Levels of Pentraxin-3 With Iron Status in Sera Children with Beta- Thalassemia Major

Ammar Kareem Madlool[1]*, Hanaa Addai Ali[2] , Rawaa Adday Ali[3], Seyed Omid Ranaei Siadat [4], Mohammed k. kahlol[5], Azhar Mohammed Brbber[6]
[1, 2, 5, 6] Department of Chemistry, Faculty of Science, University of Kufa, Najaf 54001, Iraq.
[3] Microbiology Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51001, Iraq.
[4] Department of Chemistry, Shahid Beheshti University of Medical Sciences, Iran.
E-mail: ammar.kareem@saqid.edu.iq

Abstract
Background: The genetic condition beta-thalassemia results in a poor generation of hemoglobin (Hb), which harms red blood cells. It is characterized by a decreased ability to synthesize one or more globin chains. Long pentraxin-3 is a component of the pentraxin superfamily and a potential marker for many disorders. The purpose of this study was to examine pentraxin-3 as potential biomarker for the inflammatory diseases in patients with beta-thalassemia major at range of age (5-20 years). Material and Method: The prospective case control study comprises 120 patients with β-thalassemia major (TM) and 60 healthy participants. Plasma level of C-reactive protein (CRP)و Pentraxin-3(PTX3), ferritin, transferrin, total iron-binding capacity (TIBC), iron, unsaturated iron-binding capacity (UIBC) and transferrin saturation were estimated. Results: Serum PTX3 level demonstrated significantly increasing in β-thalassemia patients (2.72 ± 0.60) as comparing with healthy controls (1.85 ± 0.40, P<0.001). similarly, CRP, ferritin, TIBC and iron exhibited statistically significant increasing while, UIBC, TS and transferrin appeared markedly decreasing for β-thalassemia patients as comparing with control groups. The comparison study of PTX3 with other biomarkers has been done. PTX3 revealed significant positive correlation with age (r = 0.792), BMI (r = 0.453), CRP (r = 0.230), ferritin (r = 0.580), iron (r = 0.580) and TS (r = 0.728), whereas demonstrated significant negative correlation with TIBC (r = -0.464), UIBC (r = -0.542) and transferrin (r = -0.464). Conclusion: The level of PTX3 increases with the severity of β-thalassemia patients, age and ferritin. It is considered as predictor for many disorders.
Keyword: β-thalassemia major, pentraxin-3, iron overload, ferritin.

1. Introduction

Beta-thalassemia is hereditary hemolytic anemia, meaning that the disorder must be present in at least one parent, characterized by the reduce or absence of globin genes resulting in reduced hemoglobin in red blood cells (RBC) [1]. Depending on genetic defect, thalassemia disorder can be classified into beta-(β) thalassemia and alph-(α-) thalassemia. Several clinical forms of β-thalassemia include major that is known severe form of β-thalassemia, intermediate (severe to moderate β-thalassemia) and minor (carrier) [2]. People with Middle Eastern, Southeast Asian, Mediterranean or Indian ancestry are more likely to have -thalassemia [3]. Every year, Beta-thalassemia affects more than 42,000 newborns worldwide. If patients with beta-thalassemia major are not treated with blood transfusions, they will die before the age of three. [4]. One of the important treatments to reduce complications of severe Beta-thalassemia major is regular blood transfusion, which lead to iron overload [5] [6]. Iron can be circulated in plasma by binding with transferrin. Non-transferrin-bound iron (NTBI), which emerges in the plasma and accumulates in several cell types including hepatocytes, pituitary, cardiomyocytes, and pancreatic cells, damages these organs when the ability of transferrin to bind iron is overcome (transferrin saturation 60–80%) [7] [8].

The pentameric structure of pentraxins, a class of proteins implicated in innate immunity, consists of five subunits [9]. The pentraxin family can be divided into two subfamilies: short pentraxins and long pentraxins, depending on how long the protein sequence is [10] [11]. The short pentraxins involve serum amyloid P and C-reactive protein (CRP), while the long pentraxin includes pentraxin-3 (PTX3) [12].Acute phase protein PTX3 regulates inflammation by controlling cell extravasation, complement activation and pathogen detection by myeloid cells. It also plays a key role against pathogens of bacterial, viral and fungal origin by mediating innate immunity, [13]. Different cell types can release PTX3 involving fibroblasts, macrophages, synovial cells, epithelial cells, monocytes , vascular endothelial cells, adipocytes, chondrocytes, granulosa cells, dendritic cells (DCs), smooth muscle cells, mesangial cells, glial cells and monocytes in response to microorganism, inflammatory cytokines (such as IL- 1β and TNFα), TLR agonists and microbial moieties [outer membrane protein A (OmpA) or LPS] [14]. In contrast to PTX3, Hepatocytes secrete serum amyloid P and CRP, which, in response to pro-inflammatory signals like IL-6, are, respectively, the main acute phase

