Contents lists available at IOCS

Science Midwifery

journal homepage: www.midwifery.iocspublisher.org

"Unveiling cellular immunity: A study on diabetes mellitus patients with gangrene wounds"

Meri Meri[1], Lisna Mesisya F[2], Yane Liswanti[3]

[1,2,3]The Health Analyst Diploma Program, Faculty of Health Sciences, Bakti Tunas Husada University, Tasikmalaya, Indonesia

ARTICLE INFO

Article history:

Received Mar 24, 2024
Revised Mar 29, 2024
Accepted Apr 17, 2024

Keywords:

Celluler Immunity
Diabetes Mellitus
Gangrene
Patiens
Wounds

ABSTRACT

Diabetes mellitus experiences disruptions in the immune system, particularly in cellular components. Cells crucial in inflammatory conditions are represented by granulocytes and lymphocytes. However, further explanation is needed regarding the quantities of these cells, especially in diabetes mellitus with gangrene. The research method employed is a quantitative descriptive cross-sectional approach. The research population consists of diabetes mellitus patients with gangrene wounds. The sample size contributing to the study is 14 individuals, representing the total population. Exclusion criteria include patients with other systemic infections. The research findings reveal the frequency distribution of the average granulocyte cell count to be 65.47%, with a standard deviation (SD) of 8.52, a minimum value of 53.3%, and a maximum value of 83.4%. The average lymphocyte cell count is 25.7%, with an SD of 7.97, a minimum value of 10.8%, and a maximum value of 38.7%. In conclusion, the study indicates an increased granulocyte count in 4 individuals or 26.6%, while 10 individuals or 71.4% exhibit normal counts. Conversely, the normal lymphocyte count is observed in 11 individuals or 78.6%, whereas 3 individuals or 21.4% exhibit low counts.

This is an open access article under the CC BY-NC license.

Corresponding Author:

Meri,
The Health Analyst Diploma Program,
Faculty of Health Sciences,
Bakti Tunas Husada University,
Cilolohan 36, Tasikmalaya, 46115, Indonesia
Email: meri@universitas-bth.ac.id

INTRODUCTION

Diabetes Mellitus (DM) is a disease caused by a lack of control over blood glucose levels. Globally, 1 in 11 adults suffers from DM (90% of whom have type 2 DM) (Petrie et al., 2018); (Sapra & Bhandari, 2023). Complications of DM, especially type 2, can lead to inflammation affecting the circulatory system, digestive tract, pancreatic beta cells, liver, skeletal muscles, rendering them dysfunctional (Daryabor et al., 2020).

Foot ulcers in poorly controlled diabetic patients often become the most commonly occurring complication. This is frequently triggered by suboptimal blood sugar control, underlying neuropathy, peripheral vascular disorders, or insufficient foot care. This condition also often serves

as a primary trigger for osteomyelitis in the foot and lower limb amputation. Patients with diabetes mellitus may also experience severe atherosclerosis in the small blood vessels of the legs and feet, resulting in circulation disturbances. These disturbances are an additional cause of foot infections in diabetic patients. Because blood supply is hindered to the wound, the healing process is delayed, ultimately leading to necrosis and gangrene (Oliver et al., 2023).

The severity of this systemic inflammatory condition depends on the extent of damage to the immune system, particularly in several corporeal areas such as adipose tissue, pancreatic islets, endothelium, and circulating leukocytes (Lisco et al., 2021). Therefore, diabetes will increase patients' vulnerability to serious and prolonged infections (Daryabor et al., 2020). Leukocytes have 2 types of cells when distinguished based on granules, namely granulocytes and agranulocytes. Neutrophils are a type of granulocyte, while lymphocytes are a type of agranulocyte. Both of these cells play crucial roles in the inflammatory process. Granulocytes, including neutrophils and eosinophils, are key components in the immune response to bacterial infection and inflammation. In patients with diabetes, the function of granulocytes can be affected by chronic hyperglycemia, leading to a decrease in phagocytic ability and morphological changes (Ead & Armstrong, 2023).

