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Research Article

# Lack of Association between Mac 387 and Atherosclerosis Development

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## Abstract

**Introduction:** At a global level, cardiovascular disease (CVD) is regarded as the primary reason for death. Atherosclerosis, which is the most common form of CVD, is characterized by cholesterol buildup and inflammation of the major arteries. The development of these issues, such as myocardial infarction (MI) and stroke, can be attributed to atherosclerosis.

**Study Objective:** The current study's objective was to investigate the role of macrophages (MAC387) in the development of atherosclerosis.

**Methodology:**

- **Study Design:** A retrospective study design was conducted to collect data and samples of autopsies.
- **Study Sample:** A total of 11 cases who were reported to have atherosclerosis, was included in this study.
- **Study Procedure:** Both H&E staining for routine histologic studies, and indirect immunoperoxidase staining for MAC 387 were carried out to study the atherosclerosis in selected samples.

**Study Findings:** Atherosclerosis was identified in many persons. Various degrees of atherosclerosis were also identified. The expression of MAC387 was weak in all sections and at various degrees of atherosclerosis.

**Conclusion and Recommendations:** The results of the current investigation indicated that there was no association between the development of atherosclerosis and MAC 387 macrophages. It is recommended to conduct further studies to investigate the localization of more subsets of macrophages such as CD86, and to study the localization of lymphocyte types.

***Keywords:*** *Cardiovascular Disease; Atherosclerosis; Macrophage (MAC 387); CD68; Immunohistochemistry; H&E*

## Introduction

The greatest cause of death worldwide is cardiovascular disease (CVD), which accounts for about 50% of death cases from non-communicable diseases [1]. Although the most common type of CVD, coronary artery disease, has well-established risk factors, such as hypertension, high cholesterol, aging, and inheritance, CVDs are increasingly understood to be chronic inflammatory disorders [2,3]. When too much cholesterol builds up inside the artery wall, it causes a chronic inflammatory state that leads to atherosclerosis [4]. To disseminate disease, inflammatory cells invade the generally quiet intimal layer of the artery wall and change the nearby endothelium, smooth muscle, and extracellular matrix elements [5].

Atherosclerosis is a persistent inflammatory condition brought on by cholesterol buildup in the artery wall [6]. Due to localized endothelial dysfunction, certain areas of arteries, such as branching points and bends, are more susceptible to the development of atherosclerotic plaques [7]. Circulating lipoprotein molecules accumulate in the subendothelial layer of the arterial wall intima after entering the arterial wall [8].

The most prevalent kind of CVD, atherosclerosis or coronary artery disease (CAD) is characterized by cholesterol buildup and main artery inflammation, which could eventually lead to its clinical repercussions, such as myocardial infarction (MI) and stroke [9]. Clinically significant atherosclerosis is a slow-progressing illness that mostly affects elderly people and despite a decline in prevalence in some nations, it remains to be the prime reason of death at global level [10]. Lipids, inflammatory cells, smooth muscle cells, and necrotic cell debris are found in the intimal space, which is located underneath a monolayer of endothelial cells (ECs) that line the inside of the artery wall, they accumulate and change throughout the course of a lifetime in atherosclerotic lesions [9].

Macrophages are essential for the progression of atherosclerosis in different stages [11]. It is commonly believed that atherosclerotic lesions attract circulating monocyte-derived cells, which then differentiate into macrophages there [12]. This notion, however, has been debunked by a number of recent investigations, which show that adult tissue precursor cells do not contribute monocytes to the development of the bulk of tissue macrophages [13-16]. Surprisingly, the subendothelial intimal layer of the human artery wall may include a population of pluripotent pericyte-like cells that can differentiate into a variety of cell types, including phagocytes that express the macrophage marker CD68 [17-19]. Macrophages actively consume lipoproteins and accumulate in atherosclerotic lesions, producing foam cells containing lipid droplets as a result [20]. The buildup of foam cells facilitates the storage of lipids and the development of atherosclerotic plaques [21]. Because atherosclerotic plaque-populating macrophages have a limited ability to move, inflammation persists and the lesion develops into a complex atherosclerotic plaque [22,23]. At this point, by secreting pro-inflammatory cytokines and chemokines and producing reactive oxygen species, macrophages assist in maintaining the local inflammatory response [24]. Death of macrophages in developing plaques results in the creation of necrotic cores [25,26]. Macrophages are a prospective target for therapeutic development due to their significant role in the pathophysiology of atherosclerosis [27].

## Study Objective

The current study's objective was to investigate the role of macrophages (MAC387) in the development of atherosclerosis.

