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LYMPHOCYTE SCATTERGRAM PROFILE IN THE ELDERLY WITH BONE PAIN AND ANEMIA

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ABSTRACT

Old age according to the World Health Organization in 2017, is someone who has entered the age of 60 years and over. Due to various aging processes that occur in the elderly, the elderly will experience many complaints, one of which is bone pain and anemia. Various conditions in the elderly can cause bone pain and anemia, including: osteoporosis, osteomalacia, renal osteodystrophy, osteonecrosis, malignancy or bone metastases. From these conditions, a lymphocyte profile can be seen using a white blood cell differential scattergram in areas A, B, C, D, and E, according to the purpose of the study, wanted to know the scattergram profile of lymphocytes in the elderly with bone pain and anemia. This research method uses descriptive observation with a cross-sectional design method. The study was conducted from February-June 2020. The research subjects were elderly patients who experienced bone pain and anemia. Bone pain was measured by the Numeric Rating Scale on a scale of 1-10. Anemia was measured by examining hemoglobin on a hematology analyzer, then scattergram analysis was carried out through WDF using Sysmex XN 1000. The results of this study showed that the scattergram profile of lymphocytes in most elderly subjects with bone pain and anemia was in the SSC (A2, A3), SFL area. (B2, C2, D3, E3), which means that there are many changes in the lymphocyte profile, more atypical lymphocyte cells and suspicion of abnormal plasma cells or lymphocyte cells.

INTRODUCTION

Elderly (elderly) according to the World Health Organization (WHO) in 2017, is someone who has entered the age of 60 years and over. Research on age-related changes is an area of interest and importance in recent times. The elderly often complain of pain, pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. (Kemenkes, 2017).

One of the most common pain complaints by the elderly is bone pain. Various conditions or diseases can cause bone pain in the elderly, including: osteoporosis, osteomalacia, renal osteodystrophy, osteonecrosis, malignancy or bone metastases. Measuring the degree of pain can use the Numeric Rating Scale (NRS) in adults (elderly). The NRS scale starts from a scale of 1 – 10, which consists of categories of mild, moderate and severe pain. Mild pain is pain that comes and goes, especially when doing daily activities and disappears during sleep (scale 1-3). Moderate pain is continuous pain, disturbed activity, which only disappears when the patient sleeps (scale 4-6). Severe pain is pain that lasts continuously throughout the day, the patient cannot sleep or is often awakened by pain disorders during sleep (scale 7-10). (Hjermstad et al., 2011).

Bone pain due to malignancy or metastases to bone is the most common in Multiple Myeloma (MM) as much as 1% of all malignancies and 10% of hematological malignancies. Patients with MM were only discovered after appearing in the Monoclonal Gammopathy of Underterminated Significance (MGUS) stage, which is a disorder that occurs due to plasma cell dyscrasias and is known as one of the precursor tumors of MM. Clinical signs and symptoms of MM are known as CRAB, which stands for Hyper-Calcemia (hypercalcemia), Renal failure (kidney failure), Anemia, Bone pain with lytic lesions (bone pain with lytic lesions) (Malanga, Yan, & Stark, 2015).

To establish the diagnosis of MM, examinations that can be done include bone marrow examination, protein electrophoresis, immunophenotyping, and cytogenetics, each of which has advantages and disadvantages. Malignancy in MM patients can cause anemia. According to WHO, the elderly are said to be anemic if the hemoglobin value is less than 12 g/dL in women and less than 13 g/dL in men. There was 85.3% anemia in MM and required transfusion. Anemia in MM patients is caused by bone marrow replacement and direct inhibition of the erythropoiesis process, this change causes a decrease in the production of vitamin B12 and folic acid.