The function of granulocytes, also known as polymorphonuclear leukocytes, is to perform the processes of phagocytosis, neutralization, and elimination of microorganisms, cellular debris, and other substances through mechanisms such as phagocytosis, degranulation, the formation of high concentrations of reactive oxygen species (ROS), and the formation of neutrophil extracellular traps (NETs). In conditions of diabetes mellitus, the activities of chemotaxis, phagocytosis, excessive release of reactive oxygen species, and granulocyte cell death at the wound site caused by high glucose levels experience a decrease (Geng et al., 2021)

Lymphocytes, such as T lymphocytes and B lymphocytes, play a crucial role in regulating immune responses, including responses to infections and wound healing processes. T lymphocytes, especially regulatory T cells (Tregs), have been shown to support the process of repair and regeneration of various types of tissues. Tregs can indirectly regulate regeneration by promoting neutrophil apoptosis, controlling helper T cells, and inducing macrophage polarization. Additionally, Tregs can also directly facilitate regeneration by stimulating local resident stem cells (Rehak et al., 2022). Lymphocytes, attracted by signaling chemicals produced at the wound site, such as CCL3, CCL4, and CCL5, are the last immune cells to enter the wound. Although historically T cells were thought to arrive late in the inflammatory process, recent research indicates that T cells are present at the rat wound within 24 hours of injury and persist for at least 30 days. CD4+ T cells are the subset of T cells most commonly found in the wound healing process (Short et al., 2022).

However, the condition of cellular immune cell counts, such as granulocytes and lymphocytes, particularly in the bloodstream, has not provided clear information in cases of diabetes mellitus with gangrene. In patients with diabetes mellitus and gangrene, dysfunction in granulocyte immune cells, especially neutrophils, leads to difficulties in wound healing (Wilkinson et al., 2020).

In the context of diabetes mellitus with gangrene, research that elucidates its impact on the quantity and function of immune cells, particularly granulocytes (neutrophils) and lymphocytes, in the bloodstream remains severely limited. Deeper knowledge regarding the roles and responses of immune cells in this condition could be crucial for understanding the underlying pathophysiological mechanisms and for developing more effective management strategies and interventions. Previous studies have measured the number of monocytes and lymphocytes in diabetes mellitus with diseased wounds. The results show that monocyte examination can see the results of wound healing, especially if there is an increase in the number of anti-inflammatory macrophage cells (M2), indicating that the wound healing process has occurred. Meanwhile, a decrease in the number of lymphocytes indicates worsening wound healing (Rehak et al., 2022). Other studies have measured the number of neutrophils and lymphocytes in diabetes mellitus

without gangrenous wounds, which shows that the results of measuring the number of cells are markers of inflammation, which is characterized by an increase in the value of the neutrophil lymphocyte ratio (NLR). This NLR marker is an indicator in controlling type 2 diabetes mellitus (Duman et al., 2019). Other studies related to granulocytes have been conducted in diabetes mellitus with ulcers, but using immunohitochemical methods on skin sections through biopsies. The results showed an increase in the number of granulocytes in skin biopsy results from the feet of patients with diabetes mellitus with ulcers (Galkowska et al., 2005). Therefore, further research focusing on the relationship between diabetes mellitus and gangrene and the alterations occurring in the immune system, particularly concerning the quantity and function of granulocytes and lymphocytes in the bloodstream, is needed.

