

## Challenging The Diagnosis of Multiple Sclerosis: Histopathological Insights Gained Through Brain Biopsies

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### ABSTRACT

**Introduction:** While tissue sampling through brain biopsy is rarely required throughout the Multiple sclerosis (MS) disease course, it remains essential in cases with atypical clinical or radiological findings where other diagnostic methods are inconclusive. This study aims to present brain biopsy findings in patients diagnosed with MS.

**Methods:** MS patients were screened from the national database. Brain MRI scans of all MS patients who underwent brain biopsy were reviewed to confirm the compliance of MAGNIMS criteria. Data on MS diagnosis, biopsy date, disease-modifying therapy (DMT) use, and the duration of DMT exposure prior to biopsy were also collected. Pathology reports of brain biopsies were reviewed with these parameters.

**Results:** Among 87,640 MS patients, 21 patients had brain biopsies after MS diagnosis, highlighting the rarity (0.02%) of this invasive procedure, with a median age at biopsy of 43 (IQR: 13) years. Pathological findings revealed 12 malignant diagnoses, including lymphoma, glioblastoma, metastasis, and nine non-malignant conditions, such as vasculitis, demyelination, and benign masses.

**Conclusion:** Brain biopsy is rarely required in patients with MS; however, it should be considered in cases of atypical lesion development, underscoring the need for meticulous clinical monitoring.

**Keywords:** Brain biopsy, demyelination, disease-modifying therapy, malignancy, multiple sclerosis, pathology

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### INTRODUCTION

Multiple sclerosis (MS) can mimic a range of other conditions, including infections, neuromyelitis optica spectrum disorder, myelin oligodendrocyte glycoprotein-associated disease, neurosarcoidosis, adult-onset leukodystrophies, primary or secondary central nervous system (CNS) vasculitis, and even neoplastic disorders (1). Over the years, advances in the understanding of MS have led to the refinement of the diagnostic criteria, enabling earlier and more accurate diagnosis (2). Furthermore, recent advances in MS treatment have promoted the use of highly effective disease-modifying therapies (DMT) in the earlier stages of the disease (3). However, the increased efficacy of these treatments is sometimes accompanied by additional risks, depending on their mechanisms of action (4).

Histopathological diagnosis remains a cornerstone for definitive diagnosis in many areas of medicine. Clinicians often rely on biopsies of accessible tissues, particularly in neoplastic, infectious, and inflammatory conditions, to confirm the underlying pathology. However, in CNS diseases, tissue sampling is not always feasible, and diagnoses are frequently made using advanced neuroimaging and cerebrospinal fluid analyses. Nevertheless, there are cases where a brain biopsy becomes essential to establish or clarify the diagnosis.

### Highlights

- Brain biopsy is rarely needed in MS, but crucial for atypical MRI findings.
- Among 87,640 MS patients, 21 underwent post-diagnosis brain biopsies.
- 12 biopsies revealed malignancies, including glioblastoma and lymphoma.
- MRI plays a vital role in detecting atypical lesions in MS patients.

An ongoing debate exists regarding the potential association between MS and cancer, as well as the role that MS treatments may play in this relationship (5–8). Additionally, throughout the course of the disease, patients undergo repeated neuroimaging evaluations. When unexpected or atypical imaging or clinical findings arise, brain biopsy remains the most reliable diagnostic method, particularly when cerebrospinal fluid analysis and imaging fail to provide a definitive answer for atypical conditions.

In this study, we aimed to present the brain biopsy findings in patients diagnosed with MS and to discuss the appropriate indications and timing for performing brain biopsies in a large cohort of MS patients.

## METHODS

### MS patient population

The data was obtained from the national database of the Ministry of Health of Türkiye. Patients meeting at least one of the following criteria were included in the study: a minimum of three instances of the G35 ICD-10 code or one to two times of G35 ICD-10 code with a MS specific drug prescription (Anatomical Therapeutic Chemical codes: L03AB13, L03AX13, L04AA31, L04AX07, L04AA27, L04AA23, L04AA36, L04AA40, L01BB04, L04AA34, L01XC04, L01XC02, L04AX01, L01BA01, L04AA06, L01DB07, prescriptions with L01AA01 codes and 8699505761978, 8699783760014, 8699809779228 transaction numbers including inpatient treatments). Based on these criteria, the MS patient cohort consisted of 87,640 individuals.

Our previous Turkish MS epidemiology study validated defining MS patients as those with at least three G35 ICD-10 codes, demonstrating a sensitivity of 97.5% and specificity of 99.5% (9, 10). Additionally, the brain MRIs of all patients who underwent brain biopsy were reviewed to confirm compliance with the MAGNIMS criteria at the time of MS diagnosis (11).

