at power 80% and C.I 95%. As seaming that the mean ± SD of (TNF-α) in acute ITP is 0.16±0.12 versus 0.08±0.05 in control group. The subjects divided into two groups: Group (A): composed of 21 children diagnosed with ITP (newly diagnosed, persistent and chronic).Group (B): composed of 21 age and sex matched healthy children as a control group.

Inclusion and exclusion criteria:

Children from 1-18 years of both sexes who diagnosed with primary ITP (newly diagnosed, persistent and chronic). However, children below 1 year or above 18 years and patients with secondary immune thrombocytopenia.

Operational interpretation:

All patients in this study were subjected to complete history taking including disease duration, bleeding tendency and complications, and family history. Complete clinical examination was done with special emphasis on site and shape of bleeding, presence of organometallic and lymphadenopathy.

Response to treatment was classified in the studied cases according to: (a) Complete response (platelet >100×109/l) at least 6 weeks post treatment. (b)Response (platelet between 30×109/l and 100) and double base line platelet count. (c) No response (platelet <30×109/l or less than double base line platelet count. Laboratory Investigations were performed including Complete blood count (CBC) was done using Sysmex Xp-300 (Sysmex Corporation. Germany).It was done Initially at diagnosis, at discharge and after 3 month to exclude secondary causes (Retics%, PT, PTT, C3, DNA, anti Dnase and H.pylori). Specific investigation for measuring of serum tumor necrosis factor – α in patient with primary ITP by ELISA kit. Blood samples were collected by venipuncture, 1 ml on EDTA for CBC and 2 ml were allowed to clot naturally and the serum separated as soon as possible. Sera were frozen at -20 C until used. Grossly hemolysed or lipemic specimens were excluded from the study.

Tumor necrosis factor-α measurment:

Serum TNF-α were assessed using ELISA Kit (sunRed Corporation china): allow the serum to

clot for 10-20 minutes at room temperature. Centrifuge (at 3000 RPM) for 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay ELISA to assay the level of Human Tumor necrosis factor α (TNF-α) in Samples. The chroma of color and the concentration of the Human Substance Tumor necrosis factor α (TNF-α) of sample were positively correlated.

Statistical analysis:

Collected data were analyzed using computer using SPSS version 22 (SPSS Inc. Chicago, IL, U.S.A). Data were presented in tables and graphs and summarized as median and mean ± standard deviation for quantitative variables and as number and percentage for qualitative variables. Shapiro-Wilk's W-test was applied for checking the normality assumption of continuous variables. Chi-square test and Fisher's exact test, as well as, independent two-sample t -tests or Mann-Whitney-U tests were applied to compare the continuous variables between the two groups. Kruskal Wallis test was used for comparing the median .Probability (P-value): P-value <0.05 was considered significant. P-value <0.001 was considered as highly significant.

RESULTS

The attainable results showed that, there was statistically significant difference between the chronic cases and control group, newly diagnosed and persistent cases regarding age where chronic cases had higher age than other groups with no statistically significant difference between the control group, newly diagnosed and persistent cases (Figure 1). Regarding sex and Consanguinity there was no statistically significant difference between all the studied groups (Figure 2). Concerning bleeding manifestation, purpuric rash was the commonest bleeding manifestation followed by ecchymosis, epistaxis, gingival bleeding, bruises and lastly sub-conjuctival hemorrhage and hematuria which were (71.4%, 66.7%, 38.1%, 33.3%, 23.8%, 4.7% and 4.7%), respectively (Figure 3). There was statistically significant increase in platelets count at discharge and after 3 months among newly diagnosed, persistent and chronic cases (7.6±4.5 versus 286.7±105.9, 12.4±11.1 versus 128±103.6 and 16.7±15.4 versus 86.6±68.9) respectively. Also, there was no statistically significant difference between the ITP cases regarding platelets count initially and at discharge but after 3 months follow up, there was statistically significant higher platelets count among newly diagnosed than persistent and chronic cases (Table 1). There was statistically significant increase among ITP cases compared to control group. Also, there were highly significant cases of TNF alpha in newly diagnosed cases compared to persistent and chronic cases but no statistically significant difference was found between chronic and persistent cases (Table 2).

