## ON-LINE APPENDIX: METHODS

### *MR Imaging Acquisition*

TOF MRA was acquired without a presaturation band, with TR/TE of 14/4.3 ms, flip angle of 70°, bandwidth of 121.094 kHz, the number of contiguous axial slices varying between 90 and 150, slice thickness of 1.5 mm, distance factor of 1.5 mm, axial resolution of 0.430 × 0.430 $mm^2$, and axial FOV of 220 × 220 $mm^2$. A sagittal localizer was used to position the MRA FOV so that all the cervical levels between C3 and C7 were imaged. Recruited subjects were instructed to breathe normally during the scans.

### *MR Imaging Analysis*

**Cervical Level Identification.** Before performing the neck vessel segmentation, the z-coordinates of the limits of each cervical level between C3 and C7 were identified. Specifically, on each sagittal localizer image, 6 markers were positioned at the center of the intervertebral spaces between the C2 and T1 vertebrae (ie, C2–C3, C3–C4...C7–T1), with the FSLView tool (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/fslview). Then, registration parameters from the localizer to the MR angiography space were computed with the FMRIB Linear Image Registration Tool (FLIRT; http://www.fmrib.ox.ac.ukfsl/fslwiki/FLIRT) (uses qform option),[1] and their reliability was verified by visual inspection of the registration outcome. Finally, the coordinates of the limits of each cervical level between C3 and C7 in the MRA space were computed by applying the registration parameters to the cervical marker coordinates defined in the localizer space.

**ROI Definition.** If no vessel hyperintense area could be identified, no contour was drawn on that slice. Above the CCA bifurcation, only the ICA was segmented because we were interested in investigating the arterial pathways for the brain supply.

**Resampling.** Because the number of slices between the C2–C3 and C7–T1 intervertebral spaces was different for each MRA image, direct slice-by-slice comparison of corresponding CSA measures between subjects and time points (baseline and follow-up) was not possible. Therefore, we interpolated and resampled CSA-to-slice curves with Matlab (Release 2013b; MathWorks, Natick, Massachusetts), setting the same number of samples for all images (ie, average number of segmented slices). Accordingly, corresponding sample-by-sample CSA measures for all the MRA scans were obtained.

### *Statistical Analysis*

Tests at each cervical level were performed by considering for each subject the CSA and ΔCSA median values computed across samples belonging to the same level, while WV analyses were performed by considering the CSA values of all the samples for each subject.

Square root transformation and the Blom formula[2] were respectively used to normalize CSA and ΔCSA data before performing linear mixed-model analysis at each cervical level. WV distributions were transformed with the Blom formula.

Linear mixed-model analysis was used to take into account the hierarchic structure of the data. In mixed-effect models implemented for group comparison, group and samples were tested as fixed effects, while subjects were tested as a random slope. Longitudinal mixed-effect models testing baseline-to-follow-up differences between 2 groups or within the same group were set, including random slope for subjects. Furthermore, demographic or clinical nonmatching factors between the considered groups were considered in the models as covariates for comparisons between groups.

## REFERENCES


1. Jenkinson M, Smith S. **A global optimization method for robust affine registration of brain images.** *Med Image Anal* 2001;5:143–56 CrossRef Medline
2. Blom G. *Statistical Estimates and Transformed Beta-Variables*. New York: John Wiley & Sons; 1958


**On-line Table 1: Demographic and clinical characteristics of HC and subjects with MS presenting and not presenting with cardiovascular disease**

