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Review Article

EFFECTS OF ANTICOAGULANTS ON BLOOD CELLS MORPHOLOGY AND BIOCHEMISTRY

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Abstract: *Blood is a particular type of connective tissue that majorly contains red blood cells (RBCs), white blood cells (WBCs), and platelets (Plts). Anticoagulants are used to preserve the fluidity of blood and retard morphological changes in blood cells. Anticoagulants like EDTA, heparin, sodium citrate, and CPDA-1 are used. However, these anticoagulants also have some disadvantages, summarized in this review. This chemical causes the lysis of many cells, including RBCs, and some of them, like EDTA, cause agglutination of platelets, which not only causes a decrease in platelet count but also causes a spurious increase in WBC count. These substances also alter the shapes of various cells present in blood. RBCs and platelets may become spherical from the normal biconcave disc and plate-like structures. Commonly used anticoagulants contribute to alterations of many hematological parameters, including hematocrit, hemoglobin concentration, and packed cell volume of RBCs. Certain chemicals used in anticoagulant solutions, such as potassium and sodium in EDTA solution, make the blood unsuitable for determining these electrolytes in the sample. They also induce many changes in the biochemical composition of the sample. The most affected biochemical changes are observed in 2,3-DPG, D-dimer concentration, blood gas estimation, and cytokine levels. As they contain different chemicals, they have varying pH, so they alter blood pH.*

Keywords: Anticoagulants, Blood Cells, Hematological Parameters, Biochemical Composition, Agglutination, pH Alterations

Introduction

Blood is the essential fluid for life, which is opaque and reddish in color, connective tissue, and has a pale yellow-colored fluid matrix known as plasma. It contains blood cells suspended in plasma, i.e., erythrocytes or red blood cells (RBCs), leukocytes or white blood cells (WBCs), and platelets or thrombocytes (Plts), along with other biochemical substances (Chohan and Davidow, 2015; Karolczak et al., 2019). These cells and cellular fragments are collectively called formed elements of plasma. The relative volume of formed elements and plasma is 45% and 55%, respectively. Normovolemia is 4-6 liters, i.e., 6-8% of body weight in normal adults (Namisnak et al., 2022). In clinical practices, blood is commonly used for diagnostic purposes because it is easy to collect and handle in the laboratory. Blood also serves as a medium to transport different chemicals throughout the body, such as glucose, hormones, vitamins, plasma proteins, and waste products. Most importantly, it transports oxygen (O2) and carbon dioxide (CO2) from the lungs to the body and back to the lungs. Besides this, blood performs another vital function for thermoregulation as it transfers heat equally to all parts of the body (Namisnak et al., 2022).

With the development of laboratory automation, centralized high-volume laboratories are also developed simultaneously. This requires transporting blood samples from remote areas to the laboratory as soon as possible. Still, transmission over long distances poses a significant pre-analytical alteration in blood cell morphology. To encounter these changes, laboratorians use suitable anticoagulants (COMITTEE) (Arnold et al., 2019). Anticoagulants are also used to store blood in blood banks.

Anticoagulants are substances that retard the clotting of whole blood; thus, it preserves the fluidity of blood both in vitro and in vivo (Dibiasi et al., 2018; Graverini et al., 2018). Not only preserves fluidity but also maintains cell viability and functionality (Levental et al., 2020). The most commonly used and recommended in vitro anticoagulant is ethylenediamine tetraacetic acid (EDTA). Calcium is a necessary cofactor for enzymes of the coagulation cascade. EDTA chelates this calcium, thus preventing the clotting of blood in tubes (Farias et al., 2016; Lima-Oliveira et al., 2021). Some anticoagulants are used for long-term preservation of blood. Besides this, anticoagulants also have pharmacological effects, which are used to prevent venous thromboembolism (Papakonstantinou et al., 2020). Different anticoagulants have different effects on blood cells and have different storage times (Hennø et al., 2017). Storing blood for an extended period requires optimum conditions such as 1-6°C temperature, which is provided in blood banks (García-Roa et al., 2017) (Mansell and Boller, 2016). Several additive solutions are added to blood along with anticoagulants to preserve blood's structural and biochemical bases, thus preserving the blood cells in a natural, normal state (Barshtein et al., 2021). However, these anticoagulants also have some disadvantages, which cause blood transfusion to be unsafe and even life-threatening. Anticoagulants like EDTA also have disadvantages in the diagnostic field because they chelate

metal ions and are used as sodium (Na+) and potassium (K+) salts; therefore, metal ions (calcium, iron, magnesium) and Na+ and K+ cannot be measured in EDTA-induced plasma (Putra and Hernaningsih, 2022). They may cause changes in blood cell morphology and biochemical constituents of blood. These pathological conditions related to prolonged blood storage are sometimes known as "Storage Lesions" (Weisel and Litvinov, 2019; Yoshida et al., 2019). EDTA is proven more efficient than oxalate and heparin, found satisfactory, and recommended for routine use (Akorsu et al., 2023; Carlquist et al., 2017). The recommended concentration of EDTA in routine use is 1mg of EDTA per 1 ml of blood 21. EDTA blood is also very efficient for differential staining of WBCs (Bills et al., 2019).

Heparin is another commonly used anticoagulant. It is a direct inhibitor of the coagulation cascade by antithrombin-mediated inhibition of the clotting factor and works in vivo and in vitro. Due to these properties, heparin is used to treat thromboembolism and in blood transfusion practices. It is a biological anticoagulant that retard the action of specific proteins, which are essential for clotting cascade. It is ideal anticoagulant for osmotic fragility test. However, when sodium heparin is used, it induces many changes in blood count and alters the rheology of blood. Heparinized blood is unsuitable for slide preparation as it causes bluish discoloration in the background (Nussbaum et al., 2022) (Bain et al., 2016).

Besides these, citric acid used with sodium citrate makes acid citrate dextrose, one of the earlier known anticoagulants. Similarly, sodium phosphate dextrose is used as an anticoagulant. Citrate phosphate dextrose (CPD) can be used with the addition of adenine citrate phosphate dextrose adenine (CPDA-1) is also one of the most commonly used anticoagulants for whole blood storage for long periods (Meledeo et al., 2019).

Anticoagulants are extensively used in blood banks. In the early days, the blood is only stored for 21 days using acid citrate dextrose (ACD). With the development of our knowledge about anticoagulants, new anticoagulants are discovered, such as CPD and CPDA-1. These new anticoagulants add more shelf life for storage of refrigerated blood up to 42 days; thus, these new anticoagulants have reduced the artifact induced by previously known anticoagulants (Sakthipriya, 2019).

HEMATOLOGICAL CHANGES

Anticoagulants Induced Artifacts in Blood Cells Count:

When blood is stored with anticoagulants in test tubes for laboratory procedures or even stored in blood banks for transfusions, these anticoagulants induce specific changes in various hematological and biochemical parameters of blood, such as changes in blood cell count, induce hemolysis, cause morphological changes and even sometimes affects standard functionality and viability of blood cells (Bogdanova et al., 2020) (Huisjes et al., 2018).

Changes in Reticulocyte Count:

Reticulocytes are immature, anucleated erythroid cells in peripheral blood with small extranuclear RNA remaining (Chakrahari, 2018). Blood stored in EDTA doesn't have significant changes in reticulocyte count until 48 hours (Rautaray and Tripathy, 2018). Researchers reported that neither EDTA nor heparin affected the reticulocyte count, but dilution of the sample could have significant effects (Kaufhold et al., 2018). Similarly, sodium citrate doesn't interfere with reticulocyte counts (Akorsu et al., 2023).

