REVIEW

Molecular Biomarkers for Spinal Muscular Atrophy

A Systematic Review

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Abstract

Background

There is an unmet need for reliable biomarkers to predict disease severity, prognosis, and treatment effect in patients with spinal muscular atrophy (SMA). The purpose of this review is to evaluate the clinical utility of blood-based biomarkers in patients with SMA.

Methods

A systematic review of MEDLINE, DARE, PEDro, PsycINFO, Cochrane Database, LILACS, OTSeeker, SpeechBITE, CINAHL, Scopus, Science Direct, clinicaltrial.gov, OpenGrey, and Google Scholar was performed with the last search data of June 30, 2019.

Results

Survival motor neuron (SMN)-related biomarkers showed an important interpatient and cell variability with a wide overlap between SMA phenotypes and healthy controls. Several plasma protein analytes correlated with motor scores; however, validation studies are needed to rule out false positives. DNA methylation analysis distinguished between patients with mild/moderate SMA and healthy controls. Plasma phosphorylated neurofilament heavy chain (pNF-H) levels increased with disease severity and declined considerably after nusinersen treatment.

Conclusion

There is no sufficient evidence to support the clinical utility of SMN-related biomarkers to predict disease severity in SMA. pNF-H appears to be a promising biomarker of disease activity and treatment effect in SMA. Further studies should include longitudinal assessments of patients with SMA across functional groups and comparisons with age-matched healthy controls to evaluate the stability of putative biomarkers over time and in response to SMA therapeutics. PROSPERO registration: CRD42019139050.

Spinal muscular atrophy (SMA) is a rare, autosomal recessive neurodegenerative disease with an incidence ranging from 4 to 10 per 100,000 live births,[1] across clinical subtypes I–IV.[2] SMA is caused by deletion or mutation of the survival motor neuron 1 gene on chromosome 5 (5q11-13),[2] resulting in reduced expression of full-length SMN protein.[2,3] Depletion of the SMN protein in alpha motor neurons results in neuronal degeneration and thus various degrees of paresis of proximal muscles.[4,5]

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Current therapeutic alternatives for patients with SMA aim to increase the amount of full-length SMN protein levels either by replacing *SMN1* through gene therapy[6,7] or by *SMN2* splicing modulators such as nusinersen (Spinraza™),[8] risdiplam,[9] and branaplam.[10] Given that new treatments are under development,[11] there is a critical need for reliable biomarkers to predict disease severity, prognosis, and treatment effect.

Several biomarkers have been proposed for SMA, including molecular,[12,13] physiologic,[14,15] structural (imaging modalities),[16] and clinical biomarkers[17]; however; no agreement has been made on the most reliable biomarkers. Physiologic, structural, and clinical biomarkers, although valuable, they can be limited by assessor bias, intra- and interrater variability, poor sensitivity, and dependency on patients' collaboration. The most reproducible, quantitative, unbiased, and minimally invasive method to characterize SMA disease state would be a blood-based biomarker. The purpose of this systematic review was to investigate the clinical utility of molecular biomarkers as indicators for disease severity, treatment effect, or predicting prognosis for patients with SMA.

Methods

This systematic review was conducted following "The Cochrane Handbook for Systematic Reviews of Interventions Guidelines"[18] and the guidelines of the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses."[19] The protocol was registered in the International prospective register of systematic reviews (PROSPERO) database (CRD42019139050), University of York, and is available at: crd.york.ac.uk/PROSPERO/.

Literature Search

A systematic review of MEDLINE, DARE, PEDro, PsycINFO, Cochrane Database, LILACS, OTSeeker, SpeechBITE, CINAHL, Scopus, Science Direct, clinicaltrial.gov, OpenGrey, and Google Scholar was performed with the last search data of June 30, 2019. The search strategy included the following keywords: "spinal muscular atrophy," and "muscular atrophies." These terms were combined with "biomarker*." Potentially eligible articles were screened by 2 independent reviewers. A third experienced reviewer resolved any disagreements.

Study Selection Criteria

Studies included satisfied all the following criteria: (1) interventional clinical trials as well as observational, longitudinal, or cross-sectional original articles in English or Spanish, (2) SMA genetically confirmed following diagnostic criteria defined by the SMA Consortium (i.e., a homozygous deletion of the *SMN1* gene or a hemizygous deletion with an additional pathogenic point mutation in the second *SMN1* allele),[20] and (3) studies compared serum markers in patients with SMA vs healthy controls. Exclusion criteria were the following: (1) the number of participants enrolled was less than 10 per group, (2) no healthy control comparator, (3) missing demographic data about SMA or healthy control group, (4) nonquantitative methods to assess molecular biomarker concentrations, (5) enrolled participants with inflammatory or neurologic comorbidities that may affect biomarker concentration, (6) animal studies, and (7) abstracts, reviews, and posters.

Data Extraction

The following information was extracted: (1) characteristic of studies: molecular candidate, biomarker type, number of SMA participants vs healthy controls, sex, age range, research design, and molecular technique (table 1) and (2) results: outcomes measures and correlation among putative biomarkers and between biomarkers and clinical motor scales (tables 2 and 3).

Quality Assessment

The quality of each selected article was assessed by the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2).[21] This tool consists of 4 key domains assessing the risk of bias for patient selection, index test, reference standard, and flow and timing of index tests.[21] Quality assessment is summarized in figure 1.

Data Availability

Data used in this systematic review are the data reported by each selected article. We do not have additional data not published within each article.

Results

Study Selection

Through database searching, 430 abstracts were found to have the relevant keywords. After duplicates were removed, abstracts were assessed by 2 independent reviewers, and 23 articles were selected for full-text review. Finally, 10 articles fulfilling the inclusion/exclusion criteria were selected for qualitative analysis (figure 2).

Study Characteristics

Based on the type of molecular biomarkers, 7 studies included SMN-related biomarkers: *SMN2* copy number,[22–24] SMN transcript levels,[12,22–27] and SMN protein expression,[12,22,23,25,27] and 3 studies included non–SMN-related biomarkers: DNA methylation profiling,[28] proteomic, metabolomic, and transcriptomic discovery platforms,[29] and plasma phosphorylated neurofilament heavy chain (pNF-H)[30] (table 1).

