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Precise Detection of Monocyte Levels as a Determinant of Health Status

Akefe Isaac Oluwatobi[1]*, Yusuf Lamidi Ibrahim[2], Adamu Andrew Musa[3], Victoria Adegoke Aderonke[4], Steven Ezekiel Nyan[4], Ogbeifun Progress Osariemen[5]

[1]*Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[2]*Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria*

[3]*Department of Veterinary Public Health, Faculty of Veterinary Medicine, University of Abuja, Nigeria*

[4]*Department of Science Laboratory Technology, Faculty of Science, Ekiti State University, Nigeria*

[5]*Department of Microbiology, Faculty of Science, University of Abuja, Nigeria*

***Corresponding Author**: Akefe Isaac Oluwatobi, Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

Received: July 17, 2018; **Publisded:** October 28, 2019

Abstract

Evaluation of monocyte levels is essential for diagnosis and management of health conditions. Monocytes are sizeable white blood cells appearing as large, smooth, indented, kidney-shaped, with notched nucleus. They shield the body against infections and combat diseases. The average monocytic cell size varies between 13 and 25 micrometers in diameter. Monocytes originate from hematopoietic stem cells in the bone marrow. Monocytes make up about 2 - 8% of the total white blood cells which undergo systemic circulation for about few days before migrating into other regions of the body such as loose connective tissues, spleen, lymph nodes, and bone. Enumeration of monocytes is conventionally by hematological cell counters which are based on the principle of functional and morphological cell variations. However, this has been misinforming and unsatisfactory owing to inadequate precision and accuracy. In recent times, flow cytometry has emerged as a more reliable alternative for the enumeration of monocytes to aid in determination of health status. This review highlights the identification and characterization of monocyte subpopulation, with emphasis on the use of proteomics for enumeration and the significance of monocyte fractions in health and disease.

Keywords: *Monocyte; Health; Cytometry; Haematology; Proteomics*

Introduction

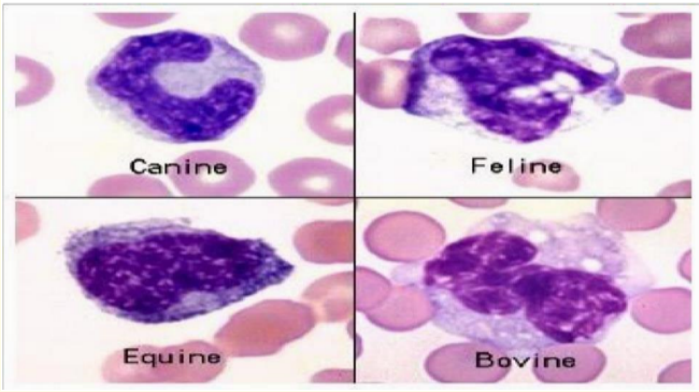
Monocyte comes from the Greek word "monos" meaning "single," and the Greek word "kytos" meaning "cell." Put the two words together and you have "single cell" [1]. A monocyte is a full-sized white blood cell (WBC) with well-defined, oval, kidney-shaped single nucleus. White blood cells play a major role in immune protection by shielding the body against infections. A complete blood count (CBC) test with differential is a vital procedure carried out to detect and enumerate monocytes [2]. The CBC supplies vital data on the types and quantities of cells in the blood. Monocytes represent the largest category of haematocytes with sizes varying between 13 to 25 micrometers in diameter [3]. Monocytes originate from the hematopoietic stem cells in the bone marrow, as premature monoblasts before transforming into the smaller sized matured monocytes. Monocytes make up 2 - 8% of the total WBC which undergo systemic circulation for about few days before migrating into other regions of the body such as loose connective tissues, lymph nodes, bone marrow and spleen [4,5]. When stained, monocyte cytoplasm appear pale blue or blue gray. The large cytoplasm of monocytes is filled with numerous fine reddish-blue particles. Vacuoles are also often present; which are clear spaces in the cytoplasm of a cell [5] appearing predominantly sequel to monocytic phagocytosis of alien materials. In addition, monocytes possess several vesicles responsible for handling foreign substances [1]. Aside playing a crucial role in our innate immune system by defending the host from intruding microbial pathogens but they also contribute to disease pathogenesis and progression as seen in liver fibrosis, atherosclerosis, multiple sclerosis, and tumor metastasis. In addition, monocytes and monocyte-derived macrophages play a crucial beneficial role in the liver fibrosis regression, muscle regeneration, and the clearance of the β-amyloid plaques in Alzheimer's disease [6].