Changes in Platelets Count:

When the blood is stored in anticoagulants such as EDTA, heparin, and even calcium oxalate and sodium citrate, they stimulate the platelets membrane against which auto-antibodies are generated and clumps of platelets are formed. So, when automatic hematology analyzers aspirate this type of blood, they count these clumps as WBCs, which results in decreased platelet count. Although the person has a normal platelet count due to anticoagulant-related artifacts, the plate count has decreased. This phenomenon is known as EDTA-dependent pseudo thrombocytopenia (PTCP). When it was observed that this artifact was also caused by other anticoagulants like magnesium sulfate, it was given a new name, "Anticoagulant-induced PTCP" (Lardinois et al., 2021; Schuff-Werner et al., 2020). Heparin is the second worst anticoagulant because it also induces thrombocytopenia both in vitro and in vivo due to clumping of platelets (Gruel et al., 2020; Lardinois et al., 2021). Another study reported that platelet count was even lower than EDTA-treated blood when a heparinized cord blood sample was used (Thanasak et al., 2022). In a publication, researchers found that EDTA and EGTA (ethylene glycol tetraacetic acid) cause aggregation of platelets. Anticoagulants like sodium citrate, sodium oxalate, and heparin show no clumping of platelets (Mannuß, 2020) (Hürmeydan et al., 2021). The platelets remain stable after blood collection in K3EDTA for four days. Thirty-seven anticoagulants like ammonium oxalate and 3.8% sodium citrate are recommended to prevent pseudo-thrombocytopenia (Sharif et al., 2023). Magnesium sulfate is a reliable anticoagulant for determining actual platelet count. Citrate (sodium citrate) is known to cause a decrease in platelet counts as compared to Na2EDTA and K3EDTA. Another anticoagulant, hirudin, is unreliable because the blood clots entirely after 4 hours of blood collection (Benjamin et al., 2018; Sugerman et al., 2021). Virtually no anticoagulant is so efficient in eliminating platelet clumping completely. EDTA, heparin, sodium citrate, sodium fluoride, sodium oxalate, and rarely hirudin were found to cause PTCP (Schuff-Werner et al., 2020) .

Changes in White Blood Cell Count:

When the blood from rats was stored in K3EDTA, the WBC count was observed to be higher after storage of 72h at 3°C. This increase is known to be caused by platelet aggregation (Layssol-Lamour et al., 2019). However, the WBC count in human blood is stable for four days (Wu et al., 2017). EDTA-induced thrombocytopenia has already been observed in humans, which may also cause pseudoleukocytosis (Lardinois et al., 2021). A team of researchers reported that the leukocyte count of blood is stable when sodium heparin is used and blood is stored in a refrigerator for 24 hours (Hennø et al., 2017). A study from the last decade shows that spurious low WBC count can be observed in blood taken into K3EDTA, sodium citrate, and heparin anticoagulants because of polymorphonuclear neutrophils and lymphocyte aggregation (Margraf et al., 2022). A Significant decrease in the total number of leukocytes was observed in heparin and K3EDTA-treated blood. Heparin was not found satisfactory for WBC counting (Akorsu et al., 2023) (Baccini et al., 2020). Sodium citrate gave a less reliable and lower count of WBCs than

other anticoagulants such as EDTA and Heparin (Akorsu et al., 2023) (Putra and Hernaningsih, 2022). Leucocytes in balanced ammonium-potassium oxalate anticoagulant stored at refrigerator temperature were stable for 24 hours (Zhou et al., 2024). EDTA showed less toxicity as compared to heparin when peripheral blood mononuclear cells are stored, which leads to decreased lysis of cells as compared to heparin (Baien et al., 2018).

Changes in Red Blood Cell Count:

In a study, the blood stored with CPDA-1 at 4-6°C is examined over time. They observed the reduction in RBCs starting from the 5th day of storage. Only a small number of RBCs are lysed; the only reasonable explanation for this is that the old-aged cells are lysed (Gong et al., 2023) (Yousef et al., 2019). Another team of researchers observed that when a prolonged stored blood unit was transfused to the recipient, the extravascular hemolysis of RBCs increased after transfusion, which was characterized by elevated direct and indirect bilirubin produced as a result of extravascular hemolysis in the liver and spleen (Phillips and Henderson, 2018). Similar results were reported in research conducted on *Schizopyge plagiostomus* (Himalayan snow trout). The RBC counts are significantly less in snow trout blood stored in K2EDTA than in lithium heparin. In the K2EDTA sample, bare nuclei indicate the destruction of RBCs (Ji, 2021). Heparin and EDTA both cause a decrease in RBC count. Heparin activates sialic acid residue on adjacent cell surfaces, which causes cells to form aggregates and decreases the viable count of RBCs.

Morphological Changes in RBCs:

Red blood cells carry oxygen from the lungs to body parts and give characteristics of red color to whole blood (Villa et al., 2016). When blood is stored in EDTA salts for laboratory analysis, various morphological changes occur in red blood cells. Due to the movement of water into the cells, the cell size increases. On baseline, the RBCs are observed to be normocytic and normochromic. But on day 4, the RBCs also have echinocytes, spherocytes, and sphero-echinocytes shapes along with increases in RBCs rouleaux formation (Recktenwald et al., 2022). RBCs showed 3% increases in cell size when stored at room temperature in anticoagulants like tri potassium EDTA, disodium EDTA, balanced potassium-ammonium oxalate, and sodium heparin which is indicated by the increased value of MCV and Hct (Mendhurwar, 2018). However, a high concentration of K3EDTA causes water to move out of the cell, and cell size decreases, which is indicated by a reduction in pack cell volume (Moxon et al., 2023). An excellent review on red cell morphology concluded that the RBCs change their shape from discoid to spherocytes when stored in ACD or CPD (Goyal, 2016).

A study of the Tibetan population shows that the shape of RBCs is altered as the storage time increases. The number of normal disc-shaped cells starts to decrease sharply after storage of 21 days in CPDA-1 anticoagulant. After 35, only 5% of cells are spherocytes and sphere-schistocytes, which are irreversible shapes of RBCs (Pretorius, 2018).

Morphological Changes in Leukocytes:

Leukocytes are different types of blood cells that help the body defend against infections (Garg et al., 2020). K2EDTA anticoagulant affects neutrophils when the blood is examined at 0, 4,12, 24 hours after sample collection significant decrease in segmented neutrophils is observed, and this decrease is justified by a relative increase in non-segmented neutrophils (band-form) (Jonas, 2020). Sodium citrate causes nuclear lobulation, nucleus degeneration, and karyolysis starting from 0 hours. The same effects had been seen with EDTA but markedly decreased up to 2 hours. Smudge cells and increased cell size of WBCs have also been observed in blood smears soon after 1 hour in sodium citrate and delayed up to 3-4 hours in EDTA-containing samples (Amir et al., 2023).

Morphological Changes in Platelets:

EDTA anticoagulant causes morphological changes in platelets. The typical shape of platelets is discoidal, but when blood is stored in an EDTA anticoagulant, it changes its morphology and becomes spherical. Potassium EDTA causes more increase in mean platelets volume than sodium EDTA. Due to this shape change, the mean platelet volume also increases in impedance-based instruments. EDTA also causes clumping of platelets, which are counted as WBCs by hematology analyzers. This results in pseudo-thrombocytopenia (Kausar et al., 2021) (Goyal and Goyal, 2023). Besides, EDTA and EGTA cause degranulation of platelets (Karhausen et al., 2020). Heparinized blood sample causes platelet volume to increase, leading to many changes in the shapes of Plts (Mannuß, 2020). Sodium citrate and EDTA cause not only swelling of platelets but spiny projections are also observed, and these artifacts increase as time passes. Sodium citrate doesn't cause aggregation of platelets (Levin, 2019).

Effects on Hematocrit Value:

In research, it is observed that the concentration of EDTA influences the hematocrit value of blood. When the EDTA concentration in the blood exceeds the recommended concentration (1mg per 1 ml of whole blood), significant changes in hematocrit values are observed. With the increase in concentration of EDTA, the value of hematocrit decreases (Reckziegel et al., 2016). Erythrocytes volume has been observed to be reduced in citrate anticoagulant (Lim et al., 2018). The ostrich blood, when stored in heparin, shows the lower value of HCT as compared with blood stored with EDTA (Vincent et al., 2023).

