Pro-inflammatory role of stem cells in abdominal aortic aneurysms

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Proinflammatory role of stem cells in abdominal aortic aneurysms

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Objective: The pathogenesis of abdominal aortic aneurysm (AAA) formation includes inflammation, vascular smooth muscle cell apoptosis, extracellular matrix degradation, and oxidative stress. That multipotent stem cells have an important role in cardiovascular health and disease has been well established, but the role of stem cells in aortic structural deterioration is poorly defined. We sought to describe the presence of stem cells in human AAA tissue and also investigated the differentiation of stem cells within the aneurysmal aorta.

Methods: Infrarenal aortic wall specimens were collected from patients (n = 7) undergoing open AAA surgical repair. Nonaneurysmal infrarenal aortic control samples (n = 4) were collected at autopsies. Using immunohistochemistry, we compared the abundance of Stro1-positive ($^+$), c-kit$^+$, and CD34$^+$ cells in aortic tissue. Using double-immunofluorescence staining, we evaluated stem cell differentiation into smooth muscle cells (SM22), fibroblasts (FSP1), and macrophages (CD68). We then investigated the colocalization of CD68$^+$ cells with the cellular marker of proliferation Ki67.

Results: The media and adventitia of infrarenal AAA samples both demonstrated a significantly greater number of c-kit$^+$ and CD34$^+$ cells compared with matched control nonaneurysmal aortic tissues; however, the abundance of Stro1$^+$ cells was not significantly different between the groups. Using double-immunofluorescence staining, we identified that AAA stem cells express the macrophage marker CD68 but not the smooth muscle cell marker SM22 or the fibroblast marker FSP1. CD68$^+$ cells within the aortic wall colocalized with the cellular marker of proliferation Ki67.

Conclusions: Stem cells are significantly elevated in infrarenal AAA tissue compared with matched control aortic tissue. Our data also demonstrate that AAA stem cells express macrophage surface antigens but not smooth muscle cell or fibroblast markers. Furthermore, CD68$^+$ cells within the aortic wall colocalized with the cellular marker of proliferation Ki67. These finding suggest an inflammatory/immune role of stem cells during AAA pathogenesis and raise the possibility of localized replenishment therapy within the aneurysm wall. (J Vasc Surg 2014;■:1-9.)

INTRODUCTION

- Numerous animal models have shown that stem cell regenerative therapy may be useful in the treatment of abdominal aortic aneurysms (AAAs).

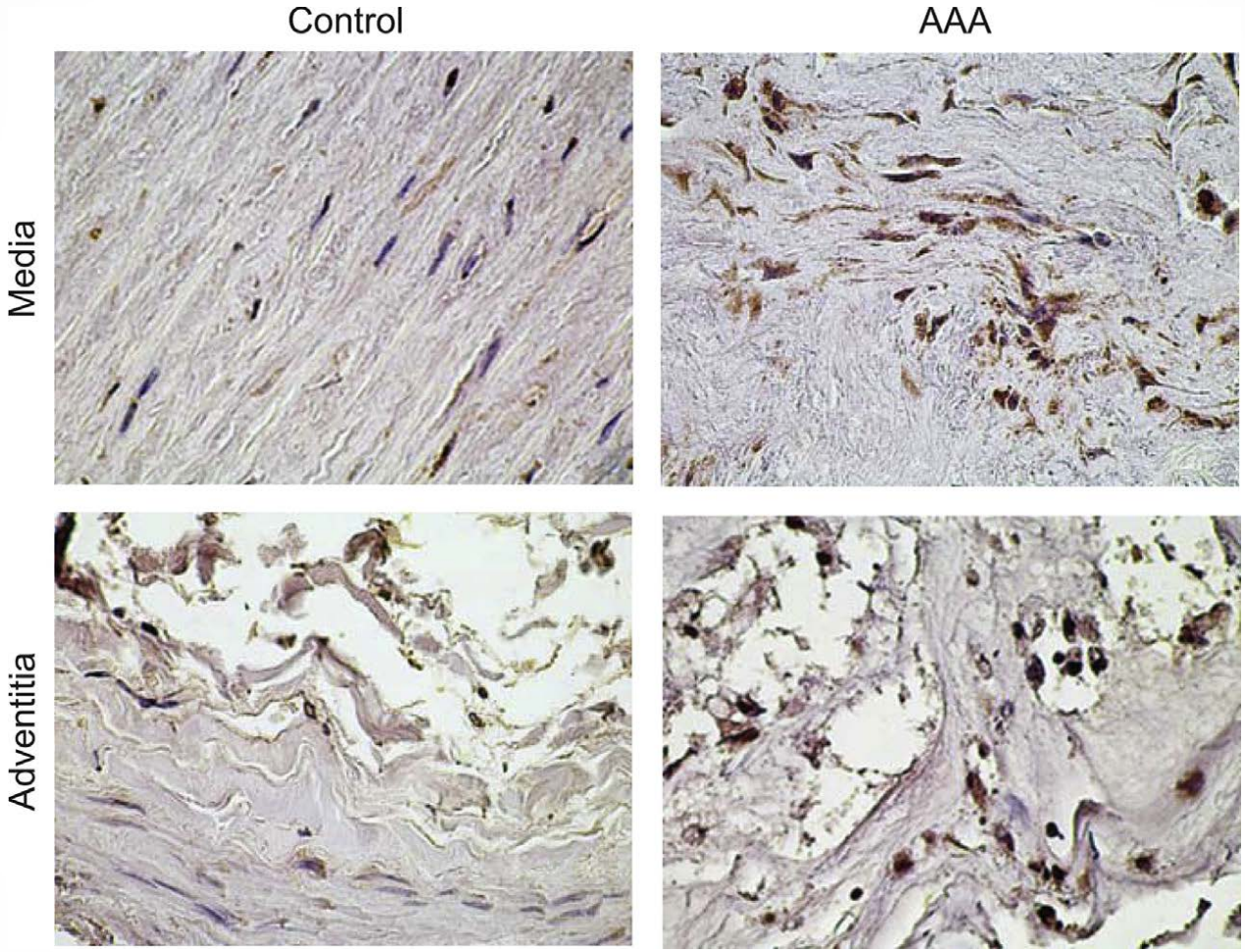
- Before AAA stem cell-based interventions are pursued, the presence and differentiation of stem cells in human AAA samples needs to be evaluated.