Because of the heterogeneity of putative biomarkers, molecular laboratory essays, and housekeeping genes for normalization in SMN transcript analysis, a meta-analysis was not conducted. Tables 2 and 3 present a summary of the outcome measures per each type of biomarker and the correspondent correlation with motor outcomes.

Table 1 Characteristics of Eligible Studies (n = 10)

Reference (y)	Biomarkers	Biomarker type	No. of cases/ controls	Sex (M/F)	Range age	Research design	Molecular technique	Blood collection time points
Crawford et al.[22] (2012)	SMN copy number SMN2–full length *SMN-Δ7* SMN protein	Disease severity biomarker	Participants with SMA = 108 (type I: 17, type II: 49, and type III: 42) HC = 22	SMA (52/56) HC (12/10)	2–12 y	Cross-sectional, single-visit, multicenter design	SMN copy number = real-time TaqMan PCR (qPCR). SMN2-FL, SMN1-FL, and SMN-Δ7 transcripts levels = real-time PCR. SMN protein = ELISA.	Once
Czech et al.[23] (2015)	SMN copy number SMN mRNA SMN protein	Disease severity biomarker	SMA = 36 (type I: 7, type II: 14, and type III: 15) HC = 96	SMA (18/18) HC = NR	SMA = 0.5–61 y HC = 18–60 y	Cross-sectional, multisite	SMN copy number = digital PCR (Bio-Rad Laboratories). SMN mRNA assay = Roche multiplex qRT-PCR. SMN protein levels = Roche SMN-ECL immunoassay	Once
Darras et al.[30] (2019)	Plasma pNF-H	Prognostic and pharmacodynamic biomarker	Infantile-onset SMA = 121 HC = 34	Infantile-onset SMA = (54/67) HC = (14/20)	SMA = 1–20 w HC = 7 w–18 y	Longitudinal study reporting the pNF-H levels of participants enrolled in the ENDEAR trial and compare it with HV.	pNF-H enzyme-linked lectin assay from ProteinSimple® platform	Baseline measurement
Finkel et al.[29] (2012)	Proteomic, metabolomic, and transcriptomic discovery platforms for non-SMA biomarkers	Disease severity biomarker	SMA = 108 (type I: 17, type II: 49, and type III: 42) HC = 22	SMA (56/52) HC (10/12)	SMA = 2–12 y HC = 2–12 y	Cross-sectional, multicenter	Proteomics: multidimensional liquid chromatography. Transcriptomics: RNA was isolated using the Ambion RiboPureTM blood kit (Austin, TX). Metabolomics profiling was conducted on organic extracts of plasma samples using multiple analytical platforms (LC/MS profiling on a QStar Elite quadrupole time-of-flight instrument, multiple reaction monitoring, and free fatty acids analysis by gas chromatography/mass spectrometry)	Once
Kolb et al.[25] (2015)	SMN protein total SMN mRNA Plasma protein analytes	Disease severity biomarker	SMA type I = 26 HC = 27	SMA (11/15) HC (13/14)	Mean (SD) SMA = 3.7 (1.7) mo HC = 3.3 (2.0) mo	Prospective, longitudinal natural history study	SMN mRNA analysis was performed using ddPCR (Bio-Rad Laboratories, Hercules, CA). SMN expression was normalized to HPRT expression using prime PCR ddPCR expression probe assay for intron-spanning human HPRT1 with HEX assay (dHsaCPE5192872, Bio-Rad). SMN protein was measured at PharmOptima (Portage, MI) using the company's proprietary electrochemiluminescence immunoassay based on the Meso Scale Discovery technology. Serum protein analytes (n = 25) were analyzed by MAP analysis.	Baseline
Sumner et al.[12] (2006)	SMN mRNA SMN protein in peripheral blood	Disease severity biomarker	SMA = 29 (type I: 6, type II: 9, and type III: 14) Carriers = 29	SMN (14/15) carriers (13/16) HC (11/16)	SMA type I = 4–53 mo SMA type II: 2–47 y	Cross-sectional design	SMN mRNA: quantitative reverse transcriptase PCR SMN protein: cell immunoassay. SMN copy number: PCR restriction	Once

Continued

Table 1 Characteristics of Eligible Studies (n = 10) *(continued)*

Reference (y)	Biomarkers	Biomarker type	No. of cases/ controls	Sex (M/F)	Range age	Research design	Molecular technique	Blood collection time points
			HC = 28		SMA type III = 2.5–56 y Carriers = 6–78 y HC = 8–55 y		fragment length polymorphism assay	
Tiziano et al.[26] (2010)	SMN mRNA in leukocytes	Disease severity biomarker	SMA = 51 (type I: 2, type II: 16, and type III: 33) Carriers = 23 HC = 28	SMA (25/26) carriers (9/14) HC (14/14)	SMA type I = 0.5–1.5 y SMA type II = 3–33 y SMA type III = 2–68 y Carriers = 18–73 y HC = 19–45 y	Cross-sectional design	Absolute real-time PCR	Once
Vezain et al.[24] (2007)	SMN mRNA in whole blood and muscle	Disease severity biomarker	SMA = 48 (type I: 14, type II: 22, and type III: 12) HC = 75	SMA (21/27) HV = NR	SMA type I = 1 mo–19 y SMA type II = 15 mo–32 y SMA type III = 6–47 y HV = NR	Cross-sectional	Multiplex fluorescent RT-PCR	Once
Wadman et al.[27] (2016)	SMN protein and SMN mRNA (SMN1, SMN2–full length [SMN2-FL], and SMN2-Δ7) in fibroblasts and PBMCs	Disease severity biomarker	PBMC study SMA = 135 (type I: 18, type II: 60, type III: 52, and type IV: 5) HC = 229 Fibroblast study SMA = 40 (type I: 5, type II: 19, type III: 10, and type IV: 6) HC = 47	PBMC study SMA (62/73) HV (114/115) Fibroblast study SMA (18/22) HC (21/26)	PBMC study SMA type I = 0.3–49.7 y SMA type II = 1–66.7 y SMA type III = 2.4–75 y SMA type IV = 41–68 y HC = 0.3–86 y Fibroblast study SMA type I = 0.4–42.2 y SMA type II = 1–66.7 y SMA type III = 6–61.9 y SMA type IV = 14–54.7 y	Cross-sectional	SMN copy number: MLPA analysis SMN transcripts: qPCR and ELISA	Once
Zheleznyakova et al.[28] (2013)	DNA methylation profiling from leukocytes	Disease severity biomarker	SMA = 12 (type I: 3, type II: 3, type III: 5, and type IV: 1) HC = 11	SMA (12/0) HC (11/0)	SMA = 5 mo–21 y HC = 1–20 y	Cross-sectional	Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA, USA)	Once