Scattergram is the result of a plot of data that comes from the results of the scatter light that passes through a cell. Forward Scattered lights (FSL) reflect cell size, Side Scattered lights (SSC) reflect cell complexity, Fluorescent light (SFL) reflect total nucleic acid content and cell organelles. These three signals are used for differentiation and counting of white blood cells, NRBC, reticulocytes, and PLT-F, as well as detecting abnormal cells and immature cells with the help of digital technology and tool algorithms. NRBC, reticulocyte, and PLT-F, as well as detecting abnormal cells and immature cells with the help of digital technology and tool algorithms. In the elderly the scattergram of lymphocytes is the same as the scattergram in general, where the position of the lymphocytes is on the WDF curve, marked with a violet color, on the scattergram the color of each type of leukocyte is determined due to the presence of a fluorescent substance. The principle of this tool is flowcytometry, the position of the lymphocytes is at the bottom of the scattergram is due to the smaller size of lymphocytes compared to other types of leukocytes and their wavelength is 380 – 488 nm, which is at FL1 (Briggs, Longair, Kumar, Singh, & Machin, 2012) (Xu, Yu, Xie, Chen, & Zhang, 2017).

White blood cell differential (WDF) is a channel on the hematology analyzer found on the Sysmex XN-series device. WDF can read leukocytes such as basophils, eosinophils, neutrophils, lymphocytes and monocytes, using specific reagents containing detergent (lysercell WDF) and fluorescent staining (fluorocell WDF). The use of WDF lysercell reagent is to lyse erythrocytes and platelets, perforating the leukocyte membrane, which will cause external and internal structural changes depending on the type of leukocyte. While the WDF fluorocell will stain the nucleic acid and cytoplasmic organelles of leukocytes. In this WDF, there is a separation between monocytes and lymphocytes so that the calculation of each type of leukocyte is more accurate.

The prepared samples were then analyzed using fluorescence flow cytometry. The measurement signals associated with side scatter (SSC) and side fluorescence (SFL) are analyzed and depicted in a scattergram. Cells with similar cytochemical properties belong to the same area in the scattergram and can be separated using advanced software algorithms. WD Scattergram F has an X or horizontal axis called Side-Scattered Light (SSC) which provides information about the internal structure of the cell and its contents (eg granules); while the Y or vertical axis is referred to as Side-Fluorescence Light (SFL) which provides information about the amount of nucleic acid content possessed by cells (Stiel et al., 2016).

The scattergram examination needs to be confirmed again by flowcytometry. T cells were confirmed by the presence of CD4 and CD8, in the elderly the CD4 and CD8 values decreased, cell memory increased, activation in the elderly decreased, oligoclonal dominance increased, and cytokine production and effector generation decreased. In the aging process, slow changes and a long life span of naive T cells can be maintained, but gradual involution of the thymus results in an inability to replace naive T cells lost from the circulation. In addition, aging is also associated with decreased function of naive T cells. Compared with young mice, 40% of CD8+CD28+ naive T cells in old mice did not express CD62L and CCR7, receptors that play a role in migration to peripheral lymphatic tissue. CD8 naive T cells appear more susceptible to receptor-mediated apoptosis of CD4 T cells. On polyclonal stimulation, CD45RA+CD28+CD8+ T cells from elderly individuals produced more IFN-1 than young people. B cells in the elderly are found to have differentiated and matured progenitor B cells in secondary lymph tissues such as the spleen and lymph nodes. Old age is associated with changes in the spleen including decreased arteries, increased stromal cells and fibroblast infiltration.

This condition causes disturbances in the number and function of the B cells that are produced. Decreased IL-7 production leads to decreased ability to support B cell expansion by bone marrow stromal cells. The number of B cells (CD19+) also decreases with advancing age. The proportion of IgG-IgA-IgD+CD27-decreased with age and showed decreased susceptibility. B cells on flow cytometry examination were confirmed by CD45+, CD3+, CD56+, CD16+ and in the elderly it increased (Keim, Hao, Dayal, Janetzko, & Bak, 2010).

