Review

Phenotypic and functional heterogeneity of monocyte subsets in chronic heart failure patients

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Simple Summary: A long-term condition known as chronic heart failure (CHF) represents a constant hindered work of heart in pumping blood enriched in oxygen and required nutrients around the body tissues. Moreover, the CHF pathogenesis can be associated with various causes, and the inflammation could be regarded as one of important factors promoting such a condition. In addition, monocytes, a certain group of cells present in blood and infiltrating tissues, are known to participate in both the pro- and anti-inflammatory processes, and thus to affect the myocardial remodeling over time. Therefore, our aim was to review current studies on the function of monocyte subsets in different CHF: with preserved and reduced left ventricle ejection fraction, and to discuss the relation of monocyte subsets to inflammatory markers. We expect that a deeper view into the pathogenesis of CHF could stimulate the search and development of individualized therapies.

Abstract: Chronic heart failure (CHF) results when heart cannot constantly supply the body tissues with oxygen and required nutrients, and it can be categorized as heart failure (HF) with preserved ejection fraction (HFpEF), and HF with reduced ejection fraction (HFrEF). There are different causes and mechanisms of the HF pathogenesis; however, the inflammation can be regarded as one of the factors promoting both HFrEF and HFpEF. Monocytes, a subgroup of leucocytes, are known as cellular mediators in response to cardiovascular injury and are closely related to inflammatory reactions. These cells are a vital component of the immune system and are the source of macrophages, which participate in cardiac tissue repair after injury. However, the monocytes are not homogenous as thought, and thus can present different functions under different cardiovascular disease conditions. In addition, there is still an open question whether the functions of monocytes and macrophages should be regarded as a cause or a consequence in CHF development. Therefore, our aim was to summarize the current studies on the function of various monocyte subsets in CHF with a focus on the role of a certain monocyte subset in HFpEF and HFrEF patients, and the relation to inflammatory markers.

Keywords: monocyte subset, heart failure, inflammation, cytokine, macrophage.
1. Introduction

Chronic heart failure (CHF) is a condition when heart cannot constantly supply the body tissues with proper amount of oxygen and nutrients. There are different causes and mechanisms of the CHF pathogenesis and thus, depending on the pathogenesis the heart failure is categorized as heart failure (HF) with preserved ejection fraction (HFP EF), and HF with reduced ejection fraction (HFrEF). HFP EF means that patient's heart extrudes close to normal amount of blood (≥50% of left ventricle ejection fraction (LVEF)) to the tissues. Whereas HFrEF means that amount of blood is lower than normal, i.e., <50% of LVEF [1]. Though the exact sequence of events contributing to the development and progress of HF remains to be elucidated, the HFrEF is known to be the outcome of myocardial ischemia and infarction. Noteworthy, the HFP EF is associated with older age, dysregulated metabolism and chronic hypertension contributing to oxidative stress and myocardial dysfunction [2]. It is also suggested that at early HF stages the systemic inflammation can induce endothelial dysfunction [3], which consequently promotes the invasion of pro-inflammatory cells like monocytes into the heart tissue and contributes to the increased stiffness of myocardium. The altered levels of matrix metalloproteinases and modified composition of the myocardial extracellular matrix can further contribute to the progress of myocardial dysfunction [4,5]. Thus, inflammation can be regarded as one of the factors promoting HF in both HFrEF and HFP EF [6]. Consequently, the efforts to reduce inflammation in blood vessel walls and to modulate the anti-inflammatory processes in monocyte/macrophage system could be a promising therapeutic approach [7]. Monocytes, a subgroup of leukocytes (white blood cells), are known as cellular mediators in response to cardiovascular injury [8,9] and are closely related to inflammatory reactions. These cells are a critical component of the innate immune system and are the source of many vital elements of the immune system such as macrophages, which sense and respond to pathogens and other environment challenges and participate in tissue repair after injury. Monocytes play a role in both the pro- and anti-inflammatory processes that take place during an immune response in tissue repair and continue even after the acute phase [5,8]. Recently it has been demonstrated that monocytes are not homogenous as previously thought, and thus can present different functions under different cardiovascular disease (CVD) conditions [9]. Additionally, HFP EF is known can change to HFrEF, and hence it is important to explore pathological mechanisms of early diastolic dysfunction, such as monocyte activity, phenotype and function. Therefore, we aimed to summarize the current studies on the function of various monocyte subsets in CHF with a special focus on the importance of a monocyte subset in HFP EF and HFrEF patients, and the relation to inflammatory markers. We think that a more accurate knowledge about the pathogenesis of CHF could help the search for individualized therapies targeted to stop harmful myocardial remodeling and consequently, improving patient's heart function.

2. Nomenclature of monocyte subsets and their formation

Monocytes are the biggest agranular leukocytes that are named circulating mononuclear phagocytes. Upon entering tissues monocytes undergo morphological and functional changes, and then they are identified as macrophages. Both monocytes and activated macrophages together with their cytokines are essential to inflammation and sustain tissue responses that lead to chronic inflammation [8]. Monocytes are grouped according to the relative expression levels of CD14 and CD16 surface proteins and chemokine receptors, and their phagocytic activity [10]. Monocyte subsets also differ in combinations of adhesion molecules and chemokine receptors, on which different monocyte functions depend. It is also known that combinations of adhesion molecules and chemokine receptors can vary during the inflammation process [11].

