Deciphering the dynamics of the splenium: A comprehensive analysis of flair hyperintensity variations

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Received December 5, 2025; Accepted February 18, 2026

DOI: 10.3892/etm.2026.13128

Abstract. The splenium of the corpus callosum plays a pivotal role in interhemispheric communication and undergoes various changes with ageing. The present study aimed to elucidate the age-related variations in splenial fluid-attenuated inversion recovery (FLAIR) hyperintensity, providing insights into the normal ageing process of the brain and enhancing the clinical interpretation of magnetic resonance (MR) imaging. In the present retrospective cohort study, cerebral MR imaging of 1,176 patients was analysed following application of strict inclusion and exclusion criteria to isolate age and sex-related changes and other factors that can affect splenium hyperintensity. The present study focused on measuring the thickness (anteroposterior) of splenial FLAIR hyperintensity, with interobserver agreement assessed to ensure measurement reliability. Statistical analyses, including the Mann-Whitney U test for sex comparisons and the Kruskal-Wallis test for age group comparisons, were employed to investigate the effects of age, sex, radiation therapy and Fazekas score on splenial FLAIR hyperintensity. The present study demonstrated a significant increase in the presence of splenial FLAIR hyperintensity and thickness ($P<0.001$; $P=0.006$) with advancing age, particularly in individuals >57 years of age. No significant differences were observed between male and female participants, suggesting that these age-related changes were consistent across sex. Splenium hyperintensity thickness was significantly higher in the group that received radiation therapy and in the patient group with a Fazekas score of 3 (0.048, 0.018). Interobserver agreement was evaluated using the intraclass correlation coefficient (ICC) to assess measurement consistency (ICC=0.977; $P<0.001$). The present study provides crucial insights into the age-related dynamics of the splenium.

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Key words: corpus callosum, splenium, FLAIR hyperintensity, age-related changes, MR imaging, ageing

Introduction

The human brain undergoes structural and functional changes throughout the aging process. Among these changes, the corpus callosum, the largest white matter structure in the brain, plays a notable role in interhemispheric communication and exhibits age-related structural and microstructural alterations (1-4). In particular, the splenium of the corpus callosum is involved in the integration of visual, auditory and somatosensory information, and its morphological and signal intensity alterations have been recognized as potential imaging biomarkers of brain aging (1).

Fluid-attenuated inversion recovery (FLAIR) hyperintensity in the splenium has been observed in a variety of neurological disorders, including ischemia, metabolic disorders and demyelinating diseases (5,6). However, limited research has investigated the presence and significance of splenial hyperintensity in otherwise healthy individuals as part of the normal aging process. Given the growing elderly population and the increasing utilization of magnetic resonance (MR) imaging in clinical practice, the understanding of the normal variations of splenial FLAIR hyperintensity across different age groups is essential for distinguishing physiological changes from pathology.

Previous studies have suggested that splenial signal abnormalities may be linked to changes noted in myelin density, axonal integrity and vascular supply, which naturally evolve with age (7-9). In addition, radiotherapy and white matter disease have been implicated as factors contributing to increased splenial hyperintensity; however, their precise impact remains unclear (10). The identification of the threshold at which these changes become statistically significant can provide a valuable reference for radiologists and clinicians interpreting brain MR imaging scans.

The present study aimed to fill this gap by systematically analysing splenial FLAIR hyperintensity in a large cohort of patients, establishing age-related reference values and assessing the influence of sex, radiation therapy and white matter disease on these findings. By using a comprehensive statistical approach, the present study aimed to refine the interpretation of MR imaging findings and contribute to the standardization of diagnostic criteria for aging-related changes in the splenium.

The subsequent sections detail the methodology used in the current retrospective cohort study, present the results derived from a thorough examination of cerebral MR imaging

and discuss the broader implications of these findings in clinical practice. By elucidating the normal aging dynamics of the splenium, the current research study sought to provide a foundation for more accurate and reliable neuroradiological assessments, ultimately improving patient care and diagnostic accuracy.

