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Selective Accumulation of the Complement Membrane Attack Complex in Aging Choriocapillaris

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Abstract

The complement membrane attack complex (MAC) shows increased abundance in the choriocapillaris during normal aging and is especially prevalent in age-related macular degeneration (AMD). While perivascular MAC accumulation occurs in the choroid, it is not well understood whether similar deposition occurs in other aging tissues. In this study we examined the abundance of MAC across multiple human tissues. For studies on fixed tissues, paraffin sections were obtained from six human donor eyes and a commercially available tissue array containing 19 different tissues.

Immunohistochemical labeling was performed using antibodies directed against the MAC and intercellular adhesion molecule-1 (ICAM-1), as well as the lectin *Ulex europaeus* agglutinin-I (UEA-I). The choriocapillaris was the only tissue with high levels of the MAC, which was not detected in any of the 38 additional samples from 19 tissues. ICAM-1 was abundantly expressed in the majority of tissues evaluated, and UEA-I labeled the vasculature in all tissues. A second experiment was performed using unfixed frozen sections of RPE-choroid and 7 extraocular tissues, which confirmed the relatively limited localization of the MAC to the choriocapillaris. In comparison to other tissues assessed, the restricted accumulation of MAC in the choriocapillaris may, in part, explain the specificity of AMD to the neural retina, RPE and choroid, and the relative absence of systemic pathology in this disease.

The membrane attack complex of complement (MAC) is a multimeric assembly of proteins consisting of C5b, C6, C7, C8 and multiple copies of C9, which forms the membrane spanning pore (Morgan, 2015). Although the function of MAC is to lyse pathogens, aberrant activation can lead to bystander injury of resident cells (Rus and Niculescu, 2001). The MAC is abundant along the walls of kidney glomerular capillaries in an experimental model of glomerulonephritis, and absent in healthy control samples (Koffler et al., 1983). In contrast, even healthy human maculae labeled with anti-MAC antibodies exhibit some degree of labeling in domains surrounding the choriocapillaris, but little or no labeling in the normal RPE or neural retina (Hageman et al., 2005; Seth et al., 2008). During normal aging,

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the levels of MAC increase in abundance. This is especially notable in the choroids of individuals with high-risk complement factor H (*CFH)* genotypes (Mullins et al., 2011a) and patients with age-related macular degeneration (AMD) (Mullins et al., 2014).

The molecular basis for MAC activation in the choroid is not fully understood, although it may be a result of the unusual composition of the outer retina (such as long chain fatty acids, phospholipids and abundant bisretinoids (Curcio et al., 2011; Zhou et al., 2009)) which creates a pro-inflammatory choroidal microenvironment. While robust anti-MAC labeling is observed in aging choriocapillaris, whether this deposition is choroid-specific has not been systematically examined. In the current study we sought to examine the abundance of the MAC in the microvasculature of a variety of human tissues to aid in our understanding of the role of its deposition in aging and AMD.

Human donor eyes were acquired from the Iowa Lions Eye Bank (Iowa City, IA) following informed consent of the family and in accordance with the Declaration of Helsinki. Posterior poles were fixed in 4% paraformaldehyde in phosphate buffered saline, and full thickness (retina to sclera), 4mm-diameter punches were collected from six donor eyes (ages 41 to 83) without any known retinal disease adjacent to the macula. Ocular tissue was processed at the University of Iowa Central Microscopy Research Facility using an RMC Paraffin Tissue Processor 1530 to dehydrate and infiltrate with paraffin before embedding in a paraffin block. Sections were cut at 5 μm using a microtome and collected on charged glass slides. Tissue array slides with 38 healthy tissue samples from 19 different anatomic sites were obtained from a commercial vendor (Pantomics; MNO381; Richmond, CA). These tissue arrays contain sections from the following tissues: breast, cerebellum, cerebral cortex, colon, esophagus, heart, kidney, liver, lung, ovary, pancreas, prostate, skin, small intestine, stomach, testis, thyroid, tonsil, and uterus. For all procedures, from deparaffinization through final coverslipping, ocular sections and tissue array sections were handled identically.