Abbreviations: ddPCR = droplet digital PCR; ECL = enhanced chemiluminescence; ENDEAR = A Study to Assess the Efficacy and Safety of Nusinersen in Infants With Spinal Muscular Atrophy; HC = healthy controls; HEX = hexosaminidase; HPRT = hypoxanthine phosphoribosyltransferase; HV = healthy volunteers; LC/MS = Liquid chromatography–mass spectrometry; MAP = model-based analysis of proteomic data; MLPA = multiplex ligation-dependent probe amplification; mRNA = messenger RNA; NR = not reported; PBMC = peripheral blood mononuclear cell; pNF-H = phosphorylated neurofilament heavy chain; qPCR = quantitative polymerase chain reaction; qRT-PCR = quantitative real-time PCR; RT-PCR = reverse transcription PCR; SMA = spinal muscular atrophy; SMN = survival motor neuron; SMN1-FL = SMN 1 full length; *SMN-Δ7* = SMN transcript lacking exon 7.

Table 2 Results of SMN-Related Biomarkers

Reference (y)	SMN2 copy number SMA vs controls mean (SD)	SMN2 copy number vs motor scores	SMN transcripts SMA and controls mean (SD)	SMN transcripts vs SMN2 copy number	SMN transcripts vs SMN protein levels	SMN transcripts vs motor scores	SMN protein SMA and controls	SMN protein vs SMN copy number	SMN protein vs motor scores
Crawford et al.[22] (2012)	Type I: 2.5 (0.9) Type II: 3.0 (0.7) Type III: 3.5 (0.8) HC: 1.7 (1.1). $p < 0.001$	No correlation (data NR)	SMN2-FL Type I: 38.3 (16.3), type II: 49.0 (18.6), type III: 58.1 (20.0), HV: 51.5 (22.3). Type I vs type II ($p = 0.031$), type I vs type III ($p < 0.001$), type II vs type III ($p = 0.024$). Type III vs HC (p value NR) SMN-Δ7 Type I: 140.6 (69.8), type II: 202.6 (73.0), type III: 200.4 (97.5) and HC: 115.2 (64.2). p value NR	No correlation (data NR)	Total SMN-FL vs SMN protein in SMA + HC ($r = 0.26$, $p = 0.021$)	SMN2-FL vs MHFMS Type II + III ($r = 0.34$, $p = 0.009$) SMN-Δ7 vs MHFMS: type III ($r = 0.60$, $p = 0.0001$)	Type I: 10.1 (4.8) pg/10^6 cell Type II: 14.0 (7.3) pg/10^6 cells Type III: 14.7 (10.0) pg/10^6 cells HC: 25.2 (19.6) pg/10^6 cells (p value NR)	All participants with SMA ($r = 0.33$, $p = 0.001$) Participants with type II SMA ($r = 0.41$, $p = 0.008$)	No correlation (data NR)
Czech et al.[23] (2015)	Type I: 2 Type II: 3.1 Type III: 3.5 HC: NR (p value NR)	N/A	SMN2-FL Overlap among SMA types (data NR) HV = 0.128, type 1 SMA = 0.66 (p value NR)	In controls, SMN2-FL 1 SMN2 copy = 0.069 2 SMN2 copies = 0.128 2 SMN1 copies = 0.31 3 SMN1 copies = 0.44 4 copies = 0.48 In SMA, SMN2-FL 2 SMN2 copies = 0.66 3 SMN2 copies = 0.82 4 SMN2 copies = 0.90 (p value NR)	SMN2-FL vs SMN protein type 1 ($R^2 = 0.93$, $p = 0.003$) Type 2: ($R^2 = 0.62$, $p = 0.02$) Type 3: ($R^2 = 0.23$, $p = 0.4$)	N/A	Data NR	No correlation (data NR)	N/A
Kolb et al.[25] (2015)	N/A	N/A	Total SMN/HPRT SMA vs HC 0.50 (0.14) vs 1.27 (0.44) ($p < 0.0001$).	N/A	Total SMN-FL vs SMN protein in either cohort ($r = -0.0184$, n = 31, $p = 0.9217$)	TIMPSI score vs SMN mRNA in HV ($r = 0.244$, n = 19, $p = 0.315$) CHOP-INTEND vs SMN in HV ($r = 0.856$, n = 7, $p = 0.014$) TIMPSI score vs SMN mRNA in SMA ($r = 0.147$, n = 19, $p = 0.547$) CHOP-INTEND vs SMN in SMA ($r = 0.158$, n = 16, $p = 0.556$)	SMA = 6,601.7 (3,592.8) pg/10^7 PBMCs HC = 8,967.8 (5441.3) pg/10^7 PBMCs, ($p = 0.12$). Data as mean (SD)	N/A	TIMPSI score vs SMN protein in HV ($r = -0.101$, $p = 0.664$) CHOP-INTEND vs SMN protein in HV ($r = -0.245$, $p = 0.559$) TIMPSI score vs SMN protein in SMA: ($r = 0.360$, $p = 0.141$) CHOP-INTEND vs SMN protein in SMA ($r = 0.176$, $p = 0.546$)
Sumner et al.[12] (2006)	N/A	N/A	SMN + 7 in SMA types, carriers, and HC ($F_{4,80} = 5.25$, $p = 0.001$) (data NR)	SMN1 and SMN2 copy number determine the expression of SMN + 7 (data NR).	N/A	N/A	SMA types, carriers, and HC ($F_{4,52} = 11.89$, $p < 0.001$) SMA types except type I,	SMN1 and SMN2 copy number determine the expression of	N/A