Patients and methods

Study design and population. The present study was approved by the Ethics Committee of Dr Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (approval no. 2022-01/27) in accordance with institutional guidelines and The Declaration of Helsinki. The present study analysed cerebral MR imaging, which was performed between January 2018 and December 2021. The most common clinical indication for MR imaging referral was headache. The patients' neurological history was evaluated from the information recorded in the hospital and exclusion criteria were applied. Initially, 1,176 patients were screened and following application of strict inclusion and exclusion criteria, 975 patients were included in the final analysis (Fig. 1).

Inclusion and exclusion criteria. The inclusion criteria were as follows: Availability of high-quality axial FLAIR MR imaging, adults aged ≥18 years with no history of neurological disorders and no contraindications to MR imaging. The exclusion criteria were as follows: Presence of intracranial mass lesions or stroke that could alter the structure and signal of the splenium, diagnosis of white matter diseases such as multiple sclerosis or leukoencephalopathy, prior traumatic brain injury with reported MR imaging abnormalities, use of medications known to affect FLAIR signal intensity such as antiepileptics and corticosteroids and severe metabolic disorders such as uncontrolled diabetes and hepatic encephalopathy (11).

MR imaging protocol. All MR imaging was performed on a 1.5T MR imaging scanner (SIGNA™ Experience; GE HealthCare) with the following standardized FLAIR protocol: Repetition time, 9,000 msec; echo time, 85 msec; inversion time, 2,500 msec; slice thickness, 5 mm (no interslice gap); field of view, 240 mm.

Measurement of splenial FLAIR hyperintensity. Splenial FLAIR hyperintensity thickness was measured in the anteroposterior direction on the axial FLAIR slice where the hyperintensity appeared most prominent, using the outer margins of the hyperintense area as anatomical reference points; a sample case is presented in Fig. 2. Two independent observers (one with 2 years and another with 10 years of neuroradiology experience) performed the measurements in a blinded manner to minimize bias. The interobserver agreement was assessed using the intraclass correlation coefficient (ICC).

Assessment of white matter hyperintensities. White matter hyperintensities were evaluated using the Fazekas scale, which classifies lesions into four categories: 0, absent; I, punctate foci; II, beginning confluence of foci; and III, large confluent areas (12).

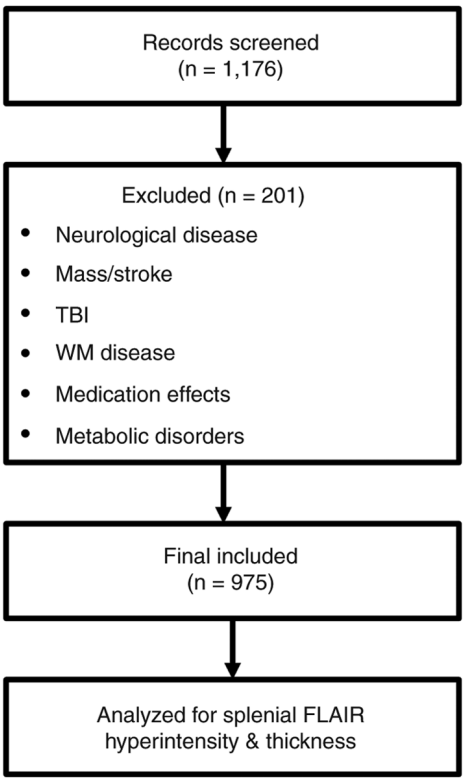

Figure 1. Flow diagram of the study population. A total of 1,176 patients were screened. After applying the inclusion and exclusion criteria, 975 patients were included in the final analysis. FLAIR, fluid-attenuated inversion recovery; TBI, traumatic brain injury; WM, white matter.

