Original Article :

Cytokine Profiles and Neutrophil Responses in Varying Severities of Streptococcus Pneumoniae Infections

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Received on Nov 12, 2024, accepted for publication on Feb 23, 2025, doi: 10.5455/mjhs.2025.03.007

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Abstract

Background and Aims:
Streptococcus pneumoniae is a significant global health threat in infants, causing high morbidity and mortality. This study analyzed the inflammatory markers TNF-α, IL-6, and IL-8 to assess immune responses in children under 5 years attending Nnamdi Azikiwe University Teaching Hospital.

Methods:
Serum cytokine levels were compared among control participants, outpatients with mild infections, and hospitalized patients with severe infections. Oxidative burst activity of leukocytes exposed to S. pneumoniae was assessed using nitro blue tetrazolium (NBT) staining.

Result:
Inflammatory markers were markedly elevated in severely infected patients compared to mildly infected and control groups. Leukocyte activity against S. pneumoniae was confirmed by the formation of formazan crystals during NBT reduction.

Conclusion:
The study highlights a strong link between infection severity and cytokine elevation, emphasizing their role in the pathogenesis of pneumococcal infections and potential as biomarkers for disease progression.

Key words:
Inflammatory markers, Streptococcus pneumoniae, infection, leucocytes

Introduction:

Pneumococcal infection is the most common cause of upper respiratory diseases and mortality in children under 5 years of age and other vulnerable groups across the global[1]. Leukocytes, especially neutrophils, are front-line immune cells that are mobilized to the site of infection to engulf pathogens or release antimicrobial chemicals that destroy the pathogens[2]. Leukocytes produce cytokines, which are signalling proteins that play important roles in cellular communication, especially in the immune system. Different types of leukocytes, such as monocytes, macrophages, lymphocytes, and dendritic cells, can regulate the immune response and other cell activities.[3, 4, 5]

Interleukin-6 (IL-6) is a multifunctional cytokine that plays crucial roles in the immune system and other physiological processes. It is produced by various cells, including T cells, B cells, fibroblasts, macrophages, and endothelial cells, in response to infections and tissue injuries[6]. IL-6 is a proinflammatory cytokine that contributes to both acute and chronic inflammation. It induces the production of acute-phase proteins (e.g., C-reactive protein), which are important markers of inflammation, in the liver[7]. It acts on the hypothalamus and induce fever, which is a common feature of inflammatory responses. Elevated IL-6 levels are associated with a variety of inflammatory diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and inflammatory bowel disease[8]. Chronic elevation of IL-6 is also associated with metabolic disorders, such as type 2 diabetes and obesity[8, 9].

Interleukin-8 (IL-8), also known as CXCL8, is a cytokine that plays a significant role in the immune response, particularly in inflammation and the chemotaxis of immune cells[10]. IL-8 is a potent chemoattractant, which means that it attracts immune cells to the site of infection or inflammation. Its primary role is to recruit neutrophils but it also attracts other immune cells, such as basophils and T cells. It helps initiate and sustain the inflammatory response by guiding these cells to sites of tissue damage or infection. It activates neutrophils and increase their ability to adhere to the endothelium, migrate through tissues, and perform functions such as phagocytosis and the release of granules containing antimicrobial proteins[11]. Elevated levels of IL-8 are associated with various inflammatory conditions such as inflammatory bowel disease, rheumatoid arthritis, and chronic obstructive pulmonary disease (COPD). In these diseases, excessive IL-8 can lead to persistent inflammation and tissue damage. IL-8 levels increase during bacterial infections as part

of the body's defense mechanism to recruit neutrophils to the infection site[12]. However, excessive IL-8 can contribute to detrimental inflammation, as evident in severe infections and sepsis. IL-8 production is induced by various stimuli, like physical stress, bacterial products, and proinflammatory cytokines. The regulation of IL-8 is crucial for balancing its beneficial roles in immune defense and wound healing, with its potential to cause excessive inflammation and tissue damage[13].