Derivation and identification of monocytes

Monocyte refers to a lineage termed as mononuclear phagocytes or macrophages which are bone marrow-derived leukocytes functionally characterized by their ability to engulf, produce cytokines, and present antigens. Previously, they were identified on the bases of morphology and glass adherence [5]. In addition, enzyme-specific cytochemistry for monocyte specific esterase has been utilized. The

standard clinical hematology approach relies on the physical characteristics of the cells including light scattering property. In the bone marrow, monocytes originate from myelomonocytic stem cells, which engender supplementary precursors such as promonocytes and monoblasts. These cells earlier identified based on morphology [7] had ill-defined cell shape. Recently, the mouse model; Ly6C+ CD115+ CD117+ monoblast-type cell, called common monocyte progenitor (cMoP), was identified in the spleen and bone marrow which has ability to proliferate into diverse monocyte subsets [4]. The circulating monocytes can rapidly surge within minutes of stress or exercise before rapidly returning to the baseline. These recruited cells congregate from the marginal pool [8] which is a compartment of reduced blood velocity near the endothelium of venules. The CD11b+ Ly6Chi monocytes can be assembled from bone marrow into the blood stream during infection [3] and may return back to bone marrow in the mouse infectious disease model. Studies are yet to ascertain if bone marrow and spleen compartments also donate to the pool of monocytes mobilized during exercise and stress. By definition, macrophages are monocytes that migrate into tissues during inflammatory or homeostatic conditions [9]. Following the arrival of monocytes in tissue, they transform into larger cells which rapidly lose their monocytic properties and have been termed "small macrophages" as they possess traits similar to blood monocytes [10,11]. More data is required before concluding on the use of the term tissue monocyte or whether we continue to use the term macrophages. At present, the term monocyte should be limited to cells in the blood, bone marrow and spleen reservoirs capable of replenishing the blood monocyte pool [10].

Morphological identification of monocytes in different species



Source: Loems., *et al* [12].

Each of the four monocytes shown above as examples are from different species but all could be found in blood of any individual animal of any species. For instance, the canine monocyte illustrated appears alike in a smear of equine blood and the feline monocyte will be similar to a canine blood. Worthy of note is how the canine lymphocyte resembles a toxic band neutrophil with less clumped chromatin. Vacuoles may be seen in some monocytes but is not a specie-dependent alteration [10,11].

Monocyte count

This test measures the amount of monocytes in blood. This test is used to evaluate and manage blood disorders, certain problems with the immune system, and cancers, including monocytic leukemia [1]. This test may also be used to evaluate for the risk of complications after a heart attack. This test can aid in the diagnosis of Heart attack, Monocytic leukemia, Monocytopenia, and Monocytosis [2].

Monocyte percentage fraction in blood

Under 4%	[0.04]	(Low)
4 - 10%	[0.04 - 0.10]	(Normal)
11 - 15%	[0.11 - 0.15]	(Elevated)
Over 15%	[0.15]	(High) (Frankenberger *et al.,* 2013).

Adults	Absolute: 0.2 - 0.95 x 10^3 cells/microL	Relative: 4% - 11%
Neonates, Birth to 2 days	Absolute: 0 - 2 x 10^3 cells/microL	
Neonates, 4 days to 28 days	Absolute: 0 - 1.7 x 10^3 cells/microL	
Infants, 1 to 4 weeks	Absolute: 0.7 x 10^3 cells/microL	
Infants, 6 months to 1 year	Absolute: 0.6 x 10^3 cells/microL	
Infants, 1 year		Relative: 4.8%
Children, 2 years	Absolute: 0 - 1 x 10^3 cells/microL	
Children, 4 to 10 years	Absolute: 0 - 0.8 x 10^3 cells/microL	Relative: 4.3% - 5% (Randolph *et al.,* 2009)

Identification of blood monocytes based on cells surface markers

As stated earlier, monocytes were initially identified by morphology and function which is a misleading criteria especially when these features have been altered by disease processes.

As a result, there have been attempts to define an unequivocal criteria for monocytes and monoclonal antibodies against cell surface molecules have been suggested. In man, CD14 has been used as a marker [13] and in the mouse, CD115 is often employed [14]. Although CD115 identifies the M-CSF receptor, it is down-regulated in mouse blood monocytes during inflammation. Furthermore, it is unknown whether these markers are sufficiently specific and do not react with other types of cells such as dendritic cells (DCs). A fraction of CD1c+ blood DCs in humans can express low-level CD14 and human B cells have also been reported to express some CD14 [1]. Hence, monocytes can be branded with markers like CD14 and CD115, but this must be supported by additional markers and functional studies. Intriguingly, when investigating for macrophage-specific transcripts, CD64 and MerTK have emerged in mouse model [15], while CD64 is lacking in non-classical monocytes in humans. MerTK may prove informative for blood monocytes in various species. Furthermore, staining for CD16 used for monocyte subset definition, will also aid in the exclusion of DCs in human blood [13].