Sections were deparaffinized and rehydrated prior to staining. Immunohistochemistry was performed with antibodies directed against the MAC (M0777; Dako, Carpinteria, CA) or ICAM-1 (BBA17; R&D, Minneapolis, MN; included as a positive control to verify preservation of antigenicity), or were labeled with the fucose-binding lectin *Ulex europaeus* agglutinin-I (UEA-I; Vector Laboratories, Burlingame, CA).

Immunohistochemical labeling and colorimetric detection (using avidin-biotin horseradish peroxidase and VIP substrate, both from Vector Laboratories) were performed as described previously (Skeie et al., 2010).

Of the 19 tissue types evaluated, the choriocapillaris was the only tissue with abundant MAC immunolabeling (Figure 1A), which was absent from all other tissues (Figure 1E, I, M, Q). ICAM-1 was present in the vascular endothelial cells of all tissues except stomach, which had milder staining overall (Figure 1G), and was highly expressed in choroid (Figure C) and kidney glomerulus (Figure O). Small intestine exhibited strong intracellular epithelial cell staining (Figure 1K), and the pancreas tissue showed high intracellular pyramidal cell staining, with minimal vascular labeling (Figure 1S). UEA-I lectin labeling was detected in

all tissues on the vasculature (Figure 1B, F, J, N, R), with some tissues showing labeling in specific cell classes (for example, gastric mucosa (Al-Marzoqee et al., 2012)). A summary of the data can be found in Table 1.

One limitation of this experiment is that choroid was not present on the commercial tissue arrays, and the processing of these tissues was outside of our control and therefore could result in different levels of antigenicity between ocular and extraocular tissues. We sought to address this potential problem by embedding our ocular tissue in paraffin, performing all labeling steps for ocular and tissue array samples together, and including positive controls for preservation of protein and carbohydrate epitopes (anti-ICAM-1 and UEA-I), both of which showed strong labeling in both ocular and tissue array sections.

In order to further assure that the differences in MAC labeling between choroid and the other tissues studied were not due to fixation and processing, we performed a second set of comparisons in which we obtained unfixed sections of human tissues from the University of Iowa Tissue Procurement Core Facility and compared them with similarly aged unfixed donor eye sections. Sections were collected, labeled with anti-MAC antibody and biotinylated UEA-I lectin, and evaluated by fluorescence microscopy as described previously (Mullins et al., 2014). As observed with the first comparison, the choriocapillaris had the most abundant MAC labeling between all the tissues (Figure 2A, A′). While low levels of the MAC were present within kidney (Figure 2C, C′) and liver (Figure 2D, D′), the microvasculature in all 7 extraocular tissue sections were negative for the MAC (Figure 2B, B′, E, E′, F, F′, G, G′, H, H′).

In summary, the selective accumulation of MAC in the choriocapillaris is a plausible explanation for the fact that individuals with high risk *CFH* genotypes—which lead to increased systemic complement activation (Hecker et al., 2010; Reynolds et al., 2009)—develop macular degeneration rather than an array of extraocular diseases. For reasons that are not yet clear, the choroid appears to be a “hot spot” for MAC deposition. The impact of ocular specific factors in AMD pathogenesis, as opposed to systemic factors, is indicated by elegant studies of genotyped liver transplant patients, in which recipient *CFH* genotype, but not donor *CFH* genotype, contributes to AMD risk (Khandhadia et al., 2013).

The increased accumulation of MAC in AMD occurs concomitantly with the microvascular injury that occurs in individuals with AMD. Morphometric and molecular studies by us (Mullins et al., 2011b; Whitmore et al., 2013) and others (Biesemeier et al., 2014; Ramrattan et al., 1994) suggest that choriocapillaris loss is one of the earliest anatomical events in AMD, and that the MAC may represent a smoking gun for early AMD (Whitmore et al., 2014). Efforts are underway to employ anti-complement therapies for AMD (Birke et al., 2013; Rohrer et al., 2009). These findings suggest that identifying the molecular basis for choroid specific MAC activation is an important question, and they further support the notion that local inhibition of the terminal complement pathway will be necessary in delaying or arresting damage to the choriocapillaris and, ultimately, RPE and photoreceptor cells, in AMD.