| | $HC_{noCVD}$ (*n* = 14) | $HC_{CVD}$ (*n* = 8) | $MS_{noCVD}$ (*n* = 49) | $MS_{CVD}$ (*n* = 20) | $HC_{noCVD}$ vs $HC_{CVD}$ (*P* Value) | $MS_{noCVD}$ vs $MS_{CVD}$ (*P* Value) |
|---|---|---|---|---|---|---|
| Female (No.) (%) | 11 (78.6) | 7 (87.5) | 36 (73.5) | 12 (60.0) | 1.000[a] | .385[a] |
| Age (yr) (median) (range) | 45.3 (17.7–73.3) | 50.2 (39.2–65.5) | 47.7 (18.9–68.3) | 56.0 (42.8–65.9) | .402[c] | <.001[b,d] |
| BMI (median) (range) | 24.4 (18.1–44.9) | 28.2 (21.6–42.0) | 26.4 (19.0–44.9) | 27.7 (23.5–36.6) | .212[c] | .358[c] |
| Disease duration (yr) (median) (range) | NA | NA | 11 (0–36) | 18 (1–37) | NA | .103[c] |
| EDSS (median) (range) | NA | NA | 2.5 (0–8) | 3.3 (1.5–7) | NA | .041[c,d] |
| Smoking status (No.) (%) | 3 (23.1) | 2 (25.0) | 20 (40.8) | 12 (60.0) | 1.000[a] | .187[a] |

**Note:**—NA indicates not applicable.

[a–c] Subjects who presented with hypertension and/or heart disease and/or hyperlipidemia and/or diabetes were classified as subjects with CVD. The Fisher exact test (a), the independent-samples Student *t* test (b), and the independent-samples Mann-Whitney *U* test (c) were used to evaluate differences between $HC_{CVD}$ and $HC_{noCVD}$ groups and between $MS_{CVD}$ and $MS_{noCVD}$, as appropriate.

[d] *P* values < .05 were considered significant.

**On-line Table 2: Group medians and IQRs of neck vessel total cross-sectional area at baseline and follow-up in patients with RRMS ($n = 44$) and PMS ($n = 25$)**

| Vessel/ Cervical Level | RRMS BL CSA ($mm^2$) (Median) (IQR) | PMS BL CSA ($mm^2$) (Median) (IQR) | BL CSA RRMS vs PMS (*P* Value) | RRMS FU CSA ($mm^2$) (Median) (IQR) | PMS FU CSA ($mm^2$) (Median) (IQR) | FU CSA RRMS vs PMS (*P* Value) | RRMS CSA BL vs FU (*P* Value) | PMS CSA BL vs FU (*P* Value) | BL-to-FU RRMS vs PMS CSA (*P* Value) |
|---|---|---|---|---|---|---|---|---|---|
| CCA–ICAs | | | | | | | | | |
| C3 | 54.0 (27.6) | 62.9 (30.8) | .890[a] | 56.2 (33.3) | 59.5 (33.7) | .685[a] | .666[c] | .459[c] | .614[e] |
| C4 | 78.4 (32.4) | 79.3 (25.1) | .956[a] | 78.1 (29.0) | 75.5 (24.2) | .915[a] | .345[c] | .182[c] | .614[e] |
| C5 | 73.7 (19.3) | 76.0 (26.7) | .956[a] | 70.3 (23.0) | 71.7 (25.8) | .915[a] | .666[c] | .345[c] | .614[e] |
| C6 | 69.5 (14.9) | 73.9 (25.5) | .947[a] | 67.1 (18.3) | 70.0 (24.8) | .915[a] | .666[c] | .182[c] | .614[e] |
| C7 | 67.7 (16.9) | 76.0 (31.0) | .890[a] | 65.7 (14.8) | 72.3 (23.4) | .685[a] | .035[c,g] | .182[c] | .614[e] |
| WV | 70.1 (25.0) | 75.2 (29.8) | .419[b] | 67.9 (26.5) | 71.5 (26.8) | .427[b] | .115[d] | .036[d,g] | .455[f] |
| VAs | | | | | | | | | |
| C3 | 31.8 (6.1) | 31.7 (8.2) | .740[a] | 29.9 (6.8) | 30.7 (8.1) | .961[a] | .070[c] | .459[c] | .696[e] |
| C4 | 29.9 (5.7) | 31.5 (8.0) | .740[a] | 29.7 (7.5) | 29.5 (6.5) | .961[a] | .073[c] | .459[c] | .696[e] |
| C5 | 29.7 (6.0) | 29.1 (6.6) | .740[a] | 28.8 (6.9) | 30.0 (5.0) | .961[a] | .103[c] | .459[c] | .696[e] |
| C6 | 29.3 (6.8) | 29.7 (6.7) | .740[a] | 28.9 (7.3) | 28.9 (6.2) | .961[a] | .082[c] | .459[c] | .696[e] |
| C7 | 29.2 (9.1) | 28.8 (8.1) | .740[a] | 28.5 (8.2) | 28.6 (8.2) | .961[a] | .180[c] | .060[c] | .696[e] |
| WV | 30.4 (7.3) | 30.6 (7.8) | .663[b] | 29.5 (7.9) | 29.9 (7.8) | .956[b] | .030[d,g] | .227[d] | .816[f] |
| IJVs | | | | | | | | | |
| C3 | 97.7 (51.2) | 117.4 (77.1) | .584[a] | 80.6 (56.6) | 113.2 (73.8) | .991[a] | .100[c] | .074[c] | .810[e] |
| C4 | 115.6 (60.4) | 147.4 (89.1) | .584[a] | 97.6 (70.5) | 119.8 (84.8) | .991[a] | .100[c] | .040[c,g] | .810[e] |
| C5 | 119.7 (54.2) | 126.1 (76.3) | .719[a] | 107.3 (72.1) | 110.6 (72.5) | .991[a] | .110[c] | .040[c,g] | .810[e] |
| C6 | 116.2 (99.4) | 122.5 (93.8) | .584[a] | 99.9 (86.9) | 101.7 (81.6) | .991[a] | .100[c] | .040[c,g] | .810[e] |
| C7 | 123.1 (88.4) | 131.9 (117.1) | .584[a] | 115.5 (96.5) | 109.1 (74.1) | .991[a] | .100[c] | .040[c,g] | .810[e] |
| WV | 116.5 (66.6) | 126.5 (88.7) | .329[b] | 100.9 (76.1) | 108.2 (77.7) | .834[b] | .032[d,g] | .021[d,g] | .667[f] |