Differentiation of monocytes from dendritic cells

Dendritic cells were first described by Steinman and Cohn as stellate cells isolated from the spleen of mouse. In the past, it has been debated as to whether these cells are a separate lineage or part of the mononuclear phagocyte system. Although a common precursor for monocytes and DCs was described in mice [16], the existence of this cell was later argued proposing that DCs and monocytes diverge at an earlier multi-potent progenitor period.

However, demonstrations of monocytes for the generation of DCs *in vitro* by adding GMCSF and IL-4 indicate a close relationship between monocytes and DCs [17]. Subsequently, transcriptome analysis revealed that such monocyte-derived DCs noticeably resemble macrophages than DCs from lymphoid tissue. Hence, the *in vitro* generated monocyte derived cells are strong antigen-presenting cells, but do not epitomize bona fide DCs; as they belong to the macrophage/monocyte lineage. In addition to DCs in tissue, cells with DC characteristics have been described on the bases of expression of CD68, CD1c, or CD141 in blood. Transcriptome analysis has shown that these cells and the monocytes belong to separate groups [18]. Data suggests that blood DCs can be separated from monocytes and macrophages as a distinct lineage. The potency of transcriptomic analysis in defining and dissecting leukocyte populations such as monocytes and DCs has also been demonstrated (Macaulay., *et al.* 2007). An illuminating example is the megakaryocyte–erythrocyte progenitor (MEP) cell, which engenders megakaryocytes and their platelet offspring or to erythroblasts and their progeny red blood cells [19]. Megakaryocytes and erythroblasts have a distinct transcriptome (Macaulay., *et al*. 2007), and are concerned with distinct functions, (blood clotting and oxygen transport) respectively [10].

Role of cytometry in the evaluation of monocyte subpopulations

Evidence for monocyte subpopulations has come from experiments using differential flotation in counter-current elutriation and from differential binding to antibody-coated red blood cells, which has defined populations with different functions [20]. In recent times, monoclonal antibodies and flow cytometry, has it possible to clearly enumerate, specify, and isolate monocyte subdivisions on the bases of differential expression of CD14 and CD16 cell surface markers. Recently, the WHO proposed a new nomenclature for monocyte subsets [21]. The major population of $CD14^{high}$ cells found in human blood were defined as classical monocytes while the minor population of cells with low CD14 and high CD16 were classed as non-classical monocytes. The population in between these two subsets was designated as intermediate monocytes [21]. This same nomenclature proposed for humans can be used in other animal species [21]. In mouse, the classical and non-classical monocyte subpopulations can be identified, but different markers like CD115, Ly6C, and CD43 are usually employed [22]. In addition, for species like rat, pig, cow, and horse, classical and non-classical monocytes can be distinguished with intermediate monocytes described in some species [21].

Role of cryomicrotome imaging in the detection and quantification of monocytes

This is a novel imaging platform used to efficiently determine the localization of monocytes in relation to the coronary microvascular network. These techniques are invaluable for investigating the role of monocyte populations in the progression of coronary neovascularization in animal models of chronic and sub-acute myocardial ischemia [23]. It involves the use of high-resolution three-dimensional (3D) imaging and quantification techniques to characterize monocytes relative to the entire coronary artery network using a novel episcopic imaging modality. It involves labelling the umbilical vein endothelial cells and CD14 + monocytes with fluorescent live cell tracker probes before infusing them into the coronary artery network of excised rat hearts by a Langendorff perfusion method. The coronary arteries are subsequently infused with fluorescent vascular cast material and processed with an imaging cryomicrotome, whereby each heart was consecutively cut (5 μm slice thickness) and block face imaged at appropriate excitation and emission wavelengths. The resulting image stacks yielded 3D reconstructions of the vascular network and the location of cells administered. Successful detection and quantification of single cells and cell clusters were achieved relative to the coronary network using customized particle detection software [24]. These methods can also be applied to an in vivo rabbit model of chronic myocardial ischemia in which autologous monocytes were isolated from peripheral blood, labeled with a fluorescent live cell tracker probe and re-infused into the host animal. The processed 3D image stacks reveal homing of monocytes to the ischemic myocardial tissue. Monocytes detected in the ischemic tissue are predominantly concentrated in the mid-myocardium. Vessel segmentation identified coronary collateral connections relative to monocyte localization [23].