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References

Al-Marzoqee FY, Khoder G, Al-Awadhi H, John R, Beg A, Vincze A, Branicki F, Karam SM. Upregulation and inhibition of the nuclear translocation of Oct4 during multistep gastric carcinogenesis. Int J Oncol. 2012; 41:1733–1743. DOI: 10.3892/ijo.2012.1608 [PubMed: 22922943]

Biesemeier A, Biesemeier A, Taubitz T, Taubitz T, Julien S, Julien S, Yoeruek E, Yoeruek E, Schraermeyer U, Schraermeyer U. Accepted Manuscript. Neurobiol Aging. 2014; :1–42. DOI: 10.1016/j.neurobiolaging.2014.05.003

Birke K, Lipo E, Birke MT, Kumar-Singh R. Topical application of PPADS inhibits complement activation and choroidal neovascularization in a model of age-related macular degeneration. PLoS ONE. 2013; 8:e76766.doi: 10.1371/journal.pone.0076766 [PubMed: 24130789]

Curcio CA, Johnson M, Rudolf M, Huang JD. The oil spill in ageing Bruch membrane. Br J Ophthalmol. 2011; 95:1638–1645. DOI: 10.1136/bjophthalmol-2011-300344 [PubMed: 21890786]

Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM, Smith RJH, Silvestri G, Russell SR, Klaver CCW, Barbazetto I, Chang S, Yannuzzi LA, Barile GR, Merriam JC, Smith RT, Olsh AK, Bergeron J, Zernant J, Merriam JE, Gold B, Dean M, Allikmets R. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA. 2005; 102:7227–7232. DOI: 10.1073/pnas.0501536102 [PubMed: 15870199]

Hecker LA, Edwards AO, Ryu E, Tosakulwong N, Baratz KH, Brown WL, Charbel Issa P, Scholl HP, Pollok-Kopp B, Schmid-Kubista KE, Bailey KR, Oppermann M. Genetic control of the alternative pathway of complement in humans and age-related macular degeneration. Hum Mol Genet. 2010; 19:209–215. DOI: 10.1093/hmg/ddp472 [PubMed: 19825847]

Khandhadia S, Hakobyan S, Heng LZ, Gibson J, Adams DH, Alexander GJ, Gibson JM, Martin KR, Menon G, Nash K, Sivaprasad S, Ennis S, Cree AJ, Morgan BP, Lotery AJ. Age-related macular degeneration and modification of systemic complement factor H production through liver transplantation. Ophthalmology. 2013; 120:1612–1618. DOI: 10.1016/j.ophtha.2013.01.004 [PubMed: 23562165]

Koffler D, Biesecker G, Noble B, Andres GA, Martinez-Hernandez A. Localization of the membrane attack complex (MAC) in experimental immune complex glomerulonephritis. Journal of Experimental Medicine. 1983; 157(6):1885–1905. [PubMed: 6343549]

Morgan BP. The membrane attack complex as an inflammatory trigger. Immunobiology. 2015; doi: 10.1016/j.imbio.2015.04.006

Mullins RF, Dewald AD, Streb LM, Wang K, Kuehn MH, Stone EM. Elevated membrane attack complex in human choroid with high risk complement factor H genotypes. Exp Eye Res. 2011a; 93:3–3. DOI: 10.1016/j.exer.2011.06.015

Mullins RF, Johnson MN, Faidley EA, Skeie JM, Huang J. Choriocapillaris vascular dropout related to density of drusen in human eyes with early age-related macular degeneration. Invest Ophthalmol Vis Sci. 2011b; 52:1606–1612. DOI: 10.1167/iovs.10-6476 [PubMed: 21398287]

Mullins RF, Schoo DP, Sohn EH, Flamme-Wiese MJ, Workamelahu G, Johnston RM, Wang K, Tucker BA, Stone EM. The Membrane Attack Complex in Aging Human Choriocapillaris: Relationship to Macular Degeneration and Choroidal Thinning. Am J Pathol. 2014; doi: 10.1016/j.ajpath.2014.07.017

Ramrattan RS, van der Schaft TL, Mooy CM, de Bruijn WC, Mulder PG, de Jong PT. Morphometric analysis of Bruch's membrane, the choriocapillaris, and the choroid in aging. Invest Ophthalmol Vis Sci. 1994; 35:2857–2864. [PubMed: 8188481]