RESEARCH METHOD

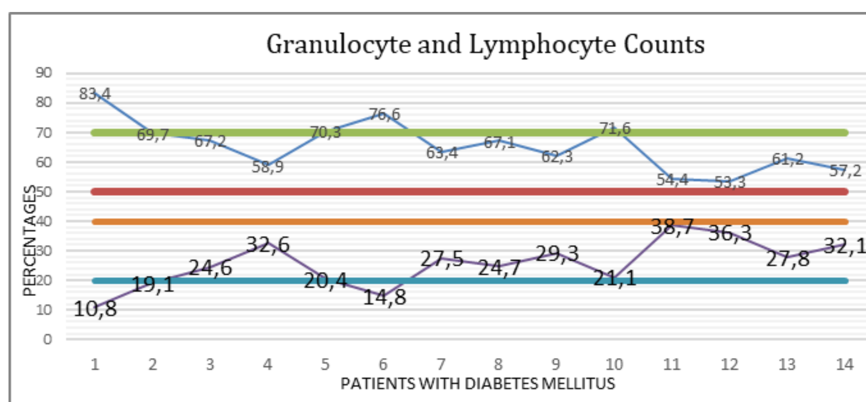
This study is descriptive in nature, utilizing primary data collected from blood tests conducted at the laboratory of Dr. Soekardjo Tasikmalaya Regional Hospital. The study included a total of 14 subjects, who were selected as the entire population. Inclusion criteria were patients with diabetes with gangrene wounds. Data were obtained using SPSS software, presenting frequency distribution, mean, minimum value, maximum value, and standard deviation (SD) in tabular form.

Research subjects provided informed consent and underwent venous blood collection, which was then examined using a hematoanalyzer. The obtained results, particularly the counts of granulocytes and lymphocytes, were recorded and compared with normal values for interpretation, determining whether they fell within normal, low, or high criteria. The normal criteria for granulocyte count is 50–70%; granulocyte count decreases if less than 50%; and granulocyte count increases if above 70%. While the number of lymphocytes is said to be normal if it has a value of 20.0–40.0%, it is said that the number of lymphocytes decreases if it is less than 20.0%, and the number of lymphocytes increases if it is more than 40.0%.

RESULTS AND DISCUSSIONS

The participants of this study were Diabetes Mellitus patients experiencing gangrene, who had several initial contributing factors such as abrasions, punctures, blisters, and stumbles. These wounds are difficult to heal due to dysfunction in granulocyte immune cells, such as neutrophils. (Berbudi et al., 2019) As a result, they are unable to heal wounds properly. The function of these cells should naturally aid in tissue repair, restoring the injured tissue to its original state. (Nguyen & Soulika, 2019).

The research findings can be observed in Figure 1, which demonstrates that granulocyte counts in diabetes mellitus patients with gangrenous wounds have values that are both normal and high compared to the normal range, which is 50.0-70.0%(Chapple et al., 2023), with the red line as the lower limit and the green line as the upper limit. The normal values used are in accordance with the criteria for normal values provided by the equipment used. Meanwhile, lymphocyte counts show low, normal, and high cell counts, referring to the normal lymphocyte count range of 20.0-40.0%, with the blue line as the lower limit and the orange line as the upper limit.



Ficture 1. Description of cellular immunology counts

In Ficture 1, the frequency distribution of each granulocyte and lymphocyte count is shown. The average granulocyte count is 65.47%, with a standard deviation (SD) of 8.52, a minimum value of 53.3%, and a maximum value of 83.4%. The average lymphocyte count is 25.7%, with an SD of 7.97, a minimum value of 10.8%, and a maximum value of 38.7%.

Based on the research findings in Table 1, the interpretation of granulocyte counts predominantly indicates a normal count, with 10 individuals or 71.4%, and an increase in 4 individuals or 26.6%. Neutrophils are one of the granulocyte types, being the main and most abundant leukocytes in the bloodstream. Neutrophils respond well to inflammation, leading to an increase in their count in the blood. The increase in granulocyte count in these 4 individuals is a response of granulocyte immune cells to inflammation in diabetes mellitus with gangrene. As a professional phagocyte, neutrophils can eliminate tissue debris at the wound site. The waste removal mechanism appears to be highly effective, as cellular debris is generally rare in physiological conditions. However, the process of identifying and removing cell debris by neutrophils is still under extensive investigation. Additionally, mature neutrophils possess over 700 proteins, including growth factors or pro-angiogenic factors, stored in their nuclei and segmented granules. Many of these proteins can be rapidly released upon activation without depending on transcription, thus directly contributing to the regeneration and reformation of blood vessels(Phillipson & Kubes, 2019). The most studied mechanism of neutrophil contribution to tissue healing is that neutrophils undergo apoptosis and are cleared by macrophages. This clearance process triggers a feed-forward pro-resolution program characterized by the release of cytokines that transform growth factor-β (TGFβ) and interleukin-10 (IL-10)(Raziyeva, 2021) to repair tissue. Therefore, drugs that stimulate neutrophil apoptosis have therapeutic potential to accelerate tissue healing (Wang, 2018).