### Brain biopsy reports

All pathology reports of the study cohort were obtained from the Ministry of Health database. Cerebrospinal fluid cytology examinations and aborted fetal brain tissues were excluded from the study. Only brain biopsy results were considered, identified by filtering records containing the term “brain.” If the same patient had more than one result within the same date, these were consolidated and recorded as a single entry. Biopsy results were classified as benign or malignant based on the International Classification of Diseases for Oncology (ICD-O) codes provided in the pathology reports (12).

### Data Collection and Analysis of DMT Exposure

The MS diagnosis date, biopsy date, and prescription details, including DMT use, were recorded for all patients. The age at the time of biopsy was calculated using the biopsy date and patient’s date of birth, while the interval between MS diagnosis and biopsy was determined based on the respective dates. The final analysis included only patients who underwent brain biopsy after their MS diagnosis. For patients whose biopsy results indicate malignancy, the duration of DMT exposure was calculated. This

duration was derived from prescription records preceding the biopsy date, accounting for the specific molecule, the quantity per box, and the number of boxes dispensed. For ocrelizumab, exposure was assessed on a session-based scale, whereas for cladribine, it was calculated annually.

The study was approved by the local ethics committee (Approval date and number: 2024/433). It was conducted in accordance with the Declaration of Helsinki and relevant national/international ethical guidelines.

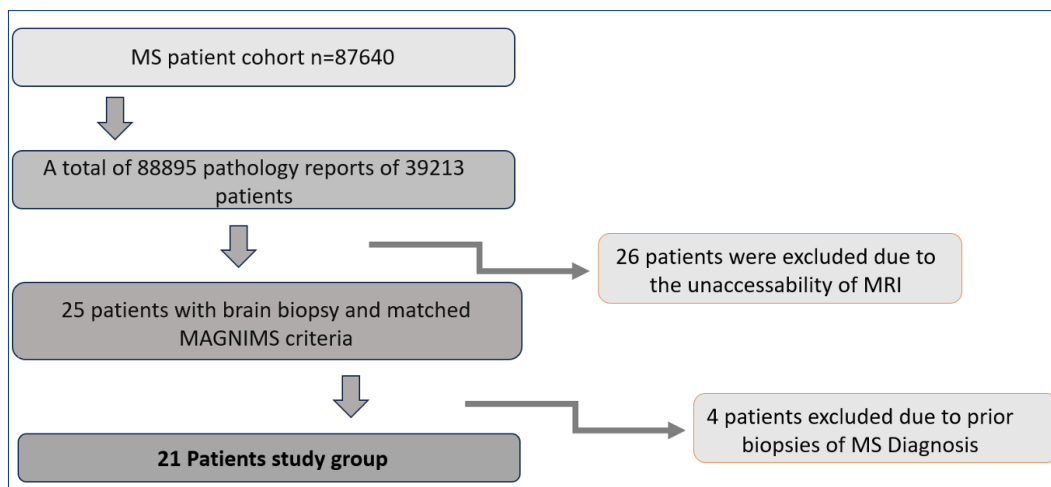
## RESULTS

The study cohort consisted of 87,640 MS patients, among whom 39,213 individuals underwent a total of 88,895 pathology examinations. From this group, 25 patients with brain biopsies fulfilling the MAGNIMS criteria were identified. After excluding four patients who had biopsies prior to their MS diagnosis, 21 patients were included in the final analysis (Figure 1).

The data of 26 MS patients who underwent brain biopsy were excluded from the study due to inadequacy of MRI examinations to confirm the MS diagnosis (e.g., single acquisition, inappropriate acquisition, or unavailability of images). The MRIs of 25 patients were deemed compatible with MAGNIMS criteria. Among these, four patients underwent brain biopsy prior to their MS diagnosis, while the remaining 21 patients underwent biopsy following their MS diagnosis (Table 1).

Among the 21 patients (8 females, 13 males), the median age at MS diagnosis was 39.0 years (IQR: 16.0). The overall median age at the time of biopsy was 43.0 years (IQR: 13.0) for the entire group, with females having a median age of 42.5 years (IQR: 6.5) and males 46.0 years (IQR: 24.0;  $p=0.42$ ).