doi.org/10.31838/hiv23.03.117 Received: 03.11.22, Revised: 10.12.22, Accepted: 20.01.23

proteins in humans and mice[15].

2. Material and method

The prospective case control study included hundred and twenty with β-thalassemia major and sixty healthy volunteers. The samples were collected through period from January 2022 to July 2022. The ages of patients in this study were in range 5-20 years which are matching of healthy controls. The β-thalassemia major disorder was registered in the "Thalassemia Unit" in "Al Zahra Teaching Hospital" Najaf, Iraq. The regional ethical committee of the University of Kufa's Faculty of Science accepted the study. The patients which undergo β-thalassemia major were diagnosed and recognized by clinical symptoms, hematological and hemoglobin electrophoresis analysis. All patients were given detailed information on the study aims and risks and they gave consent before enrolled. Diabetes mellitus, infection and inflammation, heart diseases and autoimmune disease were excluded in the study. A questionnaire was designed to obtain the information of the detailed history about the present thalassemia, history of thalassemia, family history, weight, height, age, gender and other anthropometric parameters are calculated on all enrolments. 5 ml of all patient's samples were drowned from venous by using a disposable needle and plastic syringes before treatment by blood transfusion. After drowning 5 mL of blood from patients, it was left for 10-15 minutes for clotting then centrifuged (at 5000 xg) for 5 minutes in order to separate serum from other component of blood. The Serum was distributed into five Eppendorf Tubes and stored at (-70C°) until time of analysis. PTX3 and ferritin were examined by a sandwich enzyme linked fluorescent assay (ELISA) technique using the manufacture instruction as supplied with kit from MELSIN. enzymatic colorimetric method used to determine iron levels using BIOLABO kit (France).

3. Statistical analysis

In this study, statistical analysis of the data has been done by the SPSS 26.0 (Statistical Package for Social Sciences) package program. Plasma concentrations of biomarkers, PTX3, ferritin, CRP and iron status, have normal distribution. Through findings, the descriptive statistical methods during statistical analysis are mean SD, independent t-test (uses to compare between biomarker and the significance level is considered accepting as P<0.05.) and the Pearson correlation test (uses for the assessment of the relation between variables). Receiver operating characteristic (ROC) also estimated by calculating area under the curve (AUC) and cut-off value for PTX3

4. Results

The present study includes 120 patients with β-thalassemia major and 60 healthy controls. 65 of total patients were girls (54.16%) and remainder 55 of total patients were boys (45.84%), whereas 60 participants of healthy controls were divided into two groups, 31 girls (51.66%) and 29 boys (48.33%). The mean body mass index (BMI) and age for β-thalassemia patients were (18.12 ± 2.26) Kg/m2 and (13.06 ± 4.65) years respectively, while the mean age and BMI for healthy persons were (12.57 ± 4.65) years and (20.52 ± 3.31) Kg/m2 respectively. There is significant statistical difference for BMI between patients and control (P<0.05), and no-significant difference for age (P=0.504).

Clinical parameters involving pentraxin-3, CRP and iron status such as ferritin, iron, (TIBC), (UIBC), transferrin saturation (TS) and transferrin have been investigated for patient and control groups as summarized in table.1. For iron status, ferritin, iron and transferrin saturation in β-thalassemia patients have significant difference higher than healthy controls (P<0.001), whereas TIBC, UIBC and transferrin have significant decreasing for patients as comparing with control groups (P<0.001). The increasing in both pentraxin-3 and CRP have statistically significant difference between patient and control groups (P<0.001).

As shown in table.2, the correlation relationships of pentraxin-3 with measured parameters are done in this study. Significantly positive correlation between pentraxin-3 and age (r = 0.792, P = 0.000), BMI (r = 0.453, P = 0.000), CRP (r = 0.230, P = 0.011), ferritin (r = 0.580, P = 0.000), iron (r = 0.580, P = 0.000) and TS (r = 0.728, P = 0.000) demonstrated as shown in fig.1. In other hand. Significantly negative correlation detected between pentraxin-3 and TIBC (r = -0.464, P = 0.000), UIBC (r = -0.542, P = 0.000) and transferrin (r = -0.464, P = 0.000) as illustrated in fig.2.