According to the allotment restored in 2017 by the Nomenclature Committee of the International Union of Immunologic Societies (NCIUIS) there are three distinct subsets
of monocytes, defined as classical (CD14+/CD16−, or Mon1), intermediate (CD14+/CD16+, or Mon2) and non-classical (CD14+/CD16++, or Mon3) in the blood of healthy grown-up person [10]. Noteworthy, there are reports [11-16] suggesting that an additional CD14+/CD16+ monocyte subset could be distinguished. There are also suggestions to relate this subset to the intermediate subset [17]; however, this needs further clarification. Thus, despite the discrepancies in literature on the nomenclature of human monocytes, in this article we will follow the classification system approved by the NCIUIS. Moreover, the available literature data indicate variations in levels of secreted cytokines and surface markers among and within individual monocyte subsets obtained from fresh and cryopreserved bone marrow or blood of human and mouse origin. We summarize the most overlapping results obtained after investigation of the fresh monocytes from human blood. The summarized results about monocyte subsets, certain surface markers, produced specific cytokines, physiological functions, distribution in the blood and implicit place of subset formation are presented in the Table 1.

Table 1. The subsets of human blood monocytes with certain surface markers, produced specific cytokines, activated physiological functions, distribution in the blood and implicit place of subset formation.

| Classical (Mon1) subset | Intermediate (Mon2) subset | Non-classical (Mon3) subset | Reference |
|------------------------|----------------------------|-----------------------------|-----------|
| CD14+/CD16−            | CD14+/CD16+                | CD14+/CD16++                | 18,19-35  |
| Highly expressed surface markers |                     |                             |           |
| CCR1, CCR2, CD1d, CD9, CD11b, CD33, CD36, CD62L, CD64, CLEC4D, CLEC5A, CXCRI-4 | CCR5, CD11b, CD32, CD40, CD47, CD54, CD64, CD80, CD86, CD163, GFRα2, HLA-ABC, HLA-DR, TNFR1 | CD45, CD47, CD116, CD123, CD294, CD11c, CX3CR1, P2RX1, Siglec10, SIRPα, SLAN, TNFR2 | |
| High levels of cytokines | IL-6, IL-8, IL-10, TNF-α, IL-1β, IL-6, IL-8 | TNFα, IL-1β, IL-6, IL-8 | 19,25,36,37 |
| Activated function | Phagocytosis; adhesion to the endothelium; migration; anti-microbial responses; inflammation | Antigen presentation; participation in proliferation and inflammatory responses; regulation of apoptosis; trans-endothelial migration; high ROS production | Complement and FcR-mediated phagocytosis; trans-endothelial migration; adhesion; anti-viral responses; patrolling the endothelium | 18,38 |
| Part of total monocyte count in the blood (%) | 80.1 ± 7 | 3.7 ± 2 | 6.2 ± 2.8 | 18,24,37 |
| Implicit place of formation/persistency | Bone marrow/tissues | Peripheral blood flow or tissues/blood | Peripheral blood flow or tissues | 39,40 |
| Lifespan | 1 day | 3-4 days | 4-7 days | 41 |

CD14 – a glycosylphosphatidylinositol (GPI)-anchored receptor known to serve as a co-receptor for several Toll-like Receptors (TLRs) both at the cell surface and in the
endosomal compartment; CD16 – a type I transmembrane low-affinity receptor for IgG (FcγRIIAa); CD36 – a class B scavenger receptor; CCR2 – C-C chemokine receptor type 2 (CD 192); HLA-DR – one of the key cell surface molecules expressed on antigen presenting cells; CD11c – a type I transmembrane protein expressed on monocytes, granulocytes, a subset of B cells, dendritic cells and macrophages; CXCR1 – one of more than 20 distinct chemokine receptors, a receptor to interleukin-8; CXCR2 – a member of the chemokine receptor family involved in neutrophil chemotaxis; CLEC4D – a member of the C-type lectin/C-type lectin-like domain (CTL/CTLD) superfamily with diverse functions, such as cell adhesion, cell-cell signaling, glycoprotein turnover and roles in inflammation and immune response; CLEC5A – a pattern recognition receptor for members of the Flavivirus family; CD40 – a receptor also known as TNFRSF5, a tumor necrosis factor receptor superfamily member 5; IL13RA1 – interleukin 13 Receptor Subunit Alpha 1; CD62L – L-selectin; CD86 – a type I membrane protein, which is a member of the immunoglobulin superfamily; CLEC10A – Ca²⁺-dependent lectin-type receptor family member 10A, CD301, an endocytic receptor; CD99 – a cell surface glycoprotein; GFRA2 – a glial cell line-derived neurotrophic factor receptor alpha 2; CD163 – an acute phase-regulated and signal-inducing transmembrane receptor for the hemoglobin–haptoglobin (Hb:Hp) complexes; CD74 – a cell-surface receptor for the cytokine macrophage migration inhibitory factor; P2xr1 – purinoceptor subunit; CD1d – a glycoprotein and a member of the CD1 family of Ag-presenting molecules; CXCR4 – a G-protein-coupled chemokine receptor for extracellular ubiquitin; G-CSF – granulocyte colony-stimulating factor; IL – interleukin; LPS – lipopolysaccharide; MCP-1 – monocyte chemoattractant protein 1; CCL2 and CCL3 – small cytokines that belongs to the CC chemokine family.