Tumor necrosis factor alpha (TNF-alpha) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. TNF-alpha is a potent mediator of inflammation. It is primarily produced by activated macrophages but also by other cells, such as NK cells, T cells, and certain tumor cells. It plays a crucial role in the immune system's response to infection by promoting inflammation, which helps to contain and clear pathogens. TNF-alpha binds to its receptors (TNFR1 and TNFR2) on the surface of target cells, thereby triggering various intracellular signalling pathways. These pathways can lead to diverse outcomes, which include apoptosis, cell survival, and necroptosis[12]. It is a key regulator of the acute phase reaction, a rapid inflammatory response that provides immediate defense against infections. It stimulates the production of acute-phase proteins by the liver, which help opsonize pathogens and activate other immune responses. It enhances the activity of other immune cells, including macrophages and neutrophils, by promoting their migration to sites of infection or injury and increasing their phagocytic activity. It also acts on the hypothalamus and induces fever, which is a common systemic response to infection and inflammation[14]. Fever can inhibit the growth of certain pathogens and enhance the immune response. Elevated levels of TNF-alpha are associated with a variety of inflammatory and autoimmune diseases. TNF-alpha production is highly regulated and can be induced by various stimuli, such as endotoxins, infections, and other proinflammatory cytokines[10].

Cytokines are involved in several key aspects of body immunity during infections. IL-8 and MCP-1 are secreted in response to S. pneumoniae infection and act to recruit neutrophils, monocytes, and other immune cells to the infection site. These immune cells attack and kill bacteria[15]. TNF-α can trigger the recruitment of T cells to more effectively target and destroy bacterial pathogens[16]. They can increase the ability of immune cells to swallow and remove pathogenic bacteria. This process is known as phagocytosis[15]. The aim of this study was to enhance our understanding

of the innate immune mechanisms underpinning pneumococcal infections, with a specific focus on cytokine profiles and neutrophil activities that influence disease progression and host immune defense.

Method:

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Approval was obtained from the Nnamdi Azikiwe University Teaching Hospital Ethics Committee (reference number NAUTH/66/VOL.16/VER. 3/318/2023/85), written consent was obtained from the parents and caregivers of all study participants after we thoroughly explained the objectives and purpose of the study to them. S. pneumoniae was isolated from a 5-year-old child attending the clinic at Nnamdi Azikiwe University Teaching Hospital, Nnewi. The isolate was identified via standard biochemical tests, such as Gram staining, catalase tests, optochin disc tests and bile solubility tests, as described by Cheesbrough[17].

Determining some of the key features of the innate response to pneumococcal infections

To investigate the relationships between S. pneumoniae infection and serum cytokines, tumor necrosis factor-α (TNF-α), interleukin-8 (IL-8), and interleukin-6 (IL-6) levels in the serum of 5 control participants, 5 outpatients with mild pneumococcal infection and 5 hospitalized patients with severe pneumococcal infection were measured via ELISA. After blood collection and serum extraction via centrifugation, the wells of microtiter plates were coated with the captured antibodies specific for TNF-α, IL-8, and IL-6 and incubated at 35 °C for one hour and 30 minutes. The samples were then washed with phosphate-buffered saline (PBS). Horseradish peroxidase, which is a detection enzyme, was added before the addition of tetramethylbenzidine (TMB), which acted as a substrate. The optical density was measured via a spectrophotometer. A standard curve with known cytokine concentrations was used to convert the absorbence to pg/mL, and the cytokine concentration for each study participant was calculated. The mean values and standard deviations of these serum biomarkers were calculated.

Evaluation of reactive oxygen specie (ROS)

Neutrophil isolation

Five (5) healthy children younger than 60 months whose parents/guidance provided written consent for participation in the study were recruited. Subjects who were on antibiotics or recently used non-steroidal painkillers were not included in the study. To isolate neutrophils, samples of peripheral blood were collected from the study participants and diluted with

PBS at a 1:1 ratio. The diluted blood was then centrifuged at 2000 revolutions per minute for twenty minutes at 35 ˚C. The plasma layers were discarded to isolate the RBC pellets with granulocytes. These pellets were promptly resuspended in 2 milliliters of PBS for further processing. A 6% dextran solution was added to separate the neutrophils from the RBC sediment. The supernatant rich in neutrophils was collected and subjected to centrifugation for 10 minutes. RBC lysis was subsequently performed to eliminate any remaining RBCs, resulting in a supernatant and a white pellet containing granulocytes. To assess the cell count and purity of the neutrophils, freshly isolated neutrophils were dyed with 0.4% trypan blue and assessed via an automated cell counter as described by Baisya et al.[18]. Neutrophil viability was determined via ELISA.

