

Interleukin-33 in Immune Thrombocytopenia: Relation to Disease Severity and Prognosis

Thesis

*Submitted for the fulfillment of the master degree in
Pediatrics*

By

Dr. Ahmed Said Ali Mohamed

M.B., B.CH (2009)

Under supervision of

Professor Dr. Azza Abd El-Gawad Tantawy

Professor of Pediatrics

Faculty of Medicine, Ain Shams University

Professor Dr. Amira Abd El-Moneim Adly

Professor of Pediatrics

Faculty of Medicine, Ain Shams University

Professor Dr. Eman Abd El-Rahman Ismail

Consultant of Clinical Pathology

Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University

2018

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العليم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Acknowledgment

*First and foremost, I feel always indebted to **ALLAH**, the Most Kind and Most Merciful.*

*I'd like to express my respectful thanks and profound gratitude to **Professor Dr. Azza Abd El-Gawad Tantaawy**, Professor of Pediatrics - Faculty of Medicine- Ain Shams University for her keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

*I am also delighted to express my deepest gratitude and thanks to **Professor Dr. Amira Abd El-Moneim Adly**, Professor of Pediatrics, Faculty of Medicine, Ain Shams University, for her kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

*I wish to introduce my deep respect and thanks to **Prof. Dr. Eman Abdel Rahman Ismail**, Consultant of Clinical Pathology, Faculty of Medicine, Ain Shams University, under whose supervision I had the honor and pleasure to proceed with work. Her constant guidance encouragement and foresight made all the difference.*

I would like to express my hearty thanks to all my family for their support till this work was completed.

Last but not least my sincere thanks and appreciation to all patients participated in this study.

Ahmed Said

List of Contents

Title	Page No.
List of Tables	i
List of Figures	iv
List of Abbreviations	vi
Abstract	xi
Introduction	1
Aim of the Work	5
▪ Immune Thrombocytopenia	6
▪ Interleukin-33	51
Subjects and Methods	82
Results	93
Discussion	114
Summary	122
Conclusion	126
Recommendations	127
References	128
Arabic Summary	

List of Tables

Table No.	Title	Page No.
Table (1):	ITP International Working Group proposed definitions of disease.....	10
Table (2):	Current definitions of ITP in relation to historical criteria.....	11
Table (3):	Types of ITP classified by pathophysiology.....	15
Table (4):	Bleeding symptoms are known to roughly correlate with platelet count at diagnosis.....	17
Table (5):	Grading of bleeding symptoms at presentation and at each subsequent evaluation.....	18
Table (6):	Grading and scoring systems in pediatric ITP.....	26
Table (7):	A number of other laboratory parameters in the diagnostic evaluation of ITP have been recommended by the ITP IWG	29
Table (8):	Common laboratory tests obtained in the thrombocytopenic patient at presentation.....	30
Table (9):	Differential Diagnosis of Primary Immune Thrombocytopenia	31
Table (10):	Comparison of high-dose IVIG and intravenous RhIG for management of immune thrombocytopenic purpura:	37
Table (11):	New drugs of ITP:	42
Table (12):	Treatment options for Immune Thrombocytopenia.....	46
Table (13):	Treatment options for refractory ITP	48
Table (14):	Target platelet counts during surgery in adults with ITP	50

List of Tables (cont...)

Table No.	Title	Page No.
Table (15):	Principal sources of nuclear IL-33 in human and mouse tissues	54
Table (16):	74
Table (17):	Roles of IL-33 in infectious diseases:	76
Table (18):	Roles of IL-33 in inflammatory diseases	77
Table (19):	Roles of IL-33 in some autoimmune diseases:.....	78
Table (20):	85
Table (21):	Descriptive clinical data of the studied patients with ITP	94
Table (22):	Therapy and outcome of studied ITP patients	95
Table (23):	Laboratory data of the studied ITP patients	96
Table (24):	Comparison of demographic and laboratory data between patients with ITP control	97
Table (25):	Comparison between different ITP stages as regards clinical variables	99
Table (26):	Comparison between different ITP stages as regards therapy and response	100
Table (27):	Comparison between different ITP stages as regards laboratory data	102
Table (28):	Comparison between active ITP and those in remission as regards clinical variables	103
Table (29):	Comparison between active ITP and those in remission as regards therapy and response	104
Table (30):	Comparison between active ITP and those in remission as regards laboratory data	105

List of Tables (cont...)