Patients with gangrene in this study experienced wounds ranging from 2 weeks to 8 years. These patients were undergoing treatment and showed signs of wound improvement after treatment, resulting in a relatively normal granulocyte count. Similarly, with lymphocytes, which play a role as antibody producers when the body's first line of defense is not functioning properly. In diabetes mellitus with gangrenous wounds, some individuals experienced a decrease in lymphocyte count (21.4%). This is due to the adanya penurunan daya tahan tubuh pada pasien pada saat mengalami inflamasi yang ditandai dengan jumlah granulosit yang meningkat (26,6%).

Table 1. Results of statistics analysis of granulocyte and lymphocyte frequency distribution

		Granulocyte	Limfocyte
N	Valid	14	14
	Missing	0	0
Mean		65.471	25.700
Median		65.250	26.100

		Granulocyte	Limfocyte
N	Valid	14	14
	Missing	0	0
Std. Deviation		8.5241	7.9729
Minimum		53.3	10.8
Maximum		83.4	38.7

Table 1. shows the frequency distribution of each granulocyte and lymphocyte cell count. The average granulocyte cell count is 65.47%. The average granulocyte count is normal because the normal value is 50.0-70.0%. The SD value of the granulocyte count is 8.52, the minimum value is 53.3%, and the maximum value is 83.4%. In line with previous research, the number of granulocytes in patients with diabetic ulcers is normal on average (David G. Armstrong et al., 1996). Whereas in the research of Ulandari et al., (2023), the results of the number of granulocyte cells (neutrophils) showed an average increase. The difference in results can occur due to various factors, such as the size of the wound, blood glucose levels, the presence of a secondary infection or not, and many other factors.

The mean lymphocyte cell count was 25.7%. The average lymphocyte count is normal, as the normal value is 20.0–40.0%. The SD value of the lymphocyte count is 7.97, the minimum value is 10.8%, and the maximum value is 38.7%. The normal condition of the lymphocyte cell count indicates that the average person with diabetes mellitus with gangrene is in a normal immune system state; there is no immune cell dysfunction or abnormal cell count. However, there are some people with a decreased number of lymphocyte cells, which is in line with previous research by Ulandari et al., (2023) showing the number of lymphocytes has a decreased average value (1.74 ± 0.80 x 103 cells/ul). The decrease in the number of lymphocyte cells indicates a lack of body resistance to the condition of diabetes mellitus with gangrenous wounds.

Examination of granulocytes and lymphocytes is considered important because it is an indication of the condition of the body's immune system and disease prognosis, so at this time, laboratory examination parameters related to granulocytes (especially 95% neutrophil cells) (George-Gay & Parker, 2003) and lymphocytes are often used as a ratio value as a marker of inflammation. The previous study did not show the percentage of decreasing and increasing numbers. Whereas this study describes the percentage of granulocyte counts that decrease and increase, which can describe the neutrophil-lymphocyte ratio as a value or number for inflammation. Which is in line with research on NLR parameters as a marker of inflammation ((W. Chen & Chen, 2021; Arıcan et al., 2020); and Duman et al., 2019). On the other hand, previous studies used absolute cell counts, which means that cell counts are based on a total blood count of ×109/L (Zhang et al., 2021) or 103/μL (Ulandari et al., 2023), while this study uses cell counts based on percentage units of the number of cells measured from each 100 leukocyte cell types.