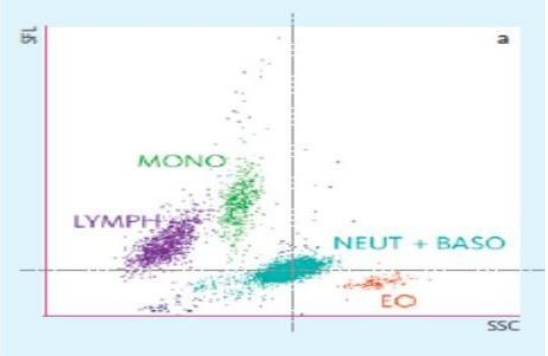
Lymphocyte scattergram in the elderly is the same as in non-geriatric adults. The process of T cell maturation is in the thymus. T cells are very important in lymphocytes to kill bacteria and help other cell types in the immune system. As we age, many T cells or T lymphocytes lose their function and ability to fight disease. The number of T cells will decrease with age so that the body is less able to control disease compared to previous times. In addition, the aging process is also associated with a decrease in the function of naive T cells. Compared with young mice, 40% of CD8+CD28+ naive T cells in old mice did not express CD62L and CCR7, receptors that play a role in migration to peripheral lymphatic tissue. CD8 naive T cells appear to be more susceptible to receptor-mediated apoptosis than CD4 T cells. On polyclonal stimulation, CD45RA+CD28+CD8+ T cells from elderly individuals produced more IFN-ı than young people (Keim et al., 2010).

The advantages and disadvantages of scattergrams

In using the scattergram we can find the advantages and disadvantages, as for the advantages of the scattergram, among others; 1). Clear data range, minimum and maximum points can be seen. 2). The data displayed is accurate because it uses dots. 3). Can display positive and negative relationships. 4). Graphics are easy to explain and view. 5). Easy method of graphing. Disadvantages in using scattergram: 1). Cannot display relations that use more than 2 variables. 2). The amount of data that can be observed is limited, because if you display a lot of data it will not be clear. 3). It is difficult to accommodate data that uses decimal values. 4). Can only use variables whose data is quantitative. 5). Cannot accommodate external data. 6). There is no object criteria for selecting the best line (Seghezzi et al., 2018).

Normal lymphocyte profile on the White Blood Cell Differential scattergram

Scattergram in healthy people shows lymphocytes in violet color, monocytes in green, neutrophils + basophils in bright blue while eosinophils in red, healthy people have lymphocyte scattegram positions in SSC/line x (A2), SFL/line y (B2).



Gambar 1. WDF scattergram plot SSC dan SFL pada orang sehat (Sale, Carone, Fumi, Pancione, & Rocco, 2016)

Profil scattegram limfosit dapat juga ditemukan pada berbagai keadaan, seperti untuk membantu menentukan keadaan inflamasi dengan lebih lebih cepat dengan mengunakan parameter RE-LYMP (Reactive Lymphocytes) dan AS-LYMP (Antibody-Synthesiszing Lymphocytes) mampu memberikan penilaian mengenai limfosit teraktivasi. Parameter ini mampu membantu klinisi untuk mendiagnosis,

memberikan terapi, dan memberikan informasi tambahan mengenai aktivasi sistem imun. Parameter Figure 1. WDF scattergram plot of SSC and SFL in healthy people

Lymphocyte scattegram profile can also be found in various circumstances, such as to help determine the state of inflammation more quickly by using the RE-LYMP (Reactive Lymphocytes) and AS-LYMP (Antibody-Synthesiszing Lymphocytes) parameters which can provide an assessment of activated lymphocytes. These parameters can help clinicians to diagnose, provide therapy, and provide additional information regarding the activation of the immune system. The RE-LYMP parameter describes the entire lymphocyte population that has a high fluorescence intensity which indicates the presence of a reactive lymphocyte population (Kawauchi, Takagi, Kono, Wada, & Morikawa, 2014).

AS-LYMP parameters provide an overview of activated B lymphocytes (plasma cells) which have a function for antibody synthesis. The combination of RE-LYMP and AS-LYMP parameters is able to provide additional information regarding cellular activation of the innate and adaptive immune systems, as shown in the 2 pictures below:

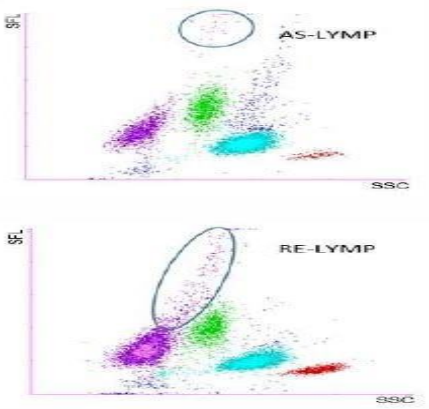


Figure 2. Side Scattered signal in the 14 . lymphocyte population

Description: a. Reactive lymphocyte population; b. population of antibody synthesizing lymphocytes

METHOD RESEARCH

This study is a descriptive observational with a cross-sectional design that is to know the profile of the WDF lymphocyte scattergram in the elderly with complaints of bone pain accompanied by anemia.