Table No.	Title	Page No.
Table (31):	IL-33 levels among active ITP and those in remission compared with healthy controls	106
Table (32):	IL33 in relation to clinical characteristics of ITP patients	107
Table (33):	Serum IL-33 in relation to therapy and outcome of ITP patients	109
Table (34):	Correlation between serum IL-33 and clinical and laboratory data of ITP patients	111
Table (35):	Multivariable linear regression analysis of factors affecting IL-33 levels in ITP patients	113

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Model of relationship of contributing factors in ITP.....	8
Figure (2):	13
Figure (3):	Schematic representation of pathophysiology of cITP	14
Figure (4):	Platelet-associated immunoglobulin and antibodies against platelets	28
Figure (5):	Roles of IVIG in innate and adaptive immunity-specific mechanisms in innate and adaptive immunity are described including areas of overlap specifically in complement, cytokine expression, and dendritic cell expression and activation	35
Figure (6):	Structure of Romiplostim and Eltrombopag and the Cellular Mechanisms of Action	40
Figure (7):	Therapeutic mechanisms of current ITP treatments.....	43
Figure (8):	Schema for an approach to ITP.....	44
Figure (9):	Using a standardized protocol was effective in reducing hospitalization and treatment use in children with newly diagnosed thrombocytopenia	45
Figure (10):	Interleukin-33 genetic study.....	53
Figure (11):	IL-33/ST2 signaling pathway	59
Figure (12):	IL-33 release and signaling via ST2L.....	60
Figure (13):	Mechanisms increasing and limiting IL-33 activity	61

List of Figures (cont...)

Fig. No.	Title	Page No.
Figure (14):	Schematic representation of the potential pro-inflammatory role of IL-33 in normal skin and in skin inflammation (atopic dermatitis and psoriasis)	65
Figure (15):	Role of IL-33 in rheumatoid arthritis	69
Figure (16):	IL-33 promotes vascular permeability and may be involved in the disruption of the blood-brain barrier	70
Figure (17):	The potential ant-inflammatory role of IL-33 in adipose tissue inflammation	72
Figure (18):	81
Figure (19):	Serum IL-33 levels in ITP patients compared with healthy controls.....	98
Figure (20):	Serum IL-33 levels in patients with active ITP and those in complete remission compared with healthy controls.	106
Figure (21):	Correlation between serum IL-33 levels and disease duration.....	112
Figure (22):	Correlation between serum IL-33 levels and platelet count at sampling.....	112

List of Abbreviations

Abb.	Full term
<i>Ab</i>	<i>Antibodies</i>
<i>ADHD</i>	<i>Attention deficit hyperactivity disorder</i>
<i>A-IPF</i>	<i>Absolute immature platelet fraction</i>
<i>ALPS</i>	<i>Autoimmune lymphoproliferative syndrome</i>
<i>AMI</i>	<i>Acute Myocardial Infarction</i>
<i>ANA</i>	<i>Antinuclear antibody</i>
<i>ANOVA</i>	<i>Analysis of Variance</i>
<i>Anti-D</i>	<i>Anti rhesus antibodies</i>
<i>Anti-Rh</i>	<i>Anti rhesus antibodies</i>
<i>Apoe</i>	<i>Apolipoprotein E</i>
<i>ATP</i>	<i>Adenosine triphosphate</i>
<i>BM</i>	<i>Bone marrow</i>
<i>BMI</i>	<i>Body mass index</i>
<i>Ca</i>	<i>Calcium</i>
<i>CBC</i>	<i>Complete blood count</i>
<i>CD</i>	<i>Crohn's disease</i>
<i>CLL</i>	<i>Chronic lymphocytic leukemia</i>
<i>CMV</i>	<i>Cytomegalovirus</i>
<i>CNS</i>	<i>Central nervous system</i>
<i>COPD</i>	<i>Chronic obstructive pulmonary disease</i>
<i>CR</i>	<i>Complete response</i>
<i>CV</i>	<i>Cardiovascular</i>
<i>CVID</i>	<i>Common variable immunodeficiency</i>
<i>CyA</i>	<i>Cyclosporin A</i>
<i>D</i>	<i>Rhesus factor</i>
<i>DAMPs</i>	<i>Damage-associated molecular patterns</i>
<i>DCs</i>	<i>Dendritic cells</i>
<i>DIC</i>	<i>Disseminated intravascular coagulation</i>
<i>DNA</i>	<i>Deoxyribonucleic acid</i>

List of Abbreviations (cont...)