**Note:**—BL indicates baseline; FU, follow-up.

[a–f] Group medians and IQRs of neck vessel total CSA at baseline and follow-up are reported for RRMS and PMS, at each cervical level and for the WV course. To evaluate CSA differences between RRMS and PMS groups at baseline and at follow-up, linear mixed models were used at each cervical level (a) and for the WV (b). Age, hypertension, disease duration, and EDSS were used as correcting factors for both analyses, a and b. To evaluate differences between baseline and follow-up within each group, the related-samples Wilcoxon singed rank test (c) was used at each cervical level, while linear mixed models were used for the WV (d). To compare RRMS and PMS CSA for 5 years, linear mixed models were used at each cervical level (e) and for the WV (f). Age, hypertension, disease duration, and EDSS were used as correcting factors for both analyses, e and f. The Benjamini-Hochberg procedure was performed to correct for multiple comparisons.

[g] An $\alpha$ level of .05 was considered significant.

**On-line Table 3: Assessment of the combined effect of MS and cardiovascular disease in the evolution of neck vessel total cross-sectional area for 5 years**

| Vessel/Cervical Level | $MS_{noCVD}$ BL CSA ($mm^2$) (Median) (IQR) | $MS_{CVD}$ BL CSA ($mm^2$) (Median) (IQR) | BL CSA $MS_{CVD}$ vs $MS_{noCVD}$ (*P* Value) | $MS_{noCVD}$ ΔCSA ($mm^2$) (Median) (IQR) | $MS_{CVD}$ ΔCSA ($mm^2$) (Median) (IQR) | ΔCSA $MS_{CVD}$ vs $MS_{noCVD}$ (*P* Value) |
|---|---|---|---|---|---|---|
| CCA–ICAs | | | | | | |
| C3 | 54.5 (29.9) | 56.4 (27.7) | .926[a] | −1.2 (12.5) | −0.1 (12.8) | .889[a] |
| C4 | 78.8 (24.6) | 80.7 (42.5) | .926[a] | −3.8 (12.9) | −0.9 (12.4) | .075[a] |
| C5 | 74.6 (16.8) | 81.6 (33.2) | .926[a] | 0.1 (8.8) | −0.5 (8.7) | .889[a] |
| C6 | 69.5 (17.4) | 74.3 (21.6) | .926[a] | −0.8 (6.5) | −0.7 (13.2) | .845[a] |
