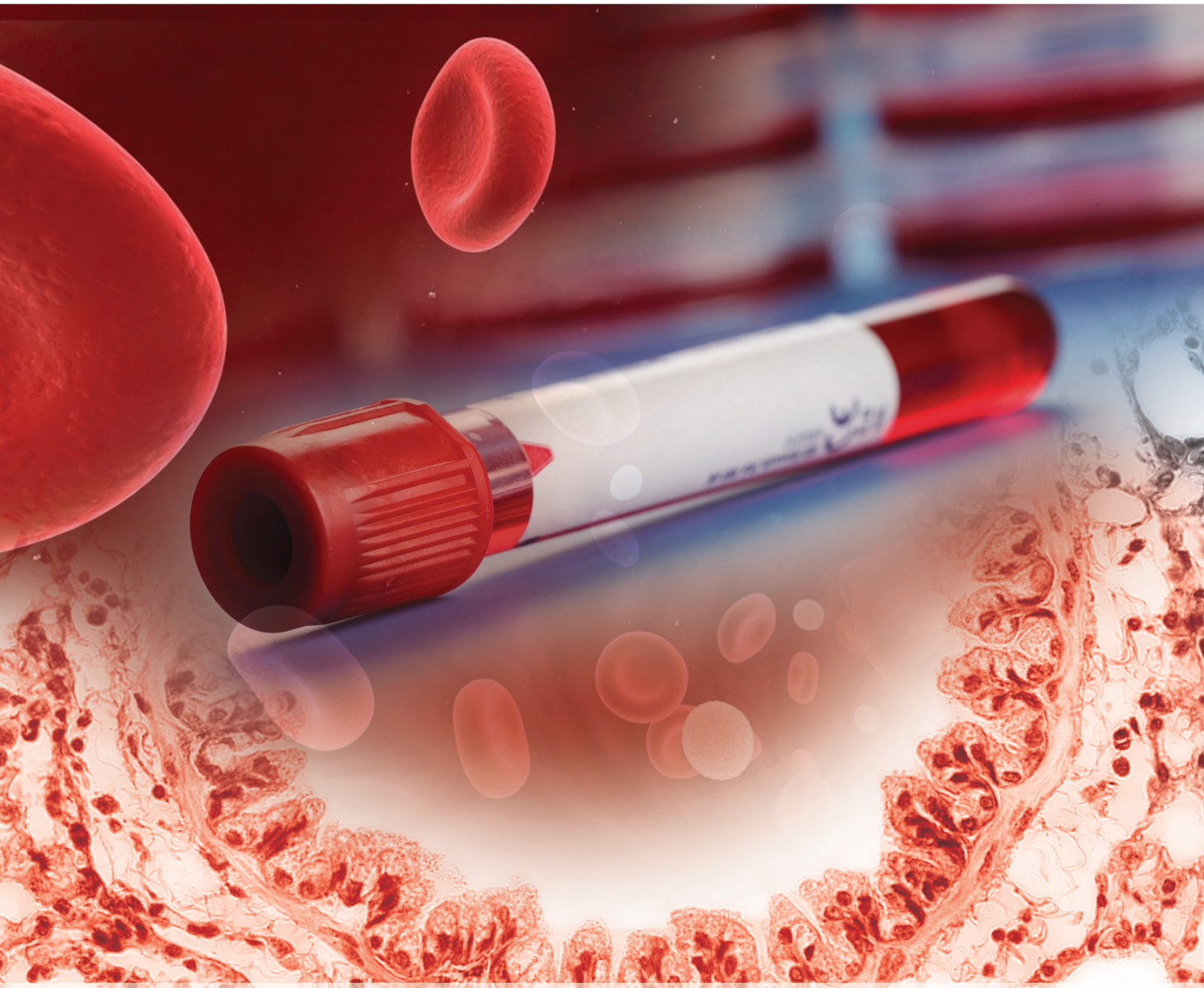
Frontiers in Clinical Drug Research

(Hematology)



Editor:
Atta-ur-Rahman, *FRS*

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Frontiers in Clinical Drug Research - Hematology

(Volume 5)

Edited by

Atta-ur-Rahman, *FRS*
Honorary Life Fellow,
Kings College, Cambridge,
University of Cambridge,
UK

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CONTENTS

PREFACE

The fifth volume of the book series: ***Frontiers in Clinical Drug Research – Hematology*** comprises seven comprehensive chapters covering various important topics. These include diagnosis and management of pulmonary embolism, treatment of thalassemia patients with iron chelators, treatment of iron overload in thalassemia patients, biological and clinical aspects of preeclampsia, haematological modulations, use of eculizumab, and hematological markers used as therapeutic targets in preeclampsia.

In Chapter 1, Gunasekaran and Rahi summarize advances in the diagnosis and management of pulmonary embolism. Babu and Panachiyil describe an evidence-based approach to treat transfusion- dependent thalassemia patients with iron chelators in chapter 2 of the book. Chattipakorn *et al*., in chapter 3, review the current and future treatments of iron overload in thalassemia patients. In the next chapter Kannan *et al*., discuss biological and clinical aspects of preeclampsia. Chattopadhyay *et al*., in chapter 5 briefly describe the haematological modulations by fixed dose combination (FDC) of tramadol hydrochloride/paracetamol (THP). Lazarowski, in chapter 6 of the book explains the possible use of eculizumab in patients infected with COVID-19 and the role of complement C5, neutrophils, and neutrophil extracellular traps (NETs) in the induction disseminated intravascular coagulation (DIC), sepsis, and multiple organ failure (MOF). Ali and Khaliq in the last chapter of the book present the role of hematological markers as emerging diagnostic and therapeutic targets in preeclampsia.

I hope that the readers will find value in this collection of reviews and draw inspiration for conducting further drug discovery research in the field of hematology.

I am grateful for the timely efforts made by the editorial personnel of Bentham Science Publishers, especially Mr. Mahmood Alam (Editorial Director), Mr. Obaid Sadiq (Incharge Books Department) and Ms. Asma Ahmed (Senior Manager Publications).

Prof. Atta-ur-Rahman, *FRS*
Honorary Life Fellow, Kings College
University of Cambridge
Cambridge
UK

List of Contributors

Adisak Tantiworawit	Division of Hematology, Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand
Alberto Lazarowski	Clinical Biochemistry Department Hospital de Clínicas J San Martín INFIBIOC-FFyB-UBA Bs As, Argentina
Anand K Purushothaman	Gene Therapy Laboratory, Department of Integrative Biology, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore – 632 014, India
Atiskumar Chattopadhyay	Faculty of Science, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India
Everette J R Nelson	Gene Therapy Laboratory, Department of Integrative Biology, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore – 632 014, India
George Mathew Panachiyil	College of Medicine, Nursing and Health Sciences, National University of Ireland, Galway, Ireland
Kausikisankar Pramanik	Department of Chemistry, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India
Kulothungan Gunasekaran	Division of Pulmonary Critical Care, Yale-New Haven Health Bridgeport Hospital, Bridgeport, CT, USA Division of Pulmonary Critical Care, Yuma Regional Medical Center, Yuma, AZ, USA
Mandeep Singh Rahi	Division of Pulmonary Critical Care, Yale-New Haven Health Bridgeport Hospital, Bridgeport, CT, USA
Meganathan Kannan	Blood and Vascular Biology Research Lab, Department of Life Sciences, School of Life Sciences, Central University of Tamil Nadu, Tiruvarur – 610 005, India
Nipon Chattipakorn	Cardiac Electrophysiology Research and Training (CERT) Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand Center of Excellence in Cardiac Electrophysiology Research, Chiang Mai University, Chiang Mai, 50200, Thailand
Saba Khaliq	Lahore Medical & Dental College, Lahore/ University of Health Sciences, Lahore, Pakistan
Siriporn C. Chattipakorn	Neurophysiology Unit, Cardiac Electrophysiology Research and Training (CERT) Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand Center of Excellence in Cardiac Electrophysiology Research, Chiang Mai University, Chiang Mai, 50200, Thailand Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand

Soumendra Darbar	Faculty of Science, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India Department of Chemistry, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India
Srimoyee Saha	Department of Physics, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India
Tamil Mani Subi	Blood and Vascular Biology Research Lab, Department of Life Sciences, School of Life Sciences, Central University of Tamil Nadu, Tiruvarur – 610 005, India
Tirin Babu	College of Medicine, Nursing and Health Sciences, National University of Ireland, Galway, Ireland
Zaima Ali	Lahore Medical & Dental College, Lahore/ University of Health Sciences, Lahore, Pakistan

CHAPTER 1

Recent Advances in the Diagnosis and Management of Pulmonary Embolism

Kulothungan Gunasekaran[*, 1,2] and **Mandeep Singh Rahi, MD**[1]

[1] *Division of Pulmonary Critical Care, Yale-New Haven Health Bridgeport Hospital, Bridgeport, 06610, USA*

[2] *Division of Pulmonary Critical Care, Yuma Regional Medical Center, Yuma, AZ, 85364, USA*

Abstract: Acute pulmonary embolism (PE) is a form of venous thromboembolism (VTE) and has varied clinical manifestations with significant morbidity and mortality. The general population's overall incidence is on the rise due to the increasing availability of D-dimer and computed tomographic pulmonary angiography. The incidence is higher in males than females (58 *versus* 48 per 100,000, respectively), increasing with age. In the United States, PE accounts for approximately 100,000 deaths annually. Specific populations, including patients with malignancy, pregnant females, hospitalized medical and surgical patients, or patients with total joint replacement, or arthroplasty, are at a higher risk for PE. Patients presenting with hemodynamic compromise due to PE need to be treated with intravenous thrombolytic therapy unless contraindicated, followed by anticoagulation. For over six decades, traditional anticoagulants like unfractionated heparin (UFH) are used for short-term anticoagulation. For patients who require long-term anticoagulation, low molecular weight heparin (LMWH) like enoxaparin and a vitamin K antagonist like warfarin are used to achieve therapeutic anticoagulation. Options for anticoagulation have been expanding steadily over the last decade with the introduction of the first direct oral anticoagulant (DOAC). Since their introduction, DOACs have changed the landscape of anticoagulation. This narrative review aims to summarize for clinicians managing pulmonary embolism (PE) the main recent advances in patient care, including risk stratification, current data regarding the use of thrombolytic treatment, and direct oral anticoagulants.

Keywords: Anticoagulation, Catheter-Directed Therapy, Pulmonary Embolism, Thrombolysis.

[*] **Corresponding author Kulothungan Gunasekaran:** Yuma Regional Medical Center, Yuma, AZ, USA 85364; Tel: 928-336-1580; E-mail: stankuloth@gmail.com

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INTRODUCTION

Hemostasis is achieved by a fine balance between coagulation and fibrinolytic factors in the blood. Imbalance due to certain inherited and acquired risk factors can predispose one to bleed or thrombose. Venous thromboembolism is one such condition with significant health and economic impact around the globe. Venous thromboembolism (VTE) encompasses deep venous thrombosis (DVT) and pulmonary embolism (PE). Virchow's triad, which includes blood stasis, hypercoagulability, and endothelial damage or dysfunction, underlies the thrombus formation. Inherited risk factors which contribute to this triad include hereditary thrombophilia like factor V Leiden mutation, antithrombin III deficiency, or deficiencies in fibrinolytic factors like protein C and protein S. Acquired risk factors that contribute to this triad include critical illness like bacterial sepsis or acute pancreatitis, immobility, orthopedic surgery, and systemic inflammatory states like coronavirus disease 2019 predispose patients to thrombus formation. Hematologic conditions, such as paroxysmal nocturnal hemoglobinuria, heparin-induced thrombocytopenia, and myeloproliferative disorders are associated with thrombosis. Malignancy is another important risk factor that can cause thrombosis by a complex interplay of endothelial damage, activation of clotting factors by cancer itself, and chemotherapeutic agents. Pulmonary embolism carries significant mortality and long-term morbidity among survivors.

Blood clots can travel to the pulmonary circulation from deeper veins in the lower extremities, pelvis, or upper extremities. Thrombosis can occur intrinsically in the pulmonary circulation as well as in conditions like sickle cell disease. Clinical manifestations range from an asymptomatic state or mild shortness of breath to hemodynamic collapse and cardiac arrest, depending on the location and burden of thrombosis in the pulmonary circulation. Prompt recognition, diagnosis, and institution of anticoagulation are the key to survival. Scoring systems and algorithmic approaches should also be followed. Patients with significant hemodynamic instability or cardiac arrest are managed with systemic thrombolysis followed by systemic anticoagulation and close monitoring in the intensive care setting. These usually require respiratory and hemodynamic support with invasive mechanical ventilation and vasopressors, respectively. In others, systemic anticoagulation should generally suffice. Given the significant risk of bleeding with thrombolytic therapy, catheter-directed therapies have been introduced, reducing the bleeding risk. Anticoagulation treatments come with a risk of bleeding, and shared decision-making discussing risks and benefits is necessary before long-term treatment is started. The duration of anticoagulation depends on the risk of recurrent PE and the presence of reversible, persistent, or non-identifiable risk factors. The long-term complication of pulmonary embolism

is chronic thromboembolic pulmonary hypertension (CTEPH), challenging to treat. Therefore, close follow-up and early referral to a CTEPH center are necessary.

Epidemiology

The exact incidence of disease will change with changing demographics, location, and the particular population being studied. A systematic review showed the significant burden of VTE across Western Europe, North America, Australia, and Argentina. The annual incidence ranged from 0.75 to 2.69 per 1,000 individuals in the population. A higher incidence of 2 to 7 per 1,000 individuals was observed in a population aged 70 years or above [1]. There are about 250,000 cases annually among United States whites [2]. Population-based studies have been conflicting in terms of incidence according to sex. Population-based research has reported a slightly higher incidence in men than women (130 *versus* 110 per 100,000) with a male:female sex ratio of 1.2:1 [3]. Higher incidence in males is supported by another population-based study with male to female incidence of 134 *versus* 115 per 100,000 [4]. On the other hand, a Norwegian population-based study demonstrated a slightly higher incidence in women than men. In the same study, the incidence of VTE was 1.43 per 1,000 person-years, DVT was 0.93 per 1,000 person-years, and PE was 0.50 per 1,000 person-years [5]. A prospective Swedish study found similar incidence in men and women [6].