Table 2. Granulocyte statistics analysis results

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	High	4	28.6	28.6	28.6
	Normal	10	71.4	71.4	100.0
	Total	14	100.0	100.0	

Based on the results of the study in Table 2, the number of granulocyte cells has a dominant interpretation of being normal, namely 10 people, or 71.4%, which is found in patients with numbers 2, 3, 4, 7, 8, 9, 11, 12, 13, and 14. Patients also have an increase in the number of granulocytes in 4 people, or 26.6%, with code numbers 1, 5, 6, and 10. Cells that include granulocytes, one of which is the neutrophil; this cell is the main and most leukocyte cell in the blood circulation. Neutrophil cells can respond well to inflammation and cause neutrophil cells to increase in the blood. The increase in the number of granulocytes in these 4 people is the response

of granulocyte immune cells to inflammation in diabetes mellitus with gangrene. As professional phagocytes, neutrophils can remove tissue debris at the wound site. This waste removal mechanism seems to be very effective, as cellular debris is generally rarely found under physiological circumstances. However, the process of identification and removal of cell debris by neutrophils is still under deep investigation. In addition, mature neutrophils have more than 700 proteins, including growth factors or pro-angiogenic factors, stored in their segmented nuclei and granules. Many of these proteins can be released rapidly after activation without relying on transcription, thus directly contributing to the process of blood vessel regeneration and remodeling (Phillipson & Kubes, 2019). The most studied mechanism of neutrophil contribution to tissue healing is that neutrophils become apoptotic and are cleared by macrophages. This clearance process triggers a feed-forward pro-resolution program characterized by the release of cytokines transforming growth factor-β (TGFβ) and interleukin-10 (IL-10) to repair tissues. Therefore, drugs that stimulate neutrophil apoptosis have therapeutic potential to accelerate tissue healing (Wang, 2018). The gangrene patients in this study had wounds ranging from 2 weeks to 8 years. Patients with gangrene are undergoing treatment and have a condition of wound repair after treatment, so the number of granulocyte cells is relatively normal.

In table 2, the number of neutrophils increased by 26.6% and decreased by 0.0%. These results are in line with research from Duman et al., (2019) which states that the ratio of neutrophil lymphocytes (RNL) will increase in people with diabetes mellitus compared to healthy controls. The RNL ratio is the number of neutrophils (ganulocytes) divided by the number of lymphocytes, which is used as a marker of inflammation. Inflammatory conditions cause cytokines to increase and cause neutrophilia and lymphonenia, which together cause an increase in NLR (Duman et al., 2019).

Likewise with lymphocyte cells, which have a role as antibody producers if the body's first defense system does not play a role properly. The number of lymphocytes in people with diabetes mellitus with gangrenous wounds decreased in some people (21.4%) (Table 3). This is due to a decrease in the patient's immune system during inflammation, which is characterized by an increased number of granulocytes (26.6%).

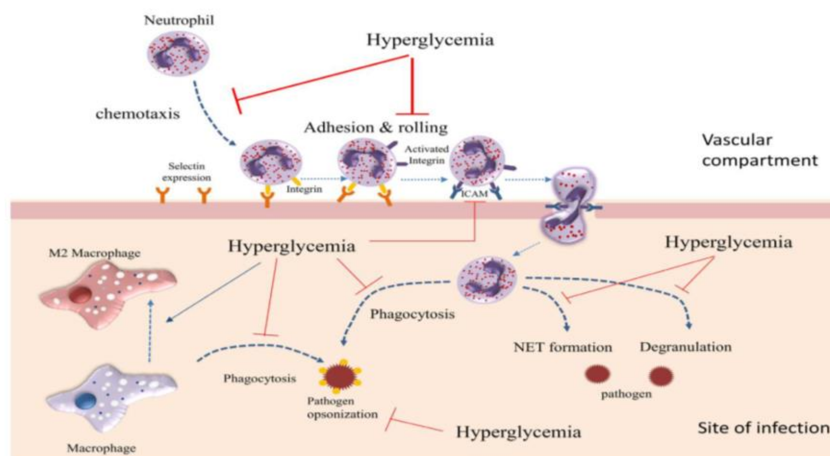
Table 3. Lymphocyte statistics analysis results

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal	11	78.6	78.6	78.6
	Low	3	21.4	21.4	100.0
	Total	14	100.0	100.0	