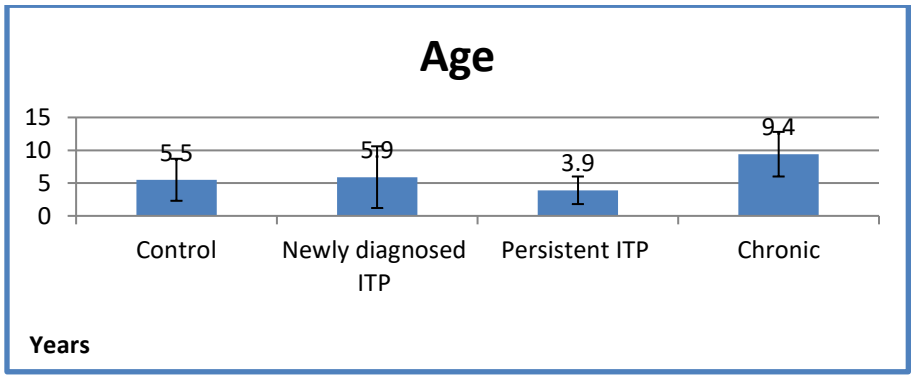

Figure 1: Bar chart for age distribution between the studied groups

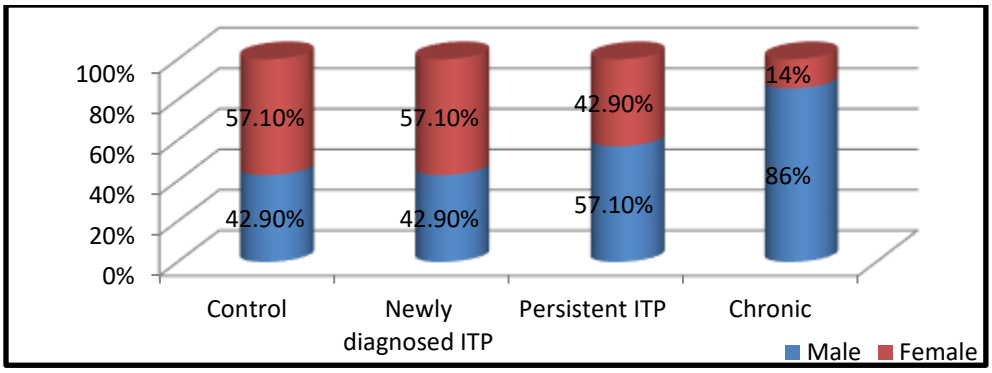

Figure 2: Bar chart for sex distribution between the studied groups

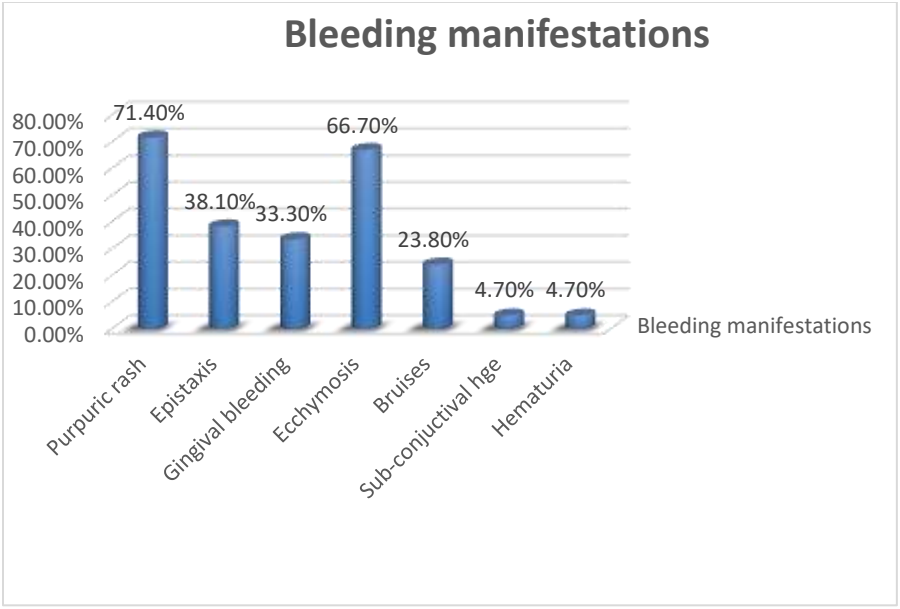

Figure 3: Bar chart for bleeding manifestations among the studied groups.