Nitroblue tetrazolium (NBT) reduction

To prepare a 0.1% nitroblue tetrazolium solution, 1 mg of NBT powder was dissolved in 10 mL of PBS, followed by filtration through 0.2 mm filter paper. The NBT assay was conducted using freshly isolated neutrophils (1x106 cells) suspended in PBS. A total of 0.1 ml of pure cell culture, which is equivalent to 0.5 McFarland standard, was added, and the mixture was incubated at 37˚C for fifteen minutes and held at 35˚C for another fifteen minutes, as described by Gooty et al [19]. A separate suspension of cells without 0.1 ml of pure cell culture was also incubated for the same duration. A smear on a clean glass slide was made before adding methanol to fix it for three minutes. The slides were then stained with Pappenheim's stain for no more than 5 minutes. Both slides were then examined under a 40X microscope.

Results:

Immunological markers

For all the inflammatory markers under study, the mean values and standard deviations were noticeably greater in the severely infected group (severely sick and hospitalized) than in the mildly infected group (positive for S. pneumoniae in blood culture but no obvious sign of sickness) and the control group (negative for S. pneumoniae in blood culture with no sign of sickness). The mean values for TNF-α, IL-8, and IL-6 in the infected group were 46.26 pg/ml, 264.5 pg/ml, and 20.62 pg/ml, respectively. For the mildly infected group, the TNF-α, IL-8, and IL-6 levels were 36.7 pg/ml, 118.74 pg/mol, and 13.28 pg/ml, respectively. The values for the mildly infected group were significantly greater than those for the control group. The values of TNF-α, IL-8, and IL-6 in the control groups were 17.32 pg/ml, 33 pg/ml, and 3.26 pg/ml, respectively. These

are represented in Figure 1 below.

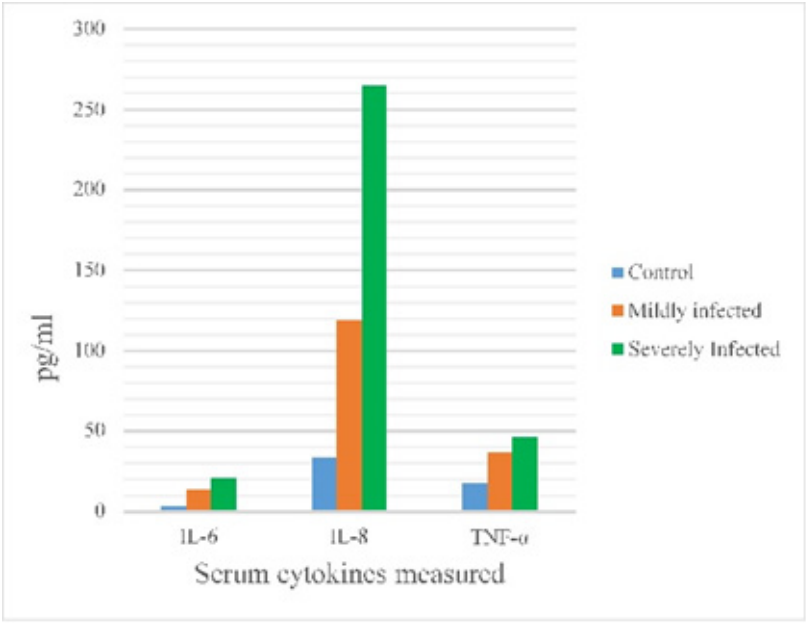


Figure 1: Mean serum levels of TNF-α, IL-8, and IL-6 in the study population

NBT reduction

The NBT assay indicated the presence of superoxide radicals generated by leukocytes upon interaction with S. pneumoniae. The formation of water-insoluble formazan crystals, represented by a purple–blue color, demonstrated the reduction of NBT by lymphocytes in response to S. pneumoniae. An increase in the intensity of the turquoise-blue color in the cells, as measured by a microplate reader at 595 nm, indicated elevated ROS production by leukocytes in the presence of S. pneumoniae. Neutrophils that took up the NBT dye and expressed an oxidative burst produced large blue–black deposits of formazan in the cytoplasm. On the basis of the morphological examination, over 96% of the isolated cells were viable neutrophils. The mean value of neutrophils stimulated with S. pneumoniae was significantly greater (0.96±0.05) (p=0.34) than that of the basal controls (0.42±0.02) (p=0.68), as represented in Table 1 below.

Table 1: Basal and S. pneumoniae-stimulated NBT reduction (mean ± SD).