Effects on Hemoglobin Concentration:

A study researcher reported that the concentration of EDTA has no significant effect on the value of hemoglobin (Varanita et al., 2022). Another study observed a slight decrease in hemoglobin concentration over time when CPDA-1 is used as an anticoagulant (Adetola et al., 2020). No significant changes in Hb concentration were recorded When heparin and EDTA were used for general hematological parameters (Akorsu et al., 2023).

Effects on Packed Cell Volume of RBCs:

Research has published that the increasing concentration of EDTA causes RBCs to shrink, thus decreasing the PCV value. At the same time, heparin was the anticoagulant of choice for calculating PCV because a fold increase in heparin concentration does not affect PCV as it has minimum effects on the morphology of RBCs (Makhro et al., 2016). Similar results were also published in a research in which blood was stored at four °C in CPDA-1 anticoagulant, and the PCV values decreased drastically after one week of storage. The apparent reason for that is red cell hemolysis (Gaur et al., 2023). A study was conducted on Green Iguana, and the results are compared with a previous study conducted on Testudo hermanni. It's found that EDTA and Heparin have no effect on PCV values of blood, but in previous studies on Testudo hermanni, the

EDTA is known to cause a decrease in PCV value (Davidson et al., 2024).

BIOCHEMICAL CHANGES:

Electrolyte imbalance:

Electrolytes are ionic fluids present in the human body and assist in the body's normal functioning (Seifter, 2019). They are ionic molecules that give cations and anions upon hydrolysis. Some crucial electrolytes are sodium (Na+), chloride (Cl-), potassium (K+), calcium (Ca2+), and bicarbonate (HCO 3-) (Gałęska et al., 2022). Due to their ionic nature, these electrolytes can conduct electricity (Zhao et al., 2020) (Li et al., 2020). Electrolytes are present in our daily meals and drinks. They are absorbed into the blood from the gastrointestinal tract. Electrolytes are in equilibrium, and certain hormones in blood control their amount (Seifter, 2019). Any malfunction in this equilibrium causes electrolyte imbalance, a severe pathophysiological condition leading to death (Dhondup and Qian, 2017). When blood is improperly stored for a long duration, the electrolytes are leaked from the intracellular compartment to the extracellular fluid, thus causing a change in RBC morphology. This altered electrolyte level can have adverse effects on the patient who receives such blood (Yazer et al., 2018).

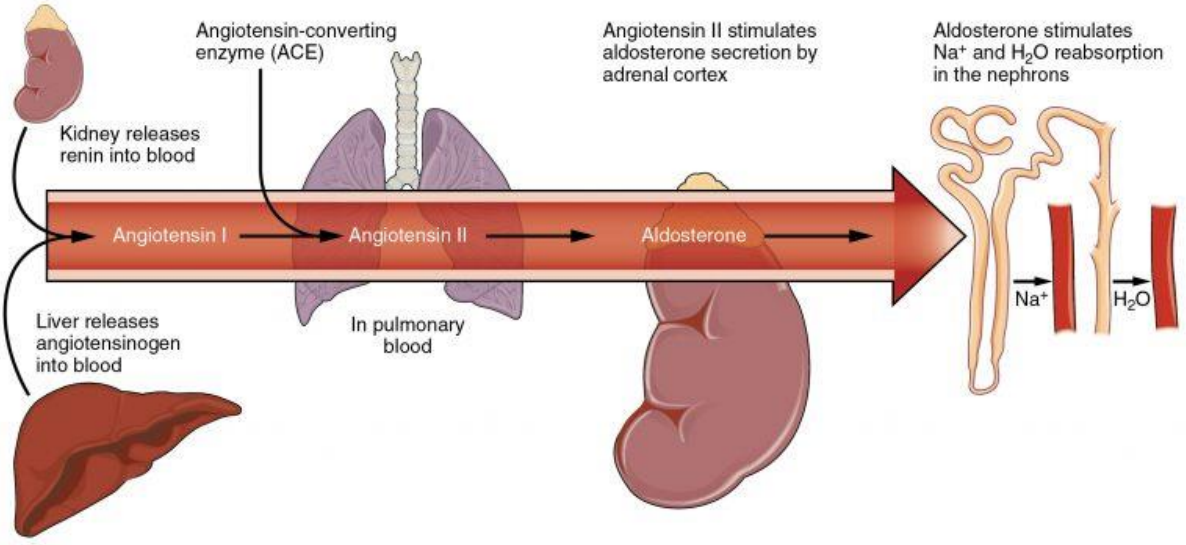

Fig.01

Sodium:

Sodium is one of the most critical and dominant extracellular cations that maintains blood volume and nerve and muscle functions (Polychronopoulou et al., 2019). Homeostasis of sodium is maintained by the kidney, especially in proximal tubules and distal convoluted tubules under aldosterone (Pearce et al., 2022). When the blood is stored in CPDA-1, the sodium moves from ECF to ICF, and its concentration drops over time. This causes various post-transfusion complications (Okereke et al., 2019). Sodium heparin causes no significant effect on the sodium electrolyte concentration as compared to serum sodium concentration within 15 minutes of sample collection (Cole et al., 2019).

Potassium:

Potassium is a principal intracellular cation, and its homeostasis with sodium is maintained by the sodium-potassium adenosine triphosphatase pump, which transfers sodium from the cell to the extracellular fluid (Danchin and Nikel, 2019). Potassium imbalance can cause platelet aggregation.77 A study observed a considerable increase in potassium and a decrease in sodium over seven days of storage while using CPDA-1 anticoagulant. It is only the change they observed in the electrolyte level (Adetola et al., 2020). When EDTA is used, the potassium concentration increases due to the presence of potassium salt in the anticoagulant being used (Jahanbani et al., 2023). Sodium heparin and lithium heparin don't have any significant effect on potassium concentration within 15 minutes of sample collection (Menta et al., 2020).

Calcium and Ferric:

Calcium is significant and plays a vital role in the mineralization of bone, muscle contractions, and conduction of nerve impulses, along with many intracellular functions. It is also a part of extracellular fluid, and an excessive amount of calcium is removed by the kidney under the influence of parathyroid hormone (Ureña-Torres et al., 2019). When plasma obtained from K2EDTA anticoagulated blood was used for biochemical tests, it caused changes in the concentration of many electrolytes, including calcium, potassium, and iron. The EDTA is divalent and trivalent, i.e., Ca+2 and Fe+3 chelating agents. So, its presence causes a decrease in the calcium concentration, especially ferric, as these ions are being chelated (Gulcin and Alwasel, 2022). Heparin does not affect Ca2+ in neonatal plasma, but plasma dilution causes a decrease in Ca2+. When the amount of diluent is the same, either heparin or a solution of sodium chloride, the same effect on Ca2+ is observed (Melkonian and Schury, 2019). The decrease in calcium concentration is also observed by the complex formation of calcium by heparin (Bedsted et al., 2003).

Changes in pH of Blood:

RBCs lack mitochondria; therefore, the only energy source is the anaerobic glycolysis end product, which is lactic acid (Constable et al., 2019). Due to this, blood pH decreases as more lactic acid accumulates in the blood, therefore changing the pH of the blood and causing many biochemical changes to stored blood (Hess and Greenwalt, 2002). It was reported that the pH of stored blood with CPD and CPDA-1 anticoagulants declines as time passes (Meledeo et al., 2019; Sivertsen et al., 2020). When heparin was used at low concentration, no changes in pH concentration were recorded, but a significant decrease in pH was observed when higher concentration was used (Derry et al., 2017).