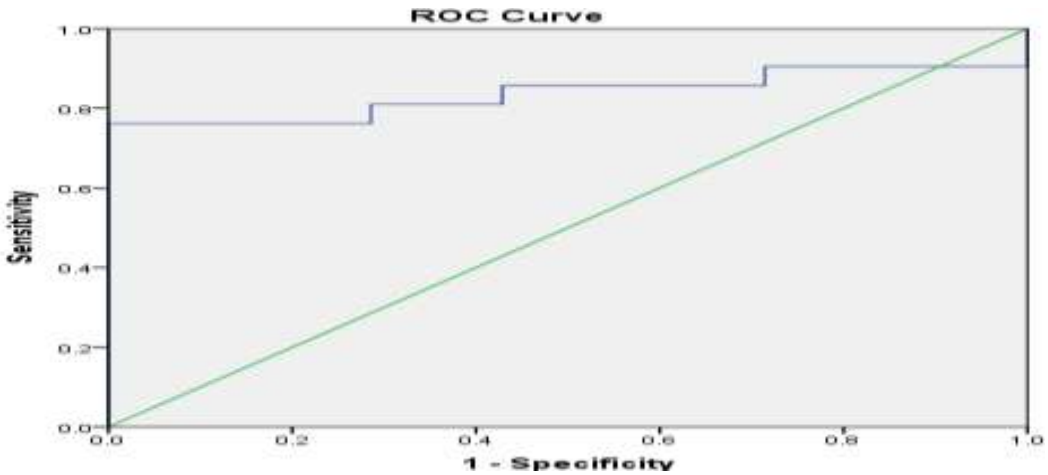

Figure 4: ROC curve for the role of TNF alpha in prognosis of ITP

Table 1: Comparison between the studied groups regarding initial and follow up platelets count of ITP cases:

Variable	Newly diagnosed ITP (NO.=7)	Persistent ITP (NO.=7)	Chronic ITP (NO.=7)	K.W test	P	LSD
Initial platelets count ($X10^9$/L) mean ± SD Range	7.6±4.5 (1-15)	12.4±11.1 (3-28)	16.7±15.4 (1-48)	1.9	0.2	0.3(1) 0.2(2) 0.6 (3)
Platelets count at discharge ($X10^9$/L) mean ± SD Range	40.0±15.5 (6-51)	44.4±24.9 (20-97)	35.3±26.4 (12-92)	0.2	0.7	0.7(1) 0.7(2) 0.5 (3)
Follow up platelets count at 3months ($X10^9$/L) mean ± SD Range	286.7±105.9 (127-400)	128±103.6 (17-270)	86.6±68.9 (14-175)	8.7	0.002*	0.006*(1) 0.001**(2) 0.4 (3)
Paired W.S	15.3	12.4	9.6			
P-value	0.001**	0.002*	0.007*			

Newly diagnosed versus persistent, (2) Newly diagnosed versus chronic and (3) Persistent versus chronic, K.W=Kruskal-Wallis test, W.S=Wilcoxon signed rank test, *Statistically significant difference (P ≤ 0.05), **Statistically highly significant difference (P ≤ 0.001).