Participant	NBT (Basal)	NBT (Stimulated)
1	0.46	1.01
2	0.39	0.88
3	0.42	0.92
4	0.43	1.02
5	0.42	0.98
Mean ± SD	0.42 ±0.02	0.96 ±0.05
P-value	0.6754	0.3395

Discussion:

For all the inflammatory markers observed, the mean values and standard deviations were noticeably greater in the severely infected group than in the mildly infected and control groups. This finding is consistent with several other studies, which reported an elevation of cytokine inflammatory markers in the infected population compared with the control group[20, 21, 22, 23]. Compared with those in mildly infected patients, immune marker levels were also greater among severely infected patients. Other studies have reported similar increases in TNF-α, IL-8, and IL-6 among severely infected humans and laboratory animals[24, 25]. The elevated inflammatory markers in the severely infected group indicate a robust immune response attempting to combat the infection, which may also contribute to the observed pathology in severe cases. For example, TNF-α, IL-8, and IL-6 are key cytokines involved in the inflammatory response, and their increased levels indicate an active but possibly dysregulated immune response, which can lead to tissue damage and worsening of the disease. Elevated levels of inflammatory markers in severely infected individuals suggest that these markers can be utilized as diagnostic tools to assess the severity of infections. This can help healthcare providers to identify high-risk patients who require more intensive care or targeted therapies.

NBT staining is a technique used to assess the functionality of phagocytes, particularly neutrophils. These phagocytes are critical members of the immune system responsible for engulfing and destroying invading S. pneumoniae and other pathogens[26]. When neutrophils encounter bacteria, they undergo a process called the respiratory burst, during which they produce ROS as part of their antimicrobial activity[27, 28]. NBT staining exploits this process by detecting the reduction of NBT to formazan, a blue–purple insoluble compound, generated by the ROS during the respiratory burst. This allows visual identification and quantification of the activity of neutrophils[29].

In this study, we detected an increase in NBT staining, which suggests robust neutrophil activity against S. pneumoniae. The use of NBT for the staining of leucocytes allows visualization of the results via light microscopy[30, 31]. The approximate viability and purity of neutrophils for the study participants were 95% and 98% respectively. This result agrees with a similar study that employed the same staining techniques to visualize neutrophils[18]. NBT penetrated the cell cytoplasm and reacted with superoxide radicals to form water-insoluble formazan crystals, which manifest-

ed as a purple–blue color, indicating the ability of neutrophils to reduce NBT. This further suggests that NBT staining can be used to monitor the efficacy of treatments aimed at enhancing the immune response against S. pneumoniae, such as antibiotics or immunomodulatory agents[32]. Changes in NBT staining over time can become more invaluable in providing data about the progression of infection and the effectiveness of treatment. NBT staining can be an important diagnostic tool for identifying patients with chronic granulomatous disease (CGD), which is a genetic disorder that affects the NADPH oxidase complex in phagocytes and leads to reduced ROS production and compromised bacterial killing. In patients with CGD, the NBT test results in reduced or no production of formazan, indicating the diagnostic utility of this assay[32]. The increased use of NBT staining as a diagnostic tool can improve the identification and assessment of neutrophil functionality in patients with infections such as S. pneumoniae. This can lead to earlier and more accurate diagnoses, particularly in resource-limited settings, improving patient outcomes and reducing the spread of infection[33]. The NBT test is a simple, cost-effective diagnostic tool suitable for early detection and management of infections, especially in low-resource settings. It also aids in monitoring treatment effectiveness, enabling healthcare providers to adjust therapies and reduce antibiotic resistance[34].

In this study, we used both NBT stain and Pappenheim's stain together. NBT staining specifically assesses the functional activity of neutrophils by detecting ROS produced during the respiratory burst. Pappenheim's stain, a combination of May-Grünwald and Giemsa stains, provides detailed morphological information about cells, including the differentiation of various leukocytes[35]. Using both stains together allows for a comprehensive assessment of neutrophil function and morphology, and offers a more complete picture of the immune response. The combination of functional and morphological evaluation can improve diagnostic accuracy. NBT staining reveals the capacity of neutrophils to produce ROS, whereas Pappenheim's stain highlights structural abnormalities and the presence of different cell types, aiding in the diagnosis of conditions such as CGD and other immunodeficiencies. Pappenheim's stain enables detailed differentiation of blood cells, aids in understanding immune responses and identifying abnormalities. Combined with NBT staining, it provides a comprehensive view of neutrophil structure and function, thereby offering valuable insights into infection pathophysiology and immune disorders[36].

