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Biomarkers Differentiating RRMS and SPMS in Multiple Sclerosis—A Systematic Review

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Abstract: Background: This systematic review searched to identify a potential biomarker in serum/plasma or cerebrospinal fluid (CSF) to differentiate between relapsing-remitting multiple sclerosis (RRMS) and secondary progressive multiple sclerosis (SPMS). There is currently no definitive method for determining whether a patient is in the RRMS course or has converted to the SPMS course. A biomarker could therefore aid the clinician to make this diagnosis. The aim of this study is to assess if there are biomarkers or combinations of biomarkers in serum/plasma or CSF that can detect secondary progression in multiple sclerosis at an early stage. Methods: The PubMed and EMBASE databases were searched to identify relevant studies. Both MeSH terms and text words in the title/abstract were used in both search strategies. The method included forward and backward citation searches. A risk of bias tool was used to assess all the studies that were included. Results: A total of 7581 articles were identified from the initial search. Additionally, 3386 articles were added after the citation search. Of these, 39 articles fulfilled the inclusion criteria and none of the exclusion criteria. The review investigated 28 different biomarkers in CSF and serum/plasma. Discussion: Of the 28 different biomarkers, six biomarkers appeared to be the most promising: neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), Galectin-9, YKL-40/CHI3L1, osteopontin, and MCP-1. This review provides new insights into potential directions for future studies to investigate biomarkers as a diagnostic tool for SPMS.

Keywords: secondary progressive multiple sclerosis; biomarker; cerebrospinal fluid; serum; plasma; systematic review

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1. Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disease affecting the central nervous system with axonal and neuronal loss [1,2]. There are three main subtypes: relapsing-remitting multiple sclerosis (RRMS), primary progressive multiple sclerosis (PPMS), and secondary progressive multiple sclerosis (SPMS). Approximately 85% of patients with multiple sclerosis initially have RRMS, of which 15–30% will eventually convert to SPMS [2]. During the progressive phase, the immune response in the central nervous system seems more active than in the periphery, which leads to increased cortical involvement. Axonal loss occurs more slowly over time in chronically demyelinated lesions than in newly acute lesions [2]. Additionally, progressive swelling and disorganization of the cytoskeleton in chronically demyelinated axons may be specific to the progressive phase [2].

The diagnosis of SPMS can be challenging to make, as there are no clear clinical, imaging, immunological, or pathological criteria to differentiate it from RRMS. The diagnosis is often made retrospectively based on a history of disability progression [3] and assessments such as the Expanded Disability Status Scale (EDSS) [4], walking tests [5,6], and patient-reported outcomes [7]. This can result in inter-individual bias in the timing of diagnosis among clinicians.

As a result, patients will not receive appropriate treatment to slow disease progression. While treatments such as disease-modifying therapies (DMTs) can be effective in treating RRMS, they may not all be effective in patients with SPMS [8]. Ocrelizumab may be helpful in treating SPMS, which highlights the need for a biomarker to differentiate between the two courses [9].

Other reasons to differentiate between the subtypes include the ability to classify patients for future studies correctly, the prevention of unnecessary side effects, and socioeconomical considerations.

In the past years, there has been a significant increase in the number of studies seeking to identify a "golden biomarker" for SPMS. Previous reviews have investigated various specific biomarkers such as neurofilaments [10,11] as well as biological biomarkers, magnetic resonance imaging (MRI), and functional tests [12]. However, it is important to concentrate on biomarkers found in only serum/plasma and cerebrospinal fluid (CSF) and to include both less-studied and well-researched biological biomarkers. Although MRI and functional tests are helpful in aiding the clinician in determining whether the patient has converted to SPMS, it has its limitations. For the MRI to be a powerful tool, it requires 3T imaging looking specifically for slowly expanding lesions and/or paramagnetic rim enhancement. Functional tests, such as walking tests and cognitive tests, are very useful, but very often it is necessary to repeat testing after 6–12 months, which delays determining if the patient has converted. This review investigates a wide variety of biomarkers in serum/plasma and CSF to determine whether these biomarkers can help clinicians differentiate between RRMS and SPMS.

The aim of this study is to assess if there are biomarkers or combinations of biomarkers in serum/plasma or CSF that can detect SPMS at an early stage.

2. Materials and Methods

This systematic review was managed by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [13].

The protocol of the review was registered in the International Prospective Register of Systematic Reviews, PROSPERO (ID: CRD42022372776).

2.1. Study Selection

PubMed and EMBASE databases were searched to identify relevant studies. The final search was performed 5 December 2023. Both MeSH terms and text words in the title/abstract were used in both search strategies (Figure 1). Forward and backward citation searches found literature that either referred to or cited the included studies using SCOPUS and Web of Science.

EMBASE search

1 multiple sclerosis/
2 multiple sclerosis.ti.
3 MS.ti.
4 1 or 2 or 3
5 biological marker/
6 biomarker*.ab,ti.
7 marker*.ab,ti.
8 marker/
9 biochemical marker/
10 serum*.ab,ti.
11 sera.ab,ti.
12 serum/
13 csf.ab,ti.
14 cerebrospinal fluid/
15 cerebrospinal fluid*.ab,ti.
16 5 or 6 or 7 or 8 or 9 or 19 or 11 or 12 or 13 or 14 or 15
17 transition*.ab,ti.
18 conversion*.ab,ti.
19 secondary progressive multiple sclerosis.ab,ti.
20 SPMS.ab,ti.
21 progression*.ab,ti.
22 predict*.ab,ti.
23 convert*.ab,ti.
24 disability.ab,ti.
25 disability/
26 disability severity/
27 disease severity/
28 severity.ab,ti.
29 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28
30 4 and 16 and 29
31 limit 30 to (conference abstract or conference paper or "conference review")
32 30 not 31
33 limit 32 to dt="20020101-20231205"

PubMed search

("multiple sclerosis"[MeSH Terms] OR "multiple sclerosis"[Title] OR "MS"[Title]) AND ("biomarkers"[MeSH Terms] OR "biomarker*"[Title/Abstract] OR "marker*"[Title/Abstract] OR "biochemical marker*"[Title/Abstract] OR "serum*"[Title/Abstract] OR "sera"[Title/Abstract] OR "CSF"[Title/Abstract] OR "cerebrospinal fluid*"[Title/Abstract] OR (cerebrospinal fluid[MeSH Terms])) AND ("multiple sclerosis, chronic progressive"[MeSH Terms] OR "conversion*"[Title/Abstract] OR "convert*"[Title/Abstract] OR "predict*"[Title/Abstract] OR "transition*"[Title/Abstract] OR "progression*"[Title/Abstract] OR "disability"[Title/Abstract] OR "severity"[Title/Abstract] OR "secondary progressive multiple sclerosis"[Title/Abstract] OR "SPMS"[Title/Abstract]) AND (2002/01:2023/12/05[Date – Publication])

Figure 1. Literature search string from EMBASE and PubMed (1 January 2002–12 May 2023).

2.2. Selected Studies

The search results were transferred to Endnote 20 [14] and afterward to Covidence [15], where duplicates were removed. Two reviewers (C.M.S. and C.T.) independently screened all titles/abstracts and full texts using Covidence. For title/abstract screening, conflicts were discussed and solved by consensus. In case of disagreement in full-text screening, a third member of the team (H.B.J.) made the final decision.

The included studies fulfilled the inclusion criteria: studies with (1) human MS patients, (2) written in the English language, (3) comparing RRMS/RRMS-remission with SPMS/PMS, and using (4) biological marker(s) from serum/plasma or CSF.