Among the 21 patients, 12 had biopsy results indicating malignant conditions, such as astrocytoma, lymphoma, glioblastoma, infiltrative glial tumors, and metastases (Figure 2). The median age at MS diagnosis was 44.0 years (IQR: 23.0) in these patients. Others had non-malignant pathologies, such as vasculitis, hematoma, pituitary adenoma, craniopharyngioma, hemangioma and meningioma, and benign glial tumors. Only two patients had biopsy results suggestive of demyelination. There was no statistically significant difference in sex, age at MS diagnosis, or age at the time of biopsy between patients with malignant and non-malignant biopsy results. Among the three patients with brain metastases, lung cancer was identified as the primary source. In the two patients with biopsy results indicating demyelination, the biopsy was performed due to the appearance of a newly enlarging lesion on neuroimaging.



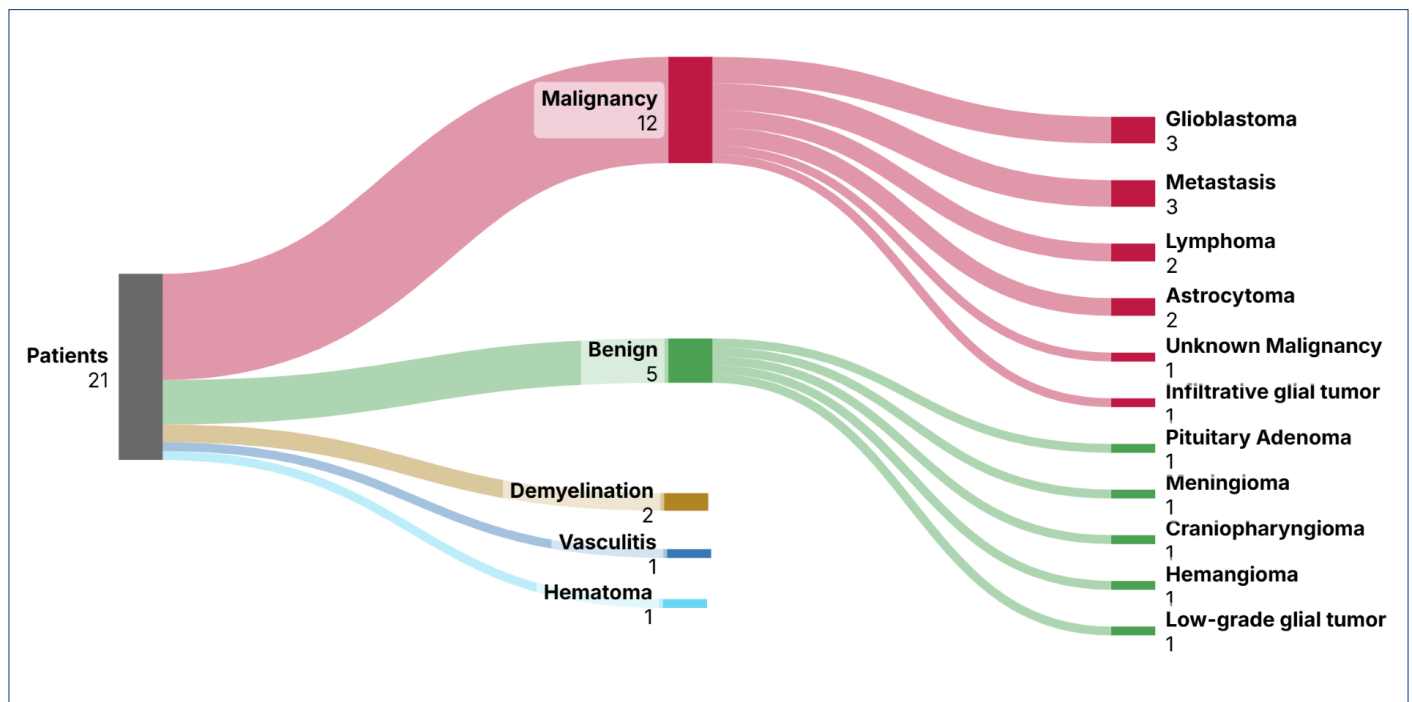
**Figure 1.** Flowchart of the study

**Table 1.** The mean interval between MS diagnosis and brain biopsy categorized by biopsy results

ID	Gender	Age at MS diagnosis	Age at brain biopsy	Final diagnosis	MS diagnosis to biopsy time (years)
1	Male	37	43	Pituitary adenoma	6.1
2	Female	40	43	Meningioma	2.6
3	Female	31	39	Craniopharyngioma	8.7
4	Male	38	38	Hemangioma	0.9
5	Male	28	31	Low-grade glial tumor	2.7
6	Female	39	46	Demyelination	6.6
7	Male	42	46	Demyelination	3.2
8	Male	64	70	Hematoma	5.8
9	Female	45	49	Astrocytoma	4.2
10	Female	30	38	Astrocytoma	7.9
11	Male	56	56	Lymphoma	0.1
12	Male	26	28	Lymphoma	1.3
13	Male	66	71	Glioblastoma	5.1
14	Male	58	60	Glioblastoma	1.9
15	Female	38	42	Infiltrative glial tumor	4.7
16	Male	30	34	Malignancy*	4.8
17	Female	21	26	Glioblastoma	5.6
18	Female	42	45	Metastasis**	3.6
19	Male	52	58	Metastasis**	6.3
20	Male	46	51	Metastasis**	5.1
21	Male	22	25	CNS vasculitis	2.7

CNS: Central nervous system ; MS:Multiple sclerosis

\*Malignant tumor with unknown primary source; \*\*Primary lung cancer

**Figure 2.** Distribution of pathologic diagnoses performed after MS diagnosis

The analysis of DMT exposures in patients with malignant tumors demonstrated that they received a variety of DMTs, with no single therapy showing a predominant association (Table 2).

## DISCUSSION

This study involved a comprehensive analysis conducted on 88,895 pathology reports from 87,640 patients diagnosed with MS. From this

cohort, 21 patients who fulfilled the MAGNIMS radiological diagnostic criteria for MS were selected for detailed evaluation. These patients, whose MS diagnosis was confirmed through MRI, underwent a thorough review of the pathological findings in their biopsy reports.

The pathological diagnoses among the 21 MS patients included a wide range of conditions, some of which were unrelated to the MS diagnosis. Specifically, one patient was found to have pathological diagnosis of

