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EDITORIAL COMMENT

# Atherosclerotic Plaque Progression to Clinical Events

## A Clue From $^{14}C$ "Bomb Pulse" Dating*

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Atherosclerosis is an inflammatory chronic disease, characterized pathologically by the formation of lipid-laden plaques in the intima of arterial wall. Initiated at a young age, the pathogenesis of atherosclerotic plaques involves years of proatherogenic events, such as lipid deposition, inflammatory infiltration, smooth muscle cell (SMC) proliferation, and fibrotic tissue accumulation. With aging, most of the adults will develop some sort of atherosclerotic lesions. The early atherosclerotic lesions in the intima, referred to as fatty streaks, contain lipid-laden foam cells likely transformed from monocyte-derived macrophages and to a lesser extent, SMCs that take up oxidized or chemically modified lipoproteins via scavenger receptors. If not treated, under the influence of risk factors (eg, hypercholesterolemia, hypertension, hyperglycemia, diabetes, obesity, smoke, and age), fatty streaks can advance to mature plaques, typically composed of a lipid-rich necrotic core covered by a fibrous cap that comprises SMCs and various numbers of T lymphocytes and fibroblasts. Cytokines produced by activated immune cells, together with cytotoxic substances generated from lipid oxidation and tissue degeneration, may trigger apoptosis, a form of programmed cell death. Advanced, progressive plaques with increased SMC apoptosis are highly vulnerable to rupture or erosion, which often triggers acute vascular syndromes, such as myocardial and cerebral infarction (1). Timing the progression of vulnerable plaques and predicting its clinical outcome, particularly vulnerable plaque disruption, is a difficult task because the advanced fibrous plaques or atheroma may take many years to form, and their structure appears highly heterogeneous. To date, no human studies have been able yet to assess, in situ, the main biological determinants and clinical relevance to different regions of vulnerable plaques at various ages.

Traditionally, radiocarbon dating has been used as an archeological tool rather than a biological one. Natural generation of radiocarbon or carbon-14 ($^{14}C$) is achieved in the atmosphere by cosmic ray interactions with nitrogen. The atmospheric concentration of natural $^{14}C$ with respect to all carbon has, over years, been relatively stable because $^{14}C$ has a radioactive half-life of 5,730 years. The natural $^{14}C$ exists at extremely low levels, and its radioactive decay is minimal within the time periods of interest in biomedical cases. However, above-ground nuclear bomb tests in the 1950s and 1960s generated huge amounts of $^{14}C$, which were distributed evenly throughout the Earth's atmosphere, peaking in 1963, thus termed the "$^{14}C$ bomb pulse." The nuclear bomb test-associated $^{14}C$ influx led to elevation of $^{14}C$ levels in all living things, including plants, animals, insects, and humans. After the treaty to ban all the above-ground nuclear bomb testing was signed in the early 1960s, the atmospheric $^{14}C$ concentrations have steadily been declining, not due to radioactive decay, but rather by mixing with large marine and terrestrial carbon reservoirs, and by certain amounts of $^{14}C$ moving out of the atmosphere. Because of $^{14}C$ incorporation into all living things, $^{14}C$ bomb pulse dating serves as an isotopic

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chronometer of the past half-century. Assessing the temporal variations of atmospheric and organic material $^{14}$C versus nonradioactive carbon or $^{12}$C (the so-called fraction modern $^{14}$C, F$^{14}$C) enables investigators to determine the physical age of live or dead forensic tissues (2).

In this issue of *JACC: Basic to Translational Science*, Edsfeldt et al (3) compared clinical characteristics between age- and sex-matched individuals with physically young and old carotid plaques, using the $^{14}$C bomb pulse methodology. They studied 52 human atherosclerotic plaques obtained by carotid endarterectomy between 2000 and 2013. The $^{14}$C contents of isolated plaque fragments were quantified by accelerator mass spectrometry, which is a highly sensitive method for detecting very low concentrations of natural isotopic abundance of $^{14}$C. The physical age of whole plaques and specific plaque regions from the living donors was determined, and then fluorescent and histochemical analyses were employed to characterize the biological components and the 6 most common processes within the plaques. The median age of all plaques was 8.7 years. The major finding of the study was that in living patients with advanced atherosclerosis younger, rapidly progressing plaques were specifically associated with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive staining for apoptosis in CD68$^+$ macrophages residing in the core or the plaque and α-actin$^+$ vascular smooth muscle cells in the cap region of the plaque, whereas apoptosis was not detected in older plaques. Edsfeldt et al (3) also compared several known pro-atherogenic risk factors, such as hypercholesterolemia, hypertension, diabetes, smoke, and body mass index, between subjects with physically young and old plaques. Among all the risk factors tested, only body mass index was significantly higher in the subjects with the young, fast progressing plaques when compared to those with older plaques.

The results from the investigation by Edsfeldt et al (3) expand upon prior observations from their group. Their first report showed the use of using $^{14}$C bomb pulse dating technology to determine the biological age of human atherosclerotic plaque components in 10 patients. The initial $^{14}$C dating estimated that plaque age in humans varied between 5 and 15 years of age, and that the cap of the plaque was the youngest part of the plaque (4). The finding in the current study that plaque cell apoptosis is the most relevant process contributing to a rapidly progressing plaque and, subsequently, clinical events provides new information. Histologically, in advanced atherosclerotic plaques, apoptotic cells are predominantly macrophages (CD68$^+$) in the core, and vascular smooth muscle cells (alpha-actin$^+$) in the cap region (5). This implies that apoptosis as a process regulating plaque progression is a global process in the lesion, unrestricted to a certain cell type or location. However, this presents a paradoxical finding in that the physically young, fast-progressing plaques with increased apoptosis should have resulted in increased cell loss with a decrease in the mass of the core of the plaque, rather than an increase in plaque size. Apoptotic cells or bodies are usually removed by tissue phagocytes, especially macrophages. Lipid loading and proapoptotic factors may block or diminish the phagocytosis of apoptotic cells, which may lead to the accumulation of TUNEL-positive apoptotic bodies in the plaque tissue. The presence of increased TUNEL staining may not only reflect increased rates of apoptosis, but also decreased removal of apoptotic cells. The dead cells or debris may become surrounded by connection tissue, and some of the plaques become calcified over time. There are likely increased cellular components in different regions of physically young plaques, including lymphocytes, mast cells, and fibroblasts, in addition to macrophages and SMCs. Physically young, fast-progressive plaques likely have increased expression of adhesion proteins, chemokines, and mechanic stress, as well as SMC immigration and proliferation. All of these may contribute to plaque remodeling, growth, and expansion. This may provide an alternative explanation for the finding from $^{14}$C-bomb pulse dating that physically young progressive plaques contain more cells undergoing apoptosis. Lastly, it should be pointed out that TUNEL detects the ends of DNA fragments in the nuclei of cells undergoing apoptosis. Increased TUNEL positivity indicates the existence of $^{14}$C-rich DNA degradation or breakdown, which likely alters the F$^{14}$C values used to calculate the plaque physical age and causes a bias towards the young one. Despite these caveats, the paper by Edsfeldt et al (3) reports a unique human data set that will allow investigators to better understand the factors that drive plaque progression, and may provide a unique opportunity for optimizing and personalizing clinical care for patients with advanced atherosclerosis, and

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potentially for developing now treatment to halt plaque progression.

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