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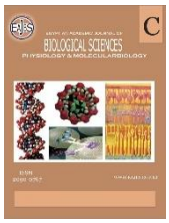

# Assessment of the Annexin A2 as a Predictive Biomarker for the Severity of Disseminated Intravascular Coagulation in Acute Leukemia Patients

**Dhuha S. Aljuboory[1*] and Intisar R. Sharba[2]**
[1]Medical Department, Faculty of Hamorabi medicine, Babylon University, Iraq.
[2]Department of Biology, Faculty of Sciences, University of Kufa, Iraq.
***E-mail:** Dhuhaaljuboory@gmail.com ; intisar.sharba@uokufa.edu.iq

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**ABSTRACT**

Annexin A2, also known as ANXA2, is a protein that controls cellular development and is reliant on calcium to bind to phospholipids. ANXA2 has a role in a variety of pathophysiological processes, including the shift from epithelial to mesenchymal cells, fibrinolysis, and cancer treatment resistance. By stimulating the MYC-HIF1A-VEGF signaling cascade, ANXA2 made it easier for esophageal squamous carcinoma cells to migrate and invade surrounding tissue. Our study showed the DIC score with D-Dimer and ANXA2 had a highly significant (p-value 0.0001) positive correlation with each acute leukemia patient group (ALL and AML), and H3 had a significant (p-value <0.05) positive correlation with AML more than ALL groups, while PDGF-BB revealed significant highly significant (p-value 0.0001) positive correlation with ALL more than AML patients. To predict the severity of DIC in acute leukemia patients we studied the association between study parameters with acute leukemia patients and we found the NNXA2 and D-Dimer, group age under 18 highly significant (p-value <0.05) increased severity of DIC in acute leukemia patients.

## INTRODUCTION

Acute leukemia is a malignant clonal disorder of blood-forming organs involving one or more cell lines in the hematopoietic system. These disorders are marked by the diffuse replacement of bone marrow with abnormal immature and undifferentiated hematopoietic cells, resulting in reduced numbers of erythrocytes and platelets in the peripheral blood. Based on the origin of the abnormal hematopoietic cells involved, such as lymphoid, myeloid, mixed or undifferentiated (Bray *et al*., 2018). Acute lymphoblastic leukemia (ALL) is seen in patients with the blastic transformation of B and T cells. It is the most common leukemia in the pediatric population, accounting for up to 80% of cases in this group vs. 20% of cases in adults. Treatment among adolescents and young adults is predominantly inspired by pediatric regimens with better survival rates (Arber *et al*., 2016). Acute myelogenous leukemia is characterized by greater than 20% myeloid blasts and is the most common acute leukemia in adults. It is the most aggressive cancer with a variable prognosis depending upon the molecular subtypes (Vardiman, 2010).Disseminated intravascular coagulation (DIC) is one of leukemia complications characterized by systemic intravascular activation of the coagulation system from various causes that can result in multiorgan failure, thrombosis, and/or excessive bleeding.

The diagnosis of DIC is challenging due to the complex underlying medical conditions of leukemia that can lead to variable presentations. (Gando *et al.,* 2006). DIC is characterized by systemic activation of blood coagulation, which results in the generation and deposition of fibrin, leading to microvascular thrombi in various organs and contributing to multiple organ dysfunction syndrome (MODS). Consumption of clotting factors and platelets in DIC can result in life-threatening hemorrhage (Papageorgiou *et al.,* 2018). Annexin A2 (also called p36, annexin II, ANXA2, calpactin I, lipocortin II, chromobindin VIII, or placental anticoagulant protein IV), (Huang *et al*., 2022). It serves as a co-receptor for plasminogen and tissue plasminogen activator (t-PA) (Flood and Hajjar, 2011), Annexin A2 calcium-dependent, phospholipid-binding protein found on the surface of many cell types including endothelial cells, macrophages, neuronal cells, and some tumor cells, (Bharadwaj *et al.,* 2013 and Huang *et al.,* 2022). It is a cell surface receptor for both PLG and t-PA, with elevated levels thought to play a role in hyperfibrinolytic events. By independently anchoring both molecules in close proximity to each other on the cell surface, annexin A2 provides an environment in which plasmin production is greatly increased. When bound to annexin A2, the fibrinolytic enzymes are safe from the destructive actions of their inhibitors. Their clearance time from the system is lengthened and, therefore, their half-lives are increased (Mican *et al.,* 2019). Annexin A2 provides an environment in which plasmin production in malignancy leads to an increased risk of thrombohaemorrhagic complications (Falanga and Barbui, 2001). In leukaemia, the risk can vary from mild to life-threatening. It has long been recognized that patients suffering from AML can often experience a secondary coagulopathy or haemostatic imbalance, which can cause major morbidity and mortality. In particular, 80–90% of those suffering from acute promyelocytic leukaemia (APL), (sub type of AML) demonstrate a severe bleeding disorder (Avvisati *et al.,* 2001) associated with disseminated intravascular coagulation (DIC) or hyperfibrinolysis (Chen *et al.,* 1995). Indeed, it is unclear which arm of the haemostatic balance is affected in APL as both the coagulation and fibrinolytic cascades can potentially trigger the bleeding complications associated with the disease (Falanga and Barbui, 2001). Overall, Annexin A2 is overexpressed in ALL (Spijkers-Hagelstein *et al.,* 2013).