Abb.	Full term
<i>DSS</i>	<i>Dextran sodium sulfate</i>
<i>DTaP</i>	<i>Diphtheria–tetanus–acellular pertussis vaccine</i>
<i>DVT</i>	<i>Deep venous thrombosis</i>
<i>ELISA</i>	<i>Enzyme linked immunosorbent assay</i>
<i>ER</i>	<i>Emergency room</i>
<i>ERK</i>	<i>Extracellular signal-regulated kinase</i>
<i>F/U</i>	<i>Follow up</i>
<i>FALC</i>	<i>Fat-Associated Lymphoid Cluster</i>
<i>g</i>	<i>Gram</i>
<i>G0</i>	<i>Gap zero period in cell cycle</i>
<i>G1</i>	<i>Gap 1 period in cell cycle</i>
<i>G6PD</i>	<i>Glucose-6-phosphate dehydrogenase</i>
<i>GI</i>	<i>Gastrointestinal</i>
<i>GTPase</i>	<i>Guanosine triphosphatase</i>
<i>GYN</i>	<i>Gynecological</i>
<i>H pylori</i>	<i>Helicobacter pylori</i>
<i>h</i>	<i>Human tissues</i>
<i>HCV</i>	<i>Hepatitis c virus</i>
<i>Hep C</i>	<i>Hepatitis C</i>
<i>HEV</i>	<i>High endothelial venules</i>
<i>HF</i>	<i>Heart failure</i>
<i>HIV</i>	<i>Human immunodeficiency virus</i>
<i>hr</i>	<i>hour</i>
<i>Hx</i>	<i>Skin</i>
<i>IBD</i>	<i>Inflammatory bowel diseases</i>
<i>IBLS</i>	<i>Immune thrombocytopenia Bleeding Scale</i>
<i>ICH</i>	<i>Intracranial hemorrhage</i>
<i>IgG</i>	<i>Gamma immunoglobulin</i>
<i>IL-1</i>	<i>Interleukin-1</i>
<i>IL-10</i>	<i>Interleukin-10</i>

List of Abbreviations (cont...)

Abb.	Full term
<i>IL-18</i>	<i>Interleukin-18</i>
<i>IL-1RAcP</i>	<i>Interleukin-1 receptor accessory protein</i>
<i>IL-1α</i>	<i>Interleukin-1 alpha</i>
<i>IL-1β</i>	<i>Interleukin-1 Beta</i>
<i>IL-33</i>	<i>Interleukin-33</i>
<i>IL-4</i>	<i>Interleukin-4</i>
<i>ILC2s</i>	<i>Type 2 innate lymphoid cells</i>
<i>IQR</i>	<i>Enter quartile range</i>
<i>IRAK-1</i>	<i>Interleukin receptor associated kinase 1</i>
<i>ITP</i>	<i>Immune thrombocytopenia</i>
<i>IVIg</i>	<i>Intravenous immunoglobulin</i>
<i>IWG</i>	<i>International Working Group</i>
<i>JAK-STAT</i>	<i>Janus kinase / Signal Transducer and Activator of Transcription</i>
<i>JNK</i>	<i>JUN N-terminal kinase</i>
<i>KC</i>	<i>Chemokine</i>
<i>kg</i>	<i>Kilogram</i>
<i>L</i>	<i>Liter</i>
<i>M</i>	<i>Macrophages</i>
<i>m</i>	<i>Mouse tissues</i>
<i>MAPK</i>	<i>Mitogen-activated protein kinase</i>
<i>MC</i>	<i>Mast cells</i>
<i>MD</i>	<i>Age-related macular degeneration</i>
<i>mg</i>	<i>Milligram</i>
<i>ml</i>	<i>milliliter</i>
<i>MMF</i>	<i>Mycophenolate mofetil</i>
<i>MMR</i>	<i>Measles–mumps–rubella vaccine</i>
<i>mRNA</i>	<i>Messenger ribonucleic acid</i>
<i>MS</i>	<i>Multiple sclerosis</i>

List of Abbreviations (cont...)

Abb.	Full term
<i>MyD88</i>	<i>Myeloid differentiation primary-response protein 88</i>
<i>N</i>	<i>Neutrophils</i>
<i>NF- κB</i>	<i>Nuclear factor-κB</i>
<i>NFAT</i>	<i>Nuclear factor of activated T cells</i>
<i>nm</i>	<i>Nanomolar</i>
<i>NMR</i>	<i>Nuclear magnetic resonance</i>
<i>NR</i>	<i>No response</i>
<i>P</i>	<i>Phosphorylation</i>
<i>p</i>	<i>Probability value</i>
<i>PCR</i>	<i>Polymerase chain reaction</i>
<i>PE</i>	<i>Oral</i>
<i>Pg</i>	<i>Picogram</i>
<i>PGE2</i>	<i>Prostaglandin E2</i>
<i>PO</i>	<i>Per oral</i>
<i>PR</i>	<i>Partial response</i>
<i>R</i>	<i>Response</i>
<i>RA</i>	<i>Rheumatoid arthritis</i>
<i>RAF</i>	<i>Rapidly accelerated fibrosarcoma kinase</i>
<i>RAS</i>	<i>Rat sarcoma GTPase</i>
<i>Rh</i>	<i>Rhesus factor</i>
<i>RhIG</i>	<i>Rhesus immunoglobulin</i>
<i>rhTPO</i>	<i>Recombinant human thrombopoietin</i>
<i>SD</i>	<i>Standard deviation</i>
<i>SF</i>	<i>Synovial fluid</i>
<i>SHC</i>	<i>Src homology collagen protein</i>
<i>SIGIRR</i>	<i>Single Ig IL-1R-related molecule</i>
<i>SLE</i>	<i>Systemic lupus erythematosus</i>
<i>SPSS</i>	<i>Statistical Program for Social Science version</i>

List of Abbreviations (cont...)