Based on the results of the study in Table 3, the number of lymphocyte cells has a dominant interpretation of being normal, namely 11 people, or 78.6%, which is found in patients with numbers 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, and 14. Patients also have a decrease in the number of lymphocytes, as many as 3 people, or 21.4%, with code numbers 1, 2, and 6. Type 2 diabetes mellitus occurs due to insulin resistance due to inhibition of insulin signaling, resulting in a series of immune responses that exacerbate the inflammatory state. Both innate immune response defects (including neutrophil and macrophage dysfunction) and adaptive immune response dysfunction, one of which is T cell lymphocytes, are considered responsible for the weak immune system against invading pathogens in diabetic subjects (Berbudi et al., 2019).

The immune response in diabetes mellitus with gangrene wounds is triggered by signals arising from the injury, be it damage-associated molecular patterns (DAMPs) released by necrotic cells and injured tissues or pathogen-associated molecular patterns (PAMPs) derived from bacterial components entering the wound. These PAMPs and DAMPs trigger the activation of resident immune cells such as mast cells, Langerhans cells, T cells, and macrophages by binding to pattern recognition receptors, initiating the inflammatory process. The release of pro-inflammatory

cytokines and chemokines then attracts leukocytes that flow to the injury site. Neutrophils, as granulocytes, are responsible for removing necrotic tissue and pathogens through phagocytosis (Peiseler & Kubes, 2019), as well as releasing reactive oxygen species (ROS), antimicrobial peptides, eicosanoids, and proteolytic enzymes. In addition, they are also able to capture and kill pathogens through extracellular traps, which are DNA networks coated with antimicrobial peptides and cytotoxic histones (Wilkinson et al., 2020).



Ficture 2. The Role of neutrophils in hyperglycemia (Berbudi et al., 2019)

In diabetes, the immune response is impaired due to insulin deficiency and hyperglycemia (L. Chen et al., 2018). Hyperglycemia is believed to weaken the activity of macrophages and other leukocytes. Neutrophils, which function to produce neutrophil extracellular traps (NETs)(Zhu et al., 2021)(Rodrigues et al., 2019)(Adib et al., 2022) (Ficture 2), will be inhibited during hyperglycemia, leading to vulnerability to infections.(Berbudi et al., 2019). Thus, patients with diabetes mellitus experiencing wounds will encounter difficulties in wound healing due to neutrophil dysfunction, resulting in prolonged healing time and susceptibility to infections, ultimately leading to gangrene.

In Table 1, it is indicated that the counts of granulocytes and lymphocytes are predominantly within the normal range, at 71.4% and 78.6% respectively. This normal condition is attributed to the treatment process undergone by patients with diabetes mellitus with gangrene, which helps to suppress excessive inflammation and restore the body's balance to normal.

CONCLUSION

Patients with diabetes mellitus with gangrene have neutrophil and lymphocyte counts that are predominantly within the normal range. In abnormal conditions, there are results in relatively small amounts. The results of the study can contribute to analyzing the presence of inflammation by measuring the number of immune cells, especially ganulocytes and lymphocytes, and knowing the prognosis. This study is expected to provide new insights into the management and care of patients with DM with gangrene and help develop more effective strategies for improving the immune response and wound healing process in these conditions. The shortcomings of this study are the relatively small number of samples, the different conditions of gangrenous wounds in each individual, and the lack of sorting of inclusion criteria for degenerative diseases. Suggestions for future research are the measurement of granulocyte/neutrophil and lymphocyte cell counts in diabetes mellitus and non-diabetes mellitus with and without complications of other degenerative diseases.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the LPPM Bakti Tunas Husada University for providing research funding, and to the students who participated in this study. May it be blessed so that this article can be beneficial to readers and contribute to further research knowledge.