Table 2: Comparison between the studied groups regarding tumor necrosis factor alpha:

The studied groups	Number of patients (21)	TNF alpha (µg/ml) mean ± SD Range	Kruskal Wallis Test	p-value	LSD
Control	21	91.1±23.8 (67.5-123.8)	24.1	0.001**	0.001**(1) 0.04*(2) 0.02* (3) 0.001**(4) 0.001**(5) 0.7 (6)
Newly diagnosed ITP	7	478.8±130.4 (301-630.3)			
Persistent ITP	7	179.8±96.1 (53.4-305.7)			
Chronic ITP	7	170.6±91.1 (49.1-310.3)			

(1) Control versus newly diagnosed, (2) Control versus persistent, (3) Control versus chronic, (4) Newly diagnosed versus persistent, (5) Newly diagnosed versus chronic and (6) Persistent versus chronic, K.W=Kruskal-Wallis test, *Statistically significant difference (P ≤ 0.05), **Statistically highly significant difference (P ≤ 0.001).

DISCUSSION

Immune thrombocytopenia (ITP) is an immune-mediated platelet disorder in which autoantibody-coated platelets are removed from the blood by monocytic phagocytes, resulting in a remarkable decrease of platelet count, accompanied by impaired platelet production. The majority of childhood cases are acute and resolve successfully without therapy within 6 months (Andersson et al. 1998 ; Čulić et al. 2013). Tumor necrosis factor-alpha is a pleiotropic cytokine produced primarily by macrophages and T cells, and has a range of inflammatory and immunomodulatory activity (Batikhan et al.2010). TNF-a have been studied as a determinant of susceptibility to numerous diseases (Chang et al. 2010). So this study was a case control study conducted on (21 ITP cases and 21 healthy controls) at inpatient and outpatient clinic of hematology and oncology unit of pediatric department at Zagazig university hospitals to evaluate the level of tumor necrosis factor-α in children with immune ITP and its contribution to

pathogenesis of the disease.

In our study there was statistically significant difference between the chronic cases with control group, newly diagnosed and persistent cases regarding age where chronic cases had higher age than other groups (table 1) and this agree with the finding of the cross-sectional case–control study, conducted at Ain Shams University, by **Fatma et al.** (2018) where patients with chronic ITP were statistically older, with mean age of 7.7±4.19, than those with acute ITP, with mean age of 3.95±2.21 and this is most probably because of the chronic nature of the disease as children with the chronic form of the disease are diagnosed for a much longer period of time, while children with acute ITP are usually diagnosed at a younger age .On the contrary, Lanzkowsky ,(2011) who reported that acute ITP is most common at age 2–10 years, with a peak between 4 and 8 years and the mean age of chronic patients was 61± 32 years. Also, many studies reported higher levels of TNF-α in the plasma/serum from ITP patients and they concluded that TNF-α is the most informative variable for discrimination between healthy children and those with ITP. This could be related to activation of macrophages, which have been reported to be stimulated in ITP patients by platelet autoantigen and lead to activation of T cells (Rodeghiero et al. 2009 ; Patel et al.,2017).

Our results were in line with the finding of Zhang et al. (2017) who reported association between TNF-α and ITP patients in Caucasian population. On the contrary Del Vecchio et al. (2012) who reported that serum levels of TNF-a did not differ significantly between ITP patients and controls.

Regarding follow-up of our patients with ITP over the study period we found that 71.4% of newly diagnosed cases had complete remission and the remaining 28.6% had partial response. Fatma et al. (2018) who reported that 70% of the patients had a complete remission, two were lost to follow-up at hematology clinic, and only one patient had a chronic progressive course. TNF alpha is a good predictor marker for prediction of the course and prognosis of ITP with a cutoff (>102.9 *µg/ml*) and (86.0%) ability to diagnose ITP truly , (72.0%) ability to exclude truly negative ones and (79.0%) total accuracy. This data agrees with Culić et al. (2013) who reported that TNF-a of 21.00 pg/mL is the most informative variable for discrimination between healthy children and children with ITP, with 81.08% of individuals correctly classified.

Correlation between TNF alpha with clinical and laboratory characteristics of our ITP cases showed that TNF alpha was statistically significantly negatively correlated with platelets count and disease duration as TNF alpha was markedly increased in newly diagnosed cases and decreased with chronicity and this data agrees with Fatma et al. (2018) who found a significant negative correlation between TNF-α and platelet count.