A retrospective study using the Nationwide Inpatient Sample (NIS) assessed the impact of computed tomography pulmonary angiogram (CTPA) on PE incidence and mortality. The incidence of PE was unchanged before CTPA but increased after CTPA (from 62.1 to 112.3 per 100,000, $p < 0.001$). Mortality due to PE decreased more before CTPA than after. Similarly, the case fatality rate decreased from 13.2% to 12.1% ($p = 0.02$) before CTPA and from 12.1% to 7.8% ($p < 0.001$) post-CTPA [7]. A study from Australia described the mortality rate from PE to be 1.73 per 100,000 population per year [8]. Analysis from the RIETE registry by Jimenez *et al*. showed a reduction in all-cause mortality from 6.6% (2001 to 2005) to 4.9% (2010 to 2013). The use of thrombolytic therapy and surgical embolectomy increased in the period of 2001 to 2013. The mean length of stay for PE patients decreased from 13.6 to 9.3 days ($p < 0.001$) in the same period [9]. In a similar study from the US analyzing the NIS sample from 1998 to 2005, the case fatality rate decreased from 12.3 to 8.2% ($p < 0.001$), and the length of stay decreased from 9.4 days to 8.6 days ($p < 0.001$) [10]. A recent study that examined mortality rates due to PE in the US from 1999 to 2018 found mortality rates reversed after an inflection point in 2008. The age-adjusted mortality rate was 5.0 per 100,000 population in 1999, decreased to 3.4 per 100,000 population in 2008, and then increased slightly to 3.5 per 100,000

CHAPTER 2

An Evidence-Based Approach to Treatment with Iron Chelators in Transfusion- Dependent Thalassemia Patients : Present Trends and Future Scenario

Dr. Tirin Babu[1] and **Dr. George Mathew Panachiyil**[1,*]

[1] *College of Medicine, Nursing and Health Sciences, National University of Ireland, Galway, Ireland*

Abstract: Treatment of hemochromatosis is a significant target-based care of transfusion-dependent thalassemia and non-transfusion dependent thalassemia patients. In some cases hemochromatosis is a secondary concern to frequent red blood cell transfusions as in transfusion-dependent thalassemia (TDT) or advances from enhanced gastrointestinal iron absorption such as in non-transfusion dependent thalassemia (NTDT), this can cause serious illness and death to the patients. When thalassemia major patients undergo frequent blood transfusions, hemochromatosis is unavoidable because the human body lacks a physiological mechanism to evacuate extra iron. Thalassemia patients with transfusional hemochromatosis regularly need treatment with iron chelators to decline the iron overload and thereby retard long-term effects related to iron accumulation in tissues. Deferoxamine, deferiprone, and deferasirox are the three currently approved iron chelators for the treatment of hemochromatosis in transfusion-dependent thalassemia patients. Today, iron chelation therapy's goal is to sustain acceptable levels of iron in the human body at all times. Correct tailoring with iron chelators and their dose modifications must implement on time.

We first describe the pathophysiology of hemochromatosis in thalassemia patients in this chapter. We then cover iron chelation therapy's general goals, the features of the permitted iron chelators, and the evidence-based practice behind the usage of iron chelators as a single drug, or as part of combination therapy, and the mechanisms by which chelators work. The guiding principles for monitoring treatment with iron chelators to reduce the toxicity risks from iron chelation are later explained. Finally, the importance of deferasirox twice-daily dose instead of a once-daily dose in transfusion-dependent thalassemia patients with inadequate response to high doses and the future directions in treating iron overload in thalassemia patients is discussed.

[*] **Corresponding author Dr. George Mathew Panachiyil**: College of Medicine, Nursing and Health Sciences, National University of Ireland, Galway, Ireland; Tel: +918281225767;
E-mail: g.mathew1993@gmail.com

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Keywords: Alpha Thalassemia, Beta-Thalassemia, Blood Transfusions, Combination Therapy, Deferasirox (DFX), Deferiprone (DFP), Deferoxamine (DFO), Exjade, Intolerance, Iron Chelation Therapy, Iron Chelator, Iron Overload, Jadenu, JAK2 Inhibitors, Luspatercept, Minihepcidins, Monotherapy, Non-Transfusion Dependent Thalassemia (NTDT) Patients, Novel Oral Iron Chelator, Poor Response, Serum Ferritin, Sotatercept, Thalassemia Guidelines, Transfusion Dependent Thalassemia (TDT) Patients, Twice-Daily Deferasirox.

INTRODUCTION

Iron burden occurs in thalassemia patients when the amount of iron taken up by the human body is increased for prolonged periods. There are two important mechanisms by which iron overload occurs in transfusion dependent thalassemia (TDT) and non-transfusion dependent thalassemia (NTDT) patients. Frequent blood transfusion therapy being the primary cause of iron overload in TDT patients and enhanced iron absorption in the human gastrointestinal tract (GI) being more predominant in NTDT patients. When TDT patients undergo frequent blood transfusions, hemochromatosis is unavoidable. The human body has deficiencies in physiological mechanisms to remove the surplus hemochromatosis due to regular blood transfusion. When a red blood cell (RBC) becomes senescent, the reticuloendothelial macrophage phagocytizes it. Inside the macrophage, the heme part of the RBC breaks down into iron and protoporphyrin. Free iron releases into the plasma. Two molecules of plasma free iron (Fe3+) then bind to one serum transferrin, the main iron transport protein. Transferrin then transports iron to the storage site by binding the transferrin receptor. In iron overload stats, transferrin saturation causes labile plasma iron (LPI) and non-transferrin bound iron (NTBI) to readily enter multiple organs through L-type calcium channels (LCC), ZIP14, and divalent metal transporter (DMT1). Excess iron in the cytoplasm produces reactive oxygen species *via* the Fenton and Haber-Weiss reaction. Increased reactive oxygen species levels cause mitochondrial damage, peroxidation of lipids, cell membrane damage, and disruption of the electron transport chain. Over time this leads to apoptosis of the target organ. Recent studies suggest that reactive oxygen species levels also impair the production of nitric oxide and damage the vessel wall. Eventually, chronic iron overload contributes to damage of multiple organs, *e.g*., cardiomyopathy, cirrhosis of the liver, endocrinopathy, arthritis, *etc* [1-3].

Iron chelation therapy is the treatment choice in thalassemia patients, thereby reducing disease and fatality in them. The main intention of treatment with iron chelators is to retain permissible levels of iron in the body at all times by maintaining the balance of iron in blood transmission and iron elimination processes. Generally, in any iron chelation therapy management, the advantage of

treatment with iron chelators must be well adjusted against harmful effects, which usually takes time to develop depending on high doses of iron chelators concerning the level of hemochromatosis. The most important US FDA (Food and Drug Administration) permitted iron chelators are Deferoxamine, Deferiprone and Deferasirox. Regular adherence to chelation is vital in the management of hemochromatosis in thalassemia patients [1, 4].

In this chapter we first briefly describe about prevalence, clinical classification, laboratory diagnosis, pathophysiology and blood transfusions in thalassemia patients. We then cover the mechanism of hemochromatosis, iron overload complications and its diagnosis in thalassemia patients. The general goals of treatment with iron chelators, the managing of hemochromatosis with monotherapy and combination therapy of iron chelators are explained in the later section. Lastly, guiding principles for overseeing treatment with iron chelators, the importance of two times a day dosing of deferasirox in unresponsive/or intolerant thalassemia patients and future outlook for the treatment of thalassemia patients are discussed.

DEFINITION AND GLOBAL DISTRIBUTION OF THE THALASSEMIAS

The medical term thalassemia refers to a set of congenital disorders of haemoglobin synthesis marked by mild to severe anaemia, resulting from the partial or complete failure in production of one or more globin chains [5]. The Detroit Paediatricians Thomas Benton Cooley and Pearl Lee first recognized thalassemia's clinical characteristics in patients of Italian origin in 1925. Later, in 1936, George Whipple and William Bradford coined the term thalassemia [6]. The term thalassemia results from two Greek words: Thalassa meaning the sea, and anaemia, the weak blood [7].

The exact prevalence of thalassemia in the world is unknown as only a few countries maintain a regular patient registry. The incidence of Mediterranean anemia and abnormal haemoglobin carriers are 5.1%, with nearly 226 million carriers in the globe, as claimed by the World Health Organization (WHO) records. Approximately 80% of global thalassemia cases are noticed in the region covering from sub-Saharan Africa to the Mediterranean Basin, the Middle East, and South and Southeast Asia. Thalassemia prevalence is now worldwide due to human migration and intermarriage between different ethnic groups, which contributed to the changing epidemiology of the disease [8 - 10].