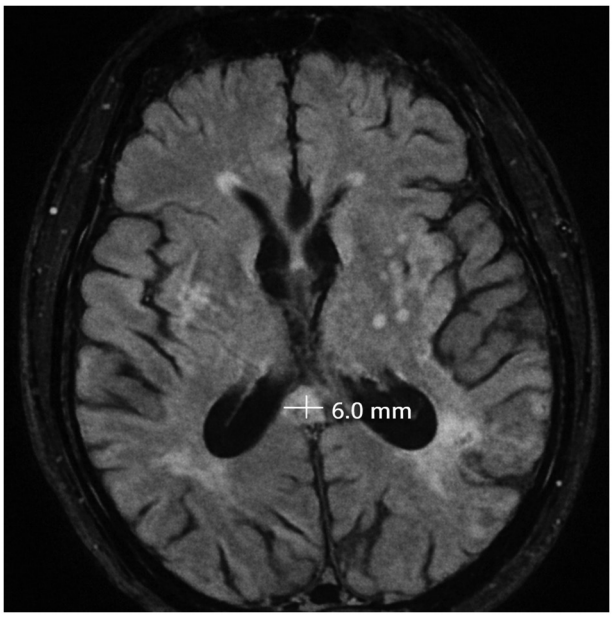

Figure 2. Anteroposterior thickness of splenium hyperintensity.

Statistical analysis. Statistical analysis was performed using IBM SPSS Statistics for Windows (version 25.0; IBM Corp.). The Kolmogorov-Smirnov test was applied to assess normality of continuous variables. The Mann-Whitney U test was used to compare sex differences in splenial hyperintensity thickness. The Kruskal-Wallis test followed by Dunn's

Table I. Presence of splenium hyperintensity according to age and sex.

Variable	Total	Yes	No	Test value	P-value
All	975 (100)	165 (16.9)	810 (83.1)		
Age, years	49.47±6.30	62.92±12.78	46.73±5.57	-11.855[a]	<0.001
Sex				0.008[b]	0.929
Male	322 (33)	54 (16.8)	268 (83.2)		
Female	653 (67)	111 (17.0)	542 (83.0)		

Values are presented as number (percentage) or mean ± standard deviation. [a]Student's t-test; [b]χ^2 test.

Table II. Thickness of splenium hyperintensity according to patient characteristics.

Variable	n	Thickness (mean ± SD)	Test value	P-value
Age	165		0.214[a]	0.006
Sex			-0.579[b]	0.562
Male	54	2.24±1.30		
Female	111	2.06±1.10		
Radiation therapy			-1.982[b]	0.048
Yes	46	2.50±1.45		
No	119	1.98±1.01		
Fazekas scale			10.026[c]	0.018
0	59	1.86±1.16		
I	63	2.12±1.07		
II	25	2.28±1.03		
III	18	2.78±1.48		

[a]Spearman correlation analysis; [b]Mann-Whitney U test; [c]Kruskal-Wallis test with Dunn's multiple comparison test. Post hoc analysis using Dunn's test revealed a significant difference between the Fazekas scale 0 and III groups.

multiple comparison test with Bonferroni correction was conducted for age group differences. Spearman correlation test was used to determine relationships between continuous variables. Receiver operating characteristic (ROC) curve analysis and Youden index were utilized to define an optimal age cut-off for hyperintensity presence [57 years; area under the curve (AUC)=0.79; P<0.001]. Interobserver reliability was assessed using the κ coefficient for categorical data and ICC for continuous measurements (ICC=0.977; P<0.001).

Results

Study population and demographics. Following application of the inclusion and exclusion criteria, 975 patients were included in the final analysis. The present study population consisted of 653 females (67%) and 322 males (33%) with an age range of 18 to 91 years (mean, 49.47±16.30 years). There were no statistically significant differences in the demographic distribution between sex (P=0.929).