Abb.	Full term
<i>sST2</i>	<i>Soluble receptor suppression of tumorigenicity</i> <i>2</i>
<i>ST2</i>	<i>Receptor suppression of tumorigenicity</i>
<i>Th</i>	<i>T-helper</i>
<i>Th1</i>	<i>T-helper type 1</i>
<i>TH17</i>	<i>T-helper type 17</i>
<i>Th2</i>	<i>T-helper type 2</i>
<i>TLR</i>	<i>Toll like receptor</i>
<i>TNBS</i>	<i>Trinitrobenzene sulfonic acid</i>
<i>TNF-α</i>	<i>Tumor necrosis factor alpha</i>
<i>TPO</i>	<i>Thrombopoietin</i>
<i>TPO-RAs</i>	<i>Thrombopoietin receptor agonists</i>
<i>TRAs</i>	<i>Thrombopoietin receptor agonists</i>
<i>Tregs</i>	<i>T regulatory cells</i>
<i>TSH</i>	<i>Thyroid stimulating hormone</i>
<i>U</i>	<i>Urinary</i>
<i>UC</i>	<i>Ulcerative colitis</i>
<i>USA</i>	<i>United State of America</i>
<i>VEGF</i>	<i>Vascular endothelial growth factor</i>
<i>WBC</i>	<i>White blood cell</i>
<i>X²</i>	<i>Chi-square</i>
μ <i>L</i>	<i>Microliters</i>

Abstract

Background: The pathogenesis of thrombocytopenia in immune thrombocytopenia (ITP) has shifted from the traditional view of increased platelet destruction mediated by autoantibodies to more complex mechanisms where both impaired platelet production and T-cell-mediated effects play a role. Interleukin-33 (IL-33) is a newly identified cytokine of the IL-1 family. There is increasing evidence to suggest that IL-33 is a key inflammatory mediator in a complex network of immune cells and non-immune cells. IL-33 signals via its ST2 receptor and is involved in several autoimmune diseases by regulating T cell immune responses. **Aim:** To assess the level of IL-33 in children and adolescents with ITP and correlate it with disease severity, treatment response and outcome. **Methods:** Fifty young patients with ITP were compared with 40 age- and sex-matched healthy controls. Patients were studied stressing on bleeding manifestations, organomegaly/lymphadenopathy and therapy. Bleeding score was calculated to each patient according to the ITP Bleeding Scale (IBLS). The studied ITP Patients were further classified into 3 subgroups: “newly diagnosed ITP” (ITP within 3 months from diagnosis), “persistent ITP” (ongoing ITP between 3 and 12 months from diagnosis), and “chronic ITP” (ITP lasting more than 12 months). ITP Patients were also classified into active ITP defined as platelet count below $100 \times 10^9/L$ accompanying with or without bleeding episode or complete response (CR) which was defined as any platelet count of at least $100 \times 10^9/L$ and absence of bleeding. **Results:** IL-33 levels were significantly lower in ITP patients than controls (median [IQR], 150 [100 – 230] versus 210 [180-260] pg/mL; $p=0.011$). Levels were also lower in chronic than newly diagnosed ITP. IL-33 levels are lower among active ITP (median [IQR], 105 [85 – 120] pg/mL) compared with those in complete remission (median [IQR], 240 [200 – 250] pg/mL) and healthy controls ($p<0.001$) while no significant difference was found among the two latter groups ($p=0.197$). Patients who had splenectomy had lower IL-33 levels than non-splenectomized ones. Lower IL-33 levels were found among patients with corticosteroid-dependence and relapse ($p<0.05$). No significant difference as regards IL-33 between treated and un-treated patients with active ITP. IL-33 was positively correlated to platelet count at sampling ($r=0.714$, $p<0.001$). **Conclusions:** Alterations of IL-33 levels in pediatric patients with ITP highlight the role of T-cell immune response in the pathogenesis of ITP. It may be considered as a potential prognostic marker for the development of chronic ITP as it correlates with disease activity. Further studies investigating the role of IL-33 in the pathogenesis of ITP may provide a new therapeutic target for ITP.