- To the best of our knowledge, this represents the first investigation to examine the prevalence and differentiation of stem cells in the aneurysmal human infra-renal aorta.

METHODS

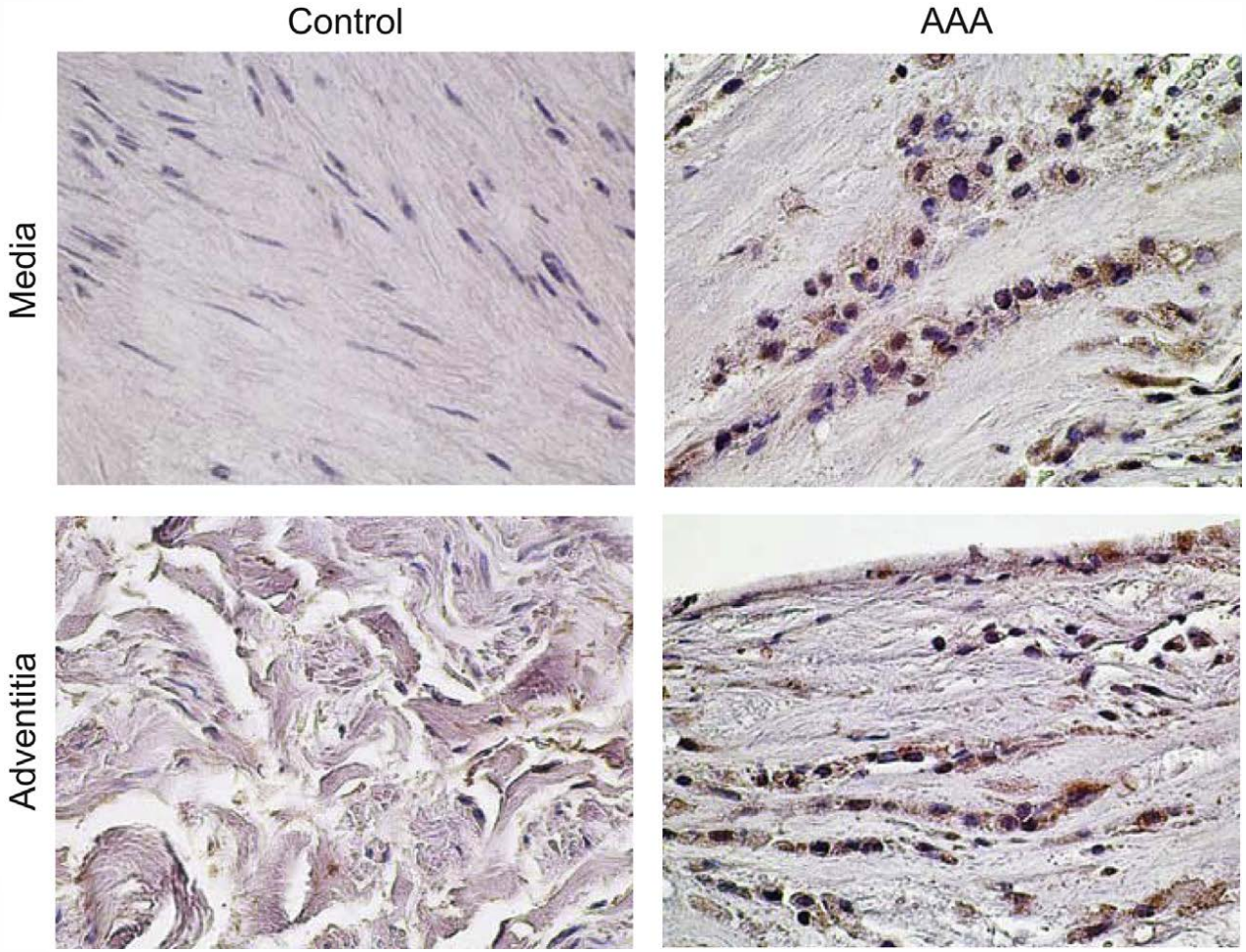
- AAA tissue (n=7) collected at time of repair and control tissue (n=4) collected at autopsy (all infra-renal aorta specimens)
- Standard IHC and double fluorescence staining
- mRNA expression profiles were generated on Sentrix Human-6 Whole Genome Expression Bead-Chips (Illumina Inc, San Diego, Calif)
- Ingenuity Pathway Analysis tool (Qiagen, Valencia, Calif) was used to perform a network analysis for the set of differentially expressed genes