Blood was taken by means of peripheral venous phlebotomy, 3 cc of blood was taken without fasting subjects, put into a tube with EDTA anticoagulant. After being drawn, the blood was homogenized manually and then examined with a hematology analyzer to see the scattergram of lymphocytes.

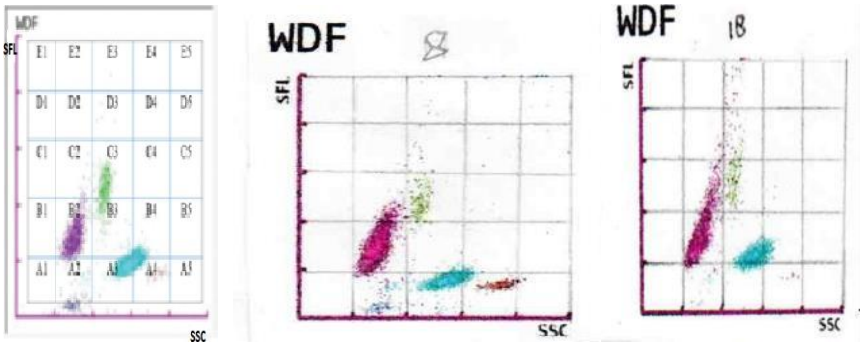


Figure 3. Lymphocyte position in the WDF scattergram.

Description of the image: 1). In healthy people, it is in the SSC (A2), SFL (B2) area. 2). On profile A SSC (A2, A3); SFL (B2, C2, D3). 3).On profile B SSC (A2, A3); SFL (B2, C2, D3, E3).

Statistical analysis used is data distribution test for numerical data, namely age. The collected data is processed descriptively, for categorical data by calculating the number and percentage, while for numerical data by presenting statistical measures of the mean, standard deviation, or median and range. To find out the lymphocyte profile is described through a scattergram. Normality test for numerical data, namely age and hemoglobin with Saphiro Wilk's test, because n = 30 (< 50). From the results of the normality test, it was found that age and hemoglobin were normally distributed, age (p= 0.077), hemoglobin (p = 0.386).

RESULT AND DISCUSSION

Found in the elderly with bone pain and anemia, most of them are male: 77%; Female: 23%), and aged between 60-72 years, the average hemoglobin includes moderate anemia (moderate anemia: 73%, severe anemia: 27%), the highest bone pain scale is 4 (moderate pain; 70%); WDF lymphocyte scattergram profile was in area B SSC (A2, A3); SFL (B2, C2, D3, E3) was found in 21 subjects (70%). More atypical lymphocytes with suspected plasma cells and abnormal lymphocytes were found than the scattergram profile in the A SSC area (A2, A3); SFL (B2, C2, D3) were found in 9 (30%) research subjects, meaning that more lymphocytes were found.

The gender of the majority who suffer from bone pain and anemia are aged more than 60 years and over (elderly) are male. This is in accordance with research conducted by Laura et al in 2015 which stated that most of the patients with bone pain and anemia were in men with an average age of 60 years, because at the time of the elderly there were changes in bone structure and bone tissue, resulting in the elderly bone structure becomes weak and painful.34-36 This is due to; 1) In the elderly, activities have begun to decrease, resulting in osteoporosis and bone pain 2) Hormonal changes, in women due to menopause, resulting in reduced calcium ions and other minerals, in men reduced testosterone, which causes osteoporosis in development 3) Decreased calcium and other minerals (Toxqui & Vaquero, 2015) (Goodnough & Schrier, 2014).