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Table 2 Results of SMN-Related Biomarkers *(continued)*

Reference (y)	SMN2 copy number SMA vs controls mean (SD)	SMN2 copy number vs motor scores	SMN transcripts SMA and controls mean (SD)	SMN transcripts vs SMN2 copy number	SMN transcripts vs SMN protein levels	SMN transcripts vs motor scores	SMN protein SMA and controls	SMN protein vs SMN copy number	SMN protein vs motor scores
			SMNΔ7 in SMA types, carriers, and HC ($F_{4,80}$ = 8.31, p < 0.001) (data NR)	SMN1 copy number has no effect on SMNΔ7 expression, but SMN2 copy number has a strong effect (data NR).			carriers, and HC ($F_{3,50}$ = 1.11, p = 0.36)	SMN protein (data NR).	
Tiziano et al.[26] (2010)	N/A	N/A	SMN2-fl SMA vs controls 67.33 ± 29.36 vs 41.65 ± 25.62 mol/ng of total RNA (p = 4.2 × 10^{-5}) SMN2-FL SMA type II vs controls 52.50 ± 24.78 vs 41.65 ± 25.62 mol/ng of total RNA (p = 2.22 × 10^{-5}) SMN2-FL SMA type III vs controls 73.67 ± 29.68 vs 41.65 ± 25.62 mol/ng of total RNA (p = 0.0042) SMN2-FL type II vs type III 52.50 ± 24.78 vs 73.67 ± 29.68 (p = 0.0034)	No correlation between SMN2-FL and SMN copy number. Data NR (p = 0.52).	N/A	HFMS vs SMN2-Fl in <12 years SMA type II (β = 0.64, p = 0.04)	N/A	N/A	N/A
Vezain et al.[24] (2007)	Type I: 2.5 Type II: 3 Type III: 3 HC: NR (p value NR)	N/A	SMN-FL in participants with SMA with 2 SMN2 vs 4 SMN2 copies (0.157 ± 0.057 vs 0.311 ± 0.035, p < 0.017) SMNΔ7 in participants with SMA with 2 SMN2 vs 4 SMN2 (0.159 ± 0.063 vs 0.361 ± 0.062, p < 0.0017) SMN-FL in controls with 1 SMN2 vs 2 SMN2 copies (0.391 ± 0.038 vs 0.451 ± 0.050, p < 0.0017) SMNΔ7 in participants with SMA with 1 SMN2 vs 2 SMN2 (0.084 ± 0.011 vs 0.160 ± 0.021, p < 0.0017) SMN-FL in participants with type I vs type II vs type III SMA (0.168 ± 0.071 vs 0.236 ± 0.041 vs 0.281 ± 0.045, p < 0.017)	Positive correlation SMN-FL in patients with SMA 2 copies: 0.157 ± 0.057 3 copies: 0.241 ± 0.040 4 copies: 0.311 ± 0.035 Controls 0 copy: 0.287 ± 0.092 1 copy: 0.391 ± 0.038 2 copies: 0.451 ± 0.050 Positive correlation SMNΔ7 in patients with SMA 2 copies: 0.159 ± 0.063 3 copies: 0.279 ± 0.041 4 copies: 0.361 ± 0.062 Controls 0 copy: 0.000 ± 0.000 1 copy: 0.084 ± 0.011 2 copies: 0.160 ± 0.021	N/A	N/A	N/A	N/A	N/A

Continued

Table 2 Results of SMN-Related Biomarkers *(continued)*

Reference (y)	SMN2 copy number SMA vs controls mean (SD)	SMN2 copy number vs motor scores	SMN transcripts SMA and controls mean (SD)	SMN transcripts vs SMN2 copy number	SMN transcripts vs SMN protein levels	SMN transcripts vs motor scores	SMN protein SMA and controls	SMN protein vs SMN copy number	SMN protein vs motor scores
			SMNΔ7 in participants with type I vs type II vs type III SMA (0.190 ± 0.100 vs 0.272 ± 0.039 vs 0.313 ± 0.066, $p < 0.0017$) SMN-FL in blood vs muscle in participants with SMA with 3 SMN2 copies (0.47 ± 0.11 vs 0.80 ± 0.18; $p < 0.005$)						
Wadman et al.[27] (2016)	N/A	N/A	SMN 1 in PBMCs vs fibroblasts in patients with SMA 0.4 ± 4.3 vs 1.1 ± 6.5 SMN2Δ7 in PBMCs vs fibroblasts in patients with SMA 1,666 ± 1,000 vs 1,745 ± 688 ($p = 0.6$) SMN2-FL PBMCs vs fibroblasts in patients with SMA 219 ± 158 vs 231 ± 78 ($p = 0.7$)	SMN2-FL vs SMN2 copy number in PBMCs No correlation ($p = 0.7$) data NR SMN2Δ7 vs SMN2 copy number in PBMCs No correlation ($p = 0.3$) SMN2-FL vs SMN2 copy number in fibroblasts No correlation ($p = 0.3$) data NR SMN2Δ7 vs SMN2 copy number in fibroblasts No correlation ($p = 0.09$)	SMN mRNA vs SMN protein No correlation ($p = 0.6$)	SMN2-FL vs MRC in PBMC No correlation ($p = 0.5$) data NR SMN2-FL vs HFMSE in PBMC No correlation ($p = 0.7$) data NR SMN2-FL vs MRC in fibroblasts No correlation ($p = 0.8$) data NR SMN2-FL vs HFMSE in fibroblasts No correlation ($p = 0.3$) data NR	PBMCs SMA vs HV (3.7 ± 2.4 vs 5.3 ± 3.6 ng/1 g total protein) ($p < 0.01$) Fibroblasts SMA vs HV: (8.8 ± 4.3 vs 13.4 ± 5.6 ng/1 g total protein) ($p < 0.01$)	SMN2 copy number vs SMN protein in fibroblasts No correlation ($p = 0.01$) SMN2 copy number vs SMN protein in PBMCs Positive correlation ($p = 0.06$)	SMN protein vs HFMSE in PBMCs No correlation ($p = 0.15$) SMN protein vs MRC in PBMCs No correlation ($p = 0.6$) SMN protein vs HFMSE in fibroblasts Positive correlation ($p = 0.004$) SMN protein vs MRC in fibroblasts Positive correlation ($p = 0.04$)