| C7 | 69.7 (18.5) | 72.8 (26.1) | .926[a] | −1.9 (10.4) | −1.7 (12.0) | .845[a] |
| WV | 71.3 (24.0) | 74.1 (36.3) | .845[b] | −1.7 (12.1) | −1.1 (13.8) | .409[b] |
| VAs | | | | | | |
| C3 | 31.7 (6.8) | 32.7 (8.2) | .988[a] | −1.2 (4.8) | −2.1 (4.7) | .869[a] |
| C4 | 29.7 (5.9) | 30.0 (6.1) | .988[a] | −0.5 (5.8) | −2.2 (4.6) | .869[a] |
| C5 | 30.0 (6.5) | 29.0 (4.1) | .988[a] | −1.0 (4.7) | −1.4 (4.1) | .869[a] |
| C6 | 29.7 (7.6) | 29.0 (5.5) | .988[a] | −0.8 (5.1) | −2.0 (6.4) | .869[a] |
| C7 | 28.8 (9.1) | 29.7 (6.3) | .988[a] | −0.3 (6.1) | −1.6 (3.6) | .869[a] |
| WV | 30.6 (7.6) | 30.3 (7.1) | .980[b] | −0.6 (6.5) | −1.7 (6.1) | .644[b] |
| IJV | | | | | | |
| C3 | 99.3 (62.6) | 114.3 (59.8) | .950[a] | −10.3 (29.7) | 0.8 (32.0) | .018[a,c] |
| C4 | 116.1 (67.5) | 141.2 (65.8) | .950[a] | −14.6 (46.5) | 7.9 (41.5) | .018[a,c] |
| C5 | 121.1 (63.3) | 126.2 (45.1) | .950[a] | −27.5 (44.3) | 2.3 (54.6) | .010[a,c] |
| C6 | 113.6 (74.1) | 136.6 (118.2) | .950[a] | −29.1 (55.3) | −0.2 (63.8) | .015[a,c] |
| C7 | 123.5 (99.8) | 163.1 (94.7) | .950[a] | −24.1 (62.2) | −4.0 (71.8) | .018[a,c] |
| WV | 115.7 (70.2) | 132.4 (76.2) | .697[b] | −17.6 (52.0) | 4.7 (56.3) | .003[b,c] |

**Note:**—ΔCSA indicates change in CSA during 5 years; BL, baseline.

[a and b] Group medians and IQR of neck vessel total CSA at baseline and of ΔCSA are reported for $MS_{noCVD}$ and $MS_{CVD}$, at each cervical level and for the WV course. To evaluate differences between the $MS_{noCVD}$ and $MS_{CVD}$ groups for CSA at baseline and for ΔCSA, linear mixed models were used at each cervical level (a) and for the WV (b). Age and EDSS were used as correcting factors for both analyses a and b. The Benjamini-Hochberg procedure was performed to correct for multiple comparisons.

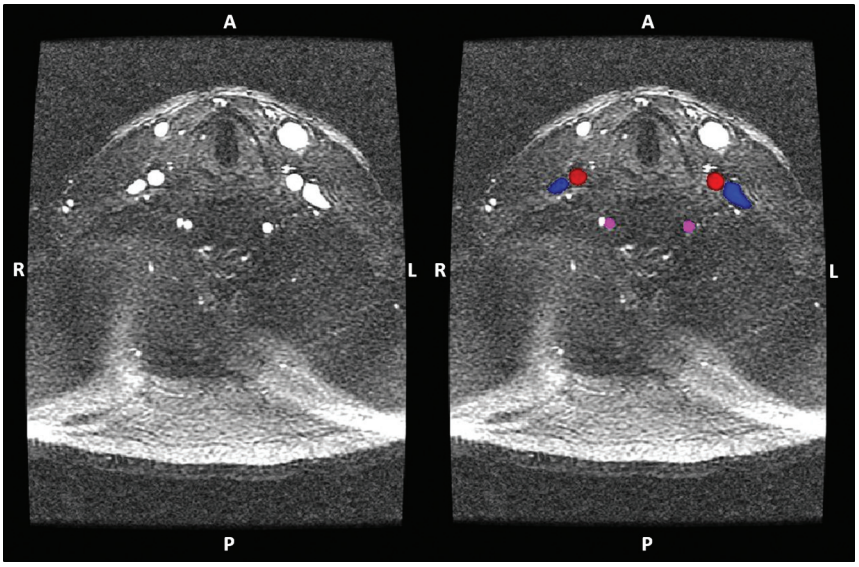
[c] An $\alpha$ level of .05 was considered significant.