Receiver operating characteristic curve (ROC) of pentraxin-3 is also investigated, since area under the ROC curve (ROCAUC) demonstrated 0.886 as shown in fig.3. Pentraxin-3 demonstrated considerably improved performance for the diagnosis and stratification (accuracy and stability) of β-thalassemia major with the cut-off value of 2.31 ng/ml (P<0.001). The sensitivity and specificity for these variables are 0.683 and 0.883, respectively.

Table.1. General characteristic of enrolled patients with control.

Parameters	Mean±SD		P-value
	β-thalassemia patients	Controls	
Age years BMI Kg/m2 CRP mg/dl Ferritin ng/ml Iron µmol/L TIBC µmol/L UIBC µmol/L TS Transferrin µmol/L Pentraxin-3 ng/ml	13.06 ± 4.65 18.12 ± 2.26 3.30 ± 1.63 3541.59 ± 1675.92 34 ± 4.01 55.01 ± 5.85 21.02 ± 7.22 62.50 ± 9.81 39 ± 4.13 2.72 ± 0.60	12.57 ± 4.65 20.52 ± 3.31 1.72 ± 0.60 106.03 ± 27.13 23.99 ± 5.64 68.15 ± 12.26 44.15 ± 12.29 36.19 ± 11.54 48.32 ± 8.69 1.85 ± 0.40	0.504 NS 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000

Table.2. Correlation relationships between pentraxin-3 and clinical biomarkers in patients with β-thalassemia major.		
Correlation Pentraxin-3 with biomarkers	r	P-value
Age years	0.792**	0.00
BMI Kg/m2	0.453**	0.000
CRP mg/dl	0.230*	0.000
Ferritin ng/ml	0.582**	0.000
Iron µmol/L	0.580**	0.000
TIBC µmol/L	-0.464**	0.000
UIBC µmol/L	-0.542**	0.000
TS	0.728**	0.000
Transferrin µmol/L	-0.464**	0.000