Presence and thickness of splenial hyperintensity. Splenium hyperintensity was detected in 165 (16.9%) of the 975 patients. Patients with hyperintensity exhibited a significantly higher mean age compared with those without hyperintensity (Z=-11.855; P<0.001; Tables I and II). A statistically significant but weak positive correlation was observed between age and splenial FLAIR hyperintensity thickness (Spearman's r=0.214; P=0.006). This relationship is visually demonstrated in the dot plot shown in Fig. 3.

Age threshold analysis. ROC analysis was performed to evaluate the potential of age to predict the presence of splenial FLAIR hyperintensity. The analysis identified an optimal age cut-off value of 57 years, with an AUC of 0.79 (95% confidence interval: 0.76-0.83; P<0.001). At this threshold, the sensitivity and specificity were 76 and 73%, respectively (Fig. 4).

Effect of sex. No significant differences were noted between males and females with regard of the splenium hyperintensity presence (P=0.929) or thickness (P=0.562), indicating that sex does not significantly influence these findings (Tables I and II).

Impact of radiation therapy and white matter disease. The patients who had undergone cranial radiation therapy exhibited significantly increased splenial hyperintensity thickness compared with those without radiation therapy (RT) history

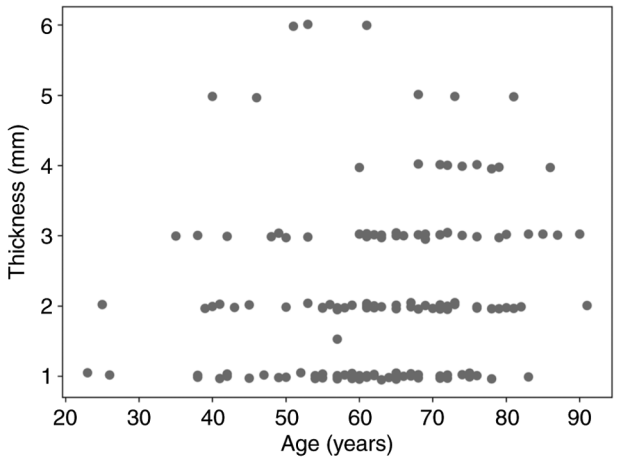

Figure 3. Scatter plot demonstrating the relationship between age and splenial FLAIR hyperintensity thickness (mm) in patients with visible hyperintensity (n=165). Each point represents an individual measurement recorded as a continuous variable in mm. Apparent clustering of points reflects measurement precision and rounding (~0.5 mm increments), as well as overlapping values in the visualization, rather than categorization of the data. A small amount of jitter was applied to improve visualization. A significant positive correlation was observed between age and splenial hyperintensity thickness (Spearman r=0.214; P=0.006).

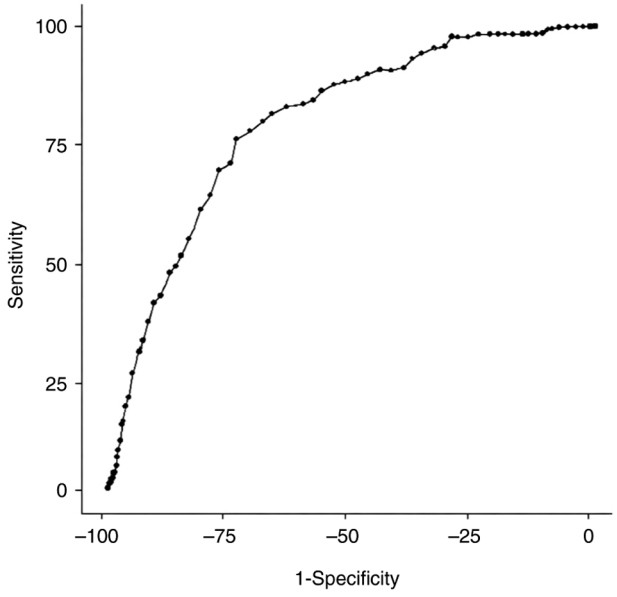

Figure 4. Receiver operating characteristic analysis.