**On-line Table 4: Assessment of the effect of cardiovascular disease in the evolution of neck vessel total cross-sectional area for 5 years in HCs**

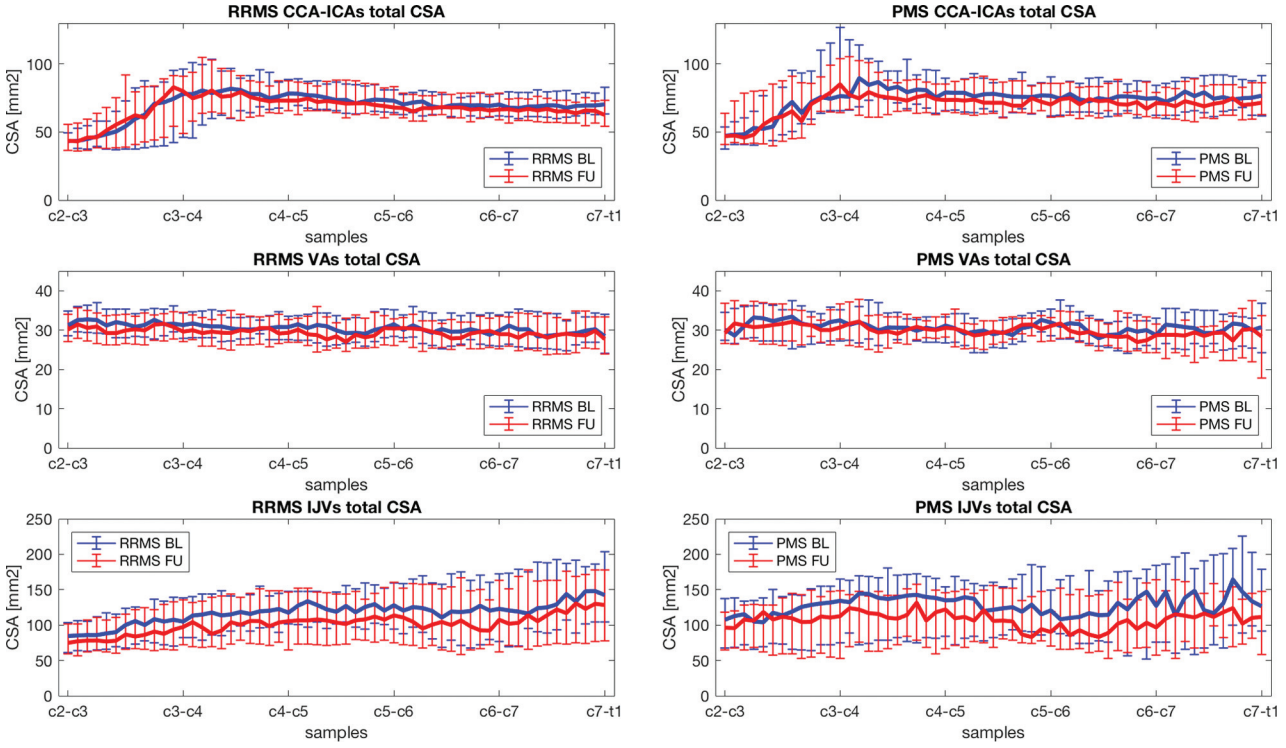
| Vessel/Cervical Level | $HC_{noCVD}$ BL CSA ($mm^2$) (Median) (IQR) | $HC_{CVD}$ BL CSA ($mm^2$) (Median) (IQR) | BL CSA $HC_{CVD}$ vs $HC_{noCVD}$ (*P* Value) | $HC_{noCVD}$ ΔCSA ($mm^2$) (Median) (IQR) | $HC_{CVD}$ ΔCSA ($mm^2$) (Median) (IQR) | ΔCSA $HC_{CVD}$ vs $HC_{noCVD}$ (*P* Value) |
|---|---|---|---|---|---|---|
| CCA–ICAs | | | | | | |
| C3 | 69.2 (30.5) | 63.9 (26.5) | .868[a] | 6.8 (10.7) | −3.6 (24.1) | .350[a] |
| C4 | 70.8 (19.6) | 73.4 (18.5) | .868[a] | 2.3 (12.7) | −0.3 (5.8) | 1.000[a] |
| C5 | 70.2 (14.6) | 68.8 (14.9) | .868[a] | −2.3 (10.1) | −3.2 (8.5) | 1.000[a] |
| C6 | 65.3 (7.8) | 69.9 (15.6) | .868[a] | −2.2 (9.1) | −2.7 (8.5) | 1.000[a] |
| C7 | 68.6 (9.0) | 69.3 (14.1) | .86[a] | −4.2 (8.4) | −3.3 (8.7) | 1.000[a] |
| WV | 67.5 (14.9) | 71.5 (16.6) | .585[b] | −0.6 (13.3) | −3.2 (10.9) | .301[b] |
| VAs | | | | | | |
| C3 | 31.9 (5.6) | 31.5 (8.1) | .714[a] | −1.2 (5.2) | −2.7 (3.9) | .950[a] |
| C4 | 30.1 (6.9) | 31.0 (10.0) | .714[a] | −1.3 (6.0) | −1.2 (3.6) | 1.000[a] |
| C5 | 29.1 (8.7) | 28.6 (9.4) | .714[a] | −0.9 (3.2) | −1.5 (7.7) | 1.000[a] |
| C6 | 27.1 (9.2) | 30.0 (9.1) | .71[a] | 0.2 (5.7) | −1.8 (5.1) | .950[a] |
| C7 | 27.4 (9.3) | 30.3 (6.0) | .714[a] | −0.9 (4.8) | 0.6 (6.2) | .950[a] |
| WV | 29.4 (7.8) | 30.3 (8.2) | .539[b] | −1.1 (6.5) | −1.3 (5.6) | .905[b] |
| IJV | | | | | | |