Abbreviations: CHOP-INTEND = The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; HC = healthy controls; HFMS = Hammersmith Functional Motor Scale; HFMSE = HFMS expanded; HPRT = hypoxanthine phosphoribosyltransferase; HV = healthy volunteers; MHFMS = Modified Hammersmith Functional Motor Scale; MRC = Medical Research Council; mRNA = messenger RNA; N/A = not applicable; NR = not reported; PBMC = peripheral blood mononuclear cell; SMA = spinal muscular atrophy; SMN = survival motor neuron; SMN-FL = SMN full length; *SMN-Δ7* = SMN transcript lacking exon 7; TIMPSI = Test of Infant Motor Performance Screening Items.

SMN-Related Biomarkers

SMN2 Copy Number as Biomarker for Disease Severity

Three studies measured *SMN2* copy number as a biomarker for disease severity.[22–24] Czech found that SMA phenotype was more related to the copy number than to SMN2 transcripts expression,[23] but statistical comparisons were not provided. Vezain reported that SMA phenotypic groups are heterogeneous regarding *SMN2* copy numbers,[24] and finally Crawford reported that *SMN2* copy number was considerably lower in healthy controls ($p < 0.001$)[22] and that *SMN2* copy number increased proportionally to SMA severity.[22] SMA types I and II most commonly had 2 and 3 *SMN2* copies, respectively, whereas type III had 3 or 4 *SMN2* copies[22] However, there were type I participants with high *SMN2* copy number and type III SMA participants with a low *SMN2* copy number, and thus, *SMN2* copy number does not predict disease severity.

SMN mRNA Levels as Biomarkers for Disease Severity

Two specific transcripts were proposed as putative biomarkers of disease severity: *SMN2*–full length (*SMN2-FL*)[12,22–27] and SMN transcript lacking exon 7 (*SMN-Δ7*).[12,22,27]

SMN2–*Full Length*

SMN2-FL transcript levels as a biomarker for disease severity showed conflicted results. Although 2 studies reported that

Table 3 Results of Non–SMN-Related Studies

Reference (y)	Biomarker	SMA vs controls mean (SD)	Biomarker vs motor score
Kolb et al.[25] (2015)	25 plasma protein analytes	Cadherin-13: 6.83 (3.18) vs 9.72 (4.39) ng/mL (p = 0.0277) Cartilage oligomeric matrix protein: 388.4 (221.2) vs 617.5 (177.7) ng/mL (p = 0.0011) Insulin-like growth factor-binding protein: 6 116.3 (48.25) vs 153.9 (40.92) ng/mL (p = 0.0135) Peptidase D: 9.39 (2.34) vs 11.15 (2.24) ug/mL (p = 0.0236) Tetranectin: 7.39 (1.62) vs 9.06 (3.20) ug/mL (p = 0.0493) Myoglobin: 32.71 (30.17) vs 14.46 (7.78) ng/mL (p = 0.0220) YKL-40: 10.11 (3.96) vs 7.58 (2.85) ng/mL (p = 0.0288)	SMA cohort: AXL receptor tyrosine kinase vs TIMPSI (r = 0.586, n = 18, p = 0.0107) Cartilage oligomeric matrix protein vs TIMPSI (r = 0.834, n = 18, p < 0.0001) Dipeptidyl peptidase IV vs TIMPSI (r = 0.603, n = 18, p = 0.0081) Endoglin vs TIMPSI (r = 0.535, n = 18, p = 0.0223) HER2 vs TIMPSI (r = 0.544, n = 18, p = 0.0196) Insulin-like growth factor-binding protein 6 vs TIMPSI (0.580, n = 18, 0.0117) PEPD vs TIMPSI (r = 0.6037, n = 18, p = 0.0080) Thrombospondin-4 vs TIMPSI (r = 0.615, n = 18, p = 0.0066) Tetranectin vs TIMPSI (r = 0.669, n = 18, p = 0.0024) C-reactive protein vs CHOP-INTEND (r = 0.776, n = 15, 0.0007) C-reactive protein vs TIMPSI (r = 0.288, n = 18, p = 0.2457)
Finkel et al.[29] (2012)	Proteomic, metabolomic, and transcriptomic discovery platforms for non-SMA biomarkers	Plasma proteomics: among the top-ranking candidate markers were plasma proteins (CILP2, TNXB, COMP, ADAMTSL4, and CLEC3B) that distinguished participants with type II from subjects with type III, subjects with type III from healthy controls, and subjects with type I SMA from each of the other groups. Raw data not presented.	A total of 200 candidate biomarkers correlate with MHFMS scores: 97 plasma proteins, 59 plasma metabolites (9 amino acids, 10 free fatty acids, 12 lipids and 28 GC/MS metabolites), and 44 urine metabolites. No transcripts correlated with the MHFMS.
Darras et al.[30] (2019)	Plasma pNF-H	Healthy controls: 167.0 (BLQ–7,030) pg/mL vs SMA: 15,400 (2,390–50,100) (p < 0.0001) Nusinersen arm: 15,200 (2,390–37,300) vs sham: 17,150 pg/mL (4,900–50,100) (p = 0.2828) Nusinersen arm vs sham at d 64: 4,212 ± 363.55 vs 13,721 ± 1,473 pg/mL (p < 0.0001) Nusinersen arm vs sham at d 302: 1,465.5 (41–5,180) vs 5,664.7 (1,100–10,600) pg/mL	pNF levels vs CHOP-INTEND score (r = -0.30; 0.0012; n = 117) pNF levels vs HINE-2 motor milestone score: no correlation (data not shown)
Zheleznyakova et al.[28] (2013)	DNA methylation profiling from leukocytes	Significant differences in the methylation level between patients with SMA and healthy controls in CpG sites close to the genes CHML (p < 0.001), ARHGAP22 (p < 0.001), CYTSB (p < 0.05), CDK2AP1 (p < 0.001), LIAS (p < 0.001), cg05712748 (p < 0.001), cg02397061 (p < 0.05), C13 rf16 (p < 0.05), cg12738248 (p < 0.001), and SLC23A2 (p < 0.001).	N/A