CLINICAL CLASSIFICATION OF THALASSEMIA SYNDROMES [1 - 3, 12, 13]

The thalassaemias are named α-, β-, γ-, δ -, δβ-, or εγδβ-thalassaemias according

CHAPTER 3

Current and Future Treatments of Iron Overload in Thalassemia Patients

Adisak Tantiworawit[1], Siriporn C. Chattipakorn[2,3,4] and **Nipon Chattipakorn[5,6,7,*]**

[1] *Division of Hematology, Department of Internal Medicine, Faculty of Medicine, Chiang Mai University,Chiang Mai, 50200, Thailand*

[2] *Neurophysiology Unit, Cardiac Electrophysiology Research and Training (CERT) Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand*

[3] *Center of Excellence in Cardiac Electrophysiology Research, Chiang Mai University, Chiang Mai, 50200, Thailand*

[4] *Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand*

[5] *Cardiac Electrophysiology Research and Training (CERT) Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand*

[6] *Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand*

[7] *Center of Excellence in Cardiac Electrophysiology Research, Chiang Mai University, Chiang Mai, 50200, Thailand*

Abstract: Iron overload is a major complication among thalassemia patients. In these patients, ineffective erythropoiesis decreases hepcidin production resulting in iron dysregulation, which leads to a number of serious complications. Damage to organs susceptible to iron overload could be prevented by effective iron chelation. Despite the efficacy of iron chelators, limitations to their use are that they are only used after the patients have suffered from iron overload, and they have also been associated with a number of side effects. New therapeutic strategies for the treatment of thalassemia have focused on addressing the pathophysiology of the disease. Drugs currently being developed to improve ineffective erythropoiesis are aimed at increasing hemoglobin levels and subsequently decreasing iron absorption. The new therapeutic drugs in this class include pegylated erythropoietin, JAK 2 inhibitors, and TGF-β activin receptor traps (sotatercept and luspatercept). Luspatercept is currently recognized as the most promising drug in this class and has completed phase III of trials. With the aim of improving iron dysregulation, these new therapeutic strategies focus on preventing the

[*] **Corresponding author Nipon Chattipakorn:** Cardiac Electrophysiology Research and Training (CERT) Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand and Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand; Tel: +66-53-945329; Fax: +66-53-945367; E mail: nchattip@gmail.com

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absorption of iron in the gastrointestinal tract. These therapies involve hepcidin agonists and specific derivatives, such as LJPC-401 and Rusfertide (formerly PTG-300), certain ferroportin inhibitors, such as Vamifeport (formerly VIT-2763) and transmembrane protease serine 6 (TMPRSS6) antisense oligonucleotides. Although the therapeutic potential of these new treatments in thalassemia patients is promising, ongoing clinical trials are needed. Importantly, these new treatment strategies may provide a new, more effective paradigm of treatment in thalassemia patients.

Keywords: Activin receptor, Antisense oligonucleotides, Ferritin, Ferroportin, Ferroportin inhibitor, Hepcidin, Hepcidin agonist, Ineffective erythropoiesis, Iron overload, Iron chelator, JAK-2 inhibitor, Luspatercept, Pegylated erythropoietin, Rusfertide, Sotatercept, Thalassemia, Thalassemia complication, TMPRSS6, TGF-β, Vamifeport.

THE PATHOPHYSIOLOGICAL BASIS OF IRON OVERLOAD

Thalassemia is a genetic disease caused by the mutation of a globin gene. Classification of the disease can be by transfusion burden into either transfusion-dependent thalassemia (TDT) or non-transfusion-dependent thalassemia (NTDT), or by type of mutation into either alpha (α)-thalassemia or beta (β)-thalassemia. The TDT patients require regular red blood cell (RBC) transfusions every 2-4 weeks with no transfusion-free period of more than eight weeks (6-24 units of packed RBC per year) [1 - 4]. The NTDT group comprises patients who do not require regular RBC transfusions for survival but who do require occasional transfusions under special circumstances such as growth retardation, pregnancy, and infection. The NTDT group does not receive intermittent RBC transfusions more than three times per year (less than 6 units of packed RBC per year) [1 - 4].

Iron overload is a major complication in thalassemia patients as a consequence of frequent blood transfusions and an increase in dietary iron absorption. TDT patients who require regular blood transfusions every 2-4 weeks often develop iron overload as a result of these repeated transfusions [1]. In NTDT patients, iron overload mainly results from a 5-10-fold increase in dietary heme iron *via* heme transporters and nonheme iron *via* divalent metal iron transporter 1 (DMT1) [2].

Iron Regulation and Homeostasis

Normally, iron content in the human body reaches about 3 to 5 grams (g). Iron in the body is mainly present in hemoglobin (Hb) of circulating RBC and myoglobin (Mb) in skeletal muscles [5]. Circulating plasma iron is mainly used in intramedullary erythropoiesis in bone marrow cells. Iron levels in the body are tightly controlled by many regulatory proteins and pathways. As regards turnover, iron in the body is mainly recycled in the reticuloendothelial (RE) system from

senescent RBC by macrophages, and only 1-2 mg of iron is absorbed into and/or lost from the body on a daily basis [6]. Iron is stored in the form of ferritin and in the circulating transferrin. Under physiological conditions, iron regulation and homeostasis are strictly regulated to prevent iron overload [6].

Thalassemia is associated with chronic hemolytic anemia, ineffective erythropoiesis and iron overload. Ineffective erythropoiesis and chronic anemia are known to decrease hepcidin production *via* an increase in hypoxia-inducible transcription factors (HIF), transmembrane protease serine-6 (TMPRSS-6), erythroferrone (ERFE) and growth differentiation factor-15 (GDF-15). Low levels of hepcidin are also associated with increasing duodenal iron absorption. Iron overload due to iron dysregulation is one of the major complications of thalassemia and as a consequence, excessive redox-active iron generates reactive oxygen species (ROS) *via* the Fenton reaction. Subsequently, this causes damage to lipids, proteins and DNA of cells and organs. The major organs that can potentially be damaged by excessive iron overload are the heart, liver, and endocrine organs [1, 2].

Intestinal Iron Absorption

The mucosa of the intestines, especially in the duodenum, plays an important role in the absorption of iron from food [7]. Duodenal cytochrome b (DCYTB) reduces ferric ion to ferrous ion, which can then be taken up by DMT1 in the mucosa of the intestines [7, 8]. After iron (Fe^{2+}) enters the cells, the iron is oxidized to the ferric form by hephaestin. This is a known ferroxidase enzyme that works in cooperation with an iron export protein known as ferroportin before the iron is exported from enterocytes and enters the blood [7].

Hepcidin is an iron regulatory protein associated with intestinal iron absorption and macrophage iron release. Low hepcidin levels allow for an increase in iron export *via* ferroportin, which then contributes to an increase in iron absorption in the intestines [9] (Fig. **1**). However, in thalassemia patients who are already experiencing iron overload, ineffective erythropoiesis and chronic anemia can lower hepcidin production to allow for an increase in iron export through ferroportin. This can also occur *via* an increase in HIF-2α that contributes to enhanced levels of iron absorption [9]. All of these mechanisms contribute to the development of iron overload in thalassemia patients [8 - 10].