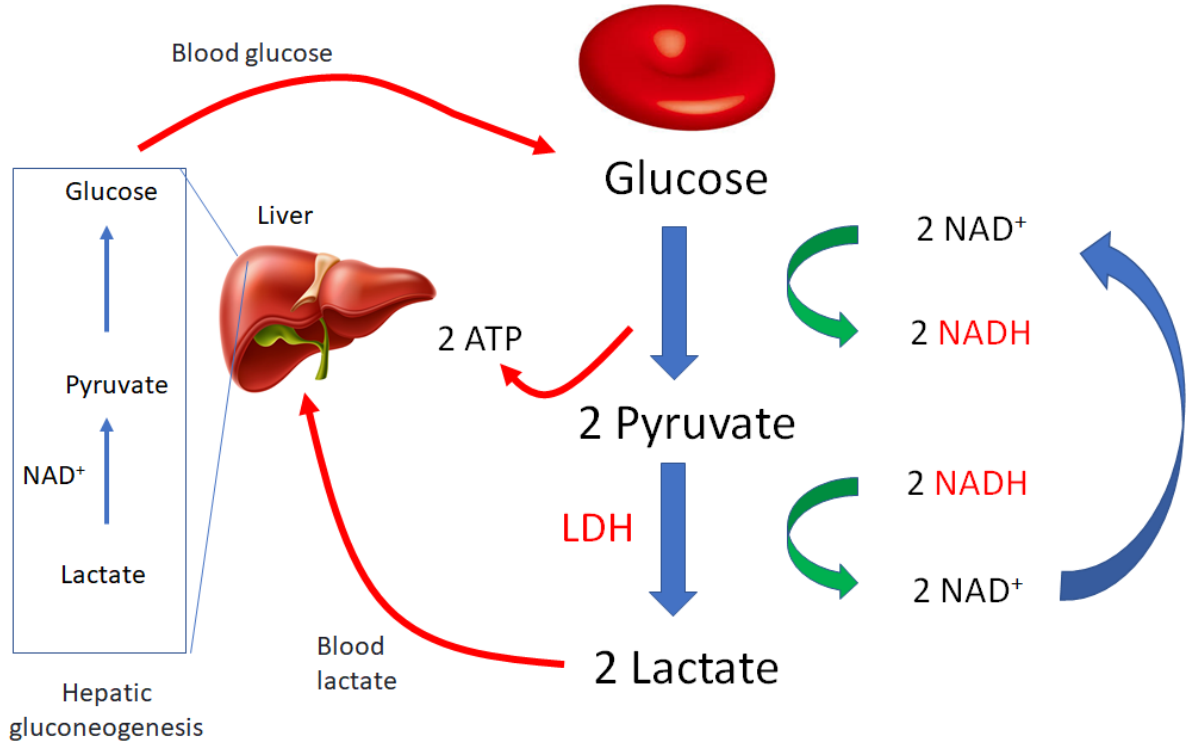

Fig.02

Changes in 2,3-Diphosphoglycerate (2,3-DPG):
When blood is stored, it produces lactic acid, which reduces the pH of blood. This causes the activation of the phosphatase three enzyme, which degrades 2,3-DPG, due to which the oxygen affinity of hemoglobin is increased, and less oxygen is transferred to tissue from the lungs by RBC (Bellelli and Brunori, 2020). Study shows that essentially no 2,3-DPG will remain after 21 days of storage in CPD and CPDA-1 (Aujla et al., 2018).

D-dimer Concentration:
In published research, it was observed that the sodium-citrate plasma, when stored for 4-24 hours at room temperature, did not show any significant change in concentration of D-dimer as compared to the reading taken at 0 hours. However, for longer durations of more than 24 hours, i.e., 48 or 72 hours, the D-dimer concentration falls proportionally with time. However, no significant change was observed in D-dimer concentration when the sample was stored at 4 or -20°C for 10 days (Kiefer et al., 2018). It is reported that the d-dimer concentration wasn't affected by lithium heparin and sodium citrate (Wauthier et al., 2023). When a comparison was made for d-dimer concentration in dog blood collected in EDTA and sodium citrate, the concentration of d-dimer was observed to be elevated (Ke et al., 2023).

Osmotic Fragility:
Blood stored in K2EDTA causes electrolyte imbalance and degeneration of intracellular components, which causes the water to move into the red cell size and increase its osmotic fragility (Kawthalkar, 2018). Similar results were also observed when the blood of common carp was treated with EDTA and stored (Afzal et al., 2022). When the blood of *Struthio camelus* was stored in K3 EDTA, the osmotic fragility of RBCs increased and caused more hemolysis compared to lithium heparin (Furtado et al., 2022).

Effect on Blood Gas Estimation:
Patients with respiratory disorders are routinely checked for blood gas concentrations. Heparin is the recommended anticoagulant. Excessive amounts of heparin can cause errors, too. However, oxalate and citrate solutions can be used when heparin is unavailable. When an EDTA anticoagulated sample is used, the result fluctuates greatly from the normal values, making the EDTA useless in blood gas estimation (Lima-Oliveira et al., 2021). EDTA causes many changes in electrolyte imbalance, such as chelating divalent cations like Ca2+, which may affect blood gas estimation (Dent and Selvaratnam, 2022).

Effects on Cytokine Level:
Chemokines and cytokines are cell signaling proteins crucial in the physiological response of growth, immunity, and inflammation. Due to their fragile nature, their concentration varies due to many external factors,

including anticoagulants. When plasma samples from EDTA anticoagulated blood from healthy and HIV-positive patients are studied, the TNF-α, IFN-γ, IL-4, IL-5, and G-CSF levels are elevated. But the level IL-6, IL-8, IL-10, IL-17,MIP-1β, GM-CSF, and MCP-1 were observed significantly higher in plasma obtained from heparin. Meanwhile, the levels of IL-7, IL-12 (P70), and IL-13 weren't affected in either group (Bæk et al., 2024). The low level of cytokines in EDTA anticoagulated blood was either due to the clumping of platelets, a major side effect of EDTA that has already been studied (Scholman et al., 2018). Heparin interaction with different cytokines, growth factors, and many other proteins has already been studied. Hence, the lower level of cytokine in heparinized blood samples may be due to the binding of cytokines with heparin and adsorption effects (Hedayati et al., 2019; Qiu et al., 2021). In research aimed to study the effects of heparin, EDTA, citrate, and hirudin on cytokine production, it is reported that EDTA and citrate both reduced cytokine production (Cedrone et al., 2017). The sample collected in citrate anticoagulant gives the same results as plasms from EDTA anticoagulated blood (Kennedy et al., 2021)

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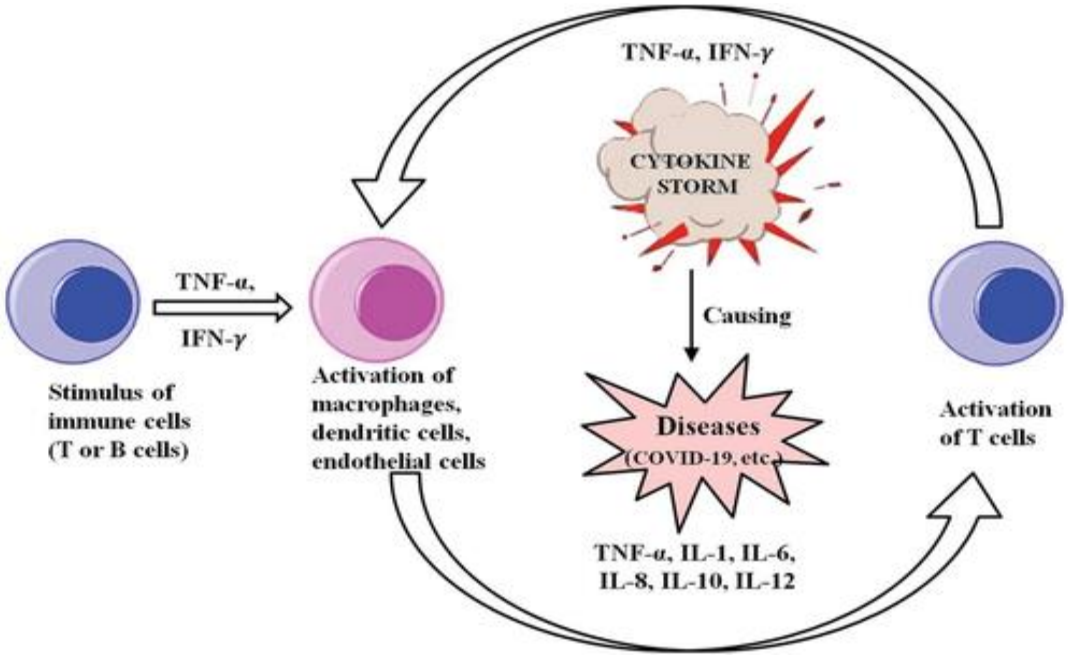

Fig.03

Effects on Plasma Ascorbic Acid:

Vitamin C is abundantly present in plasma as a reduced form known as ascorbic acid (AA). Ascorbic acid is most sensitive to oxidation, so there are more chances for rapid degradation in different anticoagulants (Zou et al., 2020). AA was reported more stable in heparin plasma than in EDTA and citrate-induced plasma. AA remained stable for 14 days when stored at -70°C and 24 hours at -20°C when the heparinized plasma was stored immediately after collection and separation (Constable et al., 2019) (Hess and Greenwalt, 2002) In another study, there was no significant difference in the AA concentration between EDTA and heparinized plasma. However, the researcher does not recommend a heparinized plasma sample of AA estimation due to poor correlation coefficient and coefficient of determination.