Studies were excluded if they were (1) published before 2002, (2) there were <2 articles investigating a specific biomarker, (3) the biomarker involved DNA or metabolism, (4) the studies involved pregnancy or the pediatric population, or (5) there was more than 60% PPMS in PMS. Furthermore, studies were excluded if they did not mention a *p*-value or comment on the significance. Statistical significance was defined as $p < 0.05$. Reviews, commentaries, conference abstracts, letters, and opinions were excluded. No studies were excluded based on the risk of bias. The authors of all studies with unclear, or conflicting results or missing data were contacted in order to confirm the correct result or to elaborate on unclear information. If there was no reply, the study was excluded.

2.3. Data Extraction and Quality Assessment

Covidence was used to extract relevant data by two reviewers (C.M.S. and C.T.) independently. Data included the first author's last name, year of publication, country, diagnostic criteria, study design, sample size, sex and distribution, age (mean/median), biomarker(s), and *p*-value.

Two reviewers (C.M.S. and C.T.) individually assessed the risks of bias in the included studies by using an adjusted version of the US National Institutes of Health (NIH) Quality Assessment Tool for Observational Cohort, Cross-Sectional, and Case–Control Studies [16]. The risk of bias was checked, and disagreements were solved by consensus. Studies were rated as good, fair, or poor.

3. Results

A total of 7581 articles were identified after checking for duplicates. Additionally, 3386 articles were added after the citation search. Of these, 39 articles fulfilled the inclusion criteria and none of the exclusion criteria (Table 1). Please refer to Figure 2 for the screening process.

In the following section, the investigated biomarkers will be introduced with the results. The results are ranked after the number of studies investigating a certain biomarker, and data are reported as they were described in the studies (IQR = interquartile range, SD = standard deviation). When data for mean/median difference were not available but shown by boxplot, the difference is described as an increase/decrease.

Table 1. Overview of the included studies investigating biomarkers to detect SPMS.

First Author, Year of Publication, Country	Diagnostic Criteria	Study Design	Total Number of Participants	Sex (Female/Male)	Age	Biomarker(s)	*p*-Value	Mean/ Median Difference	Risk of Bias
Talaat, 2023, Egypt [17]	McDonald 2017	Case–control study	RRMS (n = 30) SPMS (n = 16) PPMS (n = 6)	RRMS (24/6) SPMS (12/4) PPMS (4/2)	(Mean) RRMS 28.23 SPMS 32.31 PPMS 34.21	CSF-CHI3L1 (ng/mL)	RRMS vs. PMS $p \leq 0.001$	127%	Fair
Loonstra, 2023, The Netherlands [18]	McDonald 2017	Cross-sectional study	RRMS (n = 171) SPMS (n = 79)	RRMS (140/31) SPMS (47/32)	(Mean) RRMS 52.88 SPMS 52.9	s-NfL (pg/mL) s-GFAP (pg/mL)	RRMS vs. SPMS $p = 0.009$ RRMS vs. SPMS $p = 0.043$	17% 19%	Good
Dias de Sousa, 2022, Brazil [19]	McDonald 2010	Cross-sectional study	RRMS (n = 21) SPMS (n = 6)	RRMS (17/4) SPMS (5/1)	(Mean) F-RRMS 40.1 M-RRMS 32.8 F-SPMS 52.2 M-SPMS 60	p-IL-2 (pg/mL) p-IL-8 (pg/mL) p-IL-1β (pg/mL) p-IL-6 (pg/mL) p-IL-12 (pg/mL) p-TNF-α (pg/mL) p-IFN-γ (pg/mL) p-IL-4 (pg/mL) p-IL-10 (pg/mL) p-BDNF (pg/mL) Cultures of PBMC IL-4 (pg/mL) IFN-γ (pg/mL) IL-10 (pg/mL) IL-17 (pg/mL) TNF-α (pg/mL) IL-6 (pg/mL)	RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS $p < 0.05$ RRMS vs. SPMS $p < 0.05$ RRMS vs. SPMS ns RRMS vs. SPMS $p < 0.05$ RRMS vs. SPMS ns RRMS vs. SPMS ns	ND	Poor
Lamancová, 2022, Slovakia [20]	Lublin 2013	Cohort study	RRMS (n = 40) SPMS (n = 25)	RRMS (24/16) SPMS (10/15)	(Mean) RRMS 39 SPMS 49	s-CXCL13 (pg/mL) s-CHI3L1 (pg/mL) s-NfL (pg/mL) s-MCP-1 (pg/mL) s-MMP-2 (pg/mL) s-MMP-9 (pg/mL)	RRMS vs. SPMS $p < 0.001$ RRMS vs. SPMS $p < 0.01$ RRMS vs. SPMS $p < 0.001$ RRMS vs. SPMS $p < 0.01$ RRMS vs. SPMS $p < 0.001$ RRMS vs. SPMS ns	75% 81% 85% 31% Decrease Decrease	Poor/fair
Sağir, 2021, Turkey [21]	McDonald 2017	Cross-sectional study	RRMS (n = 12) SPMS (n = 7)	RRMS (9/3) SPMS (5/2)	(Mean) RRMS 44.0 SPMS 50.4	s-BDNF (pg/mL)	RRMS vs. SPMS $p < 0.05$	Decrease	Poor
Uphaus, 2021, Germany [22]	McDonald 2017	Cohort study	RRMS (n = 169) SPMS (n = 27)	RRMS (116/53) SPMS (21/6)	(Median) Follow-up: RRMS 39.1 SPMS 51.1	s-NfL (pg/mL)	Follow-up: RRMS vs. SPMS $p < 0.001$	51%	Fair/good
Eslami, 2020, Iran [23]	McDonald 2017	Cross-sectional study	RRMS (n = 51) SPMS (n = 25)	RRMS (41/10) SPMS (13/12)	(Mean) RRMS 33.7 SPMS 37.6	s-IL-6 (pg/mL) s-IL-8 (pg/mL)	RRMS vs. SPMS $p = 0.008$ RRMS vs. SPMS ns	92% 12%	Poor
Ferraro, 2020, Italy [24]	McDonald 2010	Cohort study	RRMS (n = 21) SPMS (n = 43) PPMS (n = 27)	RRMS (15/6) PMS (49/21)	(Median) RRMS 40 PMS 60	p-NfL (pg/mL)	RRMS vs. PMS $p = 0.007$	32%	Poor