References

Adib, Y., Bensussan, A., & Michel, L. (2022). Cutaneous Wound Healing: A Review about Innate Immune Response and Current Therapeutic Applications. *Mediators of Inflammation*, *2022*. https://doi.org/10.1155/2022/5344085

Arıcan, G., Kahraman, H. Ç., Özmeriç, A., İltar, S., & Alemdaroğlu, K. B. (2020). Monitoring the Prognosis of Diabetic Foot Ulcers: Predictive Value of Neutrophil-to-Lymphocyte Ratio and Red Blood Cell Distribution Width. *International Journal of Lower Extremity Wounds*, *19*(4), 369–376. https://doi.org/10.1177/1534734620904819

Berbudi, A., Rahmadika, N., Tjahjadi, A. I., & Ruslami, R. (2019). Type 2 Diabetes and its Impact on the Immune System. *Current Diabetes Reviews*, *16*(5), 442–449. https://doi.org/10.2174/1573399815666191024085838

Chapple, I. L. C., Hirschfeld, J., Kantarci, A., Wilensky, A., & Shapira, L. (2023). The role of the host—Neutrophil biology. *Periodontology 2000*, *April*, 1–47. https://doi.org/10.1111/prd.12490

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2018). IChen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9(6), 7204–7218. https://doi.org/10.18632/oncotarget.23208nflammatory respon. *Oncotarget*, *9*(6), 7204–7218.

Chen, W., & Chen, K. (2021). Neutrophiltolymphocyte-ratio-and-platelettolymphocyte-ratio-predict-mortality-in-patients-with-diabetic-foot-ulcers-undergoing-amputationsDiabetes-Metabolic-Syndrome-and-Obesity.pdf. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 821–829.

Daryabor, G., Atashzar, M. R., Kabelitz, D., Meri, S., & Kalantar, K. (2020). The Effects of Type 2 Diabetes Mellitus on Organ Metabolism and the Immune System. *Frontiers in Immunology*, *11*(July). https://doi.org/10.3389/fimmu.2020.01582

David G. Armstrong, Theresa A. Perales, Randall T. Murff, Gary W. Edelson, & John G. Welchon. (1996). Value of White Blood Cell Count With Differential in the Acute Diabetic Foot Infection. *Journal of the American Podiatric Medical Association*, *86*, 224–227.

Duman, T. T., Aktas, G., Atak, B. M., Kocak, M. Z., Erkus, E., & Savli, H. (2019). Neutrophil to lymphocyte ratio as an indicative of diabetic control level in type 2 diabetes mellitus. *African Health Sciences*, *19*(1), 1602–1606. https://doi.org/10.4314/ahs.v19i1.35

Ead, J. K., & Armstrong, D. G. (2023). Granulocyte-macrophage colony-stimulating factor: Conductor of the wound healing orchestra? *International Wound Journal*, *20*(4), 1229–1234. https://doi.org/10.1111/iwj.13919

Galkowska, H., Wojewodzka, U., & Olszewski, W. L. (2005). Original Research Articles – Basic Science Low recruitment of immune cells with increased expression of endothelial adhesion molecules in margins of the chronic diabetic foot ulcers. *Wound Repair and Regeneration*, *13*(3), 248–254. https://doi.org/https://doi.org/10.1111/j.1067-1927.2005.130306.x

Geng, K., Ma, X., Jiang, Z., Huang, W., Gao, C., Pu, Y., Luo, L., Xu, Y., & Xu, Y. (2021). Innate Immunity in Diabetic Wound Healing: Focus on the Mastermind Hidden in Chronic Inflammatory. *Frontiers in Pharmacology*, *12*(April), 1–19. https://doi.org/10.3389/fphar.2021.653940

George-Gay, B., & Parker, K. (2003). Understanding the complete blood count with differential. *Journal of Perianesthesia Nursing*, *18*(2), 96–117. https://doi.org/10.1053/jpan.2003.50013

Lisco, G., Giagulli, V. A., De Pergola, G., Guastamacchia, E., Jirillo, E., & Triggiani, V. (2021). Hyperglycemia-Induced Immune System Disorders in Diabetes Mellitus and the Concept of Hyperglycemic Memory of Innate Immune Cells: A Perspective. *Endocrine, Metabolic & Immune Disorders - Drug Targets*, *22*(4), 367–370. https://doi.org/10.2174/1871530321666210924124336