Changes in Blood Glucose Concentration:

Glucose is a primary energy source; all body cells use glucose as an energy reservoir. A glucose concentration test is frequently used to check diabetes. When the blood is taken to measure glucose concentration, care should be taken to reduce the amount of glucose used after collecting samples. A study measured blood glucose levels in serum and plasma from EDTA, sodium citrate, and fluoride oxalate anticoagulant samples. It was observed that the glucose concentration was reduced over time in all types of samples, irrespective of the anticoagulant used. A minor quantity reduction was observed in the fluoride oxalate sample because fluoride inhibits the action of the enzyme enolase, an essential enzyme in glycolysis. However, glucose was not stable in any sample, so immediate glucose measurement is recommended (Sivertsen et al., 2020) (Derry et al., 2017). In another study, similar results were published when NaF and citrate buffer were used as glycolytic inhibitors and heparin, citrate, and oxalate as anticoagulants. Fluoride-containing anticoagulants alone are not very adequate without acidification of the blood. For better results a combination of NaF/citrate buffer/Na2EDTA, NaF/Na-heparin, NaF/K2oxalate, and NaF/citrate is recommended (Aujla et al., 2018; Bellelli and Brunori, 2020).

HbA1c is the mean of blood glucose. When HbA1c value remains stable when measured from blood collected with sodium heparin, lithium heparin, EDTA, sodium citrate, and sodium fluoride, these anticoagulants do not affect HbA1c value. This is because the RBCs are lysed before the test is conducted (Ke et al., 2023; Kiefer et al., 2018; Wauthier et al., 2023).

Changes in Plasma Lipid Concentrations:

Lipids are organic compounds that are not miscible with

water and include fats, oils, and hormones (Kawthalkar, 2018). Human plasma contains thousands of different composition lipids. Anticoagulants affect the stability of various lipids found in human blood. Research has been conducted to observe the effects of three anticoagulants (i.e., K2EDTA, lithium heparin, and sodium citrate) on 88 lipids, of which eight are synthetic. Among them, only ten lipids show relatable peak areas in these anticoagulants, and 70 endogenous lipids showed significant differences in peak areas in citrate-heparin and EDTA-heparin comparisons. Of these 70 lipids, 53 lipid species are only comparable in terms of sodium citrate and lithium heparin. No comparison of either lithium heparin or sodium citrate with EDTA was observed, with some exceptions (Afzal et al., 2022)

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate
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Consent for publication
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Conflict of interest

The authors declared absence of conflict of interest.

Author Contribution

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Study Design, Review of Literature
Conception of Study, Development of Research Methodology Design, Study Design, Review of manuscript, final approval of manuscript
AREEJ SAFDAR
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Conception of Study, Final approval of manuscript
MUHAMMAD ATIF
Manuscript revisions, critical input.
Coordination of collaborative efforts.
HAFIZ IFTIKHAR HUSSAIN
Conception of Study, Development of Research Methodology Design, Study Design, Review of manuscript, final approval of manuscript.
MUHAMMAD WAQAS
Data entry and Data analysis, drafting article.
Data acquisition, analysis.
SAMMINA MAHMOOD
Data acquisition and analysis. Manuscript drafting.

References

Adetola, A. A., Olooto, W. E., Olaniyi, O. D., Onayemi, A. A., and Adeleke, A. D. F. (2020). Assessment of biochemical and haematological changes that occur in blood stored with CPDA-1 as an anticoagulant in a Tertiary Hospital in Nigeria. *Journal of Medicine and Biomedical Research* **19**, 13-22.

Afzal, G., Ahmad, H. I., Hussain, R., Jamal, A., Kiran, S., Hussain, T., and Saeed, S. (2022). Bisphenol A induces histopathological, hematobiochemical alterations, oxidative stress, and genotoxicity in common carp (Cyprinus carpio L.). *Oxidative Medicine and Cellular Longevity* **2022**.

Akorsu, E. E., Adjabeng, L. B., Sulleymana, M. A., and Kwadzokpui, P. K. (2023). Variations in the full blood count parameters among apparently healthy humans in the Ho municipality using ethylenediamine tetraacetic acid (EDTA), sodium citrate and lithium heparin anticoagulants: A laboratory-based cross-sectional analytical study. *Heliyon* **9**.

Amir, E., Latif, A. Q., Anwar, M., Rahat, N., Khalid, G., and Anwar, R. (2023). EDTA related Pseudothrombocytopenia (PTCP) Incidence and Resolution of Clinically Threatened Artifact. *Pakistan Journal of Medical & Health Sciences* **17**, 166-166.

Arnold, J. E., Camus, M. S., Freeman, K. P., Giori, L., Hooijberg, E. H., Jeffery, U., Korchia, J., Meindel, M. J., Moore, A. R., and Sisson, S. C. (2019). ASVCP guidelines: principles of quality assurance and standards for veterinary clinical pathology (version 3.0): developed by the American Society for Veterinary Clinical Pathology's (ASVCP) Quality Assurance and Laboratory Standards (QALS) Committee.

Aujla, H., Woźniak, M., Kumar, T., Murphy, G., Investigators, R., Cardigan, R., Deary, A., Hodge, R., Smethurst, P., and Braund, R. (2018). Rejuvenation of allogenic red cells: benefits and risks. *Vox Sanguinis* **113**, 509-529.

Baccini, V., Geneviève, F., Jacqmin, H., Chatelain, B., Girard, S., Wuilleme, S., Vedrenne, A., Guiheneuf, E., Toussaint-Hacquard, M., and Everaere, F. (2020). Platelet counting: ugly traps and good advice. Proposals from the French-Speaking Cellular Hematology Group (GFHC). *Journal of clinical medicine* **9**, 808.

Bæk, O., Rasmussen, M. B., Gerts, T., Aunsholt, L., Zachariassen, G., Sangild, P., and Nguyen, D. N. (2024). Insulin-like growth factor 1 associated with altered immune responses in preterm infants and pigs. *Pediatric Research* **95**, 120-128.

Baien, S. H., Langer, M. N., von Köckritz-Blickwede, M., and de Buhr, N. (2018). Comparison between K3EDTA and lithium heparin as anticoagulant to isolate bovine granulocytes from blood. *Frontiers in immunology* **9**, 387565.

Bain, B. J., Bates, I., Laffan, M. A., and Lewis, S. M. (2016). "Dacie and Lewis practical haematology: expert consult: online and print," Elsevier Health Sciences.

Barshtein, G., Pajic-Lijakovic, I., and Gural, A. (2021). Deformability of stored red blood cells. *Frontiers in Physiology* **12**, 722896.

Bedsted, T., Swanson, R., Chuang, Y.-J., Bock, P. E., Björk, I., and Olson, S. T. (2003). Heparin and calcium ions dramatically enhance antithrombin reactivity with factor IXa by generating new interaction exosites. *Biochemistry* **42**, 8143-8152.

Bellelli, A., and Brunori, M. (2020). Control of oxygen affinity in mammalian hemoglobins: implications for a system biology description of the respiratory properties of the red blood cell. *Current Protein and Peptide Science* **21**, 553-572.