Table 1. *Cont.*

First Author, Year of Publication, Country	Diagnostic Criteria	Study Design	Total Number of Participants	Sex (Female/Male)	Age	Biomarker(s)	*p*-Value	Mean/ Median Difference	Risk of Bias
Högel, 2020, Finland [25]	ND	Cross-sectional study	RRMS (n = 46) SPMS (n = 33)	RRMS (36/10) SPMS (20/13)	(Median) RRMS 46.32 SPMS 56.12	s-GFAP (pg/mL) s-NfL (pg/mL)	RRMS vs. SPMS $p < 0.001$ RRMS vs. SPMS $p < 0.001$	83% 71%	Fair
Naegelin, 2020, Switzerland [26]	McDonald 2001	Cohort study	RRMS (n = 178) SPMS (n = 56)	RRMS (138/40) SPMS (29/27)	(Mean) RRMS 41.74 SPMS 53.77	s-BDNF (ng/mL)	RRMS vs. SPMS $p = 0.004$	−6%	Good
Gencer, 2019, Turkey [27]	McDonald 2010	Cross-sectional study	RRMS (n = 25) SPMS (n = 15)	RRMS (19/6) SPMS (10/5)	(Median) RRMS 35 SPMS 45	s-BDNF (ng/mL)	RRMS vs. SPMS $p = 0.065$	Decrease	Poor
Gil-Perotin, 2019, Spain [28]	McDonald 2017	Cohort study	RRMS (n = 99) SPMS (n = 35) PPMS (n = 23)	RRMS (79/20) SPMS (21/14) PPMS (10/13)	(Median) RRMS 35 SPMS 45 PPMS 51	CSF-NfL (pg/mL) CSF-CHI3L1 (ng/mL)	RRMS vs. PMS ns RRMS vs. SPMS ns	−10% 17%	Fair
Ribeiro, 2019, Switzerland [29]	McDonald 2010	Cross-sectional study	RRMS (n = 147) SPMS (n = 17) PPMS (n = 4)	All MS (119/49)	(Mean) All MS 42.02	s-TNF-α (pg/mL)	RRMS vs. PMS $p = 0.802$	No difference	Poor
Barro, 2018, Switzerland [30]	Lublin 1996, McDonald 2001 and 2005	Cohort study	RRMS + CIS (n = 189) SPMS (n = 54) PPMS (n = 14)	All MS (179/78)	(Median) All MS 44.0	s-NFL (pg/mL)	RRMS + CIS vs. PMS < 0.001	41%	Good
Herman, 2018, Sweden [31]	McDonald 2005	Cohort study	RRMS (n = 30) SPMS (n = 16)	RRMS (21/9) SPMS (10/6)	(Mean) RRMS 39 SPMS 58	CSF-Galectin-9 (pg/mL) CSF-MCP-1 (pg/mL) CSF-TNF-α (pg/mL)	RRMS vs. SPMS $p = 0.007$ RRMS vs. SPMS $p = 0.006$ RRMS vs. SPMS $p = 0.08$	30% 35% 42%	Fair/good
Iacobaeus, 2018, Sweden [32]	McDonald 2010	Cross-sectional study	RRMS (n = 47) SPMS (n = 29)	RRMS (31/16) SPMS (20/9)	(Mean) RRMS 34 SPMS 60	p-CD86 p-IL-10 p-IL-17 p-TGF-beta1	RRMS-rem vs. SPMS $p < 0.01$ RRMS vs. SPMS $p < 0.05$ RRMS vs. SPMS ns RRMS vs. SPMS ns	Decrease Decrease Decrease Increase	Poor
Stein, 2018, USA [33]	McDonald 2010	Cross-sectional study	RRMS (n = 26) SPMS (n = 24)	RRMS (15/11) SPMS (12/12) PPMS (9/20)	(Median) RRMS 38 SPMS 50 PPMS 53	CSF-IL-1β (pg/mL) CSF-IL-6 (pg/mL) CSF-TNF-α (pg/mL) CSF-IL-10 (pg/mL)	RRMS vs. SPMS $p = 0.0377$ RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns	65% 33% 63% 87%	Fair
Kallaur, 2017, Brazil [34]	McDonald 2010	Cohort study	RRMS (n = 126) SPMS (n = 25) PPMS (n = 9)	All MS (99/59)	(Median) RRMS 44 PMS 53	s-IL-1β (pg/mL) s-IL-6 (pg/mL) s-TNF-α (pg/mL) s-IFN-γ (pg/mL) s-IL-12 (pg/mL) s-IL-17 (pg/mL) s-IL-4 (pg/mL) s-IL-10 (pg/mL)	RRMS vs. PMS $p = 0.0168$ RRMS vs. PMS $p = 0.6041$ RRMS vs. PMS $p = 0.8734$ RRMS vs. PMS $p = 0.0309$ RRMS vs. PMS $p = 0.0003$ RRMS vs. PMS $p = 0.9081$ RRMS vs. PMS $p = 0.0509$ RRMS vs. PMS $p = 0.2557$	125% 17% 0% 21% 0% 0% 0% 12%	Good/fair

Table 1. *Cont.*

First Author, Year of Publication, Country	Diagnostic Criteria	Study Design	Total Number of Participants	Sex (Female/Male)	Age	Biomarker(s)	*p*-Value	Mean/ Median Difference	Risk of Bias
Burman, 2016, Sweden [35]	McDonald 2010	Cross-sectional study	Cohort A: RRMS-rem (n = 18) SPMS (n = 20) Cohort B RRMS-rem (n = 11) SPMS (n = 15)	Cohort A RRMS-rem (12/6) SPMS (11/9)	(Median) Cohort A RRMS-rem 39 SPMS 59	CSF-YKL-40 (ng/mL)	Cohort A + B SPMS vs. RRMS-rem ns	Increase	Poor
Burman, 2016, Sweden [36]	McDonald 2010	Cross-sectional study	Cohort A RRMS (n = 25) SPMS (n = 22) Cohort B RRMS (n = 31) SPMS (n = 16)	Cohort A RRMS (18/7) SPMS (15/7) Cohort B RRMS (21/10) SPMS (9/7)	(Median) RRMS (A:36)(B:39) SPMS (A:59)(B:58.5)	CSF-Galectin-9 (pg/mL)	A: RRMS vs. SPMS $p <$ 0.01 B: RRMS vs. SPMS $p <$ 0.05 A+B: RRMS vs. SPMS $p <$ 0.001	31% 24% 27%	Fair
Salehi, 2016, Iran [37]	McDonald 2010	Cross-sectional study	RRMS-rem (n = 14) SPMS (n = 10)	RRMS-rem (13/1) SPMS (8/2)	(Mean) RRMS-rem 35.07 SPMS 35.50	s-CD8: IFN-γ s-CD8: IL-17 s-CD8: TNF-α	RRMS-rem vs. SPMS ns RRMS-rem vs. SPMS ns RRMS-rem vs. SPMS ns	9% −28% −3%	Poor/fair
Mañé-Martínez, 2016, Spain [38]	Poser, McDonald 2001 and 2005	Cross-sectional study	RRMS (n = 192) SPMS (n = 6)	RRMS (121/71) SPMS (4/6)	(Mean) RRMS 34.8 SPMS 43.5	CSF-NfL (ng/L) CSF-GFAP (ng/L) CSF-YKL-40 (ng/mL) CSF-MCP-1 (pg/mL) CSF-t-tau (pg/mL) CSF-p-tau (pg/mL)	RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns	−51% 47% −11% 5% 47% 33%	Fair
Pasquali, 2015, Italy [39]	McDonald 2010	Cross-sectional study	RRMS (n = 30) SPMS (n = 30)	RRMS (21/9) SPMS (19/11)	(Mean) RRMS 40.8 SPMS 56.4	s-IL-17 s-IFN-γ s-TGF-beta1 s-IL-2 s-IL-4 s-IL-6 s-IL-8 s-IL-10 s-IL-12 s-TNF-alfa	RRMS vs. SPMS $p = 0.003$ RRMS vs. SPMS $p = 0.013$ RRMS vs. SPMS $p = 0.029$ RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns RRMS vs. SPMS ns	−69% −37% 42% 22% 6% −42% 49% −17% 17% 40%	Good
Acar, 2014, Turkey [40]	McDonald 2010	Cross-sectional study	RRMS (n = 58) SPMS (n = 21) PPMS (n = 4) PRMS (n = 6)	RRMS (40/18) PMS (19/12)	(Mean) RRMS 34.5 PMS 40.4	s-TIMP1 (ng/mL) s-MMP9/TIMP1	RRMS vs. PMS ns RRMS vs. PMS ns	14% −28%	Fair
Gresle, 2014, Australia [41]	ND	Cohort study	RRMS (n = 81) SPMS (n = 13)	RRMS (55/26) SPMS (10/3)	(Median) RRMS 44.5 SPMS 55	s-pNfH (ng/mL)	RRMS vs. SPMS $p = 0.048$	Δ0.18 ng/mL	Fair
Huber, 2014, USA [42]	McDonald 2010	Cohort study	RRMS (n = 12) SPMS (n = 26)	RRMS (5/7) SPMS (11/15)	(Mean) RRMS 46.3 SPMS 53.8	Cultures of PBMC p-IL-17 (pg/mL) p-IFN-γ (pg/mL)	RRMS vs. SPMS $p = 0.114$ RRMS vs. SPMS $p = 0.792$	Increase Decrease	Good
Shimizu, 2013, Japan [43]	ND	Cross-sectional study	RRMS (n = 11) SPMS (n = 6)	All MS (6/11)	(Mean) All MS 38.3	s-osteopontin (ng/mL)	RRMS vs. SPMS $p < 0.05$	75%	Poor