CHAPTER 4

Preeclampsia: Biological and Clinical Aspects

Tamil Mani Subi[1], Anand K Purushothaman[2], Everette J R Nelson[2] and **Meganathan Kannan[1,*]**

[1] *Blood and Vascular Biology Research Lab, Department of Life Sciences, School of Life Sciences, Central University of Tamil Nadu, Tiruvarur – 610 005, India*

[2] *Gene Therapy Laboratory, Department of Integrative Biology, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore – 632 014, India*

Abstract: Preeclampsia is a complication associated with pregnancy due to an abnormal formation of placental blood vessels. Though the reason for occurrence is unclear, recent studies have enabled us to understand the pathophysiology of the condition, which has eventually improved its management. Multiple risk factors are believed to contribute to poor placentation, which includes chronic hypertension, antiphospholipid antibody syndrome, pre-gestational diabetes, chronic renal disease, previous intrauterine growth restriction, and previous placental abruption. Understanding preeclampsia at both biological and clinical levels is crucial for a proper diagnosis. Abnormally elevated plasma levels of MMP7, sFlt1, sEng, PAPP-A, VEGF, PIGF, activin A, and cell-free fetal DNA can serve as maternal markers for preeclampsia. It has been widely accepted that these changes in the plasms levels would complicate the natural course of pregnancies. Certain classical features like hypertension and proteinuria still remain the best indicators of preeclampsia. An abnormal hematological profile in preeclampsia is known to be associated with certain coagulopathies, such as disseminated intravascular coagulation. In the current chapter, we discuss both biological and clinical aspects of preeclampsia.

Keywords: Biological aspects, Clinical aspects, Diagnosis and Management, Hematological complications, Preeclampsia.

INTRODUCTION

Pregnancy is a specialized condition that demands extensive changes in the maternal system. These include changes to the hematological profile in order to support the ensuing processes for a successful delivery, deviations in which would result in one of the many complications of pregnancy. These deviations in the

* **Correspondence author Meganathan Kannan:** Blood and Vascular Biology Research Lab, Department of Life Sciences, School of Life Sciences, Central University of Tamil Nadu, Tiruvarur – 610 005, India; Email: meganathank@cutn.ac.in, kannanmd77@gmail.com

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maternal hematological parameters also result from poor maternal physiological development, which could lead to failed pregnancies due to the occurrence of preeclampsia. According to the International Society for the Study of Hypertension in Pregnancy (ISSHP), preeclampsia results from gestational hypertension (systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg) often associated with a recent onset of at least one of the following conditions, such as proteinuria, maternal organ dysfunction, or uteroplacental dysfunction at 20 weeks of gestation [1, 2].

Normal pregnancy would involve several changes in the maternal hematological parameters as a shielding and compensatory effect for successful parturition. These changes include increases in the plasma volume, RBC and WBC counts, and abnormal levels of certain coagulation factors. Additionally, a slight decrease in the number of platelets has also been observed in pregnant women compared to normal, non-pregnant women [3]. The plasma volume increases by 10-15% in 6-12 weeks of gestation and continues to increase in the following trimesters reaching 48%. The levels of plasma renin also increase along with a decrease in the atrial natriuretic peptide. This increase in plasma volume would affect the natural concentration of blood-based biomarkers such as haemoglobin, which is also known as dilution anaemia of pregnancy [3, 4]. The plasma volume increases in order to meet the circulation needs of the developing placenta and the maternal organ [5]. The RBC mass increases by 20-30% in 8-10 weeks of gestation for females with iron supplements and only 15-20% for females with non-iron supplements. This is consequent to the increase in erythropoietin levels to meet the metabolic requirement of oxygen [3], while the increase in the RBC mass is to meet the demands of the new vascular bed. This is also an important evolutionarily conserved physiological mechanism to compensate for the blood loss during delivery of the baby [6]. An increase in WBCs during normal pregnancy is largely attributed to the increase in neutrophils that is seen during the second month of pregnancy [3]. Impaired neutrophil apoptosis and bone marrow hyperplasia during the last trimester is also a reason for the neutrophilic leukocytosis, which would serve as a marker to prevent infections during the delivery of the baby [7]. The neutrophils also bear an enhanced potential to degranulate, phagocytose and form neutrophil extracellular traps (NETs) to kill or ensnare the microbes. The process of forming NETs referred to as “NETosis”, is highly regulated and only triggered when neutrophils encounter commendable challenges to protect the mother [8]. There is also an increase in certain coagulation factors which maintain the maternal system at a prothrombic state. While the levels of fibrinogen, factors II, VII, VIII, X, and VWF increase, those of protein S (PS) and factor XI decrease with the levels of factors V, IX, and protein Z (PZ) remaining unchanged [3], all of which co-ordinate in preventing an excessive loss of blood during delivery. The mean platelet counts of pregnant

women will also be slightly lower than healthy, non-pregnant women, which is called gestational or incidental thrombocytopenia accounting for 75% of thrombocytopenia in pregnancy. It is, however, mild and asymptomatic and automatically resolves post-partum [3]. This is due to increased consumption of platelets, dilution effect of plasma, lack of production of platelets, and acceleration of platelet destruction across the placenta [9]. It could also be due to high platelet clearance and splenomegaly [7].

These physiological changes would highly favour a successful pregnancy, but any deviation collapses the maternal hematological profile leading to certain complications, which could be further enhanced or supported by poor maternal physiological changes resulting in pregnancy failure. Preeclampsia is due to inadequate placentation and endothelial dysfunction, which along with an altered hematological profile, disrupts the stable maternal condition and complicates the pregnancy. About 15-20% of thrombocytopenia occurring in 8-10% of pregnancies is due to preeclampsia which could turn out to be life-threatening. Increased endothelial activation would also activate platelets and initiate the coagulation cascade, further aggravating the existing condition [9, 10]. Preeclampsia also changes the morphology of erythrocytes with early degradation and destruction of the membrane leading to lysis [11]. Preeclampsia is associated with lower plasma volume [3, 4, 12] and decreased concentration of certain coagulation factors like protein Z (PZ); it also interferes with the normal activity of neutrophils by enhancing their pro-NETotic activity [8].

PREECLAMPSIA

Preeclampsia is a hypertensive disorder of pregnancy, which remains the major reason for significant morbidity and mortality for the mother and fetus worldwide [13, 14]. Globally, preeclampsia affects 5% of pregnancies, and the-WHO estimates its incidence to be seven times higher in developing countries when compared to developed countries. In developing countries, the lack of awareness about the disease and its symptoms have also resulted in the severe form known as eclampsia, thereby worsening the disease burden in this part of the world where its incidence is reported to be 1 per 100 to 1700 pregnancies as compared to 5 to 7 per 10,000 pregnancies in developed countries. The rate of incidence in the African countries is also much higher (1.8% to 7.1%). It is difficult to define preeclampsia as it is characterised by a myriad of clinical features, but for better understanding and classification of the disorder, the ISSHP have defined preeclampsia as gestational hypertension that is associated with recent onsets of at least one of the following conditions, such as proteinuria, maternal organ dysfunction or uteroplacental dysfunction at 20 weeks of gestation [1, 2, 15]. In the past, the pathophysiology was poorly understood, which made it difficult for

CHAPTER 5

Haematological Modulations by Fixed Dose Combination (FDC) of Tramadol Hydrochloride/Paracetamol (THP)

Soumendra Darbar[1,2], Srimoyee Saha[3], Kausikisankar Pramanik[2] and **Atiskumar Chattopadhyay[1]**

[1] *Faculty of Science, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India*

[2] *Department of Chemistry, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India*

[3] *Department of Physics, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India*