CONCLUSION

Immune thrombocytopenia (ITP) is one of the most common acquired bleeding disorders of childhood. The finding of significant differences in TNF alpha levels between ITP patients and healthy controls (significantly higher in ITP cases) indicates that TNF alpha disturbances might be involved in the pathogenesis of ITP in pediatric patients.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed in all parts of the paper.

REFERENCES

Andersson J. Cytokines in idiopathic thrombocytopenic purpura (ITP). Acta Paediatr. 1998;87:61–4.

Batikhan H, Gokcan MK, Beder E, Akar N, Ozturk A, Gerceker M. Association of the tumor necrosis factor-alpha− 308 G/A polymorphism with nasal polyposis. Eur Arch oto-rhino-laryngology. 2010;267(6):903–8.

Blandizzi C, Gionchetti P, Armuzzi A, Caporali R, Chimenti S, Cimaz R, et al. The role of tumour necrosis factor in the pathogenesis of immune-mediated diseases. Int J Immunopathol Pharmacol. 2014;27(1_suppl):1–10.

Chang W-W, Su H, He L, Zhao K-F, Wu J-L, Xu Z-W. Association between transforming growth factor-β1 T869C polymorphism and rheumatoid arthritis: a meta-analysis. Rheumatology. 2010;49(4):652–6.

Čulić S, Salamunić I, Konjevoda P, Dajak S, Pavelić J. Immune thrombocytopenia: serum cytokine levels in children and adults. Med Sci Monit Int Med J Exp Clin Res. 2013;19:797.

Del Vecchio GC, Giordano P, Tesse R, Piacente L, Altomare M, De Mattia D. Clinical significance of serum cytokine levels and thrombopoietic markers in childhood idiopathic thrombocytopenic purpura. Blood Transfus. 2012;10(2):194.

El Sissy MH, El Sissy AH, Elanwary S. Tumor necrosis factor-α–308G/A gene polymorphism in Egyptian children with immune thrombocytopenic purpura. Blood Coagul Fibrinolysis. 2014;25(5):458–63.

Fatma ESE, Ahmed KS, Nihal E-KS, Salwa HY. Cytokines and immunoglobulin derangement in egyptian children with primary immune thrombocytopenic purpura. Egypt J Haematol. 2018;43(1):1.

Jernås M, Hou Y, Strömberg Célind F, Shao L, Nookaew I, Wang Q, et al. Differences in gene expression and cytokine levels between newly diagnosed and chronic pediatric ITP. Blood, J Am Soc Hematol. 2013;122(10):1789–92.

Kashiwagi H, Tomiyama Y. Pathophysiology and management of primary immune thrombocytopenia. Int J Hematol. 2013;98(1):24–33.

Lanzkowsky P. Disorders of Platelets In: Manual of pediatric hematology and oncology. 5th ed. San Diego,Academic Press Co.;2011, pp : 321-377.

Patel HJ, Patel BM. TNF-α and cancer cachexia: Molecular insights and clinical implications. Life Sci. 2017;170:56–63.

Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood. 2009;113(11):2386–93

Talaat RM, Elmaghraby AM, Barakat SS, El-Shahat M. Alterations in immune cell subsets and their cytokine secretion profile in childhood idiopathic thrombocytopenic purpura (ITP). Clin Exp Immunol. 2014;176(2):291–300.

Tomiyama Y. Differential Diagnosis: Hypoplastic Thrombocytopenia. In: Autoimmune Thrombocytopenia. Springer; 2017. p. 107–14.

Zhang J, Min Q-H, Xu Y-M, Deng L-B, Yang W-M, Wang Y, et al. Association Between TNF-α-308G/A Polymorphism and Risk of Immune Thrombocytopenia: A Meta-Analysis. Genet Test Mol Biomarkers. 2017;21(2):80–5.

Zufferey A, Kapur R, Semple JW. Pathogenesis and therapeutic mechanisms in immune thrombocytopenia (ITP). J Clin Med. 2017;6(2):16.