Different monocyte subsets also differ in their origin. Mon1 are synthesized in the bone marrow, and from there they enter the bloodstream. In literature the average lifespan of Mon1 is reported to be 1.0 (SD=0.26) day [42]. Most of Mon1 leave the circulation or die, whereas the remaining cells transform to Mon 2 [42]. The average lifespan of Mon2 was found to be 4.3 (0.36) days, and all of them transform to Mon 3. Noteworthy, the average lifespan of Mon3 was reported to be the longest, i.e., 7.4 (0.53) days. It is also thought that Mon2 may convert to Mon1 after entering the blood [18] or alternatively, the Mon1 released from bone marrow can differentiate to both Mon2 and Mon3 [26]. Thus, there are several theories of Mon3 origin [18,42-48]. One of these theories states that human monocytes mature in the bone marrow and are subsequently released into the circulation as Mon1 monocytes. These monocytes migrate to sites of injury in a CCR2 (chemokine (C-C motif) receptor) -dependent manner [43] and differentiate into macrophages [11,18,43,44]. Progressively, the Mon1 monocytes give rise to the Mon3 subset through the Mon2 subtype of monocytes [46] (Fig. 1A). Moreover, the experimental data indicate that Mon1 monocytes can turn into both Mon2 and Mon3 subsets [45] or give rise to the Mon3 through the Mon2 [42]. It is thought that a part of Mon1 monocytes from circulatory system or tissues that return to the bone marrow may be converted to the Mon3 subset as well [42] (Fig. 1B). In addition, the third possibility has also been shown suggesting that Mon2 monocytes can be formed from macrophages in vascular atherosclerotic plaques and released into the circulatory system [45] (Fig. 1C). It is also worth noting that there is scientific evidence about the co-existence of all three monocyte subsets in bone marrow [42]. Thus, a possibility that different monocytes might be formed through all of the mentioned ways cannot be ruled out.

However, it is not completely clear what exactly determines the development of different monocyte subsets. It might be that infections and human chronic or metabolic disorders could stimulate the production or prevalence of certain subsets. For instance, obesity has been found to cause the domination of the Mon2 and Mon3 subsets over the Mon1 [42,46]. The circulating monocytes in obese individuals showed an increased
expression of chemokine receptor CX3CR1, suggesting a strengthened monocytes’ interaction with chemokine receptor CX3CL1-secreting adipocytes [46]. Accordingly, obesity was characterized by a higher number of monocyte-derived adipose tissue macrophages in both mouse and human [49,50]. It is also worth noting that caloric restriction demonstrated favorable effects in several chronic metabolic disorders and CVD [48,51]. In addition, a short-term fasting reduced the count of all monocytes in healthy human subjects [52]. Moreover, it has also been found that certain growth factors can determine the formation of a particular subset of monocytes [52].

Figure 1. Scheme of putative monocyte subset formation [18,42,45,53,54].

A

B

C

Mon1 – classical monocytes; Mon2 – intermediate monocytes; Mon3 – non-classical monocytes.
Macrophage colony-stimulating factor-1 (M-CSF-1) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are cytokines known for regulating the development and function of monocytes and macrophages both in the steady-state and during inflammation [55]. Stimulation of monocytes with M-CSF-1 increases the total monocyte count, and the size [56] and count of Mon3 monocytes [57]. In addition, the surface protein CD14 expression becomes more intense among Mon1 monocytes. In contrast, stimulation of monocytes with GM-CSF has an opposite effect: the reduced CD14 expression among Mon1 monocytes [18].

Thus, although it is agreed that Mon1 monocytes originate from precursors in bone marrow, the origin of other monocyte subsets and regulatory mechanisms leading to the formation of a particular monocyte subset depend on the cellular environment and still need further experimental analysis.

3. Involvement of different monocyte subsets and monocyte-derived macrophages in the inflammatory processes

Monocytes are a part of the mononuclear phagocytosis system and are essential for the immune system. They protect tissues from harmful pathogens by direct removal of pathogens by phagocytosis, and they are also considered the major source of various cytokines and precursors of macrophages and dendritic cells. Mon1 monocytes take part in the immune response through released cytokines and by differentiation into macrophages and dendritic cells [40]. Noteworthy, under pro-inflammatory conditions the stimulated macrophages can be not only beneficial but also harmful, as they can contribute to the inflammation that is associated with chronic diseases [58].

The main function of Mon1 is the initiation of an inflammatory response and phagocytosis [39,40]. The Mon1 monocytes can enter non-inflamed tissues, where they express major histocompatibility complex MHC II [40]. During bacterial infection, Mon1 monocytes are deployed at sites of inflammation, they recognize and phagocytize pathogens, secrete high levels of pro-inflammatory (IL-1β, IL-6) and low levels of anti-inflammatory (IL-10) cytokines, and through secreted monocyte chemotactic protein-1 (MCP1) and CCL2 attract other immune cells to regulate the inflammatory response. It is known that Mon1 monocytes have higher peroxidase activity, higher ROS production and they are also linked to more pronounced expression of macrophage antigen-1 (Mac-1), scavenger receptors SR-A1 (CD204) and SR-A2 (macrophage receptor with collagenous structure, MARCO), and to stronger binding to plasma low density lipoprotein (LDL) than the Mon3 subtype [59,60-62]. Consequently, the Mon1 monocytes phagocytically are more active than Mon3 cells, and actively take place at the initiation, development and resolution stages of inflammation in tissues [63-65].

Mon2 monocytes are involved in inflammatory processes through antigen presentation, cytokine secretion, regulation of apoptosis, and angiogenic activity [39,40]. They express a stronger pro-inflammatory capacity than the Mon3 subset, since the cells produce higher levels of ROS, TNF-α, IL-1β and IL-6, CCL3 and express the highest levels of antigen presentation-related molecules [66-69]. The intermediate subset has been associated with chronic vascular and endothelial damage and atherosclerosis [60-71]. During inflammation the Mon2 and Mon3 monocytes infiltrate inflammatory tissue due to chemotaxis through CCR5/CCL5 [72].