Benjamin, J. M., Chippaux, J.-P., Sambo, B. T., and Massougbodji, A. (2018). Delayed double reading of whole blood clotting test (WBCT) results at 20 and 30 minutes enhances diagnosis and treatment of viper evenomation. *Journal of Venomous Animals and Toxins including Tropical Diseases* **24**, 14.

Bills, M. V., Nguyen, B. T., and Yoon, J.-Y. (2019). Simplified white blood cell differential: an inexpensive, smartphone-and paper-based blood cell count. *IEEE sensors Journal* **19**, 7822-7828.

Bogdanova, A., Kaestner, L., Simionato, G., Wickrema, A., and Makhro, A. (2020). Heterogeneity of red blood cells: causes and consequences. *Frontiers in physiology* **11**, 526790.

Carlquist, E., Ulleberg, P., Delle Fave, A., Nafstad, H. E., and Blakar, R. M. (2017). Everyday understandings of happiness, good life, and satisfaction: Three different facets of well-being. *Applied Research in Quality of Life* **12**, 481-505.

Cedrone, E., Neun, B. W., Rodriguez, J., Vermilya, A., Clogston, J. D., McNeil, S. E., Barenholz, Y., Szebeni, J., and Dobrovolskaia, M. A. (2017). Anticoagulants influence the performance of in vitro assays intended for characterization of nanotechnology-based formulations. *Molecules* **23**, 12.

Chakrahari, S. (2018). Evaluation Of Cord Blood Albumin, Bilirubin, Nucleated Rbc And Reticulocyte Count As Early Predictors In Neonatal Hyperbilirubinemia, BLDE (Deemed to be University).

Chohan, A. S., and Davidow, E. B. (2015). Clinical pharmacology and administration of fluid, electrolyte, and blood component solutions. *Veterinary anesthesia and analgesia: the fifth edition of Lumb and Jones*, 386-413.

Cole, N. I., Suckling, R. J., Desilva, V., He, F. J., MacGregor, G. A., and Swift, P. A. (2019). Serum sodium concentration and the progression of established chronic kidney disease. *Journal of Nephrology* **32**, 259-264.

COMITTEE, O. 5th EFLM-UEMS European Joint Congress in Laboratory Medicine Laboratory Medicine at the Clinical Interface Antalya, Turkey, October 10-13, 2018.

Constable, P., Trefz, F. M., and Stämpfli, H. (2019). Effects of pH and the plasma or serum concentrations of total calcium, chloride, magnesium, l-lactate, and albumin on the plasma ionized calcium concentration in calves. *Journal of veterinary internal medicine* **33**, 1822-1832.

Danchin, A., and Nikel, P. I. (2019). Why nature chose potassium. *Journal of Molecular Evolution* **87**, 271-288.

Davidson, A., Ryan, M., Schnelle, A. N., Graser, W., Adamovicz, L., and Allender, M. C. (2024). COMPARING THE EFFECTS OF DIPOTASSIUM ETHYLENEDIAMINETETRAACETIC ACID AND LITHIUM HEPARIN ON HEMATOLOGIC VALUES IN BLANDING'S TURTLES (EMYDOIDEA BLANDINGII), PAINTED TURTLES (CHRYSEMYS PICTA), AND COMMON SNAPPING TURTLES (CHELYDRA SERPENTINA). *Journal of Zoo and Wildlife Medicine* **55**, 92-101.

Dent, A., and Selvaratnam, R. (2022). Measuring magnesium–Physiological, clinical and analytical perspectives. *Clinical Biochemistry* **105**, 1-15.

Derry, S., Rice, A. S., Cole, P., Tan, T., and Moore, R. A. (2017). Topical capsaicin (high concentration) for chronic neuropathic pain in adults. *Cochrane Database of Systematic Reviews*.

Dhondup, T., and Qian, Q. (2017). Acid-base and electrolyte disorders in patients with and without chronic kidney disease: an update. *Kidney Diseases* **3**, 136-148.

Dibiasi, C., Plewka, J., Ploszczanski, L., Glanz, V., Lichtenegger, H., and Windberger, U. (2018). Viscoelasticity and structure of blood clots generated in-vitro by rheometry: A comparison between human, horse, rat, and camel. *Clinical hemorheology and microcirculation* **69**, 515-531.

Farias, T. H. V., Pereira, N. L., Pádua, S. B. d., Alves, L. d. O., Sakabe, R., Belo, M. A. d. A., and Pilarski, F. (2016). Na 2 EDTA anticoagulant impaired blood samples from the teleost Piaractus mesopotamicus. *Pesquisa Veterinária Brasileira* **36**, 431-435.

Furtado, Y. I. C., Monteiro, C. C., Gonzales, A. P. P. F., Costa, M. d. N. F., Brasiliense, A. R. P., and Yoshioka, E. T. O. (2022). Anticoagulants and their effects on the hematological and biochemical parameters of yellow-spotted amazon river turtle. *Ciência Rural* **52**, e20210489.

Gałęska, E., Wrzecińska, M., Kowalczyk, A., and Araujo, J. P. (2022). Reproductive consequences of electrolyte disturbances in domestic animals. *Biology* **11**, 1006.

García-Roa, M., del Carmen Vicente-Ayuso, M., Bobes, A. M., Pedraza, A. C., González-Fernández, A., Martín, M. P., Sáez, I., Seghatchian, J., and Gutiérrez, L. (2017). Red blood cell storage time and transfusion: current practice, concerns and future perspectives. *Blood Transfusion* **15**, 222.

Garg, G., Singh, S., Singh, A. K., and Rizvi, S. I. (2020). Characteristics of Healthy Blood. *Explaining Health Across the Sciences*, 179-197.

Gaur, P., Bhardwaj, A., Raturi, G., Ahmed, S., and Usmani, R. (2023). Correlation of Hemolysis by Plasma Hemoglobin with Biochemical Markers during Storage of Blood under Standard Conditions in the Blood Bank of a Tertiary Health-care Center. *Global Journal of Transfusion Medicine* **8**, 190-196.

Gong, X., Shen, H., Guo, L., Huang, C., Su, T., Wang, H., Feng, S., Yang, S., Huo, F., and Liu, H. (2023). Glycyrrhizic acid inhibits myeloid differentiation of hematopoietic stem cells by binding S100 calcium binding protein A8 to improve cognition in aged mice. *Immunity & Ageing* **20**, 12.

Goyal, I. (2016). Procedure to enhance cellular viability of blood and its components to improve its shelf life.

Goyal, S., and Goyal, S. (2023). EDTA dependent pseudo thrombocytopenia-A diagnostic dilemma! *Indian Journal of Pathology and Microbiology* **66**, 227-228.

Graverini, G., Piazzini, V., Landucci, E., Pantano, D., Nardiello, P., Casamenti, F., Pellegrini-Giampietro, D. E., Bilia, A. R., and Bergonzi, M. C. (2018). Solid lipid nanoparticles for delivery of andrographolide across the blood-brain barrier: in vitro and in vivo evaluation. *Colloids and Surfaces B: Biointerfaces* **161**, 302-313.

Gruel, Y., De Maistre, E., Pouplard, C., Mullier, F., Susen, S., Roullet, S., Blais, N., Le Gal, G., Vincentelli, A., and Lasne, D. (2020). Diagnosis and management of heparin-induced thrombocytopenia. *Anaesthesia Critical Care & Pain Medicine* **39**, 291-310.

Gulcin, İ., and Alwasel, S. H. (2022). Metal ions, metal chelators and metal chelating assay as antioxidant method. *Processes* **10**, 132.

Hedayati, M., Neufeld, M. J., Reynolds, M. M., and Kipper, M. J. (2019). The quest for blood-compatible materials: Recent advances and future technologies. *Materials Science and Engineering: R: Reports* **138**, 118-152.

Hennø, L. T., Storjord, E., Christiansen, D., Bergseth, G., Ludviksen, J. K., Fure, H., Barene, S., Nielsen, E. W., Mollnes, T. E., and Brekke, O.-L. (2017). Effect of the anticoagulant, storage time and temperature of blood samples on the concentrations of 27 multiplex assayed cytokines–consequences for defining reference values in healthy humans. *Cytokine* **97**, 86-95.