RESULTS



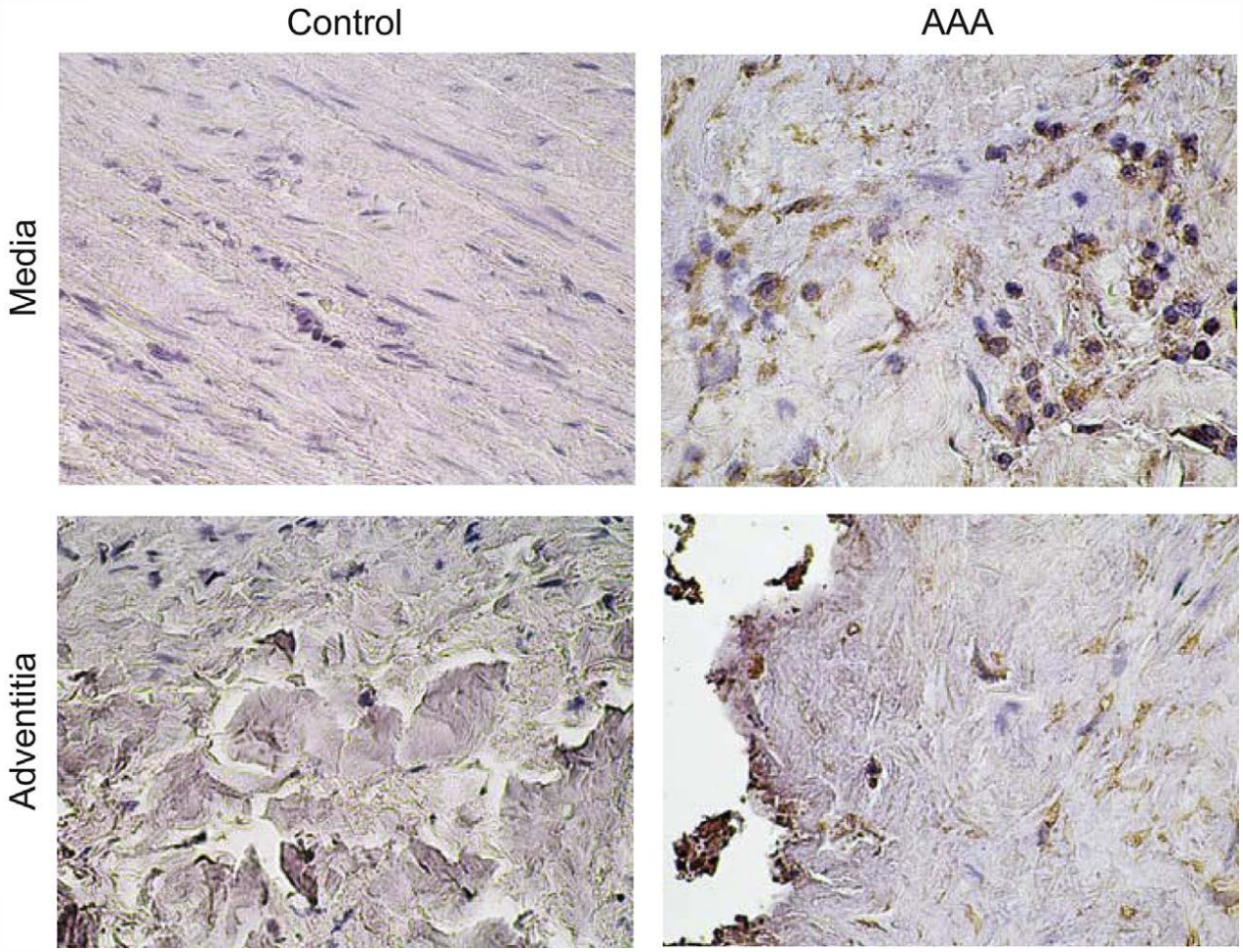
C-kit IHC performed in tissue from two 78-year-old men, one with a normal diameter aorta (control) and one with an 8-cm AAA. Magnification x400.

RESULTS



CD34 IHC performed in tissue from a 59-year-old woman with a normally sized aorta (control) and 63-year-old woman with a 5.1-cm AAA. Magnification x400.