The third subset of monocytes (Mon3) produce significantly lower levels of pro-inflammatory cytokines if compared to Mon1 [73]. Moreover, the Mon3 take place in complement and Fc γ receptor-mediated phagocytosis and neutrophil adhesion at the endothelial interface [39,66] and thus, Mon3 monocytes can be regarded as endothelial patrols [74]. Both Mon1 and Mon3 monocytes are found in the coronary vasculature of healthy person. However, the Mon1 monocytes are shown to circulate rapidly, whereas the Mon3 monocytes circulate more slowly, crawling along the endothelium [75].
It is worth noting that on entry into tissues the monocytes can develop into two additional types of cells, namely dendritic cells (Dsc) and macrophages [12]. However, there are indications that the Mon 1 monocytes are most involved in the transformation into macrophages but not into Dsc [75]. Dsc are antigen-presenting cells, and their main function is to process and present antigens to lymphocytes [76]. Dsc are classified as plasmacytoid (pDCs), conventional (cDCs) and monocyte-derived mDCs [77]. All Dsc subsets secrete different cytokines, thus, consequently, induce different immunological responses [78]. M.-T. Dieterlen and M. Collin have summarized in detail the classification of Dsc and their significance for CVD recently [77,79], therefore this topic was not covered in our review.

The changes in sets of monocyte surface receptors and secreted cytokines observed under experimental conditions in vitro suggested that Mon1 monocytes can start their transformation into macrophages before entering tissues and while still are present in the bloodstream [12, 18, 80]. Therefore, it has been acknowledged that monocytes, depending on the nature of surrounding cytokines, differentiate into two main macrophage subsets that can be distinguished by their origin, localization, and pro- or anti-inflammatory functions [81]. Thus, M1 or classically activated macrophages, which are regarded as pro-inflammatory macrophages, arise in a response to IFN-γ and TNF-α secreted by lymphocyte T helper-1 (Th1) [45,82]. Whereas the formation of M2 or anti-inflammatory macrophages is stimulated by IL-4 and IL-13 released from lymphocyte T helper-2 (Th2) [81,83,84] (see Table 2).

Noteworthy, M1 and M2 macrophage subsets in human heart tissues are defined by the expression of specific C-C chemokine receptor 2 (CCR2) [85]. CCR2- macrophages (or M2) are tissue-resident and maintained through local proliferation, while CCR2+ (or M1) macrophages are derived from both monocyte recruitment and local proliferation [81]. It has been shown that CCR2- and CCR2+ macrophages take place in tissue remodeling and reparation. Moreover, cardiac macrophage composition seems to be important for the left ventricular systolic function that may have effect on heart failure outcomes [81]. It should also be noted that both macrophage subsets are found in the distinct heart regions. CCR2- macrophages are found within the viable myocardial wall, whereas CCR2+ macrophages are found in the endocardium and areas of fibrotic tissue in the heart transplant recipients [81]. Noteworthy, macrophages (the literature sources do not specify the subset) have also been found in the region of the distal atroventricular node (AV) [86]. The authors speculated that macrophages may take place in conduction abnormalities beyond the AV node, including atrial fibrillation and ischemia-induced ventricular arrhythmias. Moreover, it has also been found that cardiac macrophages induced arrhythmias through IL-1β production [86]. Worth noting, macrophages that were obtained from the heart tissues of patients with either ischemic or dilated cardiomyopathy were found in the distinct part of cardiac tissue [87]. Thus, it could be suggested that macrophage subsets perform different functions in the heart (see Table 2 for the main macrophage characteristics in CVD patients). However, the role of cardiac tissue macrophages both in the healthy human and HF heart still needs clarification.
### Table 2. The main characteristics of macrophage subsets in humans with CVD [12,80-83,87-89].

| Macrophage subset | M1                  | M2                  |
|-------------------|---------------------|---------------------|
|                   | (CCR2+)             | (CCR2−)            |
| Factors determining the formation of macrophage subgroups | IFN-γ, TNF-α, LPS, GM-CSF, M-CSF, DAMP-DNA | IL-4 and IL-13, apoptotic cardiomyocytes |
| Monocyte receptors | CD68                | CD163, CD206        |
| Produced cytokines | IL-12, TNF-α, IL-1β, IL-27, IL-23. | TGF-β, IL-10        |
| Produced chemokines | CXCL 9-11           | CCL-17              |
| Produced MMP and growth factors | MMP-1, 2, 7, 9, 12 | Endothelial and tumor growth factor |
| Functions | Pro-inflammatory roles in acute injury: inflammation, phagocytosis and releasing compounds contribute to a pro-inflammatory environment | Anti-inflammatory: inflammation in injury resolution, fibroblast-mediated extracellular matrix fibrosis, cell proliferation, angiogenesis, promoting tissue repair and remodeling |

MMP – matrix metalloproteinases; CXCL – CXC motif chemokine ligand; CCL17 – C-C motif chemokine ligand 17; CCR2 – chemokine receptor; DAMP-DNA – damage-associated molecular patterns including double-stranded DNA; IFN-γ – cytokine interferon-γ; TNF-α – tumor necrosis factor-α; LPS – lipopolysaccharide; GM-CSF – granulocyte-macrophage colony-stimulating factor; M-CSF – hematopoietic growth factor; IL – interleukin.