## Methodology

**Study design:** A retrospective study design was conducted to collect data and samples of autopsies.

**Study sample:** A total of 11 cases who were reported to have atherosclerosis, was included in this study.

**Histological studies:** Cardiac tissue parts from atherosclerotic areas were cut into small pieces and placed in 10% formalin for 24hrs for fixative purposes. This was followed by a period of tissue processing using (Medite TPC12). This is an overnight processing period. Tissue then were embedded with paraffin using embedding station (Medite TES 99). Using Rotatory microtome (Leica RM 2125), tissue sections (4 µm thickness) were cut. The process for staining sections for H&E was as follows: racks containing tissue sections were placed in the ASI India Incubator for 60 minutes at 60°C. Sections were then immersed in Xylene for 5 minutes (twice), 100% ethanol, 90% ethanol, 80% ethanol and 70% ethanol for 5 minutes each, distilled water for 5 minutes, hematoxylin solution for 5 minutes, running tap water for 5 minutes, eosin solution for 8 minutes, 70% ethanol, 80 percent, 90% ethanol, and 100% ethanol for 5 minutes each, Xylene solution, and mounting using a solution of Distyrene (DPX). Tissue sections were then kept in special slide boxes and examined later.

### Immunohistochemical staining of MAC387

Tissue sections were stained for immunohistochemistry using the following protocol: sections were cut (4 µm thickness) and mounted into special slides for immunohistochemistry (Crystal Cruz from Santa Cruiz). Tissue sections were treated as those for H&E as described above till distilled water. After optimum conditions were reached, tissue pieces were put in a ready retrieval solution (abcam, Ab-973) that was heated using a Decloaking chamber (Biocare Medical) for 2.5 minutes. This was followed by cooling the sections till the room temperature was achieved. Five minutes were spent washing with phosphate buffered saline (Euro Clone, pH = 7.20.2). Tissue sections were dried and encircled using pap pen (abcam). The ImmunoCruz® LSAB Staining System (SC-2053) was used for the following steps: endogenous peroxidase activity was suppressed using peroxidase block for 5 minutes, followed by PBS washing, serum block incubation for 20 minutes, PBS washing, and primary antibody (MAC387, SC-66204) incubation for 2 hours (titration 1:50). Following a PBS wash, sections were incubated for 1.5 hours with a biotinylated mouse anti-goat IgG secondary antibody. Following a PBS wash, sections were incubated with the Avidin D-HRP complex for 30 minutes. Sections then were washed with PBS and incubated with HRP substrate with chromogen for 20 minutes, then sections were placed in distilled water and counterstained using hematoxylin solution for 30 seconds, dehydrated through several ascending concentrations of ethanol, and mounted using DPX.

## Results

### Histological results

Atherosclerosis was identified in different tissues. Various degrees of atherosclerosis were identified.

Atherosclerotic areas were characterized by calcification, and infiltration of some lymphocytes.

### The findings of MAC387

The expression of MAC387 was weak in all sections and at various degrees of atherosclerosis.

## Discussion

The results of this study showed that atherosclerotic lesions showed various pathological events. As an example, calcification was exhibited. However, various studies showed that the calcification of coronary is a part of the atherosclerotic process [28-30]. In the arterial wall, it has been found that coronary artery calcification (CAC) is an active process like bone creation [31-33]. The outcomes also demonstrated that lymphocytes occasionally invaded the atherosclerotic regions. Numerous investigations shown that lymphocytes are crucial to the genesis of atherosclerosis [34,35]. It is thought that immune cells have important role in accelerating the atherosclerotic lesions and different subtypes have different roles [36,37].

Immunohistochemical studies showed that there was a weak expression of macrophages (MAC 387) subtypes. The low staining intensity was observed in all parts of sclerotic lesions involving the presence of macrophages. However, these findings were not expected, because macrophages play important roles in developing atherosclerosis [38,39]. Such studies imply that atherogenesis is inflammatory disease.

The results of this study did not investigate various types of macrophages, and instead focused on MAC 387 macrophage subset. Across literature, studies showed that macrophage populations including CD68 to be included in atherosclerosis [17-19].

## Conclusion

The results of the current investigation demonstrated that there is no association between the development of atherosclerosis and MAC 387 macrophages. Numerous factors, including immunomodulation and inflammation, can be used to explain how atherosclerosis develops. The development of atherosclerosis can be explained by different mechanisms including inflammation and immunomodulation.

## Recommendations

Following the results of this study, it is recommended to conduct further studies to investigate the localization of more subsets of macrophages such as CD86, and to study the localization of lymphocyte types.

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