Table 1. *Cont.*

First Author, Year of Publication, Country	Diagnostic Criteria	Study Design	Total Number of Participants	Sex (Female/Male)	Age	Biomarker(s)	*p*-Value	Mean/ Median Difference	Risk of Bias
Jaworski, 2012, Poland [44]	McDonald 2001	Cross-sectional study	RRMS (n = 34) SPMS (n = 14)	RRMS (22/12) SPMS (5/9)	(Mean) RRMS 32.8 SPMS 44.5	CSF t-tau (pg/mL) CSF p-tau (pg/mL) s-p-tau (pg/mL)	RRMS vs. SPMS $p = 0.01$ RRMS vs. SPMS ns RRMS vs. SPMS ns	−38% −21% 24%	Poor
Axelsson, 2011, Sweden [45]	McDonald 2001	Cohort study	Baseline: RRMS (n = 15) SPMS (n = 10) 8–10 years follow-up: RRMS (n = 10) SPMS (n = 15)	RRMS (3/12) SPMS (6/4)	(Mean) RRMS 40 SPMS 43	CSF-GFAP (ng/L) CSF-NfL (ng/L)	Follow up: RRMS vs. SPMS ns RRMS vs. SPMS ns	16% −1%	Fair
Ragheb, 2011, USA [46]	McDonald 2001 or 2005	Case–control study	RRMS (n = 42) SPMS (n = 8)	All MS (39/11)	(Mean) All MS 43.9	CSF-CXCL13 (pg/mL)	RRMS vs. SPMS ns	−77%	Poor/fair
Correale, 2010, Argentina [47]	Poser and McDonald 2001	Cross-sectional study	RRMS (n = 24) SPMS (n = 24)	RRMS (16/8) SPMS (16/8)	(Mean) RRMS 37.3 SPMS 38.5	CSF-CHI3L1 (ng/mL) s-CHI3L1 (ng/mL)	RRMS vs. SPMS $p < 0.01$ RRMS vs. SPMS ns	−26% Equal	Fair
Benešová, 2009, Czech Republic [48]	McDonald 2001	Cross-sectional study	RRMS (n = 40) SPMS (n = 20)	RRMS (29/11) SPMS (14/6)	(Mean) RRMS 32.2 SPMS 47.5	s-MMP-9 (ng/mL) s-MMP-2 (ng/mL) s-MMP-9/TIMP-1 s-TIMP-1 (ng/mL)	RRMS vs. SPMS ns RRMS vs. SPMS $p < 0.002$ RRMS vs. SPMS ns RRMS vs. SPMS ns	7% 26% 4% −6%	Fair
Teunissen, 2009, The Netherlands [49]	McDonald 2001	Cross-sectional study	RRMS (n = 42) SPMS (n = 28)	RRMS (26/16) SPMS (17/11)	(Mean) RRMS 37 SPMS 54	CSF-NAA (mmol/L) CSF-NfH (Unit/mL) CSF-NfL (ng/L) CSF-t-Tau (ng/L)	RRMS vs. SPMS $p < 0.05$ RRMS vs. SPMS $p < 0.05$ RRMS vs. SPMS ns RRMS vs. SPMS ns	Decrease Increase Decrease Decrease	Fair
Jasperse, 2007, The Netherlands [50]	Poser	Cross-sectional study	RRMS (n = 26) SPMS (n = 12)	RRMS (17/9) SPMS (5/7)	(Median) RRMS 43.5 SPMS 49.6	CSF-NAA (μmol/L)	RRMS vs. SPMS $p = 0.015$	Decrease	Fair
Comabella, 2004, Spain [51]	Lublin 1996	Cross-sectional study	RRMS (n = 46) SPMS (n = 35)	RRMS (29/17) SPMS (28/7)	(Mean) RRMS 35.6 SPMS 47.5	p-osteopontin (ng/mL)	RRMS-rem vs. SPMS $p <$ 0.0005	Increase	Fair
Filion, 2003, Canada [52]	Poser	Cross-sectional study	RRMS − therapy (n = 23) RRMS + therapy (n = 23) SPMS (n = 29)	RRMS − therapy (13/10) RRMS +therapy (15/8) SPMS (18/11)	(Median) RRMS − therapy 38 RRMS +therapy 40 SPMS 50	s-CD86 (MFI) s-IL-1β (pg/mL) s-IL-6 (pg/mL) s-IL-10 (pg/mL) s-IL-12 (pg/mL) s-TNF-α (pg/mL)	RRMS− vs. SPMS $p < 0.05$ RRMS+ vs. SPMS ns RRMS− vs. SPMS ns RRMS+ vs. SPMS $p < 0.05$ RRMS− vs. SPMS ns RRMS+ vs. SPMS ns RRMS− vs. SPMS ns RRMS+ vs. SPMS ns RRMS− vs. SPMS $p < 0.001$ RRMS+ vs. SPMS $p < 0.001$ RRMS− vs. SPMS $p < 0.05$ RRMS+ vs. SPMS $p < 0.001$	Increase Increase Increase Increase Increase Increase Increase Increase Increase Increase Increase Increase	Fair

Table 1. *Cont.*

First Author, Year of Publication, Country	Diagnostic Criteria	Study Design	Total Number of Participants	Sex (Female/Male)	Age	Biomarker(s)	*p*-Value	Mean/ Median Difference	Risk of Bias
Karni, 2002, USA [53]	ND	Cross-sectional study	RRMS (n = 39) SPMS (n = 18)	RRMS (34/5) SPMS (13/5)	(Mean) RRMS 36.7 SPMS 56.5	s-IFN-γ (pg/mL)	RRMS vs. SPMS $p = 0.028$	55%	Poor
Sarchielli, 2002, Italy [54]	Poser	Cross-sectional study	RRMS (n = 20) SPMS (n = 15)	RRMS (13/7) SPMS (10/5)	(Mean) RRMS 33.5 SPMS 38.4	CSF-BDNF (pg/mL)	RRMS vs. SPMS ns	−6%	Fair
Semra, 2002, England [55]	Poser	Cross-sectional study	RRMS (n = 16) SPMS (n = 13) PPMS (n = 6)	RRMS (9/7) SPMS (8/5) PMS (2/4)	(Mean) RRMS 32.6 SPMS 45.3 PPMS 41.2	CSF-NfL (Unit/mg)	RRMS vs. PMS $p = 0.14$	Increase	Poor