In summary, different monocyte subsets differ in their surface markers, production of various cytokines and, consequently, in their functions. Mon1 monocytes are involved in phagocytosis, innate immune responses and migration within tissues. Furthermore, they can differentiate into DSc and, depending on the environment, into pro-inflammatory (M1) or reparative anti-inflammatory (M2) macrophages, and play an integral part in shaping inflammation and its resolution in tissues. Mon2 monocytes are involved in the regulation of apoptosis and show angiogenic activity. Whereas, the Mon3 monocytes can be regarded as endothelial patrols, and they also take place in immune maintenance. Noteworthy, monocyte subsets presented and discussed in this chapter represent the most common classification system; however, further categorization into transcriptionally distinct subsets is also undergoing.

### 4. Monocytes in CHF

#### 4.1. The distribution of monocytes in CHF

It is intriguing that the role of monocytes under different CHF conditions is complex and, depending on the different monocyte subset number, encompasses inflammatory processes leading to tissue damage or repair [85,90-92]. We summarized the articles, where monocyte subset (CD14++; CD16-, CD14++; CD16+, CD14++; CD16++)
distribution in CHF patients was investigated; however, we did not evaluate works in which monocyte subsets were classified differently.

Several studies have demonstrated that Mon1 was the predominant subset in HF (87-48%) and it was followed by the Mon2 (5-44%) and Mon3 subsets (7.1-8.4%) [85,93-96]. Thus, the data indicate a significant expansion of Mon2 subset in HF patients if compared to healthy controls (see also Table 3). Another study examined CHF patients with idiopathic dilated cardiomyopathy (65% of investigated population) and ischemic heart disease (the remainder part of population, in total n=20), and found a higher total leukocyte count and a higher absolute monocyte count in CHF group when compared with healthy persons, but no differences in monocyte subset ratio (but not the cells count) between healthy and diseased persons [93]. However, under ambulatory treated HF conditions the Mon1 and Mon2 subsets (50.0±17.2% and 42±17.2%, respectively) outnumbered the Mon3 monocytes (8.1±4.0%) [85]. In contrast, the Mon1 subset significantly prevailed over the other two in healthy persons (see Table 3). Moreover, the proportion of Mon2 monocytes in HF was clearly enlarged if compared with healthy persons. Noteworthy, the ratio of different monocyte subsets in HF varies and it might be dependent on different CHF conditions and hemodynamic changes [93,94]. For example, it is considered that CHFrEF can be associated with increase in the proportion of Mon3 subset when compared to healthy persons and after acute exercise [93,94,96,97], while the Mon2 subset was the most abundant in stable CHF, where patients were not classified according to LVEF [94]. Moreover, several studies found that the increased levels of Mon2 subset and decreased levels of Mon1 subset in CHF patients were related to HF severity, which was defined as New York Heart association (NYHA) functional class advancement including the reduction of LVEF [95]. Worth noting that several studies did not find correlation between monocyte subsets percentage and NYHA functional class or LVEF [85,96]. In addition, the Mon2 levels directly correlated with C-reactive protein (CRP; it reflects the increased systemic inflammation) level and with neutrophil count [95]. Recently it has also been found that Mon2 count was the highest in CHF patients who died [85]. The deceased persons were of older age, were characterized by worse NYHA functional class, and contained a higher NT-proBNP level [85]. Moreover, the NYHA functional class correlated with total monocyte count and percentage, and the CRP concentration correlated with NT-proBNP level in HF patients as well [98,99]. Therefore, it could be assumed that the more affected heart muscle is, the higher pro-inflammatory environment is related to the predominance of Mon2 subset in patients with CHF (see Table 3). It is worth adding that the elevated levels of Mon2 monocytes were also found among the HF patients, where men with chronic HF and ischemia as a cause of disease comprised the majority of cohort [85]. Again, the results could be supported by the fact that Mon2 monocytes express a higher pro-inflammatory capacity than the Mon3 subset [66]. Thus, it could be assumed that the increasing number of Mon2 monocytes within the damaged cardiac tissue is required for the earliest steps in wound healing [85]. However, the Mon2 persistence exceeding the initial repair process could lead the longer-term inflammation-related deleterious effects into healthy remote myocardial areas. Consequently, such an observation might explain the worse prognosis of HF patients with increased level of Mon2 monocytes [85]. Another study [100] has also reported a link between the amount of Mon2 subset and poorer prognosis in acute HF patients. There are also studies suggesting that a high level of Mon2 monocytes can be a risk factor for the elevation of myocardial infarction (MI) deleterious effects and cardiovascular events [101].

For the sake of correctness, it is worth mentioning a study that did not find any correlation between the percentage of Mon2 subset nor the number of Mon2 cells/μL and NYHA functional class, LVEF or estimated glomerular filtration rate, except a weak statistically significant inverse correlation between the percentage of Mon2 monocytes and LVEF (r= −0.14) in ST-segment elevation myocardial infarction (STEMI) patients [101]. It was demonstrated that the level of Mon2 subset increased in first week after
STEMI and it was associated with worsened outcomes during a 2.5-year follow-up period [101]. These findings are in agreement with the results from other study [102] showing the increased level of Mon2 monocytes in patients with worse CHF condition. Thus, it could be that Mon2 subset takes place in healing process after MI, since this is the only known subset capable of promoting angiogenesis during healing process after MI [102]. Moreover, the Mon2 was the only subset that increased in patients with stable HF, and the amount further increased under acute HF [103]. Noteworthy, a high Mon2 count was associated with better survival during the study. Therefore, it could be suggested that Mon2 monocytes demonstrate potentially protective properties in patients with failing hearts. Mon2 could also be related to the acute inflammation, and it seems that these monocytes are not desirable and even harmful after acute phase. Thus, it is important to find out what determines an increase in Mon2 count after the acute period of disease in some patients, and a decrease in others.