Reynolds R, Hartnett ME, Atkinson JP, Giclas PC, Rosner B, Seddon JM. Plasma complement components and activation fragments: associations with age-related macular degeneration

genotypes and phenotypes. Invest Ophthalmol Vis Sci. 2009; 50:5818–5827. DOI: 10.1167/iovs.09-3928 [PubMed: 19661236]

Rohrer B, Long Q, Coughlin B, Wilson RB, Huang Y, Qiao F, Tang PH, Kunchithapautham K, Gilkeson GS, Tomlinson S. A targeted inhibitor of the alternative complement pathway reduces angiogenesis in a mouse model of age-related macular degeneration. Invest Ophthalmol Vis Sci. 2009; 50:3056–3064. DOI: 10.1167/iovs.08-2222 [PubMed: 19264882]

Rus H, Niculescu F. The complement system in central nervous system diseases. Immunol Res. 2001; 24:79–86. DOI: 10.1385/IR:24:1:79 [PubMed: 11485211]

Seth A, Cui J, To E, Kwee M, Matsubara J. Complement-associated deposits in the human retina. Invest Ophthalmol Vis Sci. 2008; 49:743–750. DOI: 10.1167/iovs.07-1072 [PubMed: 18235023]

Skeie JM, Fingert JH, Russell SR, Stone EM, Mullins RF. Complement component C5a activates ICAM-1 expression on human choroidal endothelial cells. Invest Ophthalmol Vis Sci. 2010; 51:5336–5342. DOI: 10.1167/iovs.10-5322 [PubMed: 20484595]

Whitmore SS, Braun TA, Skeie JM, Haas CM, Sohn EH, Stone EM, Scheetz TE, Mullins RF. Altered gene expression in dry age-related macular degeneration suggests early loss of choroidal endothelial cells. Mol Vis. 2013; 19:2274–2297. [PubMed: 24265543]

Whitmore SS, Sohn EH, Chirco KR, Drack AV, Stone EM, Tucker BA, Mullins RF. Complement activation and choriocapillaris loss in early AMD: Implications for pathophysiology and therapy. Prog Retin Eye Res. 2014; doi: 10.1016/j.preteyeres.2014.11.005

Zhou J, Kim SR, Westlund BS, Sparrow JR. Complement activation by bisretinoid constituents of RPE lipofuscin. Invest Ophthalmol Vis Sci. 2009; 50:1392–1399. DOI: 10.1167/iovs.08-2868 [PubMed: 19029031]

AMD is a degenerative disease with increased activation of complement in the choriocapillaris.

The membrane attack complex was evaluated in human choroid and 19 other tissues.

The membrane attack complex shows specific labeling in the choriocapillaris, suggesting the choroid as a hot spot for its deposition.