RESULTS

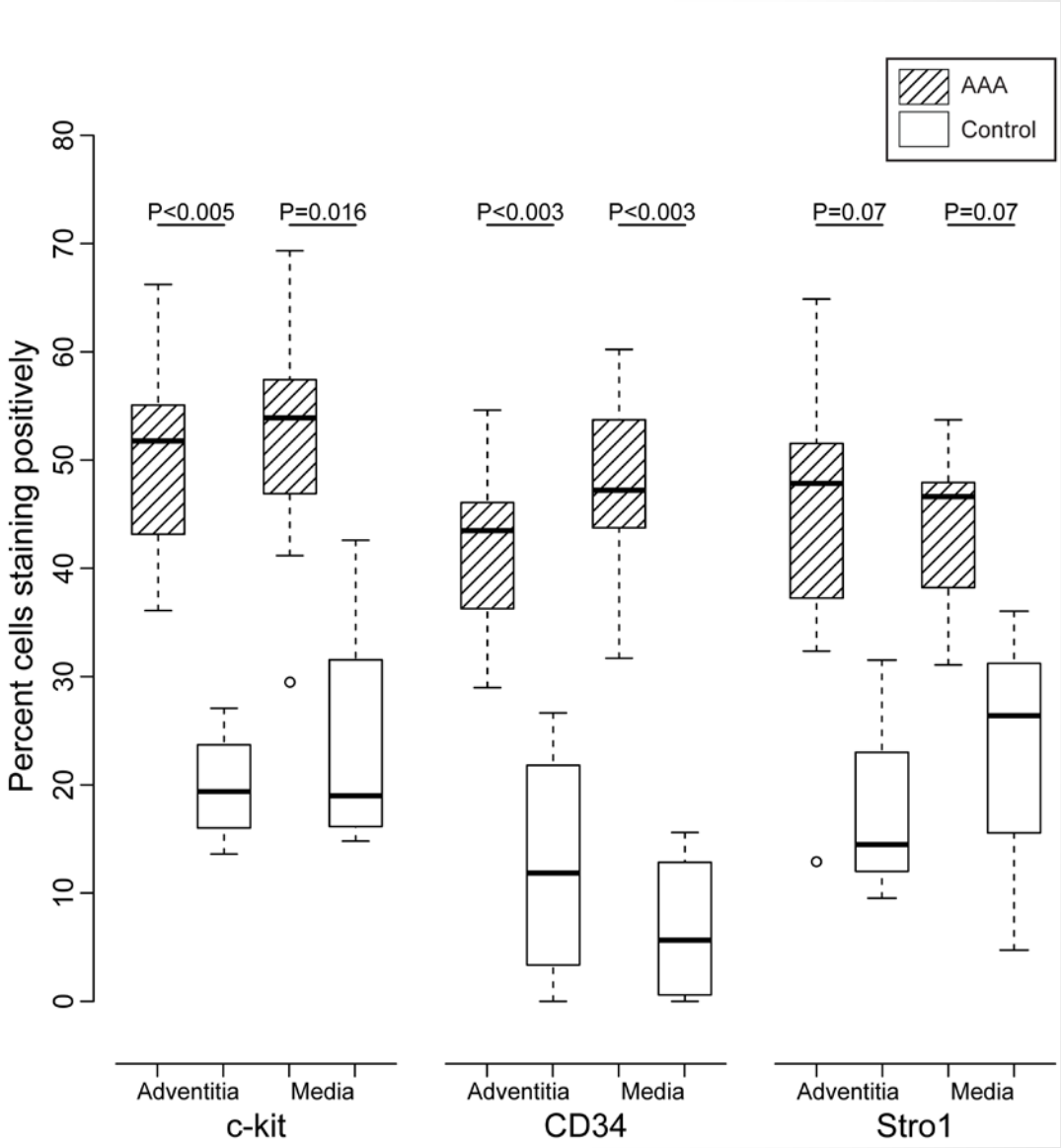


Stro1 IHC in tissue from a 53-year-old man with a normally sized aorta (control) and 55-year-old man with a 5.5-cm AAA. Magnification x400.