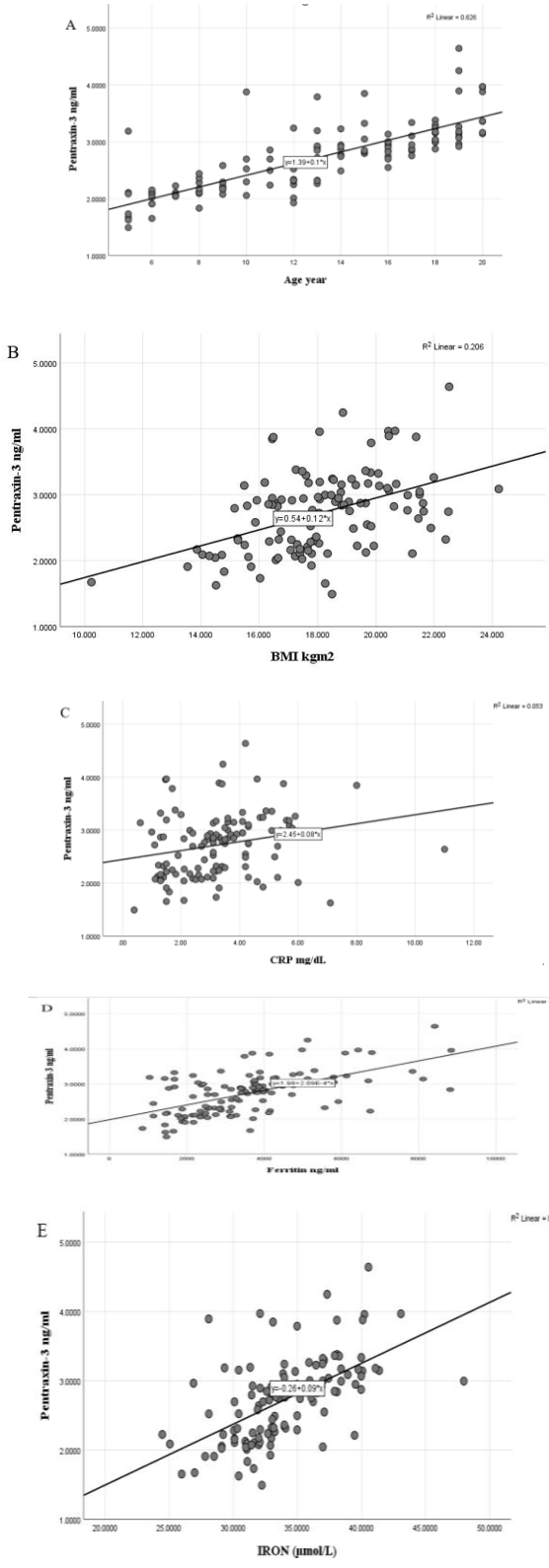

Figure.1. positive correlation of pentraxin-3 with (A) age, (B) BMI, (C) CRP, (D) ferritin, (E) iron and (F) TS in patients' group.

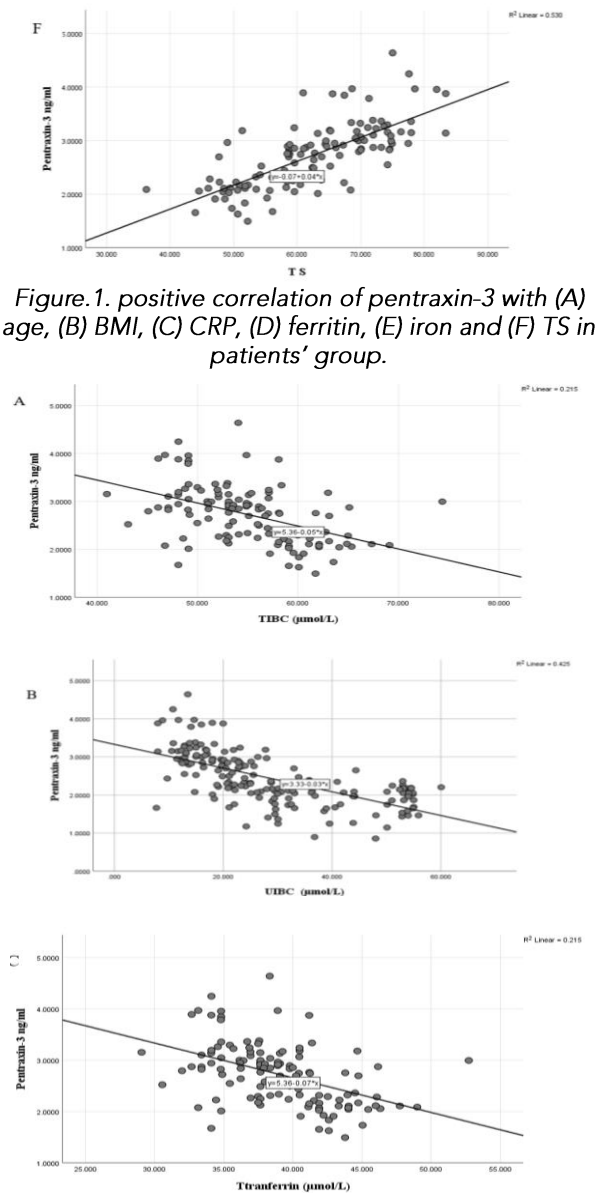

Figure.2. Negative correlation of pentraxin-3 with (A) TIBC, (B) UIBC and (C) trnasferrin in patients' group.

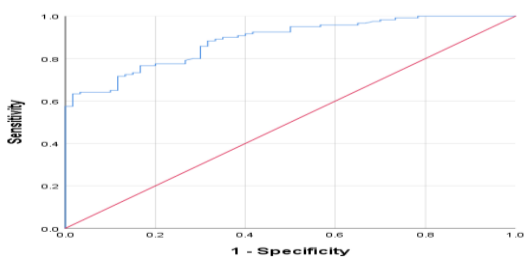

Figure.2. Receiver-operating characteristic (ROC) curves of pentraxin-3 revealing valuable discrimination of patients with thalassemia major.