Abstract: Analgesics as fixed-dose combination are very useful for fast pain relief. Overdose or chronic use of analgesics, especially fixed doses form, develop mild to severe adverse effects and sometimes damage various organs like the liver, kidney and brain. Tramadol hydrochloride/paracetamol (THP) is a fixed-dose combination (37.5 mg of Tramadol hydrochloride and 325 mg of paracetamol) extensively used for the treatment of moderate to severe pain. Administration of THP upon animal model severely disturbed hepatic and renal biochemical parameters, which leads to altering normal cellular homeostasis. In this context, our recent study established that the application of Tramadol hydrochloride/paracetamol produced deleterious effects on haematological parameters in the experimental murine model. 1.12 g/300 ml and 1.68 g/300 ml chronic administration of Tramadol hydrochloride/paracetamol (THP) decrease the packed cell volume (PCV), haemoglobin (Hb), mean cell volume (MCV), means cell haemoglobin (MCH) and increase the mean corpuscular haemoglobin concentration (MCHC) and white blood cell (WBC) in an experimental animal model. Other haematological parameters likes red blood corpuscle (RBC), reticulocyte (RT), haematocrit (HCT) did not show any significant changes upon animals. Increased MCHC inhibits the oxidation process and energy balance, whereas elevated WBC levels indicate immune system damage. Decreased haemoglobin and PCV indicate that Tramadol hydrochloride/paracetamol (THP) is an indirect cause of anaemia. It may be concluded that prolonged or chronic administration of THP may cause severe thrombocytopenia, leading to the failure of the immune system, anemia, and a very low erythrocyte count. These side effects increase according to the dosage and duration of

* **Corresponding author Atiskumar Chattopadhyay:** Principal Secretary, Faculty of Council Science, Jadavpur University, Raja S C Mallick Road, Kolkata-700032, West Bengal, India; Tell:09433144548; E-mail: atischatterjee@gmail.com

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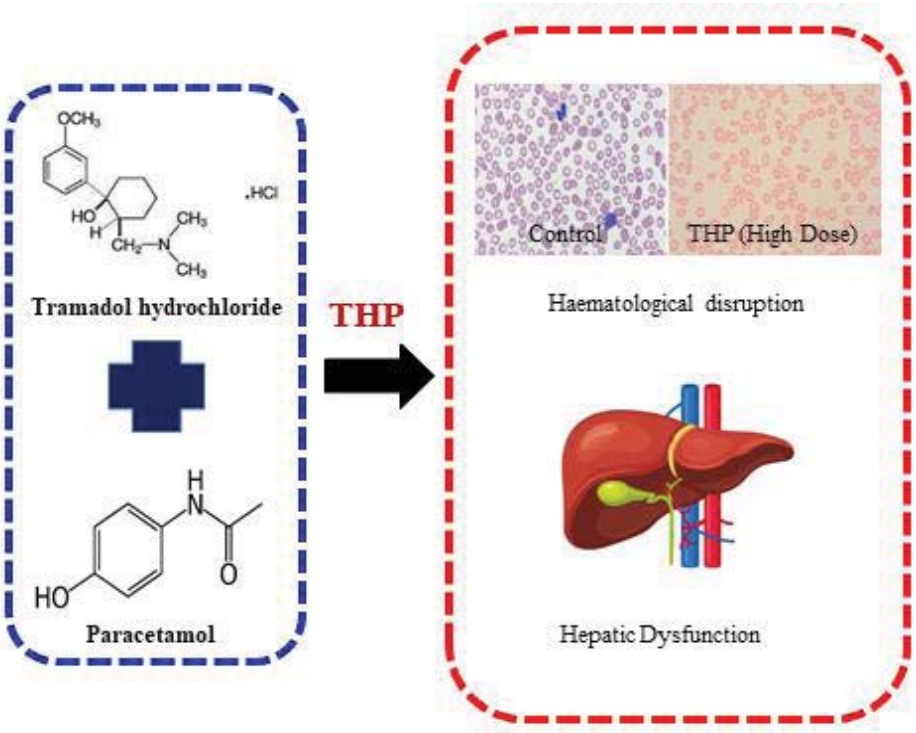
the treatment. So clinicians, patients and pharmacists should be aware of the deleterious effects of Tramadol hydrochloride/paracetamol (THP) on haematological parameters when using this combination.

Keywords: Fixed-dose combination, Hematologic parameters, Murine model, Tramadol hydrochloride/paracetamol.



Graphical Abstract

INTRODUCTION

Tramadol Hydrochloride/Paracetamol (THP) is a fixed dose combination (FDC) extensively used for the symptomatic treatment of moderate to severe pain [1]. The use of Tramadol hydrochloride/paracetamol should be restricted to patients whose moderate to severe pain is considered to require a combination of Tramadol and paracetamol. The dose should be adjusted to the intensity of pain and sensitivity of the individual patient. The lowest effective dose for analgesia should generally be selected [2, 3].

The tablet available in the market normally contains 37.5 mg of Tramadol hydrochloride and 325 mg of paracetamol. Chronic use and over dosage of THP developed moderate to severe side effects on patients [4]. Different scientific reports based on animal studies demonstrate that Tramadol has antinociceptive reactions on acute and chronic pain [5, 6]. The prolonged use of Tramadol at high dosage causes physical dependence and withdrawal syndrome [7, 8].

The adverse effect of fixed-dose combination (FDC) Tramadol hydrochloride/paracetamol (THP) in high dose in the blood is well established. Our recent study depicted that the administration of THP reduced red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb) disturbed haematopoiesis [9]. Various ultrastructural abnormalities in the leukocytes in the blood were visible under electron microscopy of mice treated with THP clearly demonstrated that this notorious chemical makes structural deformities in blood [10]. In view of the above, there is a need to know the various side effects of Tramadol hydrochloride/paracetamol (THP), mainly focused on haematological alteration and systemic hepatic disturbance in the body when system exposed with fixed-dose combination.

MATERIALS AND METHODS

Chemicals

NaCl, NaOH, TRIS buffer, *etc*., were obtained from Merck, India. PBS pH 7.4 was procured from Sigma-Aldrich. Biochemical determination kits, *i.e*., ALT and AST were procured from Accurex Biomedical Pvt. Ltd, Thane, India. All others reagents used in this study are of laboratory grade.

Animals

Eighteen young adult, healthy male swiss albino mice weighing 24g ± 4g have been randomly included in the study. The animals were housed in healthy atmospheric conditions (12 h light and dark cycles, at 25±2 ºC and 50-60% humidity), normal feeding, drinking, and medical care based on the CPCSEA guidelines. Mice were kept under observation for one week before the onset of the experiment for acclimatization and to exclude any undercurrent infection. The experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) (Approval No. 26/Dey's/s/IAEC/Pharma/2019).

Experimental Procedure

The mice were randomly assigned to three major groups of six mice according to their body weights, such that each group was made up of mice within the close

CHAPTER 6

Possible Use of Eculizumab in Critically Ill Patients Infected with Covid-19 Role of Complement C5, Neutrophils, and NETs in the Induction DIC, Sepsis, and MOF

Alberto Lazarowski[1,*]

[1] *Clinical Biochemistry Department, Hospital de Clínicas J San Martín, INFIBIOC-FFyB-UBA, Argentina*

Abstract: The most relevant critical clinical picture in CVID19 patients is respiratory failure, micro clots in various organs, disseminated intravascular coagulation (DIC) and progressive multiple organ failure (MOF). The "hyperinflammation" or "cytokine storm " is the scenario in which the complement (included C5) attack is triggered. This activity can be detected by the loss of expression of CD88 in the membrane circulating neutrophils (PMN), and this loss of expression reflects the attack of the complement to the rest of the organs and tissues, which is accompanied by a state of unmunoparalysis that favors bacterial infection and sepsis. The drop in the expression of CD88 in circulating PMNs can be a biomarker that announces this sequence of events, and is anticipated between 48-72 hours before the installation of sepsis and / or the multiple failure of organism. Under these conditions, the monitoring of PMN-CD88 can allow the concomitant use of Eculizumab as a therapeutic strategy that aborts the complement attack and avoids multiple organ failure in the critical patient, infected by COVID-19.