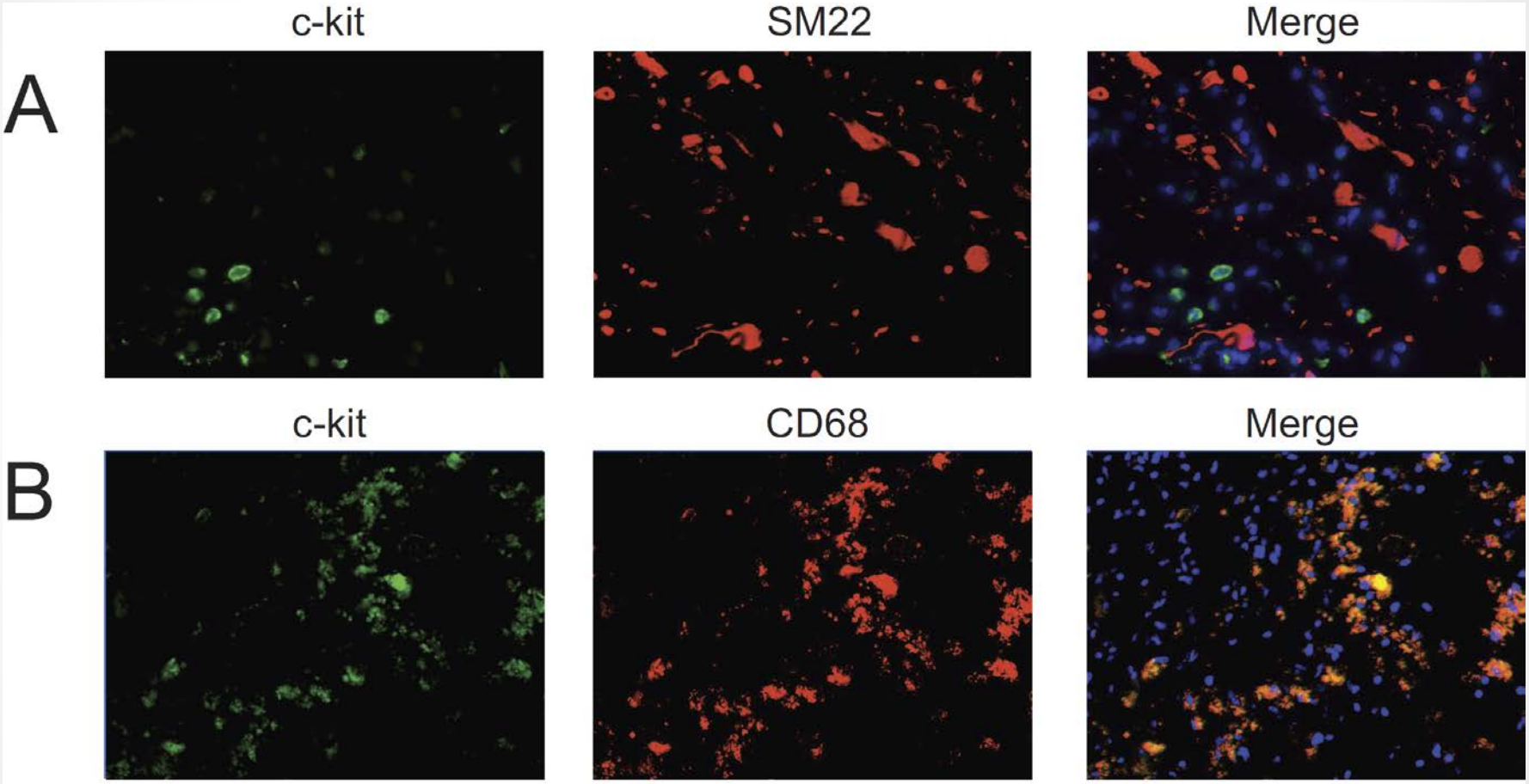
RESULTS

Quantification of IHC

- Results are presented as box and whiskers plots showing median and interquartile range (whiskers at 1.5X IQR) of the percentages of positively stained cells.
- Open circles are outliers beyond 1.5X IQR.
- P values were obtained by using Wilcoxon rank sum test (Mann-Whitney U).