When the monocyte subset distribution was assessed according to the different etiologies of HF, there were no statistically significant differences when percentages of subsets were considered [85]. It is interesting that a statistically significant difference was observed in the Mon3 subset when a number of cells was determined (cells/μL). Moreover, Mon3 monocytes showed a protective significant association with all-cause death [85]. Worth adding that the number of Mon 3 was found unchanged or reduced in the first week after STEMI [101], or increased in CHF [85,93], and increased even after acute exercise in CHF [93]. In a specific comparison related to the levels of this monocyte subset, valvular patients showed lower percentage (6.7 ± 3.4 versus 8.2 ± 4, p = 0.04) and number of cells (35.2 (23.7–63) versus 49.1 (34.7–70), p = 0.01) than ischemic patients, and also than patients with dilated cardiomyopathy (8.3 ± 3.3, p = 0.03 and 49.7 (36.5–77.3), p = 0.006, respectively). Noteworthy, there were no differences in the level of Mon2 subset among CHF etiologies. However, a statistically higher percentage of Mon3 monocytes was found in deceased patients [85].

It is worth noting that the amount of Mon1 monocytes in patients with ischemic HF was close to the values observed in patients with coronary artery disease without HF (a control group) [103]. However, the level of Mon1 increased during the HF decompensation. Noteworthy, Mon1 monocytes are known to be involved in myocardial remodeling at the site of dying cardiomyocytes.
Table 3. The distribution of monocyte subsets in CHF.

| Investigated person | CHF (idiopathic dilated cardiomyopathy (65% of investigated population) and ischemic heart disease) | Healthy Ambulatory treated CHF I-IV NYHA functional class Alive Deceased | CHF I-III NYHA functional class | Healthy Stabile CVD (with angiographically documented one-three-vessel disease), where 43% NYHA class III. | Healthy Systolic CHF II-IV NYHA functional class | Healthy Alive Deceased |
|---------------------|-------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|-----------------|---------------------------------------------------------------------------------|---------------------------|------------------------|
| Reference           | 93                                                                                             | 85                                                                     | 95               | 94                                                                              | 96                        | 59                     | 29                     |
| n                   | 20                                                                                             | 15                                                                     | 293              | 107                                                                             | 30                        | 26                     | 14                     | 13                   | 59                     | 29                     |
| Leukocyte count (10$^6$/mL) | 8.24(1.82)                                                                      | 7.17(1.60)                                                             | 8.34(0.62)       | 6.45(0.26)                                                                    | 7.0(4.2-9.4)             | 6.7(4.3-15.6)         |
| Monocyte % of leukocytes | 7.72(1.88)                                                                      | 6.28(1.24)                                                             | 5.1(3.6-10.8)    | 3.7(3.2-8.0)                                                                   |
| Count (cells/μL)    | 628(159)                                                                                   | 450(128)                                                               | 629(61)          | 509(34)                                                                        | 354(131-452)             | 308(187-440)           |
| Monocyte subsets (% of monocytes) | % Mon1 87.34(3.54)                                                                  | 88.09(4.73)                                                            | 50.4(16.5)       | 48.9(19.08)                                                                    | 73.5(1.8)                | 84.3(1.9)              |
|                      | % Mon2 4.74(2.46)                                                                         | 4.51(2.05)                                                             | 41.2(16.5)       | 44.0(18.8)                                                                     | 12.3(8.7-14.8)           | 5.9(4.7-6.9)           |
|                      | % Mon3 7.92(2.19)                                                                         | 7.39(3.17)                                                             | 8.42(4.0)        | 7.1(4.0)                                                                       |
| Monocyte subsets (cells/L) | Mon1 550.3(143.9)                                                                     | 395.2(107)                                                             | 327(222-435)     | 363(227-451)                                                                   | 303(113-437)             | 266(161-412)           |
|                      | Mon2 29.3(17.1)                                                                           | 20.7(13.5)                                                             | 253(170-374)     | 303(186-470)                                                                   |
|                      | Mon3 49.3(17.3)                                                                           | 34.1(20.9)                                                             | 48(35-71)        | 44(27-73)                                                                      |

The readings with statistically significant difference between the healthy and patients’ groups marked in bold. CHF-chronic heart failure, CVD-cardiovascular disease.
Worth adding that the presented studies about the distribution of monocyte subsets in CHF possess limitations related to: i) a small number of patients, ii) unequal size of analyzed groups and iii) the lack for comorbidities that might be responsible for the abnormal release of monocytes. Moreover, it is complicated to compare the results because of different causes of CHF and different patient conditions. Nevertheless, it is suggested that Mon2 monocytes predominate in the presence of surviving HF, and the cytokines and chemokines they release lead to fibrosis of healthy heart tissue. Thus, as a result, the left ventricular relaxation in diastole is impaired. In contrast, the Mon1 monocytes are found to be involved in myocardial remodeling at the site of dead cardiomyocytes. But it is also possible that a different subset of monocytes involved in myocardial remodeling can change over time with different causes of heart failure and differences in its pathogenesis. Thus, the monocyte distribution in patients with different CHF reasons and conditions is still not yet well understood.