Keywords: Complement C5, COVID-19, Eculizumab, MOF, PMN-CD88.

PROPOSAL FOR COMPASSIONATE USE IN CRITICAL CASES

The most relevant critical clinical pictures in patients with COVID19 are respiratory failure, micro clots in various organs, disseminated intravascular coagulation (DIC), and progressive multiple organ failure (MOF). These characteristics are very similar to what occurs in posttraumatic patients, in whom the progression of a massive and "self-destructive" inflammatory process that leads to MOF is observed [1 - 4].

[*] **Corresponding author Alberto Lazarowski:** Clinical Biochemistry Department, Hospital de Clínicas J San Martín, INFIBIOC-FFyB-UBA, Argentina; E-mail:nadiatom@ffyb.uba.ar

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Organ dysfunction is a major feature in the post-trauma "systemic autodestructive inflammation," as well as it is also part of sepsis pathophysiology. These generalized stresses are characterized by an increased inflammatory response in the bloodstream that causes diffuse damage to the capillary endothelium, resulting in tissue hypoperfusion and organ failure that contributes to its high mortality rate. In this context, sepsis can be developed, leading to a septic shock under the presence of hypotension unresponsive to vasoconstrictors [5].

In severe COVID-19 condition occurs hyper inflammation or "cytokine storm" that might lead to the acute respiratory distress syndrome (ARDS). The pathogen-associated molecular patterns (PAMPs) are the tool for the host identification of the virus, playing a central role in the host-viral interactions [6]. Through host pattern recognition receptors (PRRs) expressed on innate immune cells (*e.g.*, neutrophils, dendritic cells, epithelial cells, and macrophages) together with viral danger-associated molecular patterns (DAMPs) mark the first line of defense against pathogens, involving toll-like receptors (TLRs) [7].

Neutrophils are innate immune cells with a brief lifespan after leaving the bone marrow, and they can exist in different states as quiescent, primed to act, or fully active, and they are among the first line of leukocytes recruited during infections. Irrespective of the classical phagocytosis, PMNs have a singular stratagem to trap and kill microorganisms, developing a sophisticated mechanism called "*NETosis"*, by liberation of nucleic acids and enzymes wrapped with histones that can detain viral particles [8].

NETs and DAMPs have been described to be related to the COVID19 progress and severity (9,10) (Fig. **1**). The presence of neutrophils at the site of infection is essential for controlling the bacterial and fungal burden and avoiding the systemic spread of the infection. Neutrophils induce the killing of pathogens *via* phagocytosis, degranulation, or even the mentioned release of intracellular components such as DNA, histones, and lytic proteins, which form "*Neutrophil Extracellular Traps*" (NETs) [11].

NETs have been implicated in this process, with endothelial damage, and take part in the pathogenesis of organ dysfunction in several conditions. NETs also have an important role in counteracting invading microorganisms during infection. These findings are associated with high levels of circulating free DNA (cfDNA) in plasma. Furthermore, higher cfDNA concentrations were detected in septic patients in comparison with healthy controls, and levels were correlated with sepsis severity and organ dysfunction [12, 13]. In this regard, a wide spectrum of evidence indicates that neutrophils can play either beneficial or deleterious roles in the outcome of sepsis [14]. Furthermore, in addition to their

host-protective role during infection, the excessive formation of NETs has also been observed in many pathological conditions, such as appendicitis, cystic fibrosis, thrombosis, acute lung injury, systemic lupus erythematosus, small vessel vasculitis, diabetes, and sepsis [15], all conditions related with high death risk under COVID19 infection.

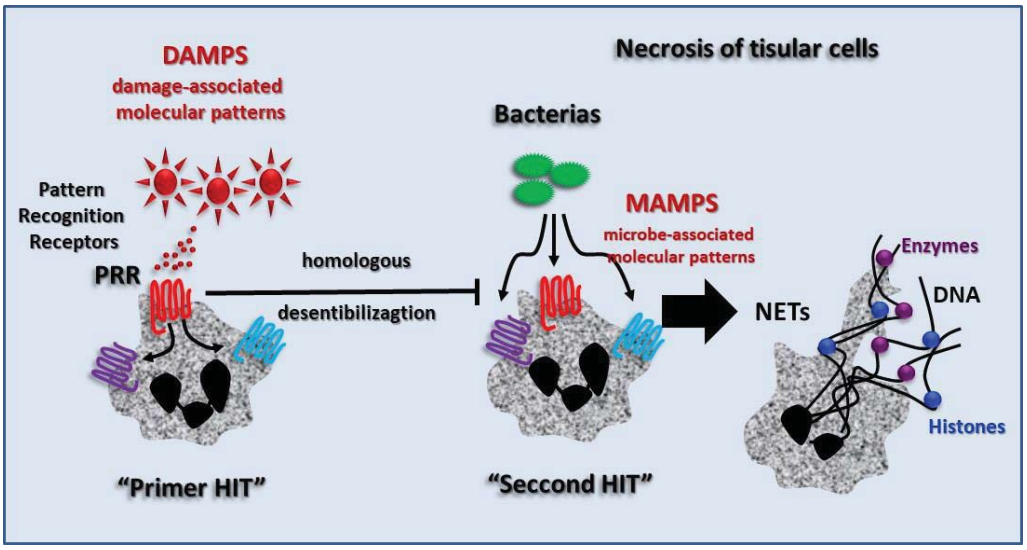

Fig. (1). DAMPS induces an abnormal activation of neutrophils that will lead to NETs formation.

Additionally, it is known now that in severe cases of coronavirus disease-2019 (COVID-19), viral pneumonia progresses to respiratory failure. In this context, NETs can propagate inflammation and microvascular thrombosis — including in the lungs of patients with acute respiratory distress syndrome (ARDS).

These mechanisms also induce the complement activation that potentiates the "*platelet/NET/tissue factor/thrombin axis"* during SARS-CoV-2 infection [16].

It was reported that sera from patients with COVID-19 have elevated circulating levels of cell-free DNA, myeloperoxidase (MPO)-DNAc complexes, and citrullinated histone H3 (Cit-H3). Interestingly, in this report, the authors demonstrated that direct exposure of normal control neutrophils to sera of patients with COVID19 (without any additional agonist) robustly promoted NETosis [17].