Abbreviations: CHOP-INTEND = Children Hospital of Philadelphia Infant Test of Neuromuscular Disorders; HINE-2 = Hammersmith Infant Neurological Examination; MHFMS = Modified Hammersmith Functional Motor Scale; N/A = not applicable; pNF-H = phosphorylated neurofilament heavy chain; SMA = spinal muscular atrophy; SMN = survival motor neuron; TIMPSI = Test of Infant Motor Performance Screening Items.

SMN2 mRNA levels were able to distinguish between patients with SMA and healthy controls and among SMA phenotypes, a correlation between *SMN2-FL* levels and Hammersmith Functional Motor Scale score was only found in SMA type II participants.[26] Similarly, Vezain found that *SMN2-FL* transcript levels were inversely correlated with disease severity in peripheral blood cells but not in muscle samples.[24] Sumner found differences in *SMN* transcript between participants with SMA and healthy controls (p < 0.001); however, type I participants were mostly responsible for this difference; omitting this phenotype resulted in no differences among groups.[12] Of interest, Czech reported that *SMN2* expression is differently regulated in participants with SMA compared with healthy controls.[23] In healthy controls, *SMN* mRNA expression is proportional to the number of gene copies, whereas in participants with SMA, there is no correlation.[23] Finally, 3 studies found no correlation between *SMN2-FL* expression and disease phenotype.[22,25,27]

SMN Transcript Lacking Exon 7

Sumner[12] and Crawford found that *SMN-Δ7* expression levels are reduced in type I participants compared with type II and III, but they were similar to healthy controls.[22] Finally, Wadman found no association between SMA phenotype and *SMN-Δ7* expression in peripheral blood cells and fibroblasts.[27]

SMN Protein Levels as Biomarkers for Disease Severity

Two studies found that SMN protein levels were greater in healthy controls than participants with SMA.[22,27] However, most studies show that SMN protein cannot distinguish between SMA phenotypes.[12,22,23,27]

Non–SMN-Related Biomarkers

Plasma pNF-H as Prognostic and Pharmacodynamic Biomarker

Darras measured baseline pNF-H concentrations in healthy controls and nusinersen-treated type I infants enrolled in the ENDEAR trial.[30] There were a 10-fold greater plasma pNF-H levels in type I infants vs non-neurologic disease controls aged <1 year. Moreover, there was an inverse correlation between pNF-H levels and several markers of disease severity including age at first dose and SMA diagnosis, symptom onset, and Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders score. Although pNF-H levels declined over time in both nusinersen-treated and healthy control groups, pNF-H concentration declined to a greater extent in the nusinersen-treated arm.[30]

Plasma Protein Analytes as Biomarkers for Disease Severity

Although Kolb found lower concentration of 5 protein analytes in the SMA cohort group vs healthy controls, only 1 analyte correlated with both the Test of Infant Motor Performance Screening Items (TIMPSI) and the CHOP-INTEND score.[25] In contrast, Finkel identified 97 plasma proteins that regressed against the Modified Hammersmith Functional Motor Scale (MHFMS) and were able to distinguish among SMA type II, III, and controls.[29]

DNA Methylation Profiling From Leukocytes as a Biomarker for Disease Severity

Zheleznyakova conducted a whole-genome methylation pattern analysis from peripheral whole blood leukocytes.[28]

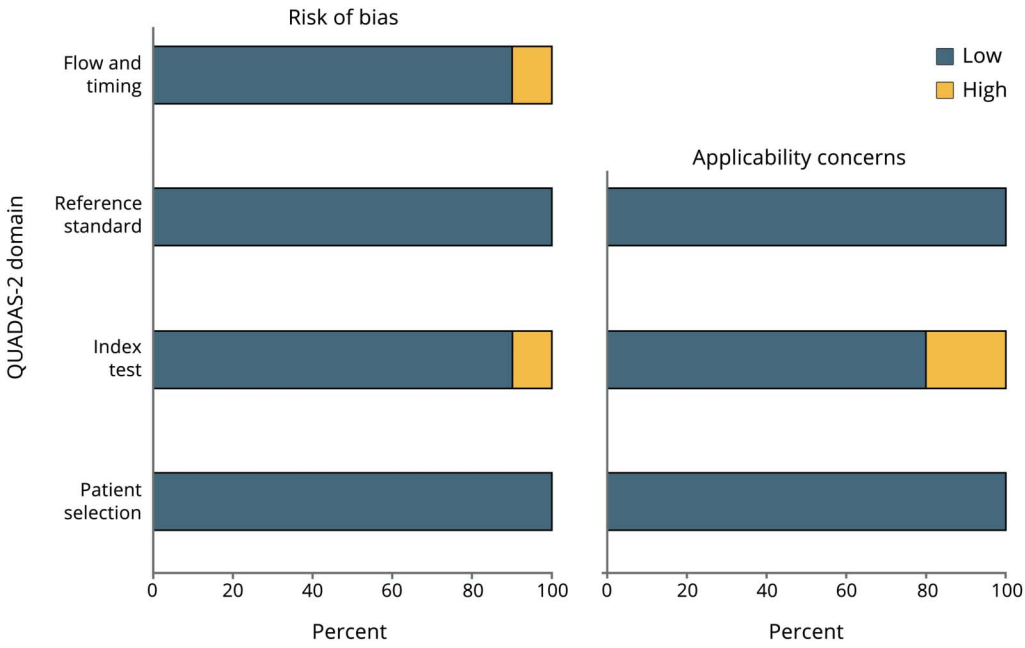
Based on this qualitative systematic review, there is no sufficient evidence to support the clinical utility of SMN-related putative biomarkers to predict disease severity.