RRMS = Relapsing-remitting multiple sclerosis, SPMS = Secondary progressive multiple sclerosis, PPMS = Primary progressive multiple sclerosis, PMS = SPMS + PPMS, RRMS-rem = RRMS in remission, RRMS-rel = RRMS during relapse, RMS = relapsing multiple sclerosis, PRMS = progressive relapsing multiple sclerosis, CIS = Clinically isolated syndrome, ns = Not statistically significant ($p > 0.05$), s = Serum, *p* = Plasms, CSF = Cerebrospinal fluid, NfL = Neurofilament light chain, GFAP = Glial fibrillary acidic protein, BDNF = Brain-derived neurotrophic factor, t-tau = Total tau-protein, p-tau = Phosphorylated tau-protein, MCP-1 = Monocyte chemoattractant protein-1, MMP = Matrix metallopeptidase, NAA = N-acetylaspartate, NfH = Neurofilament heavy chain, pNfH = Phosphorylated neurofilament heavy chain, Sm134/135 = Antibodies to recognize NfH phosphoforms, MFI = Mean fluorescent intensity, PBMC = Peripheral blood mononuclear cells, IFN = interferon, TGF = Transforming growth factor, ND = Not described, Mean/median difference (%) = (SPMS-RRMS)/RRMS*100. Δ = mean/median difference when RRMS = 0.

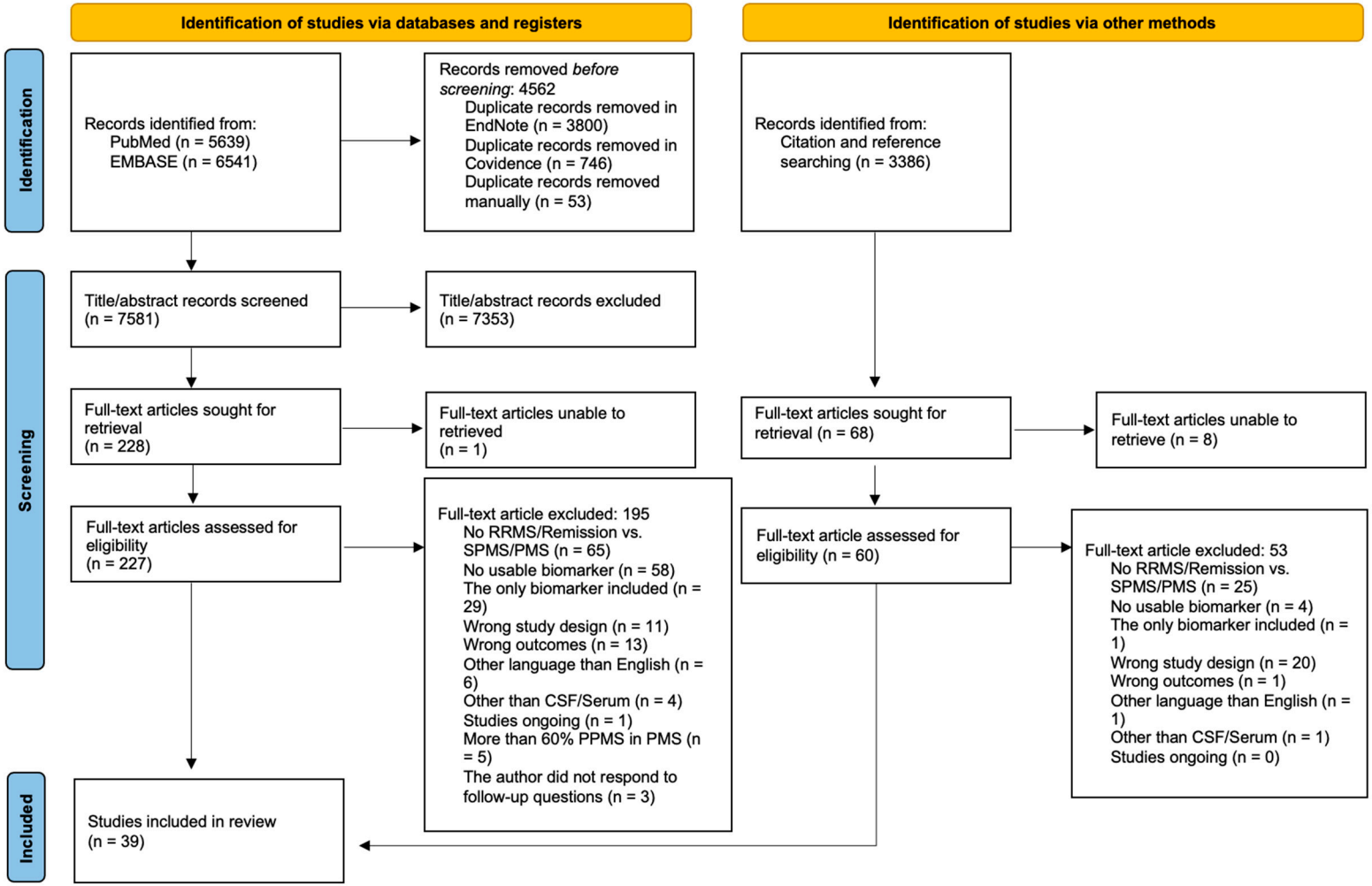

Figure 2. PRISMA 2020 flow diagram with included and excluded studies and the reasons for exclusion.

3.1. Neurofilament Light Chain (NfL)

Six studies [18,20,22,24,25,30] investigated NfL in serum and five studies [28,38,45,49,55] in CSF. None of the studies investigating CSF found a significant difference between RRMS and SPMS. These results can be seen in Table 1. Barro et al. found a significant difference in serum between relapsing multiple sclerosis (RMS) (median 29.7 [IQR 21.2–42.2] pg/mL) and progressive multiple sclerosis (PMS) (median 41.9 [IQR 31.9–55.7] pg/mL), $p < 0.001$, also after adjusting for age [30]. Ferraro et al. investigated NfL in plasma between RRMS (median 9.7 [IQR 8.3–11.2] pg/mL) and PMS (median 12.8 [IQR 10–16] pg/mL) $p = 0.007$ and did not adjust for age [24]. Högel et al. found a difference in serum between RRMS (mean 20.62 (SD 10.6) pg/mL) and SPMS (mean 35.26 (SD 20.23) pg/mL), $p < 0.001$. After adjusting for age, the difference was still significant, $p = 0.044$. With a cut-off value of 31.39 pg/mL they found a sensitivity = 54.55%, specificity = 84.78%, and area under the curve (AUC) = 0.761 [25]. Cut-off values were not available in their manuscript but were received by request. Lamancová et al. investigated NfL in serum between RRMS (mean 428.94 ± 108.13 pg/mL) and SPMS (mean 793.67 ± 180.80 pg/mL), $p \leq 0.001$, but did not adjust for age [20]. Uphaus et al. investigated a difference in serum between RRMS (median 6.9 [IQR 5.0–9.2] pg/mL) and SPMS (median 10.4 [IQR 6.9–17.6] pg/mL), $p < 0.001$. They did not adjust for age even though they found a significant difference in age between RRMS and SPMS [22]. Loonstra et al. only included participants born in 1966 and found a significant difference in serum between RRMS (median 9.3 [IQR 7.4–11.5] pg/mL) and SPMS (median 10.90 [IQR 8.9–14.0] pg/mL), $p = 0.009$ [18].

Loonstra, Uphaus, Ferraro, Högel, and Barro et al. [18,22,24,25,30] all used a newer biomarker detector SIMOA (single molecule array), instead of the standard ELISA (enzyme-linked immunosorbent assay). SIMOA is more sensitive to detect even the very smallest amounts of NfL, down to fg/mL. Lamancová, Gil-Perotin, Mañé-Martínez, Axelsson, Teunissen, and Semra et al. used ELISA [20,28,38,45,49,55].