5. Discussion

A set of inherited blood disorders known as β-thalassemia are defined by an abnormality in the production of the -chains of hemoglobin., which leads to an imbalance between α- and β-globin chains [16]. This study included the investigation of iron status, RCP and pentraxin-3 for β-thalassemia major patient and control groups. In iron status, the value of ferritin, iron and TS demonstrated significant elevation, while significant decreasing of TIBC, UIBC and transferrin (P<0.001) in β-thalassemia patients as comparing with healthy volunteers revealed. These an increase and decrease in findings are in the agreement with Several reports [17] [18] [19]. Serum transferrin and serum ferritin are principally responsible for maintaining the iron overload in -thalassemia patients brought on by the repetition of transfusion-dependent thalassemia and enhancing gastrointestinal absorption [20]. When transferrin becomes fully saturated, the deposition of non-transferrin bound iron (NTBI) will occur in several organs such as liver, heart, multiple endocrinal glands, etc leading to the to damage of these organs [21] [22]. Moreover, several studies have shown that iron overload in patients with β-thalassemia major cause reduced cellular enzymatic antioxidants, oxidative stress and antioxidant molecules, especially GSH which plays the important roles of intracellular antioxidants [23] [24].

Pentrax-3 and CRP are also investigated. The comparison of the serum pentraxin-3 and CRP levels of β-thalassemia patients with those of healthy participants were done. The relationship between pentraxin-3 and CRP, and disease activity was assessed, since the results demonstrated that although there was a statistically significant difference in the pentraxin-3 and CRP levels of β-thalassemia patients as compared to those of the healthy control group (P<0.001). It has been determined in previous studies that the levels of pentraxin-3 increase in inflammatory and ischemic states [25] [26] and decrease with obese persons [27]. Pentraxin-3 is considered to be an early marker of diseases and several clinical studies have demonstrated that height plasma Pentraxin-3 levels are early predictor of many disorders such as acute Myocardial Infarction [28], Unstable angina Pectoris [29], chronic kidney disease [30]. The conducting study by Yasemin Isik et al. showed that pentraxin-3 increases in response to the oxidative stress so that it is considered one of the important the indicator markers of vascular endothelial damage, since also can be used as an early diagnostic marker for inflammation [31]. Huan Chen et. al studied the correlation of panteraxin-3 with sepsis and septic shock. They found that the expression of PTX3 was strongly correlated with the severity and outcome of sepsis and septic shock. Through the Sepsis 3.0 definitions, they stated that PTX3 may be an early biomarker to distinguish sepsis and septic shock from other critically sick patients and that PTX3 may be important in predicting 90-day mortality of sepsis for the direction of management and advanced therapy [32].

6. Conclusion

The purpose of this study was to clarify the function of PTX3 in β-thalassemia major patients as a diagnostic and prognostic marker. It can be used as predictors for many disorders. The increasing of PTX3 is associated with severity of disease, age and ferritin.

7. Acknowledgment

The authors thank the patients for their cooperation and medical staffs in the laboratories of Thalassemia Unit" in "Al Zahra Teaching Hospital" Najaf, Iraq.

Declaration of interests

The authors declare no found conflict of interests.

Founding: None

References

1. Abu-Shaheen, A., et al., Epidemiology of thalassemia in Gulf Cooperation Council countries: a systematic review. BioMed research international, 2020. 2020.
2. Baird, D.C., S.H. Batten, and S.K. Sparks, Alpha- and Beta-thalassemia: Rapid Evidence Review. Am Fam Physician, 2022. 105(3): p. 272-280.
3. Baird, D.C., S.H. Batten, and S.K. Sparks, Alpha-and Beta-thalassemia: Rapid Evidence Review. American Family Physician, 2022. 105(3): p. 272-280.
4. Modell, B. and M. Darlison, Global epidemiology of haemoglobin disorders and derived service indicators. Bulletin of the World Health Organization, 2008. 86(6): p. 480-487.
5. Musallam, K.M., et al., Non-transfusion-dependent thalassemias. Haematologica, 2013. 98(6): p. 833-44.
6. Cappellini, M.D. and I. Motta, New therapeutic targets in transfusion-dependent and -independent thalassemia. Hematology Am Soc Hematol Educ Program, 2017. 2017(1): p. 278-283.
7. Coates, T.D., Physiology and pathophysiology of iron in hemoglobin-associated diseases. Free Radical Biology and Medicine, 2014. 72: p. 23-40.
8. Pinto, V.M. and G.L. Forni, Management of Iron Overload in Beta-Thalassemia Patients: Clinical Practice Update Based on Case Series. Int J Mol Sci, 2020. 21(22).
9. Wang, Z., et al., The basic characteristics of the pentraxin family and their functions in tumor progression. Frontiers in immunology, 2020. 11: p. 1757.
10. Du Clos, T.W., Pentraxins: structure, function, and role in inflammation. ISRN Inflamm, 2013. 2013: p. 379040.