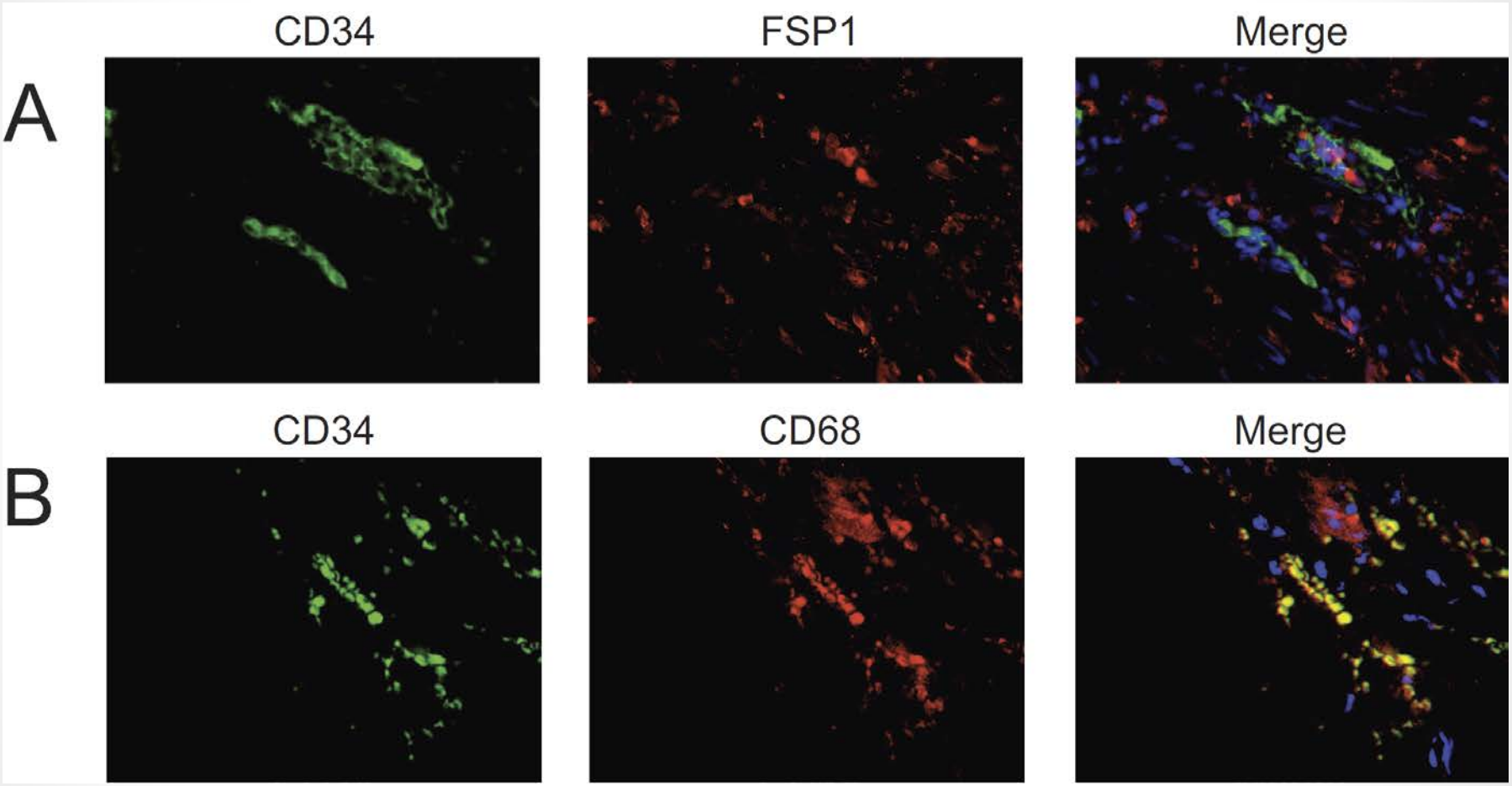


RESULTS



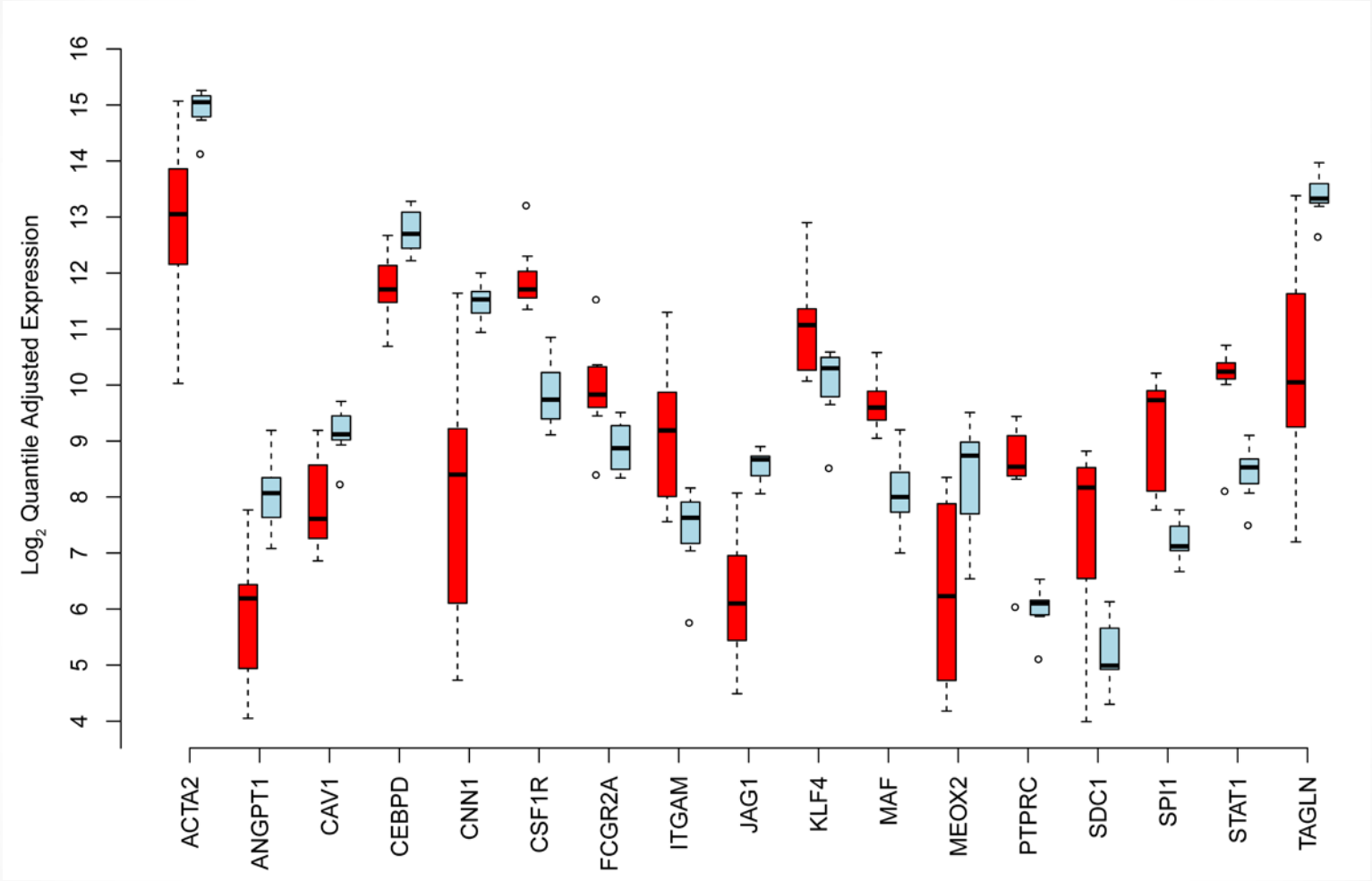
Double immunofluorescence staining with (A) SM22, (B) CD68, and the (A and B) c-kit in a 78-year-old man with an 8-cm AAA. Magnification x400.

RESULTS



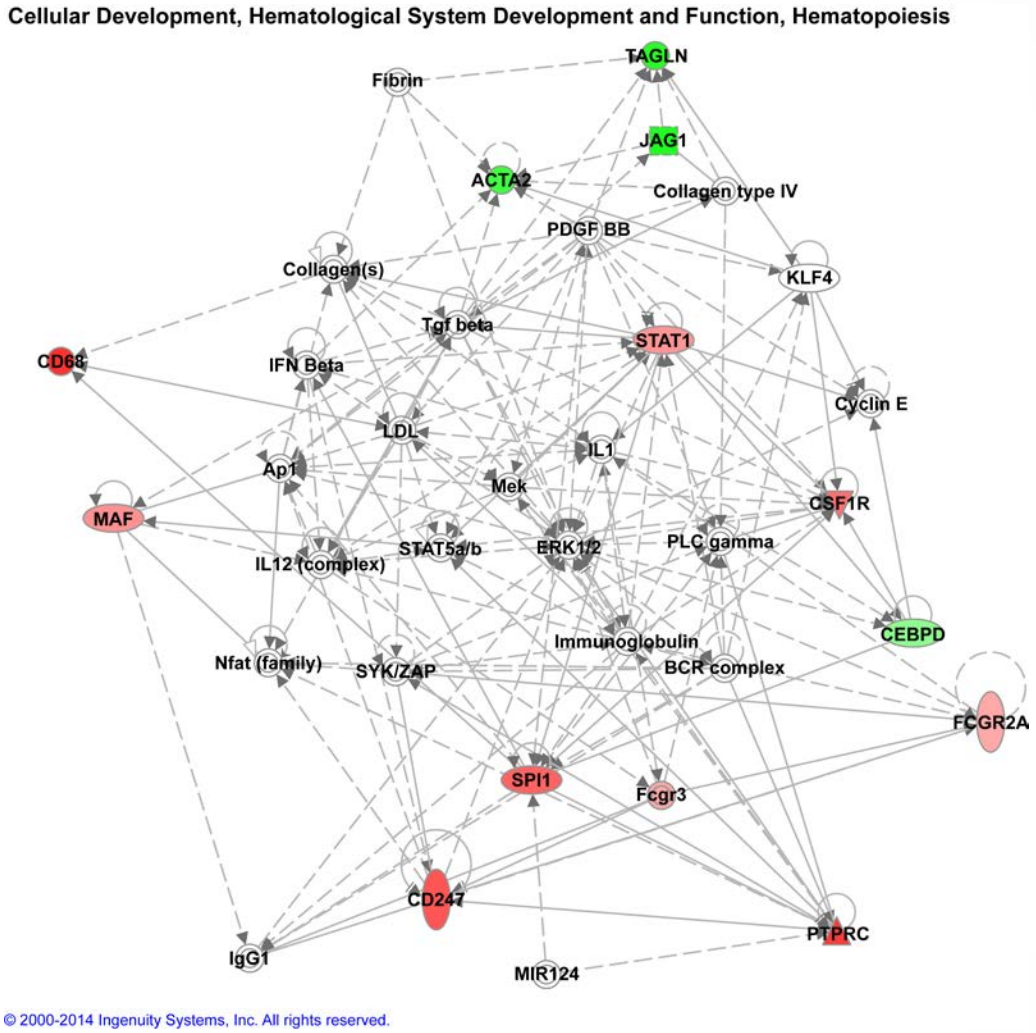
Double immunofluorescence with (A) FSP1, (B) CD68, and (A and B) CD34 in a 61-year-old woman with a 5.1-cm AAA. Magnification x400.