(Z=-1.982; P=0.048). In addition, a significant difference was observed between the Fazekas score groups (χ^2=10.026, P=0.018). Post-hoc analysis revealed that patients with a Fazekas score of III exhibited significantly higher splenial hyperintensity thickness compared with those with a score of 0 (P<0.05).

Information regarding the radiation therapy subgroup, including primary diagnoses, radiation dose parameters and the time interval since treatment, was not consistently available due to the retrospective design of the study; therefore, the observed association between cranial radiation therapy and increased splenial hyperintensity thickness should be interpreted with caution.

Interobserver agreement. The interobserver agreement was found to be high for the presence of splenial hyperintensity (κ=0.821; P<0.001) and excellent for thickness measurements (ICC=0.977; P<0.001), confirming the reliability of the measurement methodology used in the present study.

Discussion

The findings of the present study provide compelling evidence that splenial FLAIR hyperintensity is a common feature of the aging brain, with a significant increase observed in individuals >57 years. This threshold, determined via ROC analysis, underscores the requirement for age-adjusted interpretation of MR imaging findings to differentiate normal age-related changes from pathological conditions.

There are certain studies in the literature explaining the causes of splenium involvement, but the specific cause of involvement of this region and pathophysiological changes have not yet been explained. Cytotoxic oedema, focal inflammatory changes, electrolyte changes in the cell membrane and focal demyelination due to antiepileptic drugs are considered responsible for the involvement of this area (3,11,13). It has been postulated that isolated involvement may be due to the histopathological difference between the other parts of the corpus callosum or the difference in vascular circulation (3,11,13). Studies have further highlighted the importance of age-adjusted interpretation of white matter signal changes on MR imaging, emphasizing that age-related FLAIR hyperintensities may overlap with imaging features of subclinical pathology, notably in older adults. The distinction of physiological aging from early or subclinical disease therefore requires careful consideration of patient age, lesion distribution and associated imaging findings, underscoring the clinical value of age-specific reference thresholds in routine neuroradiological practice.

Splenium abnormalities on MR imaging have been reported commonly and unexpectedly in various cases. Various studies have been conducted on this topic; however, the majority of the studies are limited to case reports (7,9,14,15). Age-related white matter alterations have been widely studied, with previous literature suggesting a decline in myelin density and axonal integrity contributing to increased signal intensities in FLAIR imaging (1-3). The significant association between splenial hyperintensity and Fazekas grade III further reinforces the link between white matter degeneration and hyperintensity formation in the corpus callosum. These findings align with earlier reports that have associated deep white matter disease with microvascular dysfunction and gliosis, both of which may play a role in splenial hyperintensity progression (6,7). The absence of significant sex differences in the current findings suggests that these age-related alterations are a universal aspect of human ageing rather than being influenced by sex-specific factors. This universality necessitates a nuanced approach to interpret MR imaging, where age-related changes are considered alongside potential pathological findings.

Due to the retrospective and cross-sectional design of the present study, the observed association between age and splenial FLAIR hyperintensity reflects differences across age groups rather than longitudinal progression within the same individuals; therefore, prospective longitudinal studies are

required to confirm the development and temporal progression of these changes. Given the retrospective and cross-sectional design of the present study, the observed association between age and splenial FLAIR hyperintensity represents inter-individual differences across distinct age groups rather than a true temporal progression within the same individuals. Therefore, these findings should not be interpreted as evidence of progressive development of splenial hyperintensity over time. Confirmation of the natural evolution and progression of these changes would require well-designed longitudinal studies with follow-up imaging of the same subjects.

In addition to age-related changes, the present study highlights the influence of RT on splenial hyperintensity thickness. Patients with a history of RT exhibited significantly increased hyperintensity, suggesting a potential impact of radiation-induced demyelination or gliosis in the corpus callosum. This aligns with previous studies that have identified RT-related white matter changes, notably in patients undergoing cranial irradiation for malignancies (10). Future research with longitudinal imaging follow-ups is warranted to delineate the temporal progression of RT-associated splenial changes.