This process is a regulated form of neutrophil cell death that contributes to the host's defense against pathogens, and it is currently linked to various diseases. The released NETs can capture and kill bacteria and other pathogens to prevent them from spreading. Although substantial progress has been made in the understanding of NETosis, precise mechanism underlying NETosis is still a

CHAPTER 7

Hematological Markers: Emerging Diagnostic and Therapeutic Targets in Preeclampsia

Zaima Ali[1,2,*] and **Saba Khaliq**[2]

[1] *Lahore Medical & Dental College, Lahore, Pakistan*

[2] *University of Health Sciences, Lahore, Pakistan*

Abstract: Preeclampsia is a morbid hypertensive disease with an onset at >20 weeks of gestation. It has a global incidence of 2-10%, contributing to a large share of fetal and maternal mortality. Pathophysiology of the disease is multifaceted with the involvement of immunological, genetic, and inflammatory factors. Faulty placentation with abnormal angiogenesis and apoptosis is pivotal with subsequent hypoxia and oxidative stress leading to widespread systemic inflammation. For years placenta has been the center of research to elucidate the pathogenesis and treatment strategies of preeclampsia. Recently numerous circulating immunological, genetic, and inflammatory markers have emerged as a focus of interest and intrigued the researchers to mark their contribution to the pathogenesis of the disease. Elevated levels of pro-inflammatory factors like Tumor Necrosis Factor-alpha (TNF-α), soluble Endoglin (sENG), soluble Flt-1 (sFlt-1), Nuclear factor kappa B (NFκB), Interleukin 6 (IL-6), Interleukin 8 (IL-8), and Angiotensin II, *etc.* have been labeled as contributors to the maternal endothelial dysfunction, a hallmark of preeclampsia. MicroRNAs (the emerging field of research) belong to the noncoding RNAs that can modulate the expression of these inflammatory markers. Recent reports on the differential profile of various microRNAs in preeclampsia have focused on using these as diagnostic and therapeutic targets. Modifying the expression levels of different genes involved in inflammation, angiogenesis, and apoptosis can change the clinical picture and prognosis of the disease.

Keywords: Angiogenesis, Apoptosis, Endothelial dysfunction, Inflammation, Inter Leukins, MiRNAs, Oxidative stress, Preeclampsia, sEndoglin, sFlt-1, Syncytiotrophoblasts, TNF-α.

INTRODUCTION

Preeclampsia (PE), a morbid hypertensive disorder of pregnancy affects 2-10% of the pregnancies worldwide. This is the most common of the hypertensive disorders of pregnancy and the number of women who develop PE every year

* **Corresponding author Zaima Ali:** Lahore Medical & Dental College, Lahore, Pakistan; Tel: 03244215272; E-mail: zaima.ali@hotmail.com

exceeds 4 million, with a high mortality number of 50 thousand to 76 thousand.Similarly, it shares a large burden of fetal and infant mortality with five hundred thousand deaths per year [1]. The disease is defined as hypertension and proteinuria, starting from the mid-trimester evolving as a two-stage model. The diagnostic criteria includes new onset of hypertension with proteinuria starting at twenty weeks of pregnancy with blood pressure greater than or equal to 140mmHg / 90mmHg. Proteinuria is associated with protein excretion of more than 300mg in 24 hours [2]. It is caused by placental and maternal vascular dysfunction and is resolved after delivery over a variable period of time. Although approximately 90 percent of cases present in the late preterm (≥34 to <37 weeks), term (≥37 to <42 weeks), or postpartum (≥42 weeks) period, they have good maternal, fetal, and newborn outcomes, the mother and child are still at increased risk for serious morbidity or mortality. The remaining 10 percent of cases have an early presentation (<34 weeks) and carry the additional high risks associated with moderately preterm, very preterm, or extremely preterm birth. The common clinical presentation includes swelling of the feet and hands along with weight gain and puffiness of the face. The severe form of the disease presents headaches, nausea, vomiting, decreased urine output, abdominal pain, and visual problems. The blood tests show thrombocytopenia with a Platelet count of 100,000/mm3 in severe cases. Liver enzymes (aspartate aminotransferase or alanine aminotransferase) as well as Lactic dehydrogenase levels are deranged and may be elevated to 2 times the upper limit of normal. Serum creatinine levels may also increase in severe PE [3]. An altered coagulation profile is observed in severe PE with increased prothrombin time (PT), activated partial thromboplastin time (APTT), and D-dimer (DD) [4]. Because of its pronounced effects on maternal and fetal outcomes, PE is one of the most threatening complications of pregnancy [5]. There is transformation and remodeling of uterine spiral arteries in normal placentation from high resistance, low flow into a high flow, and low resistance vessels. In the process, the interstitial cytotrophoblasts (CTs) release several proapoptotic factors with resultant apoptosis of the vascular smooth muscle cells (VSMC) and endothelial cells (EC) lining these vessels in the myometrium. The endovascular CTs replace the endothelial cells and acquire all the receptors normally present on the endothelial cells lining the lumen of the vessels. Poor placentation with resultant hypoperfusion and oxidative stress in the placental bed in the first stage is central to preeclampsia. Fig. (**1**) summarizes the first stage of PE.

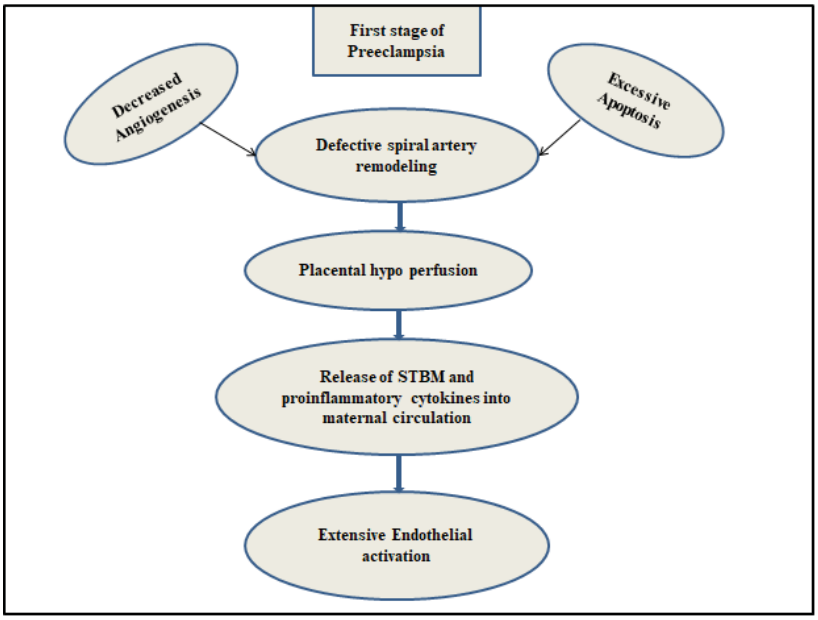

Fig. (1). First Stage of Preeclampsia, poor placental development leads to the release of pro-inflammatory markers into the maternal circulation. STBM (Syncytiotrophoblast microparticles).

The first stage is followed by generalized maternal systemic inflammation and extensive endothelial activation, characteristic of the second stage of the disease. Endothelial activation, in turn, results in vasospasm, capillary leakage, and activation of coagulation. Vasospasm contributes to the development of hypertension, abruption of the placenta, seizures, oliguria, and ischemia of the liver, while activation of coagulation can lead to and present as thrombocytopenia. Capillary leakage presents as edema, proteinuria, and hemoconcentration. Fig. (**2**) summarizes the second stage of PE.

DEVELOPMENT OF PLACENTA IN NORMAL GESTATION

Fertilization of the ovum usually takes place in the ampulla of the fallopian tubes. This is followed by segmentation, and within a couple of days, it develops into a blastocyst. The blastocyst is spherical in structure and made up of trophoblast present on the outside and a group of inner mass cells that develops into the fetus. Trophoblast cells secrete Human Chorionic Gonadotropin (hCG) and take part in implantation by invading deeper into the endometrium. The complex process of fecundation is regulated beautifully by perplexed coaction between endometrium and trophoblast. Invasion of the endometrium by the trophoblast has to be monitored because of the highly invasive capacity of these cells. The process is

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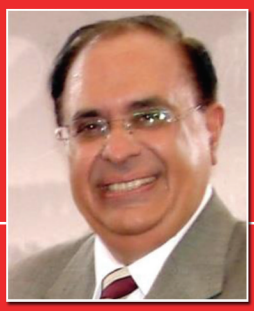

PROF. DR. ATTA-UR-RAHMAN, *FRS*

Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,232 international publications (45 international patents and 341 books). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University (2007), Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009). He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.