3.2. YKL-40/CHI3L1

Six studies [17,20,28,35,38,47] investigated levels of YKL-40/CHI3L1. Two studies [20,47] investigated levels in serum and five studies [17,28,35,38,47] in CSF. Lamancová et al. found a significant increase in serum between RRMS (mean 41.10 ± 14.10 pg/mL) and SPMS (mean 74.37 ± 16.10 pg/mL), $p < 0.01$. They did not adjust for age [20]. Correale et al. found a significant difference in CSF between RRMS (mean 111 ± 21 ng/mL) and SPMS (mean 82 ± 18.6 ng/mL), $p < 0.01$. The difference in serum was not significant and was visualized by boxplot [47]. Talaat et al. found a significant difference in CSF between RRMS (mean 107.37 ± 19.23 ng/mL) and PMS (mean 244.27 ± 52.99 ng/mL), $p < 0.001$. They found a cut-off value of 154 ng/mL, $r^2 = 1$ [17]. Gil-Perotin et al. found no significant difference in CSF between RRMS (median 118.97 [IQR 81–186] ng/mL) and SPMS (median 139.55 [IQR 96–212] ng/mL) after adjusting for age [28]. This result was confirmed after email correspondences. Burman et al. found no significant difference in CSF between RRMS remission and SPMS after adjusting for age, though a modest increase was visualized by a boxplot. Interestingly, they found a significant increase between RRMS remission and RRMS relapse visualized by the boxplot, $p < 0.01$ [35]. Mañé-Martínez et al. found no significant difference between RRMS and SPMS in CSF [38].

3.3. Glial Fibrillary Acidic Protein (GFAP)

Four studies [18,25,38,45] investigated GFAP. Two studies [18,25] investigated levels in serum, and both found a significant difference. Högel et al. investigated levels in serum between RRMS (mean 89.24 (SD 34.97) pg/mL) and SPMS (mean 162.88 (SD 107.61) pg/mL), $p < 0.001$. This was also significant when adjusted for age, $p = 0.019$. With a cut-off value of 129.36 pg/mL, they found a sensitivity = 57.58%, specificity = 89.13%, and AUC = 0.766 [25]. Loonstra et al. found a significant difference in serum between RRMS (median 59.7 [IQR 45.8–77.1] pg/mL) and SPMS (median 70.8 [IQR 48.8–103.6] pg/mL),

$p = 0.043$ [18]. Axelsson et al. and Mañé-Martínez et al. found a higher CSF GFAP level in SPMS than in RRMS. However, this was not significant after adjusting for age [38,45].

Högel et al. and Loonstra et al. used SIMOA. Mañé-Martínez et al. and Axelsson et al. used ELISA.

3.4. Brain-Derived Neurotrophic Factor (BDNF)

Four studies [19,21,26,27] investigated BDNF in serum/plasma, and one study [54] investigated BDNF in CSF. Sarchielli et al. found no significant difference in CSF between RRMS remission (mean 26.2 ± 6.2 pg/mL) and SPMS (mean 24.6 ± 4.93 pg/mL) [54]. Gencer et al. and Dias de Sousa et al. found a decrease from RRMS to SPMS in serum/plasma, but it was not significant [19,27]. Naegelin et al. found a significant difference in serum between RRMS (mean 29.52 (SD 6.73) ng/mL) and SPMS (mean 27.87 (SD 7.55) ng/mL), $p = 0.004$ [26]. Sağir et al. found a significant decrease in serum from RRMS to SPMS, $p < 0.05$ [21].

3.5. Immunological Cytokines

Most of the studies investigating immunological cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, CXCL13, TGF-β1, TNF-α) found no significant differences between the subgroups. However, this was uncertain for three biomarkers: IFN-γ, IL-12, and IL-1β. Four studies [19,34,39,53] investigated IFN-γ in serum. Karni et al. found a significant difference between RRMS (mean 3377.2 ± 251 pg/mL) and SPMS (mean 5233.9 ± 624.5 pg/mL), $p = 0.028$ [53]. Kallaur et al. found a significant difference between RRMS (median 39.5 [IQR 23.0–70.7] pg/mL) and PMS (median 47.8 [IQR 32.2–114.9] pg/mL), $p = 0.031$ [34]. Pasquali et al. found a significant difference between RRMS (mean 8.12 ± 6.96 pg/mL) and SPMS (mean 5.12 ± 3.92 pg/mL), $p = 0.013$ [39]. However, this difference was inverted compared to those of Karni et al. and Kallaur et al. Dias de Sousa et al. found no significant difference [19]. Four studies [19,34,39,52] investigated IL-12 in serum. Dias de Sousa et al. and Pasquali et al. found no significant difference [19,39]; Filion et al. found a significant increase from RRMS to SPMS, $p < 0.001$ [52]; and Kallaur et al. found a significant decrease, $p = 0.0003$ [34]. Four studies [19,33,34,52] investigated IL-1β. Three [33,34,52] found a significant increase between RRMS and SPMS. Kallaur et al. found a significant increase from RRMS to PMS in serum, $p = 0.017$ [34], and so did Filion et al. with RRMS + therapy and SPMS, $p < 0.05$, but they found no difference when participants with RRMS did not receive treatment [52]. Filion et al. did not mention if the SPMS group received treatment. RRMS + therapy all received treatment with IFN-β. Stein et al. investigated IL-1β in CSF and found a significant difference between RRMS (mean 8.2 (SD 7.3) pg/mL) and SPMS (mean 13.5 (SD 12.6) pg/mL), $p = 0.038$ [33].

3.6. Monocyte Chemoattractant Protein-1 (MCP-1/CCL2)

Three studies [20,31,38] investigated MCP-1 levels in serum and CSF. Two studies [20,31] found a significant difference. Herman et al. found a significant difference in CSF between RRMS (mean 399.8 (SD 145.3) pg/mL) and SPMS (mean 540.3 (SD 154.4) pg/mL), $p < 0.006$ [31]. Mañé-Martínez et al. found no significance in CSF between RRMS (median 370 [IQR 292–462] pg/mL) and SPMS (median 387 [IQR 264–452] pg/mL) [38]. As the only authors investigating MCP-1 in serum, Lamancová et al. found a significant difference between RRMS (mean 91.5 ± 27.4 pg/mL) and SPMS (mean 119.8 ± 23.5 pg/mL), $p < 0.01$ [20].

3.7. Tau-Protein

Three studies [38,44,49] investigated total tau (t-tau) levels, and two of these also investigated the phosphorylated form, p-tau, but found no significant difference. Only Jaworski et al. found a significant difference in t-tau with decreased CSF levels in RRMS compared to SPMS, $p = 0.01$. However, they could only detect t-tau in CSF in 64.6% of participants and rejected the others for further analysis [44].

3.8. Neurofilament Heavy Chain (NfH)

One study [49] investigated levels of NfH in CSF, and one study [41] in serum. Both studies found a significant increase. Teunissen et al. investigated NfH unphosphorylated in CSF and found a significant increase in SPMS compared to RRMS visualized by a boxplot, $p < 0.05$ [49]. Gresle et al. investigated NfH in a phosphorylated form in serum and found a significant difference between RRMS (median 0 [IQR 0–0.16] ng/mL) and SPMS (median 0.18 [IQR 0.04–0.63] ng/mL), $p = 0.048$ [41].