(Birgegård, Gascón, & Ludwig, 2006) Hemoglobin examination, in this study found moderate anemia, with Hb levels of 8 – 10.9%, this is in accordance with research conducted by Birgegard in 2006 in Sweden, moderate anemia in the elderly with bone pain caused by the use of a lot of iron, low levels of iron in the blood. low erythropoietin and low bone marrow response to erythropoietin. 17-19 Bone pain found in this study was a scale of 3, a scale of 4 and a scale of 5 from a pain scale of 1 – 10. The results showed a scale of 3:17%, a scale of 4: 70% and a scale of 5:13%. Scales 4 and 5 belong to the moderate pain category, namely pain that is continuous, activity is disturbed, and only disappears when waking up due to osteoclast activity pressing on the tissue, this is in accordance with a study conducted by George on osteoclasts in patients with bone pain in 2019 in the United States.20 Differences on a scale of 4 and 5 occur when measuring the degree of pain, where the face shown during the anamnesis, the face of the subject on a scale of 5 shows more pain (Stauder, Valent, & Theurl, 2018).

Lymphocyte association with bone pain; During human life, bone will undergo a process of formation (which is carried out by osteoblast cells) and re-destruction (which is carried out by osteoclast cells) which runs in a balanced way in the process of bone remodeling. However, for some reason, the balance is disturbed, which can result in reduced formation, increased destruction, or a combination of both. Osteoblasts as well as osteoclasts react to calcium levels in the blood. Calcium circulating in the blood will be deposited by osteocalcin to form hydroxyapatite crystals in the formation of bone matrix osteoclasts are multinucleated giant cells originating from hematopoietic stem cells in the bone marrow, branching off lineages that produce macrophages and neutrophils. Osteoclast activation is regulated by a variety of molecular signals, of which RANKL is the most

clearly studied role. RANKL is produced by osteoblasts as well as other cells (eg lymphocytes), and stimulates RANK. Osteoprotegerin (OPG) binds to RANKL, before RANKL binds to RANK, and thereby suppresses its ability to perform bone resorption, RANKL, RANK, and OPG have a close relationship with TNF and its receptors. If this is disturbed between osteoblasts and osteoclasts, it can cause disturbances in the sensory nerve fibers of the bone and the nocireceptors in the bone, which are located in the central nervous system, which causes pain in the bones. (Briggs et al., 2012) (Longanbach, Miers, Keohane, Smith, & Walenga, 2016).

Based on the WDF lymphocyte scattegram profile in elderly subjects with bone pain and anemia, the most WDF lymphocyte scattegram profiles were in the SSC (A2, A3), SFL (B2, C2, D3, E3) areas, this is in accordance with research conducted by David et al, scattergram of WDF lymphocytes with a predominantly violet color in the lymphocyte area and spread to areas suspected of increasing the number of atypical lymphocytes and suspected towards plasma cells and abnormal lymphocytes, from the Sysmex XN 1000 instrument, several types of parameters whose lymphocytes were in SSC (A2, A3), SFL (B2, C2, D3, E3) are known as Reactive Lymphocytes (RE-LYMP) and Antibody - Synthesizing Lymphocytes (AS-LYMP) to help determine the inflammatory state more quickly, RE-LYMP and AS-LYMP are able to provide assessment of activated lymphocytes. These parameters help clinicians to diagnose, provide fire, and provides additional information regarding immune system activation. The RE-LYMP parameter describes the entire lymphocyte population that has a high fluorescence intensity which indicates the presence of a reactive lymphocyte population. AS-LYMP parameters in the elderly show low levels, because the production of B cell antibodies has decreased, the combination of RE-LYMP and AS-LYMP parameters in the elderly is able to provide additional information regarding cellular activation of the innate and adaptive immune systems.20,22,25 The limitations of this study were: there was no confirmation of the lymphocyte profile using a peripheral blood smear and no confirmation of other causes of bone pain with other supporting methods.

CONCLUSION

The scattergram profile of lymphocytes in most elderly subjects with bone pain and anemia was in the SSC area (A2, A3), SFL (B2, C2, D3, E3).), SFL (B2) which shows an increase in the number of atypical lymphocytes which can be plasma cells or abnormal lymphocytes. Based on the results of this study, the scattergram examination can be used as a screening examination in the elderly who suffer from bone pain and anemia, for that it is necessary to continue with examination of peripheral blood smears, protein electrophoresis examination to increase awareness of the possibility of Multiple Myeloma. In this study, it is also suggested to continue the validity test to measure the area of SSC (A2, A3), SFL (B2, C2, D3, E3).

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