Conclusion:

Cytokines and neutrophil are critical in the innate immune response to S. pneumoniae infections. The findings of this study contribute to the understanding of host-pathogen interactions, with implications for improving therapeutic strategies and vaccine development. Future research should focus on the interplay between cytokines and adaptive immunity, the impact of genetic variations, and the dynamics of co-infections, to provide a foundation for innovative approaches to mitigate pneumococcal disease.

Ethical approval statement:

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References:

1. Cillóniz C, Dominedò C, Garcia-Vidal C, Torres A. Community-acquired pneumonia as an emergency condition. Curr Opin Crit Care. 2018;24(6):531-539.
2. Monteith AJ, Miller JM, Beavers WN, Maloney KN, Seifert EL, Hajnoczky G, et al. Mitochondrial Calcium Uniporter Affects Neutrophil Bactericidal Activity during Staphylococcus aureus Infection. Infect Immun. 2022;90(2):e0055121.
3. Vezzani A, Viviani B. Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. Neuropharmacolog.y 2015;96:70–82.
4. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nat Immunol 2015;16:448–57.
5. Hirayama D, Iida T, Nakase H. The phagocytic function of macrophage-enforcing innate immunity and tissue homeostasis. Int J Mol Sci. 2017;19(1):92.
6. Scheller J, Garbers C, Rose-John S. Interleukin-6: from basic biology to selective blockade of pro-inflammatory activities. Semin Immunol. 2014;26(1):2–12.
7. Silva NM, Gonçalves RA, Pascoal TA, Lima-Filho RAS, Resende EPF, Vieira ELM, et al. Pro-inflammatory interleukin-6 signalling links cognitive impairments and peripheral metabolic alterations in Alzheimer's disease. Transl Psychiatry. 2021;11(1):251.
8. Heink S, Yogev N, Garbers C, Herwerth M, Aly L, Gasperi C, et al. Trans-presentation of IL-6 by dendrit-

ic cells is required for the priming of pathogenic TH17 cells. Nat Immunol. 2017;18(1):74–85.

9. Choy EH, De Benedetti F, Takeuchi T, Hashizume M, John MR, Kishimoto T, et al. Translating IL-6 biology into effective treatments. Nat Rev Rheumatol. 2020;16:335–45.
10. Campbell LM, Maxwell PJ, Waugh DJ. Rationale and means to target proinflammatory interleukin-8 (CXCL8) signalling in cancer. Pharmaceuticals (Basel) 2013;6:929–959.
11. Rasool M, Natesan Pushparaj P, Karim S. Saudi. Overexpression of CXCL8 gene in Saudi colon cancer patients. J Biol Sci. 2021;28:6045–6049.
12. Tsai CW, Chang WS, Yueh TC, Wang YC, Chin YT, Yang MD, et al. The Significant Impacts of Interleukin-8 Genotypes on the Risk of Colorectal Cancer in Taiwan. Cancers (Basel). 2023;15(20):4921.
13. Najdaghi S., Razi S., Rezaei N. An overview of the role of interleukin-8 in colorectal cancer. Cytokine. 2020;135:155205.
14. Bazzichetto C., Milella M., Zampiva I., Simionato F., Amoreo C.A., Buglioni S., et al. Interleukin-8 in Colorectal Cancer: A Systematic Review and Meta-Analysis of Its Potential Role as a Prognostic Biomarker. Biomedicines. 2022;10:2631
15. Recalde G, Moreno-Sosa T, Yúdica F, et al. Contribution of sex steroids and prolactin to the modulation of T and B cells during autoimmunity. Autoimmun Rev. 2018;17(5):504–512.
16. Sun L., Middleton D. R., Wantuch P. L., Ozdilek A., Avci F. Y. Carbohydrates as T-Cell Antigens with Implications in Health and Disease. Glycobiology 2022;26:1029–1040.
17. Cheesbrough, M. District Laboratory Practice in Tropical Countries. Part 2, 2nd Edition, Cambridge University Press Publication, South Africa, 2006;1-434
18. Baisya R, Katkam SK, Sreejitha KS, Devarasetti PK, Kutala VK, Rajasekhar L. Evaluation of NADPH Oxidase (NOX) Activity by nitro blue tetrazolium (NBT) Test in SLE Patients. Mediterr J Rheumatol. 2023;34(2):163-71.
19. Gooty JR, Shashirekha A, Guntakala VR, Palaparthi R. Estimation of phagocytic activity of polymorphonuclear leukocytes in chronic and aggressive periodontitis patients with nitroblue tetrazolium test. J Indian Soc Periodontol. 2019;23(4):316-321.
20. Narazaki M, Kishimoto T. The two-faced cytokine IL-6 in host defence and diseases. Int J Mol Sci. 2018;19:e3528
21. Graham RM, Paton JC. Differential