3.9. MMP-2

Two studies [20,48] investigated serum levels of MMP-2 and found a significant difference; however, this difference was inverted: Benešová et al. found an increase between RRMS (median 1377 [5–95% 1083–1828] ng/mL) and SPMS (median 1738 [5–95% 914–2848] ng/mL), $p < 0.002$ [48], and Lamancová et al. found a decrease from RRMS to SPMS, as visualized by a boxplot, $p < 0.001$ [20].

3.10. Galectin-9

Burman et al. and Herman et al. investigated CSF levels of Galectin-9. Both found a significant difference. The difference was found between RRMS (mean 315 ± 92.4 pg/mL) and SPMS (mean 400 ± 93 pg/mL), $p < 0.001$, and between RRMS (mean 293 (SD 66.4) pg/mL) and SPMS (mean 380 (SD 70.9) pg/mL), $p < 0.007$ [31,36].

3.11. N-Acetylaspartate (NAA)

Teunissen et al. and Jasperse et al. investigated CSF levels of NAA and found a significant decrease in SPMS compared to RRMS, $p < 0.05$ and $p = 0.015$ (both visualized by boxplot) [49,50].

3.12. CD86

Two studies [32,52] investigated expression of the surface marker CD86 in serum/plasma. Filion et al. found an increase in the level of CD86 from RRMS to SPMS. Nevertheless, it was only significant when participants did not receive therapy, $p < 0.05$ [52]. Iacobaeus et al. found a significant decrease in SPMS compared to RRMS, $p < 0.01$ [32].

3.13. Osteopontin

Two studies [43,51] investigated osteopontin in plasma. Both studies found a significant increase in SPMS compared to RRMS. Shimizu et al. found a significant difference between RRMS (mean 53.39 ± 4.54 ng/mL) and SPMS (mean 93.28 ± 19.76 ng/mL), $p < 0.05$ [43]. Comabella et al. found a significant difference between RRMS and SPMS, $p < 0.0005$ [51].

3.14. TIMP-1, MMP-9, and MMP-9/TIMP-1

No studies found significant differences between RRMS and SPMS.

4. Discussion

4.1. Neurofilament Light Chain (NfL)

NfL is correlated with age due to neuronal degeneration [11,56] and increases during an acute clinical relapse [57]. Therefore, it would be a confounder not to adjust for age and not to differentiate RRMS in relapse and remission [30,58].

Six studies [18,20,22,24,25,30] found a significant difference in serum. Ferraro et al. and Lamancová et al. did not adjust for age [20,24]. This lack of adjustment degrades their external validity. It is therefore unlikely that these results are reliable. Uphaus et al. did not exactly adjust for age but investigated the correlation of age and sNfL. Also, they conducted a 6-year follow up, during which they calculated the risk of conversion to SPMS when taking age into consideration. They concluded that patients transitioning to SPMS were more likely to show elevated sNFL levels at follow-up compared to baseline. This

indicates that longitudinal monitoring of sNfL levels might be helpful in shortening the time necessary to diagnose SPMS as early as possible [22]. Barro et al. and Högel et al. adjusted for age and found a significant difference [25,30]. Both studies used the SIMOA technology when detecting NfL, were rated as good or fair in terms of the risk of bias and defined their RRMS group as free of clinical relapses. This indicates that NfL in serum might be a potential biomarker in differentiating between RRMS and SPMS. Loonstra et al. was rated as good in terms of the risk of bias and found a significant difference between RRMS and SPMS in serum. They only included participants from the same year of birth and eliminated age as a confounder. They included both relapse (n = 7) and remission (n = 164) but did not find a significant difference in the level of NfL between the two subgroups [18]. Therefore, the results of the study are reliable and strengthen the potential of NfL being a good biomarker for differentiating between RRMS and SPMS.

A previous study showed a correlation between NfL in serum and CSF [56]; hence, it is striking that none of the included studies investigating NfL in CSF found a significant difference between RRMS and SPMS.

Högel et al. calculated a sensitivity of 54.55% and a specificity of 84.78%. This indicates that a value above the cut-off (31.39 pg/mL) is not diagnostic for SPMS. However, the relatively high specificity suggests that patients with a lower value than the cut-off are unlikely to have converted to SPMS. For accurate diagnosis, multiple samples may be needed to improve confidence, and longitudinal sampling over time is important to track changes in the biomarker levels and confirm the diagnosis.

When comparing the studies focusing on the method used to measure the biomarker, four out of five with a significant difference used SIMOA. However, five out of six that did not find a significant difference used ELISA. Interestingly, Lamancová et al. has remarkably high levels of NfL compared to the other studies. This is explained by a dilution ratio of 1:9.

Based on the results, it appears unnecessary to collect NfL in CSF rather than a blood sample, especially considering that lumbar puncture is a more invasive procedure.

4.2. Glial Fibrillary Protein (GFAP)

When investigating GFAP in CSF, none of the studies found a significant difference when adjusting for age. Two studies investigated GFAP in serum and found a significant difference [18,25]. However, Högel et al. had some potential risks of bias with a deficient method section, which reduced their internal validity. Loonstra et al. had low risks of bias and found a significant difference in serum.

In summary, GFAP in CSF may not be a potential biomarker, but it appears more promising in serum because both studies investigating GFAP in serum found a significant difference. The difference between the results from serum and CSF could also be due to the difference in the measurement method. The two studies that investigated GFAP in serum used SIMOA, while the two studies investigating GFAP in CSF used ELISA.

4.3. Chitinase-3-like Protein 1 (CHI3L1/YKL-40)

CHI3L1 is a secreted glycoprotein which is involved in inflammation, macrophage polarization, apoptosis and carcinogenesis. Results of YKL-40/CHI3L1 should be interpreted with caution when evaluating the studies because of several risks of bias concerning the validity.

Burman et al. showed a correlation between age and YKL-40 in CSF, $r = 0.68$, $p < 0.0001$. They found a significant difference between RRMS remission and RRMS relapse, which might indicate an increase during relapse [35]. This conflicts with knowledge from a meta-analysis suggesting that YKL-40 increases during remission [59]. Therefore, it is important to differentiate RRMS in relapse and remission when comparing with SPMS in future studies. Correale et al. found a significant difference in CSF. However, it was unclear if the study differentiated RRMS in remission and relapse. The study did not adjust by age but matched their participants. Correale et al. did not find a significant difference in serum. It is noteworthy that Correale et al. found a decreasing SPMS value compared to RRMS,

whereas other studies find an increase from RRMS to SPMS. One possible reason could be that the study included patients in relapse, which might have resulted in elevated values for the RRMS group. Lamancová et al. found a significant difference in serum but did not adjust for age, and it was unclear if they differentiated between remission and relapse [20]. Only Burman et al. adjusted for age and differentiated RRMS but did not find a significant difference [35]. In CSF, Talaat et al. found a significant difference between RRMS and PMS. They only included patients in remission but did not adjust for age [17].

Future studies should consider separating RRMS remission and relapse when investigating this biomarker.

4.4. IL-1β

IL-1β is a key mediator of the inflammatory response and is involved in the pathogenies of MS [60]. In addition, an acute clinical relapse could be a potential confounder, as well as receiving DMT. Filion et al. and Kallaur et al. found a significant difference in serum [34,52], and both had a low risk of bias. Filion et al. differentiated patients with RRMS receiving therapy (IFN-β) and RRMS not receiving therapy. There was a big difference when comparing these two groups. Dias de Sousa et al. did not find a difference between RRMS and SPMS in serum [19]. This study had a high potential risk of bias with a small sample size and did not differentiate between relapse and remission. Stein et al. found a significant difference as the only study investigating the biomarker in CSF. It was unclear which treatment the patients were receiving.