11. Inforzato, A., et al., Structure and function of the long pentraxin PTX3 glycosidic moiety: fine-tuning of the interaction with C1q and complement activation. Biochemistry, 2006. 45(38): p. 11540-11551.
12. Parente, R., et al., Complementary roles of short and long pentraxins in the complement-mediated immune response to Aspergillus fumigatus infections. Frontiers in Immunology, 2021. 12.
13. Porte, R., et al., The Long Pentraxin PTX3 as a Humoral Innate Immunity Functional Player and Biomarker of Infections and Sepsis. Front Immunol, 2019. 10: p. 794.
14. Garlanda, C., et al., Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. Annual review of immunology, 2005. 23(1): p. 337-366.
15. Garlanda, C., et al., PTX3, a humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. Physiological reviews, 2018. 98(2): p. 623-639.
16. Ribeil, J.-A., et al., Ineffective erythropoiesis in β-thalassemia. The Scientific World Journal, 2013. 2013.
17. Yousefian, S., et al., Association of Body mass index and serum ferritin level in pediatrics with Beta-thalassemia major disease. Iranian Journal of Pediatric Hematology & Oncology, 2022. 12(1).
18. Saad, H.K.M., et al., Reduced hepcidin expression enhances iron overload in patients with HbE/β-thalassemia: A comparative cross-sectional study. Experimental and Therapeutic Medicine, 2021. 22(6): p. 1-8.
19. Basu, D., et al., Role of malonaldehyde as a surrogate biomarker for iron overload in the β-thalassemia patient: A systematic meta-analysis. Advances in Redox Research, 2021. 3: p. 100017.
20. Charoenkwan, P., S. Srichairatanakool, and K. Fanhchaksai, Correlation of hepcidin and serum ferritin levels in thalassemia patients at Chiang Mai.
21. Gardenghi, S., et al., Ineffective erythropoiesis in β-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin. Blood, The Journal of the American Society of Hematology, 2007. 109(11): p. 5027-5035.
22. Taher, A.T. and A.N. Saliba, Iron overload in thalassemia: different organs at different rates. Hematology Am Soc Hematol Educ Program, 2017. 2017(1): p. 265-271.
23. Patpan, N., et al., The Effect of Transfusion-Dependent Thalassemia Patient's Serum on Peripheral Blood Mononuclear Cell Viability. Journal of cell death, 2019. 12: p. 1179066018823534.
24. Rasool, M., et al., Effect of iron overload on renal functions and oxidative stress in beta thalassemia patients. Saudi Medical Journal, 2016. 37(11): p. 1239.
25. Jonasdottir, A., et al., Pentraxin-3–a potential biomarker in ANCA-associated vasculitis. Scandinavian Journal of Rheumatology, 2022: p. 1-9.
26. Pasqualini, F., et al., Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction: comparison with C-reactive protein, NT-proBNP and troponin T. Minerva Biotecnologica, 2004. 16(1): p. 71.
27. Santilli, F., et al., Pentraxin 3 and platelet activation in obese patients after gastric banding. Circulation Journal, 2016. 80(2): p. 502-511.
28. Latini, R., et al., Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction. Circulation, 2004. 110(16): p. 2349-2354.
29. Inoue, K., et al., Establishment of a high sensitivity plasma assay for human pentraxin3 as a marker for unstable angina pectoris. Arteriosclerosis, thrombosis, and vascular biology, 2007. 27(1): p. 161-167.
30. Tong, M., et al., Plasma pentraxin 3 in patients with chronic kidney disease: associations with renal function, protein-energy wasting, cardiovascular disease, and mortality. Clinical Journal of the American Society of Nephrology, 2007. 2(5): p. 889-897.
31. Isik Balci, Y., et al., Pentraxin-3 levels in beta thalassemia major and minor patients and its relationship with antioxidant capacity and total oxidant stress. Journal of pediatric hematology/oncology, 2016. 38(1): p. 12-16.
32. Chen, H., et al., Pentraxin-3 Is a Strong Biomarker of Sepsis Severity Identification and Predictor of 90-Day Mortality in Intensive Care Units via Sepsis 3.0 Definitions. Diagnostics, 2021. 11(10): p. 1906.