## MATERIALS AND METHODS

The study population consists of 80 acute leukemia patient samples (53 male and 27 female), 32 samples of acute myelocytic leukemia AML and 48 samples of Acute lymphocytic leukemia. The two groups divided their ages into three categories under 18 years children, 18-39 years adults and youth and above 40 years older adults (Telama et al., 2005). The patients without any liver disease or inherited coagulopathies were also the patients included in the study before taking the therapeutic dose or after ending the effect of the therapeutic dose. They attended the National Hospital for Oncology and Hematology Disease in Al-najaf Al-ashraf from April 2022 to November 2022. Informed consent was obtained from all patients.

**Blood Sample:**

Five milliliters of venous blood were obtained from each subject, three milliliters were put into EDTA tubes and the remaining two milliliters were pushed slowly into sodium citrate tube. Blood in the EDTA tubes was divided into 1 ml and placed in a tube or an Eppendorf tube for the purpose of using it immediately in the complete blood count method, and the remaining 2 ml in the EDTA tube was separated by centrifuge at 3000 rpm for 5 minutes then were abstained plasma and stored at -20 ºc for later use in the human (ANXA2 and D-Dimer) ELISA kit procedure. Blood in a sodium citrate tube was centrifuged at 3000 rpm for 5 minutes to abstained plasma and stored at -20 ºc for later use in the human PT and APTT kit methods.

**Inclusion Criteria:**

Diagnostic patients with AML and ALL should be not taking therapeutic or after ending the effects of the therapeutic dose.

**Medical History:**

A thorough medical history is critical to help determine the type of acute leukemia, the Date of leukemia and take or not leukemia treatment, if they take therapeutic dosage ask them for the date of the therapeutic dose.

**Estimation DIC Score:**

The ISTH group produced a simple scoring system for the diagnosis of DIC depending on the Platelet count, the PT\ INR, the fibrinogen level and critically the D-Dimer results, a total score of ≥5 = DIC as long as the score is associated with a clinical disorder known to cause DIC. If the score is ≥5 you must ring the ward/medic and make them aware of the risk of DIC (Thacil *et al*., 2010).

**Counts Platelet Number and White Blood Cell Number:**

The platelet count is performed by an automated hematology analyzer (Sysmex XP-300) by counting whole blood or completed blood count procedures (CBC).

**-Determine prothrombin time (PT) and activated partial thrombin time (APTT) \INR:**

Used kits and manual procedure. Calculation of INR (result) = (Patient's time/ MNPT) $^{ISI}$, (Goguel,1985).

**Statistical Analysis:**

All data were statistically analyzed with software programs SPSS v.28 and Microsoft software Excel 2021 for graphics. Using Independent T-tests to compare continuous variables between groups. Whenever the multiple comparisons between groups were performed by one-way ANOVA with Tukey`s post hoc. The significance of differences was detected at $p<0.05$ (Sullivan, 2017).