There were substantial differences in the methylation level between participants with SMA and healthy controls in CpG sites of genes involved in SMA development.[28]

Quality Assessment

The quality of selected articles was evaluated by the QUADAS-2 (figure 1).[21] Patient selection presented a low bias across studies, with all studies including a patient-vs-healthy control design as well as genetic confirmation of participants with SMA with no other neurologic comorbidities. The main index test–related bias is the lack of longitudinal measurements across studies, which prevent the assessment of the stability of the putative biomarker over time. In addition, 1 study described assay technical difficulties,[25] and another one did not report the raw data of healthy controls.[29] None of the clinical motor function scales used as a reference test have been validated for the entire range of SMA phenotypes, and thus, associations between biomarker expression and motor function scores were restricted to

Figure 1 Evaluation of Study Quality Using QUADAS Risk of Bias



Risk of bias was categorized as low (gray) or high (white). QUADAS-2 = Quality Assessment of Diagnostic Accuracy Study.

a particular SMA phenotype. In terms of statistical reports, only 1 study presented a high-risk bias since the statistical comparison between participants with SMA and healthy controls, and correlations with motor function scales were not reported.[29]

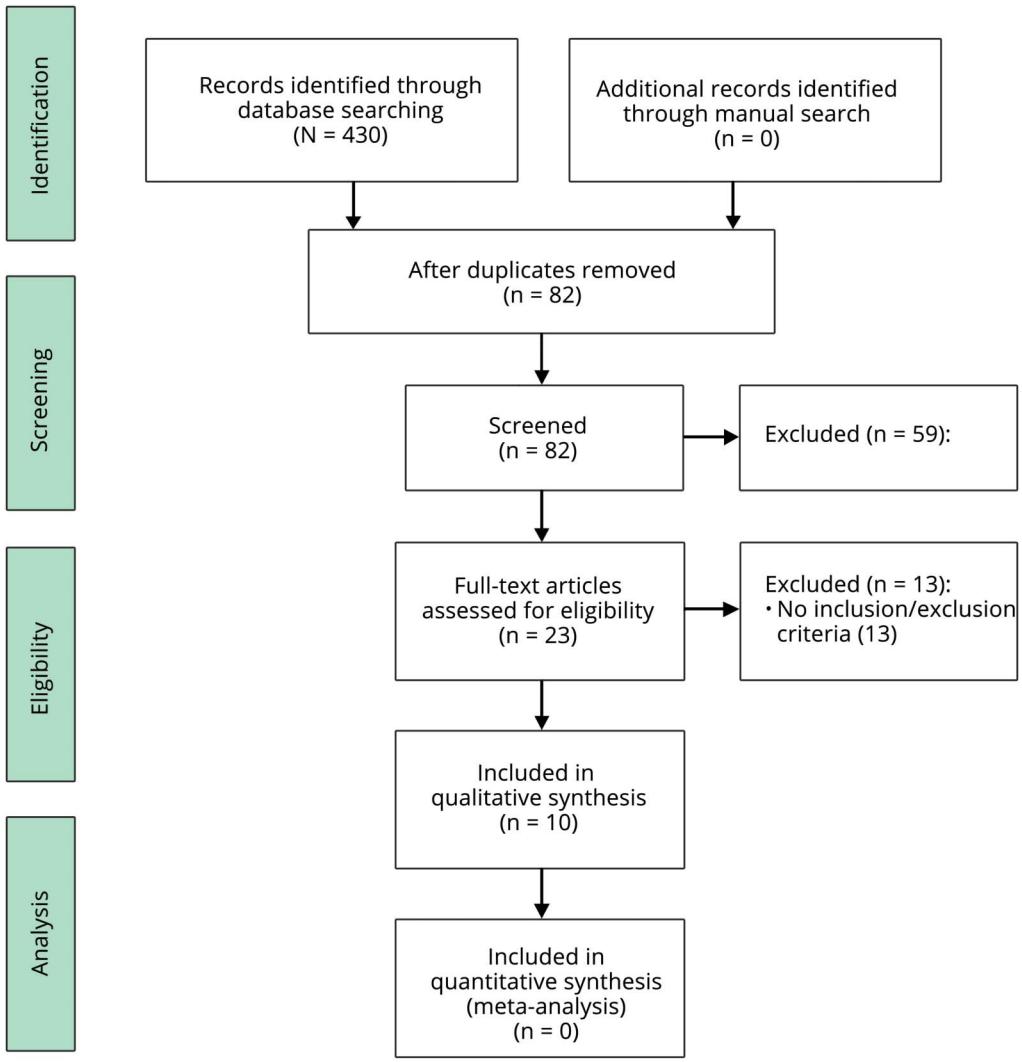
Discussion

Based on this qualitative systematic review, there is no sufficient evidence to support the clinical utility of SMN-related putative biomarkers to predict disease severity. Among non–SMN-related biomarkers, pNF-H may be a promising biomarker of disease activity and treatment effect in SMA.