**Table 2.** Pre-biopsy DMT exposure of patients with a malignant brain tumor.

ID	Biopsy result	1 <sup>st</sup> DMT	Exposure time to 1 <sup>st</sup> DMT (months)	2 <sup>nd</sup> DMT	Exposure time to 2 <sup>nd</sup> DMT (months)
9	Astrocytoma	Fingolimod	19		
10	Astrocytoma	Glatiramer acetate	21		
11	Lymphoma	Fingolimod	1		
12	Lymphoma	Interferon	14		
13	Glioblastoma	Interferon	57		
14	Glioblastoma	Cladribine	12		
15	Infiltrative glial tumor	Fingolimod	13	Ocrelizumab	18
16	Malignancy*	Interferon	15	Fingolimod	22
17	Glioblastoma	Glatiramer acetate	5		
18	Metastasis**	None			
19	Metastasis**	Glatiramer acetate	5	Interferon	4
20	Metastasis**	Fingolimod	35		

DMT: Disease-modifying therapy

\*Malignant tumor with unknown primary source; \*\*Primary lung cancer

vasculitis while another had hematoma. Two patients biopsies were reported with demyelination, a finding potentially consistent with MS but not specific to the disease. Moreover, other significant findings were observed including one case of pituitary adenoma, two cases of astrocytoma, and one case each of craniopharyngioma, meningioma, and a low-grade glial tumor. The patient with a pathological diagnosis of hematoma had been preliminarily diagnosed with glioblastoma due to the presence of intraparenchymal hemorrhage, accompanied by heterogeneous contrast enhancement and vasogenic edema surrounding the lesion. The two other cases reported as demyelination had new hyperintense lesion in T2 weighted imaging which was considered as atypical lesions due to their size, shape, and location.

Pathological analysis revealed benign masses in seven patients and malignant tumors in 12 patients. The immunopathogenesis of autoimmune diseases or autoimmune-related diseases, such as MS, is characterized by impaired self-tolerance, whereas cancer is marked by enhanced self-tolerance and uncontrolled cellular proliferation (4). Immunosuppression induced by treatment may facilitate the evasion of immune surveillance. However, the underlying mechanisms of these diseases are highly complex, involving intricate interactions within the immune system in addition to genetic, environmental, and treatment-related factors. Notably, two patients with metastatic brain tumors had been on fingolimod treatment for approximately two to three years. However, the available evidence remains insufficient to establish a definitive causal relationship.

A Mendelian randomization study examining the association between MS and 15 types of cancer, including brain tumors, found no evidence of a causal relationship, except for a slightly increased risk of cervical cancer (13). Additionally, large cohort studies have evaluated various DMTs to assess their potential association with cancer risk (4, 8). Interferons and glatiramer acetate were not found to be associated with increased cancer risk (4). Anecdotes of multiple types of cancers in all DMTs have been reported (4, 8).