| C3 | 93.0 (42.9) | 119.9 (109.3) | .365[a] | −8.6 (72.1) | −9.7 (39.5) | 1.000[a] |
| C4 | 106.1 (25.6) | 127.2 (107.4) | .365[a] | −15.0 (60.8) | 3.5 (73.6) | .371[a] |
| C5 | 114.8 (70.8) | 132.1 (133.1) | .365[a] | −22.9 (72.9) | −10.5 (132.6) | .371[a] |
| C6 | 98.1 (107.5) | 146.2 (172.8) | .365[a] | −21.6 (47.4) | 7.5 (194.8) | .371[a] |
| C7 | 93.5 (132.4) | 183.0 (163.8) | .365[a] | −11.3 (94.2) | 25.4 (140.8) | .371[a] |
| WV | 101.8 (62.1) | 138.1 (106.3) | .231[b] | −16.0 (63.7) | 6.4 (96.1) | .154[b] |

**Note:**—BL indicates baseline.

[a and b] Group medians and IQR of neck vessel total CSA at baseline and of ΔCSA are reported for $HC_{noCVD}$ and $HC_{CVD}$, at each cervical level and for the WV course. To evaluate differences between $HC_{noCVD}$ and $HC_{CVD}$ groups for CSA at baseline and for ΔCSA, the independent samples Mann-Whitney *U* test (a) was performed at each cervical level and linear mixed models were used for the WV (b). The Benjamini-Hochberg procedure was performed to correct for multiple comparisons.




**ON-LINE FIG 1.** Axial view (C5 level) of a MRA image of a healthy control with the segmented ROIs for CCA–ICAs (red), VAs (purple), and IJVs (blue) are shown. A indicates anterior; L, left; P, posterior; R, right.




**ON-LINE FIG 2.** Total CSA of CCA–ICAs, VAs, and IJVs at baseline (blue) and follow-up (red) for patients with RRMS (*left*) and PMS (*right*). The median CSA values (*lines*) and the respective IQRs (*bars*) are represented for all the samples, along C3-to-C7 cervical levels. BL indicates baseline; FU, follow-up.