## RESULTS AND DISCUSSION

In Figure (1), ANXA2 level was highly significant difference $p<0.05$ elevated in plasma ANXA2 level, in acute leukemia patients with DIC mean±SE (9.29±0.4), as compared with the non-DIC group (5.04±0.27), (p=0.0001). D-Dimer levels showed a highly significant difference $p<0.05$ elevated in plasma ANXA2 level, in acute leukemia patients with DIC mean±SE (0.96±0.04), as compared with the non-DIC group (5.04±0.27), (p=0.0001), Show figure (2). The examined risk factor for DIC. Table (1) shows the results of the multiple logistic regression analyses performed using DIC score as a reference category variable. This multiple logistic regression analysis revealed that age was a highly significant risk factor in severity DIC (B, 95% CI: 0.063 (0.045-0.082), p=0.0001), more than D-Dimer (B, 95% CI: 3.028 (1.874-4.182), p=0.0001), and ANXA2 (B, 95% CI: 0.252 (0.150-0.355), p=0.0001). According to these results, age was available as an independent predictor as a risk factor of severity in acute leukemia more than D-Dimer and ANXA2 While, WBC count is not a significant risk factor ($P>0.05$) in severity DIC in acute leukemia patients. Clinical characterize parameters were analyzed in the age groups illustrated in Table (2). The results showed a significant (p= 0.0001) increase proportion of patients in an older age group patients >41 years was mean of about (53.89±2.35) compared with younger acute leukemia patients (≤18 and 19-40) years of age groups (11.6±0.73 and 27.55±1.01), respectively. The results of WBC count in acute leukemia patients showed a significant decrease ($p<0.05$) in older age groups >41 years about (23.43±1.1) when compared with (≤18 and 19-40) years of age groups (9.68±1.12 and 26.86±0.82), p=0.001, respectively. In similar outcomes of PLT and fibrinogen, approximately, the average of PLT counts and fibrinogen level in acute leukemia patients observed significantly decrease ($p<0.05$) in older age group, in older age groups >41 years about (33.47±3.4 and 0.84±0.09), when compared with (≤18 and 19-40) years of age groups (49.05±3.38 and 46.87±3.2, 1.27±0.06 and 1.17±0.07), p=0.008 and p=0.001, respectively. PT and INR of acute leukemia patients indicated to significant increase ($p<0.05$) in older age groups, >41 years about (16.48±0.32 and 1.88±0.06), when compared

with (≤18 and 19-40) years of age groups (PT 15.12±0.3and 15.21±0.25, INR 1.67±0.05 and 1.66±0.05 b), p=0.005 and p=0.017, respectively. DIC score, ANXA2 and D-DIMER levels of acute leukemia patients showed a significant increase (p<0.05) in older age groups, >41 years about (6.53±0.35, 9.46±0.66 and 0.92±0.08) when compared with (≤18 and 19-40) years of age groups (DIC score 3.87±0.26 and 4.39±0.3, ANXA2 5.41±0.41 and 6.22±0.42 and-Dimer 0.55±0.03 and 0.67±0.05 ball of them p=0.0001, respectively. No significant differences p>0.05 in the mean of APPT parameter at all age groups of acute leukemia patients p=0.087, p=0.128 and p=0.309, respectively. The examined risk factor for DIC status. Table (3) shows the results of the multiple logistic regression analyses performed using non-DIC score as a reference category variable. This multiple logistic regression analysis revealed that age >41 was a highly significant risk factor in the severity of DIC (OR, 95% CI: 16.000 (2.122-120.648), p=0.007), more than AML group (OR, 95% CI: 2.556 (1.183-5.523), p=0.017), ALL group (OR, 95% CI: 0.200 0.252 (0.094-0.427), p=0.0001) and ages ≤18(OR, 95% CI: 0.148 (0.052-0.423), p=0.0001. According to these results, age>41 was available as an independent predictor as a risk factor of severity acute leukemia more than AML, ALL groups and ages ≤18. While the gender males, females and ages 19-40 were not significant risk factors (P>0.05) in severity DIC in acute leukemia patients. The examined risk factor for DIC status. Table (4) shows the results of the multiple logistic regression analyses performed using non-DIC score as a reference category variable. This multiple logistic regression analysis revealed that ANXA2 level was a highly significant risk factor in severity of DIC (OR, 95% CI: 192.504 (1.793-20670.5), p=0.027), more than D-Dimer level (OR, 95% CI: 1.851 (1.140-3.005), p=0.013) in acute leukemia patients groups.