Gliomas are malignant brain tumors, accounting for approximately 81% of all malignant brain tumors and 26% of all CNS tumors (14). Concurrent glial tumors and MS cases have been reported for more than 50 years (14). Studies investigating the association between MS and glial tumors

have reported conflicting findings. Evidence from large database cohorts indicates both decreased and increased risks of CNS tumors in patients with MS compared to the general population (4–6, 15). It has also been suggested that the higher rate of CNS tumors in MS may be attributed to the frequent imaging used to monitor disease activity. In our study, we identified three patients with high grade glial tumors and one patient with an infiltrative glial tumor. The proportion of glial tumors among the malignant brain tumors observed in our MS cohort aligns with findings reported in the literature (14). There remains a need for population-based controlled prevalence studies to clarify the relative risk of glial tumors in MS patients across different regions and ethnicities. Clinicians should remain vigilant for the presence of glial tumors when observing newly developing or growing lesions with atypical localization and characteristics.

One of the patients diagnosed with lymphoma was treated with fingolimod, while the other was treated with interferon. According to the literature, several cases of primary CNS lymphoma have been reported following the use of fingolimod in MS patients. These patients had been on fingolimod for periods ranging from eight months to four years (16–18). However, establishing a causal relationship remains challenging. On the other hand, patient #11 was diagnosed with CNS lymphoma after only one month of fingolimod use, raising further questions about the potential causal link between MS, fingolimod treatment, and the development of lymphoma.

Numerous studies have investigated the relationship between cancer and interferons, concluding that no significant causal association exists between interferon treatment and cancer (4, 8). Notably, only a single case of primary CNS lymphoma was reported in an MS patient participating in the CombiRx trial. However, the pre-lymphoma lesion was present prior to the patient's enrollment in the study (19).

Primary or secondary CNS vasculitis can radiologically and clinically mimic MS. The occurrence of primary CNS vasculitis in MS is exceedingly rare, with only a few cases reported, particularly following alemtuzumab treatment. However, this was not the case for patient #21 (20, 21).

Evaluation of the MR images revealed that while the lesions were initially consistent with an MS diagnosis, the development of atypical lesions

over time necessitated a biopsy. Considering the patient population, the rate of brain biopsies was notably low (21 brain biopsies in 87,640 MS patients), underscoring the critical role of MRI in diagnosis. This highlights the importance of carefully evaluating atypical MRI findings in patients with an established diagnosis of MS. Not every new MRI lesion should be automatically attributed to MS, as this may lead to alternative, new, or additional diagnoses, including malignancies, which could potentially be identified without the need for invasive procedures like brain biopsy.

A key limitation of our dataset is the absence of longitudinal clinical follow-up, detailed treatment history beyond DMT exposure, and radiological progression data. These limitations may affect the interpretation of disease evolution and diagnostic outcomes. Furthermore, the absence of clinical data precluded an assessment of the relationship between DMT use and malignancies. To better understand the relative risk of developing CNS malignant tumors, future research should examine the prevalence of these tumors in a population matched to the MS cohort, with particular emphasis on the impact of DMT use.

Brain biopsy, while providing definitive histopathological insight, raises ethical considerations, particularly in the context of evolving non-invasive diagnostic modalities. Informed consent, risk-benefit assessment, and clinical justification must be carefully weighed when considering biopsy in atypical MS presentations.

In light of these results, the crucial role of MRI in the follow-up of MS patients is evident. Physicians should be attentive not only to new T2 plaques, active plaques, smoldering plaques, and atrophy, but also to lesions that are atypical for MS. Although not limited to these features, atypical lesions may present with rapid enlargement, vasogenic edema with or without mass effect, prolonged contrast enhancement in repeated imaging.

Based on the findings of our study, we conclude that although brain biopsy is not generally required for the diagnosis of multiple sclerosis due to its highly invasive nature, it remains an important diagnostic tool in patients with established MS who present with atypical clinical and radiological features. Our study also underscores the importance of conducting further research with matched populations to better elucidate the comorbidities associated with MS. MRI remains a cornerstone in the follow-up of MS patients, facilitating early detection of atypical lesions and additional diagnoses, such as cancer, that may complicate the disease course. Brain biopsy should be considered in cases with atypical clinical or radiological findings, particularly when lesions exhibit rapid enlargement, mass effect, or persistent contrast enhancement. This underscores the importance of vigilant monitoring and a thorough diagnostic approach.

**Ethics Committee Approval:** This study was approved by the Gülhane Scientific Research Ethics Committee (Approval date and number: 2024/433)

**Peer Review:** Externally Independent.

**Conflict of Interest:** The authors declare that they have no conflict of interest regarding this work.

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