Hess, J. R., and Greenwalt, T. G. (2002). Storage of red blood cells: new approaches. *Transfusion medicine reviews* **16**, 283-295.

Huisjes, R., Bogdanova, A., Van Solinge, W. W., Schiffelers, R. M., Kaestner, L., and Van Wijk, R. (2018). Squeezing for life–properties of red blood cell deformability. *Frontiers in physiology* **9**, 350140.

Hürmeydan, Ö., Madenci, Ö. Ç., Yıldız, Z., Gültürk, E., and Orcun, A. (2021). Management of ethylenediaminetetraacetic acid and citrate-dependent pseudothrombocytopenia in the laboratory. *International Journal of Medical Biochemistry* **4**, 56.

Jahanbani, A., Goodarzi, M., Sajjadi Dezfouli, S. M., Yourdkhani, M. R., and Eskandari Roozbahani, N. (2023). A novel anticoagulation method based on electrochemical

characteristics of blood: An in vitro study. *Blood purification* **52**, 122-131.
Ji, Y. (2021). Characterisation of red blood cell Phagocytosis and assessment of nanoparticle uptake by Monocytic cells, Queensland University of Technology.
Jonas, D. N. (2020). Možnosti využití kaskád neuronových sítí pro klasifikaci krvetvorných buněk, České vysoké učení technické v Praze. Vypočetní a informační centrum.
Karhausen, J., Choi, H. W., Maddipati, K. R., Mathew, J. P., Ma, Q., Boulaftali, Y., Lee, R. H., Bergmeier, W., and Abraham, S. N. (2020). Platelets trigger perivascular mast cell degranulation to cause inflammatory responses and tissue injury. *Science advances* **6**, eaay6314.
Karolczak, K., Soltysik, B., Kostka, T., Witas, P. J., and Watala, C. (2019). Platelet and red blood cell counts, as well as the concentrations of uric acid, but not homocysteinaemia or oxidative stress, contribute mostly to platelet reactivity in older adults. *Oxidative Medicine and Cellular Longevity* **2019**.
Kaufhold, A. E., Hirschberger, J., Reese, S., Foerster, G., and Hein, J. (2018). A comparison of manual counting of rabbit reticulocytes with ADVIA 2120i analyzer counting. *Journal of veterinary diagnostic investigation* **30**, 337-341.
Kausar, S., Fateen, T., and Chaudary, H. T. (2021). Frequency of Causes of Spurious Platelets Count on Routine Complete Blood Count by an Automated Hematology Cell Analyser. *LIAQUAT MEDICAL RESEARCH JOURNAL* **3**.
Kawthalkar, S. M. (2018). "Essentials of clinical pathology," JP Medical Ltd.
Ke, C.-H., Liu, C.-C., Wang, S.-L., and Lin, C.-S. (2023). Paired analysis of D-Dimer and its correlated hemostatic parameters in 30 dogs with neoplasms after tumorectomy. *Animals* **13**, 969.
Kennedy, A. D., Ford, L., Wittmann, B., Conner, J., Wulff, J., Mitchell, M., Evans, A. M., and Toal, D. R. (2021). Global biochemical analysis of plasma, serum and whole blood collected using various anticoagulant additives. *PloS one* **16**, e0249797.
Kiefer, E., Hoover, D. R., Shi, Q., Dusingize, J. C., Sinayobye, J., and Anastos, K. (2018). Longitudinal evaluation of markers of inflammation in HIV-positive and HIV-negative Rwandan women. *HIV medicine* **19**, 734-744.
Lardinois, B., Favresse, J., Chatelain, B., Lippi, G., and Mullier, F. (2021). Pseudothrombocytopenia—a review on causes, occurrence and clinical implications. *Journal of clinical medicine* **10**, 594.
Layssol-Lamour, C., Lavabre, T., Braun, J. P., Trumel, C., and Bourgès-Abella, N. (2019). The effects of storage at 4 C and 20 C on the hemograms of C57BL/6 mice and Wistar rats using the IDEXX ProCyte Dx and blood smear evaluations. *Veterinary clinical pathology* **48**, 652-667.
Levental, K. R., Malmberg, E., Symons, J. L., Fan, Y.-Y., Chapkin, R. S., Ernst, R., and Levental, I. (2020). Lipidomic and biophysical homeostasis of mammalian membranes counteracts dietary lipid perturbations to maintain cellular fitness. *Nature communications* **11**, 1339.
Levin, J. (2019). The evolution of mammalian platelets. *In* "Platelets", pp. 1-23. Elsevier.
Li, M., Wang, C., Chen, Z., Xu, K., and Lu, J. (2020). New concepts in electrolytes. *Chemical reviews* **120**, 6783-6819.
Lim, E. K., Seow, Y.-y. T., Chen, S. E., Yang, G., Liaw, M. E., and Isaac, S. (2018). Simple citrate anticoagulation protocol for low flux haemodialysis. *BMC nephrology* **19**, 1-6.
Lima-Oliveira, G., Brennan-Bourdon, L., Varela, B., Arredondo, M., Aranda, E., Flores, S., and Ochoa, P. (2021). Clot activators and anticoagulant additives for blood collection. A critical review on behalf of COLABIOCLI WG-PRE-LATAM. *Critical Reviews in Clinical Laboratory Sciences* **58**, 207-224.
Makhro, A., Huisjes, R., Petkova-Kirova, P., Bogdanova, A., van Wijk, R., Vives-Corrons, J.-L., and Kaestner, L. (2016). Red cell properties after different modes of blood transportation. *Frontiers in physiology* **7**, 189952.
Mannuß, S. (2020). Influence of different methods and anticoagulants on platelet parameter measurement. *Journal of Laboratory Medicine* **44**, 255-272.
Mansell, C. L., and Boller, M. (2016). Blood component processing and storage. *Manual of veterinary transfusion medicine and blood banking*, 237-255.
Margraf, A., Liu, C., Küllmar, M., Meersch, M., Rossaint, J., and Zarbock, A. (2022). Analysis of leukocyte recruitment in continuous veno-venous hemofiltration with regional citrate vs. systemic heparin anticoagulation. *Cells* **11**, 1815.
Meledeo, M. A., Peltier, G. C., McIntosh, C. S., Bynum, J. A., and Cap, A. P. (2019). Optimizing whole blood storage: hemostatic function of 35-day stored product in CPD, CP2D, and CPDA-1 anticoagulants. *Transfusion* **59**, 1549-1559.
Melkonian, E. A., and Schury, M. P. (2019). Biochemistry, anaerobic glycolysis.
Mendhurwar, S. J. (2018). "Physiology: Prep Manual for Undergraduates," Elsevier Health Sciences.
Menta, P., Batchelder, T., and Neves, R. (2020). Effect of delayed analysis of cooled lithium-heparinized whole blood on the stability of ionized calcium, ionized magnesium, sodium, potassium, chloride, glucose, and lactate in samples from dairy cows. *Journal of dairy science* **103**, 5509-5513.
Moxon, R., Dutton Worsfold, R., Davis, J., Adams, W., and England, G. C. (2023). Luteal phase decrease in packed cell volume in healthy non-pregnant and pregnant bitches. *Veterinary Medicine and Science* **9**, 1989-1997.
Namisnak, L. H., Haghayegh, S., Khoshnevis, S., and Diller, K. R. (2022). Bioheat transfer basis of human thermoregulation: Principles and applications. *Journal of Heat Transfer* **144**, 031203.
Nussbaum, R., Robinson, K. J., Soda, Y., and Bakker, E. (2022). Optical detection of heparin in whole blood samples using nanosensors embedded in an agarose hydrogel. *ACS sensors* **7**, 3956-3962.
Okereke, N., Udegbunam, R., and Nnaji, T. (2019). Selected serum biochemical and electrolyte findings of splenectomised dogs transfused with whole blood, Isoplasma® and Haemaccel®. *Nigerian Veterinary Journal* **40**, 127-135.
Papakonstantinou, P. E., Tsioufis, C., Konstantinidis, D., Iliakis, P., Leontsinis, I., and Tousoulis, D. (2020). Anticoagulation in deep venous thrombosis: current trends in the era of non-vitamin K antagonists oral anticoagulants. *Current pharmaceutical design* **26**, 2692-2702.
Pearce, D., Manis, A. D., Nesterov, V., and Korbmacher, C. (2022). Regulation of distal tubule sodium transport: mechanisms and roles in homeostasis and pathophysiology. *Pflügers Archiv-European Journal of Physiology* **474**, 869-884.
Phillips, J., and Henderson, A. C. (2018). Hemolytic anemia: evaluation and differential diagnosis. *American family physician* **98**, 354-361.
Polychronopoulou, E., Braconnier, P., and Burnier, M. (2019). New insights on the role of sodium in the physiological regulation of blood pressure and development of hypertension. *Frontiers in Cardiovascular Medicine* **6**, 136.
Pretorius, E. (2018). Erythrocyte deformability and eryptosis during inflammation, and impaired blood rheology. *Clinical hemorheology and microcirculation* **69**, 545-550.