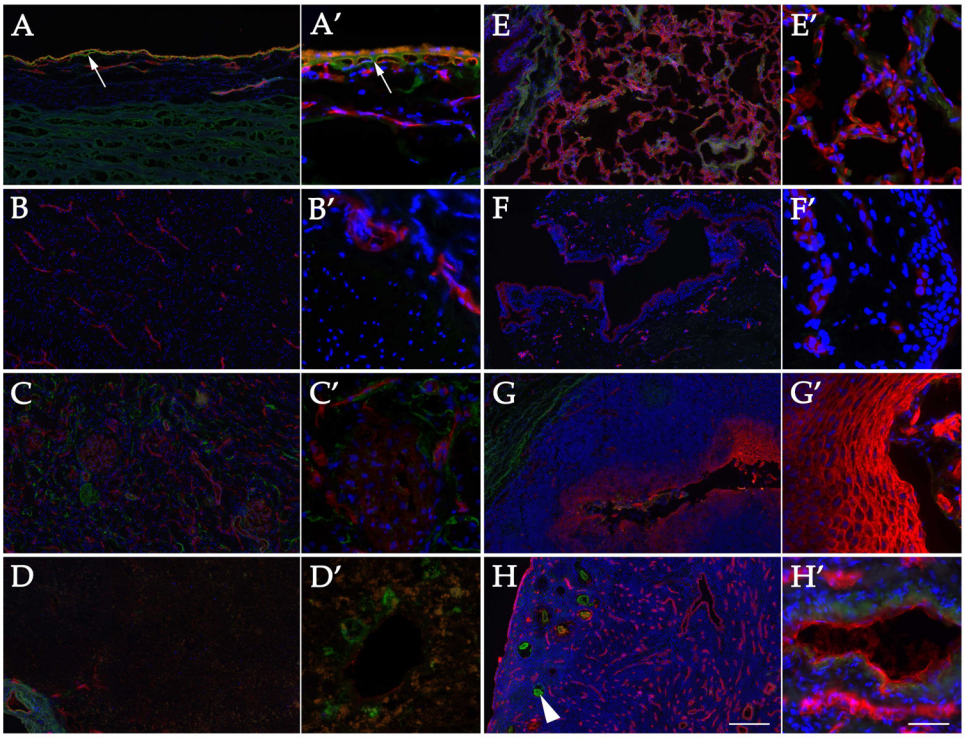

Figure 1.

Choroid (A–D), stomach (E–H), small intestine (I–L), kidney (M–P), and pancreas (Q–T) tissue labeling for MAC (A, E, I, M, Q), UEA-I (B, F, J, N, R), ICAM-1 (C, G, K, O, S), and secondary control (D, H, L, P, T). Positive reactivity is indicated by purple reaction product. The choriocapillaris showed abundant MAC (A, arrow) and ICAM-1 (C, arrow) labeling, while the stomach had mild ICAM-1 (G) and no MAC labeling (E). Intracellular ICAM-1 labeling was present in the small intestine epithelium (K, arrow), while UEA-I was only present around the outer layer of the epithelium (J, white arrow) and around the vessels (J, arrow). Kidney tissue exhibited vascular staining with UEA-I (N, arrow) and ICAM-1 (O, arrow), while pancreatic pyramidal cells showed intracellular UEA-I (R) and ICAM-1 (S) staining. No MAC labeling was present for any of the tissues in the array (E, I, M, Q). Scale bar = 50 μm.