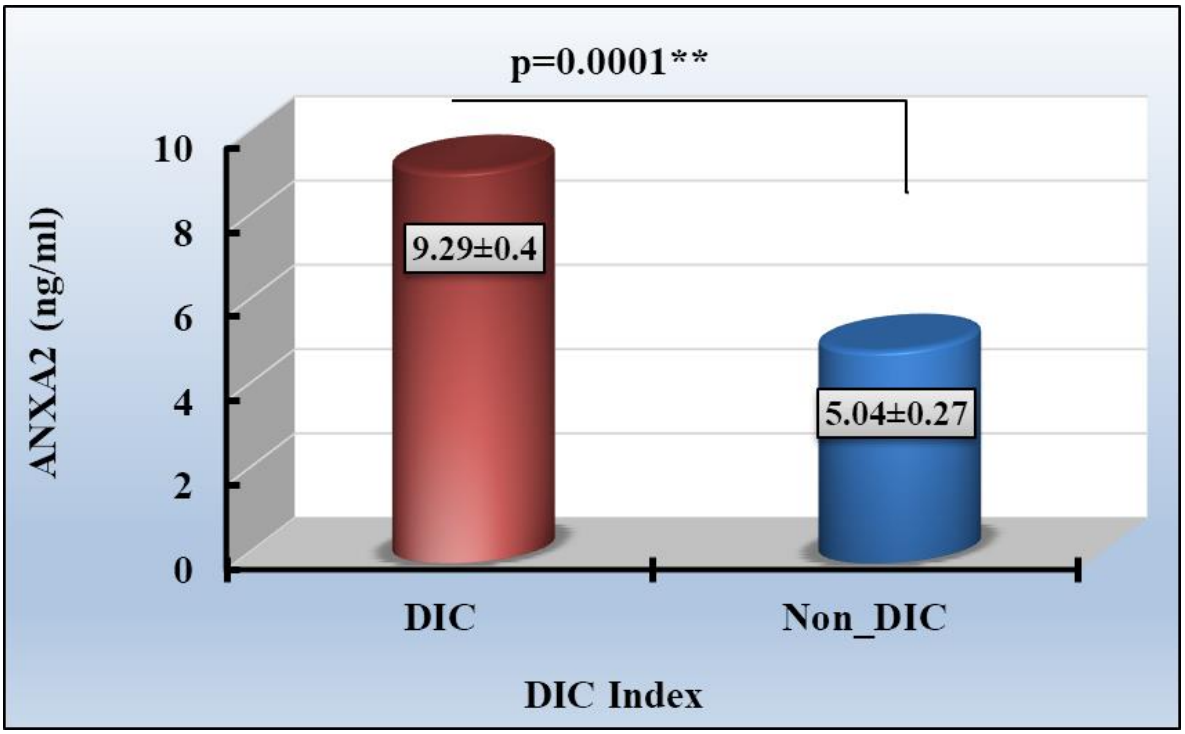


**Fig.1:** Comparison of serum ANXA2 (ng/mL) levels according to DIC index in acute leukemias (ALL and AML) patients.

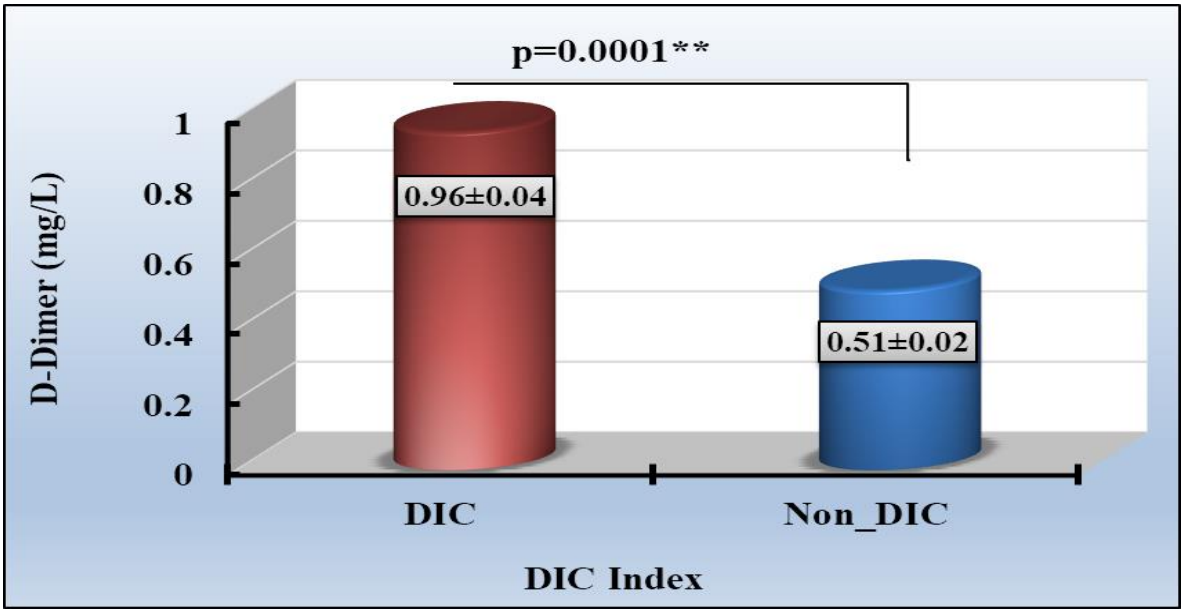


**Fig. 2:** Comparison of serum D-Dimer (mg/L) levels according to DIC index in acute leukemias (ALL and AML) patients. *Significant differences at p-value * <0.05, and ** <0.01. data expressed as Mean ±SE. independent T-test. Ns: non- significant. Different letters were significant between age groups.*

**Table 1:** Linear regression for predicting the independent variables associated with dependent variables (DIC score) in ALL and AML patients.

| Independent variables | B | Std. Error | T | p-value | 95% CI |
|---|---|---|---|---|---|
| **Age (year)** | 0.063 | 0.009 | 6.859 | 0.0001* | 0.045-0.082 |
| **WBC (× 10^9/L)** | 0.050 | 0.035 | 1.424 | 0.159 ns | -0.020-0.121 |
| **ANXA2 (ng/mL)** | 0.252 | 0.051 | 4.930 | 0.0001* | 0.150-0.355 |
| **D-Dimer (mg/L)** | 3.028 | 0.579 | 5.227 | 0.0001* | 1.874-4.182 |

The dependent variable is the DIC score. Significant differences at p-value * <0.05. ns: non-significant B: effect size. 95%CI: confidence interval.

**Table 2:** Clinical characterize parameters associated with age of acute leukemia patients.

| | ≤ 18 year | 19-40 year | > 41 year | p-value |
|---|---|---|---|---|
| **Age (year)** | 11.6±0.73 c | 27.55±1.01 b | 53.89±2.35 a | 0.0001** |
| **WBC (× 10^9/L)** | 29.68±1.12 a | 26.86±0.82 a | 23.43±1.1 b | 0.001* |
| **PLT (10^9/L)** | 49.05±3.38 a | 46.87±3.2 a | 33.47±3.4 b | 0.008* |