4.2. Influence of monocyte secreted cytokines and inflammatory readings on HFrEF and HFpEF development.

Inflammation plays different roles in the onset and progression of HF in HFrEF and HFpEF [85,104-106]. Fibrosis occurs and is differentially managed between these two HF groups. Ischemic heart disease and cardiomyocyte loss leads to the HFrEF [2]. It is documented that MI and subsequently the heart muscle necrosis causes the systemic and cardiac inflammation, which involves activation of monocytes. Activated monocytes produce cytokines and chemokines, and thus further promote inflammation [107]. Late cardiac remodeling after MI includes remodeling of both infarcted and non-infarcted myocardium since: i) unaffected myocardium strives to compensate function of impaired heart area, ii) damaged myocardium is replaced by collagen scar, and iii) the scar expands into the healthy area [108]. In addition, TNF-α secreted by monocytes triggers uncontrolled oxidative stress, cardiomyocyte apoptosis and even tissue necrosis [109,110]. The loss of cardiomyocytes contributes to the deterioration of heart muscle contractile function, and thus to the development of HFrEF [111]. Moreover, the excessive and prolonged infiltration of monocytes/macrophages into the damaged myocardium causes harmful inflammatory responses that can lead to cardiac fibrosis and adverse myocardium remodeling when LVEF become reduced and is insufficient to provide the tissues with necessary supplements and oxygen [112].

Chronic hypertension, cardiomyopathy and valvular heart disease alter metabolism in cardiac tissue and can cause the HFpEF. It has been proposed that HFpEF can be regarded as low-grade chronic systemic inflammation with activated nuclear factor kappa B (NFkB) pathway and synthesis of pro-inflammatory cytokines and chemokines [113]. The released molecules subsequently activate hematopoetic cells in bone marrow and spleen, and the process leads to the systemic low inflammation with the increased number of blood leukocytes, neutrophils and monocytes [114]. Worth remembering that Mon1 monocytes enter the heart tissue and become the pro-fibrotic macrophage subset (M2) [75], which activates fibroblasts (Table 2). Activated fibroblasts synthesize more collagen and fibronectin, and that leads to the increased myocardial stiffness [2,114]. In this case, the LVEF is normal but the diastolic function becomes impaired, i.e., the prolonged LV relaxation and filling, the increased diastolic stiffness and elevated LV end-diastolic pressure [115]. It is thought that the LV stiffness is caused by the reduced Ca²⁺ signaling and titin modifications [113]. Noteworthy, cardiac stiffness leads to extracellular matrix changes, cardiac fibrosis and hypertrophy of cardiomyocytes, and consequently, the hypertrophic changes result in the diastolic dysfunction [116].

The functional diversity of monocytes and macrophages, and their ability to contribute to different cardiac processes depend on their phenotypic plasticity [89]. However, it is still unclear how the balance of monocyte subset both in HFrEF and
HFpEF is achieved. It seems, certain cytokines and chemokines are the factors that determine monocyte subsets and the clinical outcome. For instance, 1.3- to 2.4-fold increased systemic levels of inflammatory markers (TNF-α, IL-6) and chemokine CCL2 in worsening HFpEF, when compared to stable disease, suggest that intensified inflammation may contribute to clinical worsening in HFpEF patients [117,118]. Moreover, the twofold increased level of macrophages in myocardial biopsies from HFpEF patients, and by 59% stimulated expression of profibrotic cytokine transforming growth factor beta (TGF-β) (compared to control) were associated with fibroblast activation and excess deposition of collagen [119,120]. Importantly, HFpEF patients also had two to four-fold elevated circulating levels of neutrophils and of Mon1, Mon2, and Mon3 monocytes, while the levels of circulating lymphocytes were not affected [119,121]. Thus, the results suggest a development of chronic inflammation during HFpEF.

In another study it was shown that a seven-day incubation of primary monocytes from healthy subjects in cell media containing 10% serum from HFpEF patients stimulated differentiation of monocytes into the IL-10-expressing M2 macrophages [121]. Thus, it could be proposed that a long-lasting stimulation in HFpEF patients could push emerging macrophages towards a fibrogenic phenotype, which promotes myocardial collagen deposition and diastolic dysfunction. Noteworthy, the synthesis of IL-10 has been found to be beneficial in heart tissue repair and the resolution of inflammation following acute injury, thus preventing the HFpEF after MI [122,123]. In contrast, IL-10 was found to evoke the adverse effects in a case of chronic condition by promoting myocardial fibrosis and diastolic dysfunction in HFpEF [119]. Thus, the fact that the same pathways may result in a positive outcome in HFrEF and could lead to a pathology in HFpEF should be kept in mind while designing novel therapeutic strategies to limit the disease progression in HF of diverse etiologies.

It has also been shown that in HFrEF patients, separated into two groups according to neutrophil count (relatively low and high), the CRP and fibrinogen concentrations and monocyte count were higher in the group with a higher neutrophil count [97,99]. These observations are in line with low inflammatory environment in HFrEF patients. Regardless of the cause of HFrEF, inflammation results in cardiac remodeling evoked by cardiomyocyte damage and loss due to cardiomyocyte autophagy, apoptosis and necrosis [97]. In addition, metabolic risk factors in HFpEF take place in the chronic low systemic inflammation together with the stimulated expression of adhesion molecules on the endothelial cells, what lead to systemic and local inflammation. Moreover, the circulating levels of pro-inflammatory markers (IL-6, TNF-α) and acute inflammatory CRP were higher in HFpEF when compared to HFrEF [124,125]. Noteworthy, we have recently found that in HFrEF patients the monocyte percentage and count were statistically significantly the highest in the NYHA IV group, and the NYHA functional class correlated with the total monocyte count and percentage (r=0.172). In addition, CRP concentration correlated with NT-proBNP (r=0.203) as well [98]. Therefore, it could be assumed the more affected heart muscle is, the higher pro-inflammatory environment patients with chronic HFrEF have.