RESULTS



mRNA expression of genes involved in macrophage activation and TGF-β induced differentiation of stem cells into vascular smooth muscle cells.

RESULTS



A network of the interactions of selected differentially expressed genes created using the Ingenuity Pathway Analysis tool (Qiagen, Valencia, Calif).

CONCLUSIONS

- Stem cells are significantly elevated in infra-renal AAA tissue compared with matched control aortic tissue.
- AAA stem cells express macrophage surface antigens but not smooth muscle cell or fibroblast markers.
- CD68 positive cells within AAA wall co-localized with the cellular marker of proliferation Ki67 (data not shown).
- Our findings suggest an inflammatory/immune role of stem cells during AAA pathogenesis.

QUESTIONS?

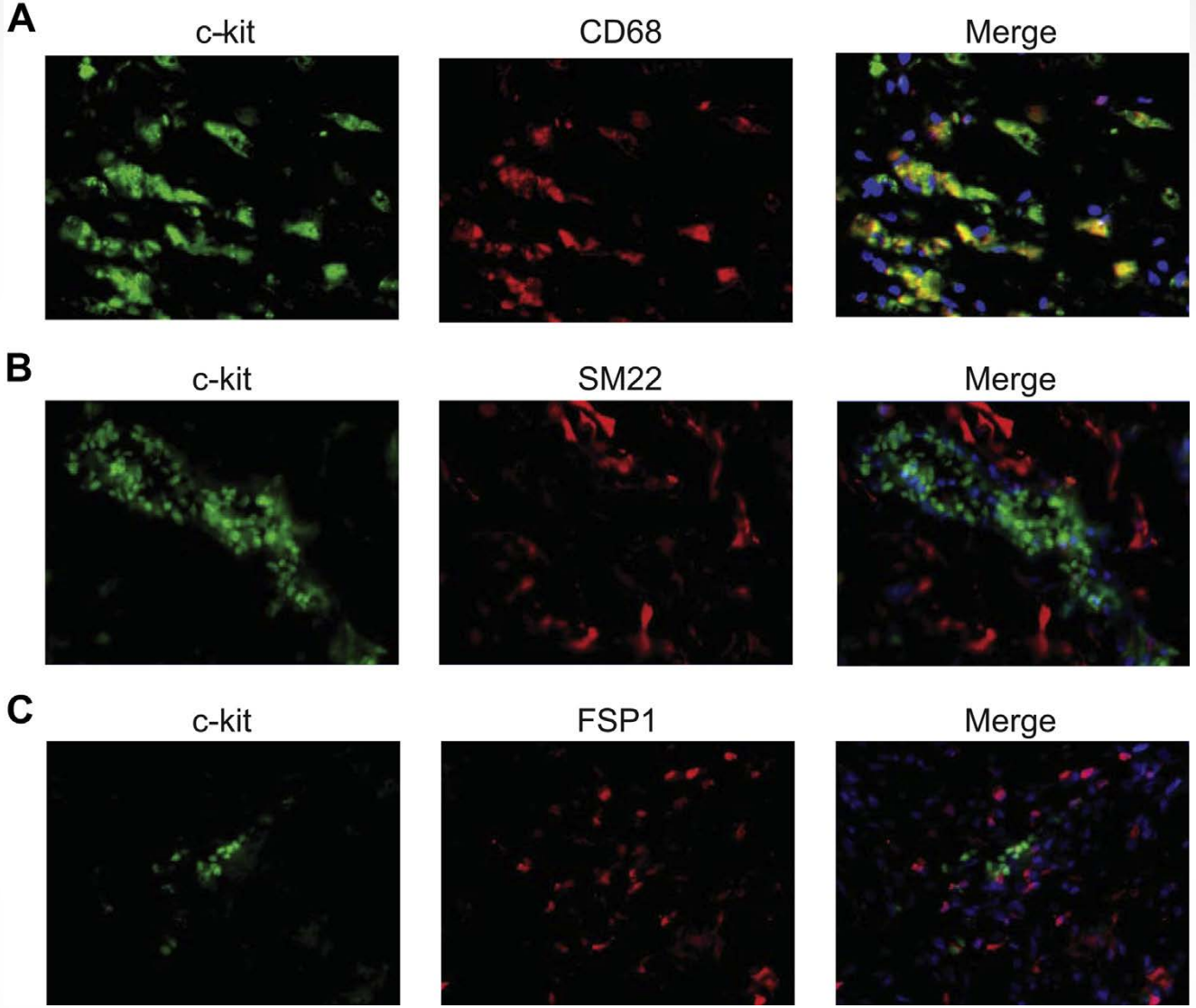
RESULTS

Table. Characteristics of abdominal aortic aneurysm (AAA) patients and control specimens of normal aorta[a]