| **PT (second)** | 15.12±0.3 b | 15.21±0.25 b | 16.48±0.32 a | 0.005* |
| **APTT (second)** | 36.18±0.56 a | 37.27±0.47 a | 37.94±0.58 a | 0.087 ns |
| **Fibrinogen (g/L)** | 1.27±0.06 a | 1.17±0.07 a | 0.84±0.09 b | 0.001* |
| **INR** | 1.67±0.05 b | 1.66±0.05 b | 1.88±0.06 a | 0.017* |
| **DIC SCORE** | 3.87±0.26 b | 4.39±0.3 b | 6.53±0.35 a | 0.0001** |
| **ANXA2 (ng/ml)** | 5.41±0.41 b | 6.22±0.42 b | 9.46±0.66 a | 0.0001** |
| **D-Dimer (mg/L)** | 0.55±0.03 b | 0.67±0.05 b | 0.92±0.08 a | 0.0001** |

Significant differences at p-value * <0.05, and ** <0.01. data expressed as Mean ±SE. ANOVA and Tukey's test. Ns: non- significant. Different letters were significant between age groups.

**Table 3:** Multinominal Regression for predicting the risk factors associated with (DIC index) in ALL and ALM patients.

| Predicted variable for DIC [a] | | B | Std. Error | p-value | OR | 95% CI |
|---|---|---|---|---|---|---|
| **Groups** | **ALL** | -1.609 | 0.387 | 0.0001* | 0.200 | 0.094-0.427 |
| | **AML** | 0.938 | 0.393 | 0.017* | 2.556 | 1.183-5.523 |
| **Gender** | **Male** | -0.501 | 0.283 | 0.077 ns | 0.606 | 0.348-1.056 |
| | **Female** | -0.375 | 0.392 | 0.339 ns | 0.688 | 0.319-1.481 |
| **Age** | **≤ 18 year** | -1.910 | 0.536 | 0.0001* | 0.148 | 0.052-0.423 |
| | **19-40 year** | -0.647 | 0.372 | 0.082 ns | 0.524 | 0.253-1.086 |
| | **> 41 year** | 2.773 | 1.031 | 0.007 * | 16.000 | 2.122-120.648 |

Significant differences at p-value * <0.05. ns: non-significant B: effect size. OR: odds ratio. 95%CI: confidence interval. a. The reference category is Non_DIC.