Compounds, released into the environment from dying cardiac cells stimulate M2 macrophages (arisen from Mon1) to produce anti-inflammatory cytokines, including IL-10 and TGF-β, which preserve neighboring tissue and cardiac function. Dying cardiomyocytes also secrete damage-associated molecular patterns (DAMPs) including double-stranded DNA. Recognition of DAMPs by M1 macrophages promotes secretion of pro-inflammatory cytokines, including IL-1β (Table 2), and leads to collateral tissue damage, adverse ventricular remodeling and systolic dysfunction [88]. Over months to years systemic neuroendocrine activation and compensatory mechanisms such as LV wall thinning and chamber dilation lead to HFrEF and its progression [126].

It is also known that in HFrEF patients the levels of IL-1β and TNF-α can increase two- to six-fold as compared to control subjects, and thus signal worsened outcomes
Therefore, it is proposed that the macrophage-mediated inflammation plays a crucial role in HFrEF pathogenesis. Moreover, a failure to clear apoptotic cardiomyocytes can lead to the secondary necrosis and the release of DAMPs, which further stimulate the pro-inflammatory reactions and collateral tissue injury. Therefore, the increased population of cardiac M1 macrophages in the ischemic heart and persistent inflammation transform the hematopoietic compartment and lead to further macrophage infiltration into the heart causing harmful remodeling, systolic dysfunction and HFrEF progression (Table 4).

It has also to be mentioned that macrophage migration inhibitory factor (MIF; the inflammatory cytokine) mediates the pro-inflammatory effects leading to fibrotic remodeling in HF due to non-ischemic cardiomyopathy with reduced LVEF. Furthermore, MIF expression statistically significantly correlated with a degree of myocardial fibrosis (r=0.51) [129]. In addition, the main differences between HFPpEF and HFrEF in monocyte and macrophage subsets, pathogenesis and myocardial changes are presented in Table 4.

Altogether, there is still an ongoing debate whether the functions of monocytes and macrophages should be regarded as a cause or a consequence in human CHF development. Despite the findings that Mon2 is more important in HFPpEF and Mon1 - in HFrEF, further investigation defining the interchange of signals between macrophages and other cardiac resident cells like monocytes, fibroblasts and cardiomyocytes is needed. Moreover, the influence of comorbidities and risk factors on the levels of monocyte and macrophage subsets and their functions are also needed to revise the existent therapeutic strategies. Thus, the choice of inflammatory cytokines as a therapeutic target, as well as the monocytes themselves necessitate more research on this topic, as the existing ones are not sufficient to prove certain targets for the treatment under consideration.

Table 4. The differences between HFPpEF and HFrEF in monocyte and macrophage subsets, pathogenesis and myocardial changes [113,117-122,124-126].

|                          | HFPpEF                                                                 | HFrEF                                                                 |
|--------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------|
| The predominant monocyte subset in myocardium | CD14 ++, CD16 + (Mon2)                                                  | CD14 ++, CD16- (Mon1)                                                  |
| Differences in pathogenesis | Low-grade systemic inflammation; Monocytes produce chemokines (MCP-1, TNF-α, TGF-β, IL-6). | Cardiac inflammation; Fibrosis is associated with monocyte surface TLRs and migration of Mon1 monocytes into the myocardium due to increased levels of IL-1β and CCR2 expression. |
| Macrophage subset        | M2                                                                     | M1                                                                     |
| Myocardial changes       | LV stiffness is caused by reduced Ca\(^2+\) signaling; Conversion of titin to a less flexible form; Perivascular and interstitial fibrosis; Fibrotic changes in extracellular matrix and cardiomyocyte hypertrophy; Impaired relaxation of the heart muscle | Collagen scar formation; Cardiomyocytes’ apoptosis; Impaired myocardial contraction |

CD14 – a glycosylphosphatidylinositol (GPI)-anchored receptor known to serve as a co-receptor for several Toll-like Receptors (TLRs) both at the cell surface and in the
5. Conclusions

According to the available literature data it could be suggested that monocytes together with macrophages take place both in acute and chronic HF processes. Moreover, every patient’s organism, depending on the severity of heart tissue damage and general condition of the body, reacts differently by releasing different cytokines, what afterwards leads to the specific organism responses including the formation of different monocyte subsets, the formation of specific subsets of macrophages, and the release of certain additional cytokines. Finally, the processes lead to the inhibition or maintenance of inflammation in the damaged heart tissue. Consequently, whether the patient’s condition improves or worsens depends on whether the inflammation is suppressed or sustained. Therefore, elucidation of the mechanisms of pathogenesis would allow to search for ways and to develop the means to direct the patient’s immune system only towards healing. On the whole, there is still and open question regarding the pathways of monocyte subset formation. Moreover, the Mon1 monocytes can be characterized by pronounced phagocytosis, by ability to differentiate into macrophages and to synthesize the pro-inflammatory cytokines, and thus they are involved in myocardial remodeling at the site of dying cardiomyocytes. The Mon2 monocytes are involved in apoptosis regulation and angiogenesis, and they secrete both the anti- and pro-inflammatory cytokines, whereas the Mon3 monocytes express the anti-inflammatory potential and patrol the endothelium. In addition, it is suggested the Mon2 subtype predominates over other two subtypes under surviving CHF conditions, whereas the Mon1 monocytes dominate in HFrEF patients, and the Mon3 subtype predominates in HFrEF patients. However, a general picture about the distribution of various subtypes in patients with different CHF conditions is still obscure, and further research is certainly needed.

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