Clinical implications of monocyte numbers

Identification of monocytes using light scatter properties have contributed little to diagnosis and monitoring of disease, however, flow cytometry, has provided more informative patterns. For instance, severe infection will raise the number of non-classical and intermediate monocytes [25]. It however remains to be analyzed whether such an upsurge can be used to ascertain prognosis, as earlier suggested. Furthermore, therapy with glucocorticoids stimulates a decrease in number of non-classical monocytes, which appears to be as a result of selective induction of apoptosis in the non-classical monocytes while classical monocytes may increase in number under glucocorticoids [26]. In addition, blockade of the M-CSF pathway leads to direct depletion of non-classical monocytes. A likely explanation is that M-CSF signaling via the CD115 M-CSF receptor is needed for the classical monocytes to mature into non-classical monocytes. Still to be determined is whether such a drug-induced depletion can be used to foretell therapeutic response in inflammatory conditions. The mechanism

of depletion of non-classical monocytes in three siblings within one family still remains unresolved [1] as more families with this defect need to be tested to identify the gene and mechanisms involved. Finally, the absolute count of intermediate monocytes was shown to aid in predicting cardiovascular events [27]. Therefore, analysis of monocyte subsets by flow cytometry provides clinically valuable parameters in various settings. Yet to be established in this circumstance is an unequivocal distinction of the non-classical and the intermediate monocytes [1].

Functions of monocytes

Monocytes have the ability to change into another cell form called macrophages before facing the germs. They can actually consume, or munch, on harmful bacteria, fungi and viruses. Then enzymes in the monocyte's body kill and break down the germs into pieces (Macaulay., *et al.* 2007).

Monocytes help other white blood cells identify the type of germs that have invaded the body. After consuming the germs, the monocytes take parts of those germs, called antigens, and mount them outside their body like flags. Other white blood cells see the antigens and make antibodies designed to kill those specific types of germs. Monocytes help mend damaged tissue by stopping the inflammation process. They remove dead cells from the sites of infection, which repairs wounds. They have also shown to influence the formation of some organs, like the heart and brain, by helping the components (Macaulay., *et al.* 2007).

Monocyte disorders

High monocytes counts and allied conditions

In adults, values above 0.8 x 10(9)/L have been specified as abnormally high levels. Some conditions associated with higher levels of these monocytes include the following: Autoimmune diseases such as: Systemic lupus erythematosus, inflammatory bowel disease, heart attack rheumatoid arthritis, sarcoidosis, depression, obesity, severe pneumonia, alcoholic liver disease [28], HIV infection, childbirth, and appendicitis. Hematologic malignancies such as myelodysplastic disorder, acute monocytic leukemia, chronic myelomonocytic leukemia and Hodgkin and non-Hodgkin lymphoma [29]. Cancers, exclusively of the breast, ovary, and rectum. Infections like bacterial endocarditis, tuberculosis, brucellosis, malaria, syphilis and viral infections [23].

Health implications of high monocyte counts

High or low monocyte counts are not associated with any symptoms. However, there are some recognizable symptoms of disorders that accompany high monocytic cells. These conditions include atherosclerosis which overtime can lead to stroke, heart attack, and heart failure. Absolute monocytes counts are also significantly increased in subjects with diabetes and this has been associated with increased occurrence of inflammation. Cardiovascular- and cancer-related deaths in the elderly have also been reported [24].

Low monocytes counts and allied conditions

In adults, values of less than 0.2 × 10(9)/L is considered as abnormally low. This can be caused by conditions that decreases overall white blood cell count such as bloodstream infection, chemotherapy, and bone marrow disorder. Skin infections and HPV have also been implicated. Other conditions associated with monocytopenia include: Aplastic anemia, administration of INF-alpha and TNF-alpha, severe burn injuries, leukemia, monoMAC syndrome, HIV infection, systemic lupus erythematosus, radiation therapy, vitamin B12 deficiency, rheumatoid arthritis, and corticosteroid therapy [30-34].

Health implications of monocytopenia

Blood disorders like monocytopenia or MonoMAC syndrome pose different challenges, but there are no symptoms regularly found with low monocytic cell counts [30]. However, patients may see signs of infection, as normal monocytic cell counts would predictably be able to repair and remove this dead tissue. Monocytopenia lowers the risk of cardiovascular diseases, but increases susceptibility to infections such as mycobacterial, fungal, or human papillomavirus infection. Higher risk of hematologic disorders and cervical cancer has also been highlighted [28].

Conclusion

The detection of high levels of monocyte is an indicator of physical or oxidative stress in animals and humans. Proteomics has afforded more reliable test solutions with better accuracy and cost effectiveness via the availability of auto-haematological analysers based on cytometric techniques as well as the use of rapid monocyte tests kits in the measurement of monocyte levels. Accurate evaluation of monocyte levels facilitates diagnosis and management of blood and immune disorders, including monocytic leukemia, monocytopenia, and monocytosis [2].

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Volume 3 Issue 1 November 2019