Nguyen, A. V., & Soulika, A. M. (2019). The dynamics of the skin's immune system. *International Journal of Molecular Sciences*, *20*(8), 1–53. https://doi.org/10.3390/ijms20081811

Oliver, T. I., Mutluoglu., M., & Author. (2023). Diabetic foot ulcer. *NCBI Bookshelf. A Service of the National Library of Medicine, National Institutes of Health.*, 1–7. https://doi.org/10.12968/jowc.2020.29.Sup10a.S17

Peiseler, M., & Kubes, P. (2019). More friend than foe: The emerging role of neutrophils in tissue repair. *Journal of Clinical Investigation*, *129*(7), 2629–2639. https://doi.org/10.1172/JCI124616

Petrie, J. R., Guzik, T. J., & Touyz, R. M. (2018). Diabetes, Hypertension, and Cardiovascular Disease: Clinical Insights and Vascular Mechanisms. *Canadian Journal of Cardiology*, *34*(5), 575–584. https://doi.org/10.1016/j.cjca.2017.12.005

Phillipson, M., & Kubes, P. (2019). The Healing Power of Neutrophils. *Trends in Immunology*, *40*(7), 635–647. https://doi.org/10.1016/j.it.2019.05.001

Raziyeva, K. (2021). Immunology of acute and chronic wound healing. In *Biomolecules* (Vol. 11, Issue 5). https://doi.org/10.3390/biom11050700

Rehak, L., Giurato, L., Meloni, M., Panunzi, A., Manti, G. M., & Uccioli, L. (2022). The Immune-Centric Revolution in the Diabetic Foot: Monocytes and Lymphocytes Role in Wound Healing and Tissue Regeneration—A Narrative Review. *Journal of Clinical Medicine*, *11*(3). https://doi.org/10.3390/jcm11030889

Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2019). Wound healing: A cellular perspective. *Physiological Reviews*, *99*(1), 665–706. https://doi.org/10.1152/physrev.00067.2017

Sapra, D. A., & Bhandari, P. (2023). Diabetes. In *NCBI* (pp. 1–10). StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK559263/

Short, W. D., Wang, X., & Keswani, S. G. (2022). The Role of T Lymphocytes in Cutaneous Scarring. *Advances in Wound Care*, *11*(3), 121–131. https://doi.org/10.1089/wound.2021.0059

Ulandari, R., Kurniawan, L. B., Nurahmi, N., & Muhadi, D. (2023). Analysis of NLR in Type 2 Diabetes Mellitus with and without Diabetic Foot Ulcer. *Indonesian Journal of Clinical Pathology and Medical Laboratory*, *29*(2), 185–188. https://doi.org/10.24293/ijcpml.v29i2.1971

Wang, J. (2018). Neutrophils in tissue injury and repair. *Cell and Tissue Research*, *371*(3), 531–539. https://doi.org/10.1007/s00441-017-2785-7

Wilkinson, H. N., Hardman, M. J., & Wilkinson, H. N. (2020). Wound healing : cellular mechanisms and pathological outcomes. *Open Biology*, *10*, 1–14. https://doi.org/http://dx.doi.org/10.1098/rsob.200223

Zhang, K., Ding, S., Lyu, X., Tan, Q., & Wang, Z. (2021). Clinical Laboratory Analysis - 2021 - Zhang - Correlation between the platelet-to-lymphocyte ratio and diabetic foot ulcer.pdf. *J Clin Lab Anal.*, 1–6. https://doi.org/https://doi.org/10.1002/jcla.23719 wileyonlinelibrary.com/journal/jcla

Zhu, S., Yu, Y., Ren, Y., Xu, L., Wang, H., Ling, X., Jin, L., Hu, Y., Zhang, H., Miao, C., & Guo, K. (2021). The emerging roles of neutrophil extracellular traps in wound healing. *Cell Death and Disease*, *12*(11), 1–9. https://doi.org/10.1038/s41419-021-04294-3