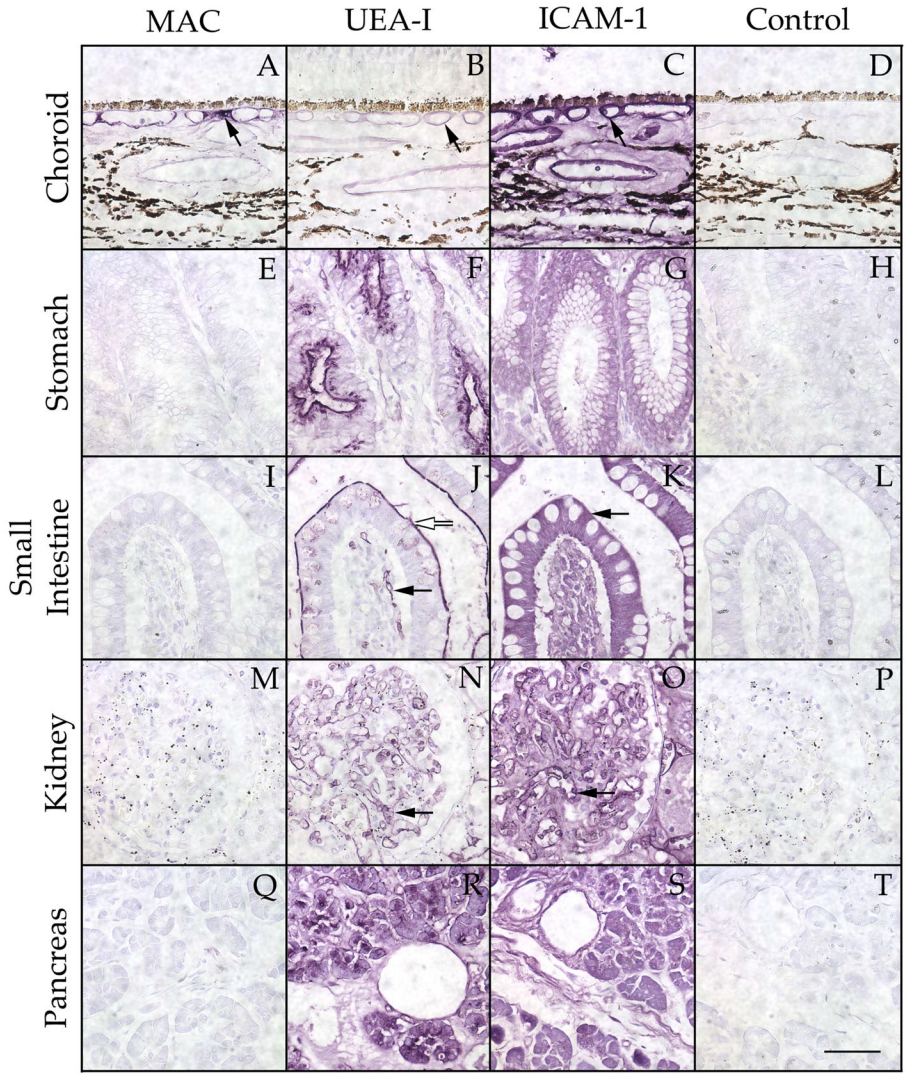

Figure 2.

Immunofluorescent labeling of unfixed sections with antibodies directed against the MAC (green) and UEA-I (red) in choroid (A, A′), colon (B, B′), kidney (C, C′), liver (D, D′), lung (E, E′), skin (F, F′), tonsil (G, G′), and uterus (H, H′). Abundant MAC labeling was present in the choriocapillaris (A, A′, arrow). While mild MAC labeling was observed throughout the tissue, kidney glomerular capillaries were negative for MAC (C, C′). Mild MAC labeling was present surrounding the portal triads and central veins of the liver, leaving the microvessels unlabeled (D, D′). For all other tissues, MAC immunolabeling was absent (B, B′, E, E′, F, F′, G, G′, H, H′). Nonspecific labeling within the endometrial glands was visible in the stained uterus tissue (H, arrowhead), as well as the secondary control (not shown). Donor ages and gender are as follows: choroid = 62, F; colon = 75, F; kidney = 61, F; liver = 70, M; lung = 65, F; skin = 55, F; tonsil = 68, M; and uterus = 86, F. Scale bar in H = 200 μm, scale bar in H′ = 50 μm.

Table 1

Tissue samples and their qualitative immunolabeling levels.

Tissue Sample	Age	Sex	MAC	UEA-I	ICAM-1
Breast	52	F	−	−	+
Breast	39	F	−	−/+	++
Cerebellum	16	M	−	++	+
Cerebellum	58	M	−	+	++
Cerebral Cortex	16	M	−	++	++
Cerebral Cortex	46	M	−	+	++
Choroid	41	F	+	+	++
Choroid	48	F	++	++	−/+
Choroid	51	F	+++	++	++
Choroid	63	M	+	++	+++
Choroid	74	F	++	+	++
Choroid	83	M	−	+	+
Colon	51	M	−	−/+	+
Colon	55	F	−	+	++
Esophagus	16	M	−	+	++
Esophagus	46	M	−	++	++
Heart	46	M	−	++	++
Heart	16	M	−	−/+	++
Kidney	16	M	−	++	+
Kidney	46	M	−	++	++
Liver	43	M	−	++	+
Liver	52	M	−	+	−/+
Lung	16	M	−	++	+++
Lung	50	M	−	++	++
Ovary	51	F	−	+	+
Ovary	41	F	−	−/+	++
Pancreas	55	M	−	++	+
Pancreas	58	M	−	++	+
Prostate	16	M	−	++	−/+
Prostate	46	M	−	+	++
Skin	47	F	−	++	+
Skin	57	F	−	++	+
Stomach	32	M	−	++	−
Stomach	50	F	−	++	−/+

Tissue Sample	Age	Sex	MAC	UEA-I	ICAM-1
Small Intestine	17	M	–	+	++
Small Intestine	68	M	–	+	++
Testis	16	M	–	++	+
Testis	73	M	–	++	++
Thyroid	16	M	–	+	+
Thyroid	52	F	–	–/+	–/+
Tonsil	7	M	–	+	+
Tonsil	8	M	–	–/+	+
Uterus	71	F	–	++	++
Uterus	46	F	–	++	++

– = no labeling; –/+ = little to no labeling; + = mild labeling; ++ = moderate labeling; +++ = intense labeling.