Putra, A. A. M. S., and Hernaningsih, Y. (2022). Comparison of complete blood count parameters using EDTA, sodium citrate, and heparin anticoagulants. *Research Journal of Pharmacy and Technology* **15**, 4687-4691.

Qiu, M., Huang, S., Luo, C., Wu, Z., Liang, B., Huang, H., Ci, Z., Zhang, D., Han, L., and Lin, J. (2021). Pharmacological and clinical application of heparin progress: An essential drug for modern medicine. *Biomedicine & Pharmacotherapy* **139**, 111561.

Rautaray, B., and Tripathy, S. (2018). Variation of CBC Parameters with Storage Time and Temperature. *International journal of science and research, IJSR* **7**.

Recktenwald, S. M., Simionato, G., Lopes, M. G., Gamboni, F., Dzieciatkowska, M., Meybohm, P., Zacharowski, K., von Knethen, A., Wagner, C., and Kaestner, L. (2022). Cross-talk between red blood cells and plasma influences blood flow and omics phenotypes in severe COVID-19. *Elife* **11**, e81316.

Reckziegel, P., Dias, V. T., Benvegnú, D. M., Boufleur, N., Barcelos, R. C. S., Segat, H. J., Pase, C. S., Dos Santos, C. M. M., Flores, É. M. M., and Bürger, M. E. (2016). Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver and kidney of rats. *Toxicology reports* **3**, 351-356.

Sakthipriya, B. (2019). Evaluation of the Level of Coagulation Factors V and VIII on Storing Fresh Frozen Plasma at Different Temperatures: A Study at Regional Blood Bank and CEmONC Centre, The Tamilnadu Dr. MGR Medical University, Chennai.

Scholman, R. C., Giovannone, B., Hiddingh, S., Meerding, J. M., Fernandez, B. M., van Dijk, M. E., Tempelman, M. J., Prakken, B. J., and de Jager, W. (2018). Effect of anticoagulants on 162 circulating immune related proteins in healthy subjects. *Cytokine* **106**, 114-124.

Schuff-Werner, P., Mansour, J., and Gropp, A. (2020). Pseudo-thrombocytopenia (PTCP). A challenge in the daily laboratory routine? *Journal of Laboratory Medicine* **44**, 295-304.

Seifter, J. L. (2019). Body fluid compartments, cell membrane ion transport, electrolyte concentrations, and acid-base balance. *In* "Seminars in nephrology", Vol. 39, pp. 368-379. Elsevier.

Sharif, F., Junaid, A., Ashraf, K., Ijaz, M., Saeed, M., Farhat, T., and Rehman, N. (2023). Evaluation of sodium citrate anticoagulant for the Resolution of edta-dependent pseudo Thrombocytopenia. *Journal of Ayub Medical College Abbottabad-Pakistan* **35**.

Sivertsen, J., Braathen, H., Lunde, T. H. F., Kristoffersen, E. K., Hervig, T., Strandenes, G., and Apelseth, T. O. (2020). Cold-stored leukoreduced CPDA-1 whole blood: in vitro quality and hemostatic properties. *Transfusion* **60**, 1042-1049.

Sugerman, G. P., Chokshi, A., and Rausch, M. K. (2021). Preparation and mounting of whole blood clot samples for mechanical testing. *Current Protocols* **1**, e197.

Thanasak, J., Tansatit, T., Taowan, J., Hirunwiroj, N., Chitthichanonte, S., and Wongmack, T. (2022). The peripheral blood mononuclear cells preparation and the hematology of Varanus salvator. *PLoS One* **17**, e0269108.

Ureña-Torres, P. A., Vervloet, M., Mazzaferro, S., Oury, F., Brandenburg, V., Bover, J., Cavalier, E., Cohen-Solal, M., Covic, A., and Drüeke, T. B. (2019). Novel insights into parathyroid hormone: report of The Parathyroid Day in Chronic Kidney Disease. *Clinical kidney journal* **12**, 269-280.

Varanita, S. V., Kahar, H., and Fitriah, M. (2022). Compatibility of two EDTA tubes for hemoglobin (Hb) hematocrit (Hct) tests with aptus equipment. *Research Journal of Pharmacy and Technology* **15**, 4447-4450.

Villa, C. H., Anselmo, A. C., Mitragotri, S., and Muzykantov, V. (2016). Red blood cells: Supercarriers for drugs, biologicals, and nanoparticles and inspiration for advanced delivery systems. *Advanced drug delivery reviews* **106**, 88-103.

Vincent, L. M., Allender, M. C., Talley, A., Davidson, A., Roy, L., Durante, K., Waligora, M., Sander, S. J., McEntire, M., and Schnelle, A. N. (2023). COMPARISON OF HEMATOLOGIC DIFFERENCES WITH LITHIUM HEPARIN AND DIPOTASSIUM ETHYLENEDIAMINETETRAACETIC ACID IN EUROPEAN STARLINGS (STURNUS VULGARIS). *Journal of Zoo and Wildlife Medicine* **54**, 538-544.

Wauthier, L., Favresse, J., Hardy, M., Douxfils, J., Le Gal, G., Roy, P., van Es, N., Ay, C., ten Cate, H., and Lecompte, T. (2023). D-dimer testing: A narrative.

Weisel, J., and Litvinov, R. (2019). Red blood cells: the forgotten player in hemostasis and thrombosis. *Journal of Thrombosis and Haemostasis* **17**, 271-282.

Wu, D.-w., Li, Y.-m., and Wang, F. (2017). How long can we store blood samples: a systematic review and meta-analysis. *EBioMedicine* **24**, 277-285.

Yazer, M. H., Cap, A. P., Spinella, P. C., Alarcon, L., and Triulzi, D. J. (2018). How do I implement a whole blood program for massively bleeding patients? *Transfusion* **58**, 622-628.

Yoshida, T., Prudent, M., and D'Alessandro, A. (2019). Red blood cell storage lesion: causes and potential clinical consequences. *Blood Transfusion* **17**, 27.

Yousef, H., Czupalla, C. J., Lee, D., Chen, M. B., Burke, A. N., Zera, K. A., Zandstra, J., Berber, E., Lehallier, B., and Mathur, V. (2019). Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1. *Nature medicine* **25**, 988-1000.

Zhao, Q., Stalin, S., Zhao, C.-Z., and Archer, L. A. (2020). Designing solid-state electrolytes for safe, energy-dense batteries. *Nature Reviews Materials* **5**, 229-252.

Zhou, J., Fabros, A., Lam, S. J., Coro, A., Selvaratnam, R., Brinc, D., and Di Meo, A. (2024). The stability of 65 biochemistry analytes in plasma, serum, and whole blood. *Clinical Chemistry and Laboratory Medicine (CCLM)*.

Zou, M.-Y., Nie, S.-P., Yin, J.-Y., and Xie, M.-Y. (2020). Ascorbic acid induced degradation of polysaccharide from natural products: a review. *International journal of biological macromolecules* **151**, 483-491.