Patient	*Age, years*	*Sex*	*AAA size, cm*	*Cause of death*
1	63	F	5.1	NA
2	55	M	5.4	NA
3	65	F	5.1	NA
4	70	M	Unknown	NA
5	78	M	8	NA
6	55	M	5.5	NA
7	61	F	5.1	NA
8	59	F	NA	Coronary artery disease
9	54	M	NA	Cardiac arrest
11	78	M	NA	Cardiac arrest
12	53	M	NA	Unknown

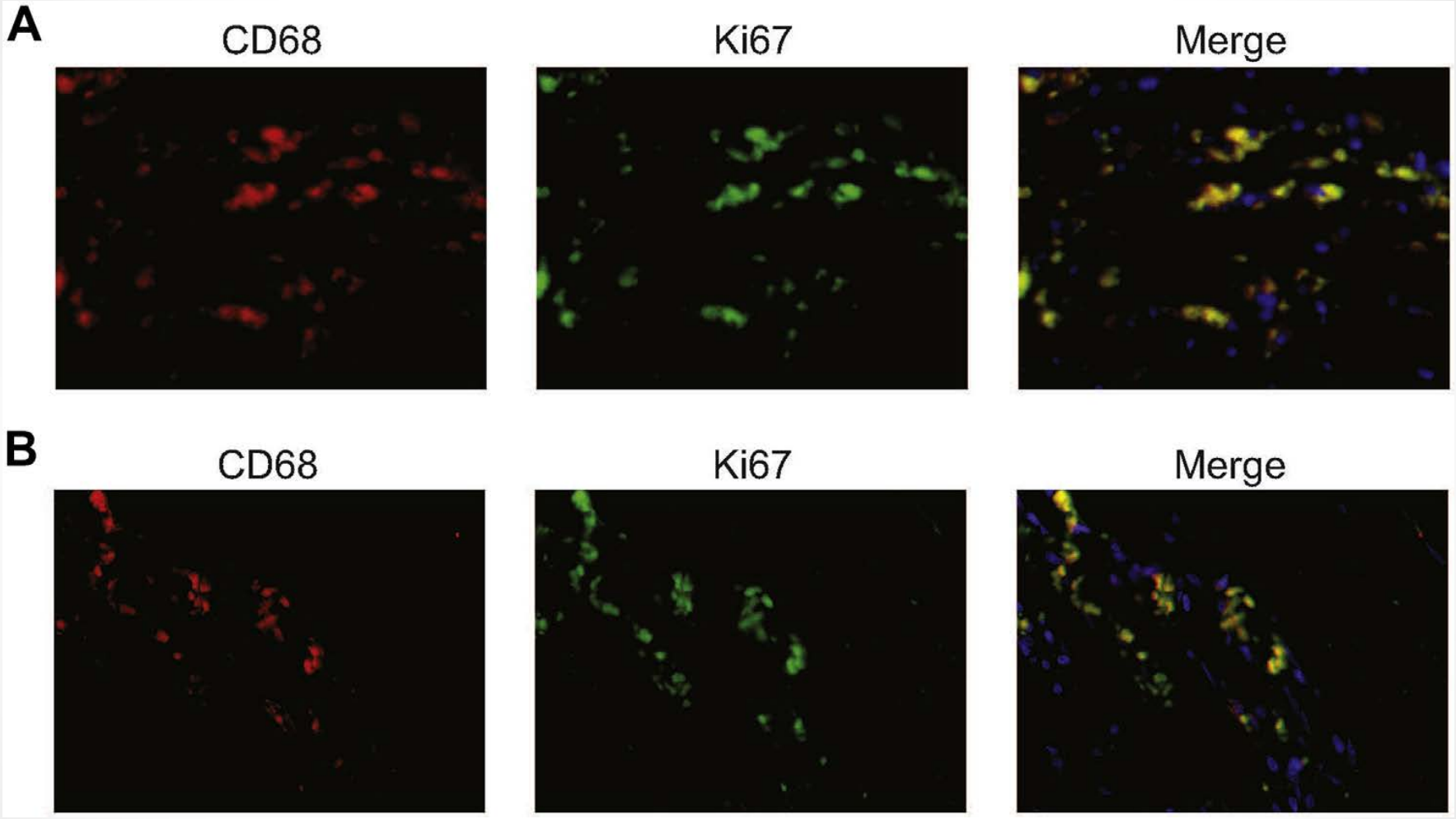
F, Female; *M*, male; *NA*, not available.

[a]Tissue samples were obtained at autopsy (controls) or at operation (AAA), and were taken from the infrarenal abdominal aorta. All donors and patients were Caucasian.



Double immunofluorescence staining with (A) CD68, (B) SM22, (C) FSP1, and (A, B, and C) c-kit in a 70-year-old man with 5.5 cm AAA. Magnification x400.

RESULTS



Double-immunofluorescence with Ki-67 and CD68 in a 78-year-old man with an 8-cm AAA (A) and in a 70-year-old man with a 5.5-cm AAA (B). Magnification x400.