**Table 4:** Multinominal Regression for predicting the biomarkers as risk factors that are associated with (DIC index) in ALL and ALM patients.

| Predicted variable for DIC [a] | B | Std. Error | p-value | OR | 95% CI |
|---|---|---|---|---|---|
| **D-Dimer (mg/L)** | 0.616 | 0.247 | 0.013* | 1.851 | 1.140-3.005 |
| **ANXA2 (ng/mL)** | 5.260 | 2.386 | 0.027* | 192.504 | 1.793-20670.5 |

Significant differences at p-value * <0.05. ns: non-significant B: effect size. OR: odds ratio. 95%CI: confidence interval. a. The reference category is Non_DIC.

The results in Figures (1 & 2) showed highly significant differences $p<0.05$ elevated in plasma ANXA2 level and D-Dimer level, in acute leukemia patients with DIC mean±SE ANXA2 (9.29±0.4), D-Dimer(0.96±0.04), as compared with the non-DIC group ANXA2 (5.04±0.27), D-Dimer (5.04±0.27), respectively. Due to the Increased expression of Annexin A2 is frequently observed in a broad spectrum of cancer cells, hyperfibrinolysis is caused by the overproduction of the fibrinolytic enzyme plasmin. Plasminogen (PLG), the precursor of plasmin, circulates in the blood in an inactive form but when converted to plasmin by its activators, tissue plasminogen activator (t-PA) or urokinase plasminogen activator (uPA), acquires the potential to digest fibrinogen and dissolve fibrin clots. This has been hypothesised to be the cause of abnormal bleeding in APL. Low levels of PLG and the fibrinolytic inhibitors, α2-plasmin inhibitor and plasminogen activator inhibitor (PAI), together with the detection of elevated amounts of D-dimers and fibrin degradation products (FDP), (Falanga and Barbui,2001 and Hugo and Avi, 2021). Therefore these biomarkers concede predictive parameters for the severity of DIC shown in Table (1) in agreement with the study (Wada *et al.*, 2022). While gender and age with a range of 19-40 years are shown in Tables (2 & 3) respectively, no significant difference in predictive severity of DIC in acute leukemia because DIC incidence decrease in middle age patients and increases in younger and older patients due to limited reserves of coagulation factors including pro-coagulative and anti-coagulative factors (Salonvaara *et al.*, 2003) this founding agree with (Geyer-Roberts *et al.*, 2022). Gender does not affect on severity of DIC because of the number of male and female closed-in samples, also the DIC is predictive with AML group more than ALL due to AML leukemic cells sharing several common procoagulant mechanisms with other tumor cells, including the potential to express tissue factor upon inflammatory stimulation and to liberate extracellular vesicles that further drive coagulation (Levi, 2018). This finding is compatible with previous studies (Nur *et al.*, 1995 and Dixit *et al.*, 2007). The result in Table (2) showed significant decrees in WBC, PLT and fibrinogen with older age patients > 41 years due to this age representing the most

AML patients and these patients suffer from severe thrombohemmorhage because of several coagulopathies. Table (4) shows a highly significant risk factor in severity of DIC (OR, 95% CI: 192.504 (1.793-20670.5), p=0.027), more than D-Dimer level. These results agree with (Geyer-Roberts *et al.*, 2022). However, the same table shows a significant increase in INR, DIC scores with older age due to the same causes.

**Conclusion:**

The study found that ANXA2 has an important role in predicting the severity of DIC in acute leukemia. And the DIC has more occurrences with AML than ALL.

**Ethical Approval:**

This study was performed in accordance with the ethical committee of Al-Kufa University, Iraq.

**REFERENCES**


Arber, D. A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M. J., Le Beau, M. M., ... & Vardiman, J. W. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood, *The Journal of the American Society of Hematology*, 127(20), 2391-2405.

Avvisati G, Lo Coco F, Mandelli F(2001). Acute promyelocytic leukemia: clinical and morphologic features and prognostic factors. *Seminars in Hematology*, 38:4 -12.