From a clinical perspective, differentiating between normal ageing processes and pathological changes is paramount. The increased splenial FLAIR hyperintensity observed in older individuals may mimic or obscure pathological hyperintensities, potentially leading to misdiagnosis or overdiagnosis of conditions such as mild cognitive impairment or early dementia. Therefore, the findings advocate for incorporating age-specific reference ranges in assessing splenial FLAIR hyperintensity, diagnostic accuracy and patient management.

One of the key strengths of the present study is the high interobserver agreement, ensuring the reliability of the splenial hyperintensity measurements. This consistency strengthens the validity of the present findings and supports the potential integration of splenial hyperintensity as a neuroimaging biomarker for age-related white matter changes. However, several limitations should be acknowledged in the present study. Firstly, the retrospective nature of the present study limits the ability to establish causality between aging and hyperintensity development. Future prospective studies with longitudinal assessments could provide a clearer picture of the progression of splenial changes over time. Secondly, while patients with known white matter diseases were excluded, subclinical neurodegenerative processes may have introduced a confounding factor despite exclusion criteria. Incorporating cognitive assessments and advanced neuroimaging techniques, such as diffusion tensor imaging or MR spectroscopy could enhance the current understanding of the underlying mechanisms.

While headache was the primary indication for MR imaging referral, rigorous exclusion criteria were used to minimize confounding pathology and this limitation inherent to retrospective, clinically referred cohorts has been considered when interpreting the results. In addition, the retrospective design limited the availability of detailed radiation therapy-related clinical parameters, which may have influenced the interpretation of its effect on splenial hyperintensity.

A further limitation is the use of 1.5T MR imaging scanners, which, while widely used in clinical practice, may not capture the full extent of subtle signal changes compared with higher-resolution 3T imaging. Future studies using higher-field MR scanners could improve sensitivity in detecting microstructural alterations in the splenium. In addition, expanding the study population to include diverse ethnic backgrounds and larger datasets from multiple institutions would enhance the generalizability of these findings.

Despite these limitations, the findings of the present study provide critical insights into the normal aging process of the splenium and emphasize the importance of contextualizing hyperintensity findings in older adults. The integration of these imaging biomarkers with volumetric analysis and machine learning-based pattern recognition may aid the refinement of the diagnostic criteria and the improvement of the differentiation between physiological aging and neurodegenerative disorders.

In conclusion, the present study demonstrated that splenial FLAIR hyperintensity was a frequent finding in aging individuals, with a marked increase observed beyond the age of 57. These findings could assist geriatricians and radiologists in collaborative decision-making, notably in distinguishing benign imaging findings from early neurodegenerative conditions. Future research integrating multimodal imaging and longitudinal assessments will be crucial in further understanding the clinical implications of these changes and their potential role in neurodegenerative disease prediction.

The present study demonstrated that splenial FLAIR hyperintensity was a common finding in aging, significantly increasing following 57 years. These hyperintensities likely reflect normal aging rather than pathology, necessitating an age-adjusted approach to MR imaging interpretation. Given the aging global population, recognizing normative imaging changes in older adults is essential to avoid overdiagnosis of white matter pathology and unnecessary interventions in geriatric care. The association between splenial hyperintensity and Fazekas grade III suggests an association with white matter changes and vascular factors. In addition, radiation therapy history was associated with increased hyperintensity thickness, highlighting the requirement to consider patient history in MR imaging interpretation.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present may be requested from the corresponding author.

Authors' contributions

EYB and YÖ designed the study and analysed the data. ÖÜ and OŞ analysed the data and prepared the manuscript. EYB and YÖ confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present retrospective study was approved by the local Ethics Committee (approval no. 2022-01/27). According to institutional and national regulations, the requirement for informed consent was waived.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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