In summary, IL-1β does not seem to be a potential biomarker in serum, but there is a need for future studies with a larger sample size and with participants only in RRMS remission and SPMS. Furthermore, it is important to clarify which treatment the patients are receiving, as it could influence the results.

4.5. N-Acetylaspartate (NAA)

NAA is an amino acid synthesized in neurons. Both studies investigating NAA found a significant difference between RRMS and SPMS [49,50]. The studies had an older date, and notably no studies have investigated NAA since. This could indicate publication bias or a deficient search strategy in the present review. Nevertheless, both studies were rated as fair with few limitations; Jasperse et al. had a small sample size, and Teunissen et al. did not have available patient characteristics, which made it difficult to assess if the population was representative.

4.6. Galectin-9

Galectin-9 is a protein involved in immune regulation and tumor pathogenesis. Burman et al. and Herman et al. investigated Galectin-9 and found a significant increase from RRMS to SPMS. Both studies found that treatment had no impact on the concentration of Galectin-9 [31,36]. Both were ranked as fair or good for risk of bias; hence, Galectin-9 might be a potential biomarker for detecting SPMS. Nevertheless, none of the studies investigated the impact of having a relapse on the concentration of Galectin-9. Both included participants with recent relapses, which could be a potential confounder.

4.7. Neurofilament Heavy Chain (NfH)

Both studies investigating NfH found a significant difference between RRMS and SPMS. Gresle et al. suggested NfH as a fluctuating biomarker that should be repeated regularly [41]. Hence, it is not sufficient for cross-sectional samplings and not suitable to aid the clinician in detecting SPMS. Therefore, the difference between RRMS and SPMS might not be clinically significant even though all the studies found a statistically significant difference. The quality of the studies was doubtful, and the studies were of an older date.

4.8. Monocyte Chemoattractant Protein-1 (MCP-1/CCL2)

MCP-1 is a chemokine which is a key mediator promoting inflammation. Overall, studies found an increase from RRMS to SPMS. Lamancová et al. was the only study to investigate MCP-1 in serum [20] but had a high risks of bias, including a small sample size without any control group and insufficient time to follow up. The biomarker was more promising in studies investigating CSF. Herman et al. found a strong significant difference [31] and was rated as fair/good. Mañe-Martinez et al. found a strong significant decrease in RRMS relapse compared to RRMS remission, $p < 0.0001$ [38]. A decrease in MCP-1 might be an expression of a recent or an acute relapse. Hence, this is striking, as MCP-1 is known to be proinflammatory and was expected to increase during relapse. Herman et al. included participants with relapses [31], which could potentially be a confounder in detecting conversion to SPMS.

4.9. IFN-γ

Three studies found a significant difference while three other studies did not. Two of the studies which found a significant difference [34,39] were rated as good and only included participants in remission. However, the difference was inverted, which currently makes IFN-γ unsuitable to detect SPMS from RRMS.

4.10. Osteopontin

Osteopontin is a protein with functions in cell-mediated immunity, inflammation, tissue repair, and cell survival. Two studies investigated osteopontin in plasma and found a significant increase from RRMS to SPMS [43,51]. However, as both studies are of older dates, it is questionable why no recent studies have reinvestigated it, which may be due to publication bias. Shimizu et al. found a significant difference in osteopontin between remission and relapse. They did not adjust for this confounder, which could have had an impact on the results. Comabella et al. also mentioned an increase in osteopontin during a relapse [51]. This may indicate that osteopontin in serum has the potential to differentiate between remission and relapse.

4.11. Main Findings and Perspectives

In summary, we investigated 28 different biomarkers. GFAP, NfL, and CHI3L1 are well-researched. It was surprising that none of the included studies investigating NfL found a significant difference in CSF. This could be explained by the different metabolic processes in CSF and serum or by the influence of the blood–brain barrier. It might be more effective and less invasive to investigate NfL in serum instead. There was no clear direction for GFAP and CHI3L1, largely due to the poor quality of the studies. These biomarkers should be further investigated in longitudinal studies with larger sample sizes. Future studies should consider relapse and age as confounders and clearly define inclusion criteria for the SPMS subgroup. Furthermore, future studies should consider using SIMOA instead of ELISA when analyzing NfL, as there is a trend towards studies using SIMOA finding significant differences.

The focus of this review was placed on *p*-values as a measure to assess the ability of the biomarker to differentiate between RRMS and SPMS. Despite this, a low *p*-value does not indicate if the biomarker is clinically significant. Few studies [22,25] calculated sensitivity, specificity, and AUC to evaluate the performance of the biomarker in differentiating between RRMS and SPMS. It might be relevant to include AUC and cut-off values in future studies, as this could assist clinicians in better determining whether a patient with RRMS has converted to SPMS.

Over the past years, there has been major attention to finding "the golden biomarker" for guidance in the diagnosis of SPMS. A recent study investigated a new promising biomarker—macrophage migration inhibitory factor (MIF)—and found a significant difference between RRMS and SPMS, $p < 0.001$ [61]. It could be interesting to investigate this biomarker further.

Some studies combined biomarkers in the search for a biomarker that could differentiate between RRMS and SPMS. For example, Huss et al. (which was excluded) calculated a Glia Score involving GFAP, CHI3L1, and NfL and found a strong significant difference between RRMS and PMS, $p < 0.0001$ [62]. Högel et al. investigated an increased predictive power when combining both GFAP and NfL in serum, AUC = 0.814 [25]. Further investigations of combinations of biomarkers could be interesting, as there might not be one specific "golden biomarker" but combinations thereof that could aid the clinician in detecting SPMS.

Galectin-9, osteopontin, and MCP-1 were less investigated biomarkers, but the results are promising, and it may therefore be interesting to further investigate or combine these biomarkers.

4.12. Strengths and Limitations

The strengths of this review are the systematic and reproducible approach. It was registered in PROSPERO to prevent publication bias. The search string provided a wide study selection, generating a high sensitivity for the search strategy. However, this led to a significant variation and heterogeneity between the included studies, which may be a limitation. Specifically, the variation in the number of participants made it challenging to compare the different studies and their outcomes. Additionally, both median and mean values were used. Some studies likely used median values due to a few outliers. They could have chosen to remove these outliers for better comparability. No studies were excluded based on study design, which resulted in inclusion of both case–control, cross-sectional, and cohort studies, which made it challenging to compare the studies but also led us to explore the entire field.

A second limitation was the inclusion of studies only published after 2002. As a result, it is possible that relevant studies were excluded. However, we expected that any promising biomarkers were investigated again in more recent studies.

A third limitation was the exclusion of any study that was the only one investigating a specific biomarker. Potentially, this could have caused important information about certain biomarkers to be missed. However, this was also assessed as a strength as it might improve the generalizability.

A fourth limitation was the omission of metabolic biomarkers. This decision was made because inflammatory and neurodegenerative biomarkers are better established and due to the fact that there are certain methodological issues to studying the metabolomics of MS as well as in the implementation into daily clinical practice. For further information, please refer to Zahoor et al. [63].

5. Conclusions

To our knowledge, no other review has systematically investigated such a wide variety of biomarkers in both CSF and serum to detect conversion from RRMS to SPMS. Although this review was unable to identify a specific biomarker, it provides insights into potential directions for future studies to investigate biomarkers as a diagnostic tool for future guidelines in the diagnosis of SPMS.

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