roles of CbpA and PspA in the modulation of the in vitro CXC chemokine response of respiratory epithelial cells to Streptococcus pneumoniae infection. Infect Immun. 2006;74(12):6739-6749

22. Kirby AC, Raynes JG, Kaye PM.The role played by tumor necrotic factor during localized and systemic infection with Streptococcus pneumoniae. J Infect Dis. 2005;191:1538-1547
23. Tsao N, Chang WW, Liu CC, Lei HY. Development of hematogenous pneumococcal meningitis in adult mice: the role of TNF-alpha. FEM Immunol Med Microbiol. 2002;32:133-140.
24. Kung E, Coward WR, Neill DR, Malak HA, Muhlemann K,Kadioglu A, et al. The pneumococcal polysaccharide capsule and pneumolysin differentially affect CXCL8 and IL-6 release from cells of the upper and lower respiratory tract. PloS One. 2014;9(3):e92355.
25. Ortqvist AI, Hedlund J, Wretlind B, Carlstrom A, Kalin M. Diagnostic and prognostic value of interlukin- 6 and C-reactive protein in community acquired pneumonia. Scand J Infect Dis. 1995 27:457-62.
26. Kalyanaraman, B., Darley-Usmar, V., Davies, K. J., Dennery, P. A., Forman, H. J., Grisham, M. B., et al. Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. Free Radic. Biol. Med. 2012;52:1–6.
27. Stockert, J. C., Horobin, R. W., Colombo, L. L., and Blázquez-Castro, A. Tetrazolium salts and formazan products in cell biology: viability assessment, fluorescence imaging, and labelling perspectives. Acta Histochem. 2018;120:159–167.
28. Esfandiari, N., Sharma, R. K., Saleh, R. A., Thomas, A. J. Jr., Agarwal, A. Utility of the nitroblue tetrazolium reduction test for assessment of reactive oxygen species production by seminal leukocytes and spermatozoa. J. Androl. 2003;24:862–887.
29. Campbell TW, Appendix A. Stains and solutions used in hematology and cytology. In: TW Campbell, ed. Exotic Animal Hematology and Cytology. 4th ed. Wiley Blackwell; 2015:377-381.
30. Saha, M. Hossain, G. Suresh Kumar. Sequence-selective binding of phenazinium dyes phenosafranin and safranin O to guanine-cytosine deoxyribopolynucleotides: spectroscopic and thermodynamic studies, J. Phys. Chem. B 2010;114(46):15278–15287.
31. Antonio MB, Alma YA, Edgar IM, Rubi CC, Hector AV, Junior AH, et al. A novel methodology for NETs visualization under light microscopy. Biochemistry and Biophysics Reports.

2023;34:101437.

32. Tunc O., Thompson J., Tremellen K. Development of the NBT assay as a marker of sperm oxidative stress. Int. J. Androl. 2010;33:13–21.
33. Wang G, Pierangeli SS, Papalardo E, Ansari GA, Khan MF. Markers of oxidative and nitrosative stress in systemic lupus erythematosus: correlation with disease activity. Arthritis Rheum. 2010;62(7):2064-72.
34. Berridge MV, Herst PM, Tan AS. Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. Biotechnol Annu Rev 2005;11:127–152.
35. Kim MJA, André PR, Ingrid MCE, Jan WW, Paul JWD. A Patient with Multiorgan Failure and Fusiform Rod-Shaped Bacteria in the Blood Smear, Clinical Infectious Diseases. 2018;67(7):1139–1141
36. Javorniczky, N.R., Grishina, O., Hund, I. Long-term decitabine/retinoic acid maintenance treatment in an elderly sAML patient with high-risk genetics. Clin Epigenet 2023;15: 185