Bharadwaj, A., Bydoun, M., Holloway, R., & Waisman, D. (2013). Annexin A2 heterotetramer: structure and function .*International journal of molecular sciences*, 14(3), 6259-6305.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer. *journal for clinicians*, 68(6), 394-424.

Dixit, A., Chatterjee, T., Mishra, P., Kannan, M., Choudhry, D.R., Mahapatra, M., Choudhry, V.P. and Saxena, R. (2007) Disseminated Intravascular Coagulation in Acute Leukemia at Presentation and during Induction Therapy. *Clinical and Applied Thrombosis/Hemostasis*, 13, 292-298.

Falanga A, Barbui T. Coagulopathy of acute promyelocytic leukemia. *Haematologica*, 2001;106:43 - 51.

Falanga, A., & Barbui, T. (2001). Coagulopathy of acute promyelocytic leukemia. *Acta haematologica*, 106(1-2), 43-51.

Flood, E. C., & Hajjar, K. A. (2011). The annexin A2 system and vascular homeostasis.*Vascular pharmacology*, 54(3-6), 59-67.

Gando, S., Iba, T., Eguchi, Y., Ohtomo, Y., Okamoto, K., Koseki, K., ... & Japanese(2006): Association for Acute Medicine Disseminated Intravascular Coagulation (JAAM DIC) Study Group. A multicenter, prospective validation of disseminated intravascular coagulation diagnostic criteria for critically ill patients: comparing current criteria. *Critical care medicine*, 34(3), 625-631.

Geyer-Roberts, E., Akhand, T., Blanco, A., Jose, R., Chowdhury, N., Ea, M. & Henderson, C. C. (2022). Disseminated Intravascular Coagulation in Varying Age Groups Based on Clinical Conditions. *The Cureus Journal of Medical Science*, 14(4).

Huang, Y., Jia, M., Yang, X., Han, H., Hou, G., Bi, L., ... & Ouyang, X. (2022). Annexin A2: The diversity of pathological effects in tumorigenesis and immune response. *International Journal of Cancer*, 151(4), 497-509.

Hugo ten Cate , Avi Leader (2010): Management of Disseminated Intravascular Coagulation in Acute Leukemias .*Hämostaseologie*, 41: 120–126.

Levi, M., & Sivapalaratnam, S. (2018). Disseminated intravascular coagulation: an update on

pathogenesis and diagnosis. *Expert review of hematology*, 11(8), 663-672.

Nur S, Anwar M, Saleem M, Ahmad PA (1995). Disseminated intravascular coagulation in acute leukemias at first diagnosis. *European Journal of Haematology*, 55:78-82.

Papageorgiou C, Jourdi G, Adjambri E, Walborn A, Patel P, Fareed J, Elalamy I, Hoppensteadt D, Grigoris T Gerotziafas (2018): Disseminated Intravascular Coagulation: An Update on Pathogenesis, Diagnosis, and Therapeutic Strategies. Clinical and Applied Thrombosis/hemostasis OCT 8 .1067029618806424

Salonvaara M, Riikonen P, Kekomäki R, et al. Arch Dis Child Fetal Neonatal (2003): Effects of gestational age and prenatal and perinatal events on the coagulation status in premature infants. 88:0–23. Thacil J et al (2010): Appropriate use of D-dimer in hospital patients. *The American Journal of Medicine*, 2010, 123, 17-9.

Spijkers-Hagelstein, J. A., Mimoso Pinhancos, S., Schneider, P., Pieters, R., & Stam, R. W. (2013). Src kinase-induced phosphorylation of annexin A2 mediates glucocorticoid resistance in MLL-rearranged infant acute lymphoblastic leukemia. *Leukemia*, 27(5), 1063-1071.

Telama, R., Yang, X., Viikari, J., Välimäki, I., Wanne, O., & Raitakari, O. (2005). Physical activity from childhood to adulthood: a 21-year tracking study. *American journal of preventive medicine*, 28(3), 267-273.

Vardiman, J. W. (2010). The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues: an overview with emphasis on the myeloid neoplasms. *Chemico-biological interactions*, 184 (1-2), 16-20.

Wada, H., Yamamoto, A., Tomida, M., Ichikawa, Y., Ezaki, M., Masuda, J., ... & Shimpo, H. (2022). Proposal of quick diagnostic criteria for disseminated intravascular coagulation. *Journal of Clinical Medicine*, 11(4), 1028.