The *SMN2* gene is proposed to be the main modifier of disease activity. However, the selected studies found that SMA phenotypes were either heterogeneous regarding *SMN2* copy numbers[24] or had a modest predictive value for individual patients.[22] *SMN2* copy number does not explain SMA severity, as patients with the same gene copy number have different SMA types,[26] which suggests that additional genetic or epigenetic factors may be affecting SMA phenotype.[31]

SMN2 transcript levels as a biomarker for disease severity showed inconsistent results. The correlation between *SMN2-FL* mRNA expression and SMA phenotype seems to be influenced by the heterogeneity of genotypes within each SMA type. For both *SMN2-FL* and *SMN-Δ7* transcript levels, there was a wide overlap among SMA phenotypes and controls[12,22,24,26] and limited correlation with clinical motor function.[22,25,27] An important finding of Czech and Crawford was that the regulation of *SMN2* expression in patients with SMA is different from healthy controls.[22,23] *SMN* mRNA expression is related to the number of *SMN* gene copies in healthy participants but not in patients with SMA,

Figure 2 Flowchart Showing Preferred Reporting Items for Systematic Reviews and Meta-analyses Study Selection Process



Overall, studies showed that although SMN transcripts, *SMN2* copy number, and SMN protein are measurable in peripheral blood, several limitations prevent their value as a clinical tool.

which suggest that SMN expression and SMA severity are regulated by additional factors.[22,23]

Although it has been widely accepted that SMA is caused by lower levels of SMN protein, the selected studies showed a great overlap of SMN protein levels among SMA phenotypes.[12,22,23,27] A plausible explanation is that SMN protein concentration may be different in blood, muscles, and the spinal cord and that the *SMN2* gene may be less transcriptionally active in alpha motor neurons. Alternatively, SMN protein levels may be reduced during early development but not later on.

Overall, studies showed that although SMN transcripts, *SMN2* copy number, and SMN protein are measurable in peripheral blood, several limitations prevent their value as a clinical tool. There significant interpatient and cell-type variability, and most importantly, neither SMN copy number, transcripts, or protein can per se predict SMA phenotype and thus, they cannot serve as a biomarker for disease severity.

Conventionally, the quest for biomarkers in SMA includes a hypothesis-based study design related to the pathophysiology of SMA. In contrast, the search for serum analytes is hypothesis generating, and includes assessing a broad set of disparate analytes, for the ability to distinguish between SMA and controls and correlate with motor outcomes. Although this approach may be considered unbiased in nature, it has the risk of increased false positives and results that are difficult to interpret. Finkel reported analytes that correlated with the MHFMS and that distinguished between type I, II, and III,[29] although this motor scale is only validated for children with type II SMA. Moreover, some analytes differentiated between type III and healthy controls,[29] but differences between controls and other subtypes were not reported. Kolb found additional factors affecting the interpretation of findings. Analyte concentration may be dependent on the number of *SMN2* copy numbers. For instance, complement component C1q receptor (C1qR1) in participants with SMA was only different than controls with 2 *SMN2* copy numbers ($p = 0.02$) but not in the entire cohort ($p = 0.1$).[25] Furthermore, correlation between age and targeted analytes needs to be previously established because the authors found that 9 analytes had a negative correlation with age in the control cohort[25] and other 10 analytes had a negative correlation with age in the SMA cohort.[25] Only 6 analytes showed this correlation in both the healthy control and SMA cohorts.[25] Finally, because analyte concentrations are quite downstream from the origin of the damage in spinal motor neurons, changes in serum may reflect concurrent biological processes in other peripheral tissues and not be indicative of a specific pathologic process in alpha motor neurons. Taken together, it would be preliminary to speculate which of these analytes has the most potential to serve as a biomarker for SMA. Additional longitudinal, validation studies are needed to determine whether serum analytes can serve as markers for SMA.

Neurofilament proteins have been proposed as potential biomarkers for several neurologic conditions including multiple sclerosis, amyotrophic lateral sclerosis, Parkinson disease, and Alzheimer disease, all of them characterized by axonal degeneration.[32] Postmortem studies in humans with SMA have found severe degeneration of axons and alpha motorneurons in the spinal cord.[33] Darras demonstrated that pNF-H levels were considerably greater in patients with SMA type I vs healthy controls and that baseline pNF-H levels were positively correlated with disease severity. Moreover, nusinersen treatment reduced pNF-H levels, which may suggest that pNF-H can serve as a pharmacodynamic marker for SMN-targeted therapies.[30] The authors observed high pNF-H levels in healthy controls younger than 1 year, which may reflect normal neuronal pruning, and therefore, an important methodological consideration for future studies is to include age-matched control cohort for every SMA phenotype.

DNA methylation is a common mechanism of epigenetic regulation altering gene expression, and abnormal methylation patterns have been associated with the development and progression of various diseases.[34] Zheleznyakova conducted the first genome-wide methylation study in patients with SMA and found that 10 CpG sites had different methylation levels compared with age-matched controls.[28] This is supported by previous studies showing different methylation patterns in CpG sites between patients with type I and III SMA in DNA extracted from leukocytes and fibroblasts.[35] Further longitudinal studies are needed to determine whether DNA methylation patterns can be altered by SMA therapy agents, as they may possess DNA-demethylase activity.

Although not a molecular biomarker, the ulnar compound muscle action potential (CMAP), investigated by Kolb et al.,[25] deserves a special mention as a potential biomarker for SMA. The authors found that CMAP peak amplitude in participants with type I SMA was considerably lower than healthy controls ($p < 0.001$) and that CMAP correlated with the TIMPSI ($r = 0.785$, $p < 0.0001$) and CHOP-INTEND ($r = 0.556$, $p = 0.0088$) motor scores. Although evidence suggests that CMAP can serve as a marker for disease severity,[36] it is unclear whether it can serve as a marker for treatment

effect. A clinical trial testing the safety and efficacy of olesoxime in participants with type II and III SMA showed an important difference between the treated and placebo cohort for Motor Function Score change across all visits (p = 0.0084), but not for CMAP values.[37]

There are several methodological limitations of the selected studies that should be considered in the development of future studies, including the use of endogenous controls to report SMN transcript levels, tissue differences, and age as a confounder factor.

The discrepancies found among studies can be partially explained for by the use of different endogenous internal controls across studies to report *SMN* transcript levels. Most studies report *SMN* transcript results by normalizing *SMN* mRNA in relation to housekeeping gene transcripts.[12,22,24,25,27] However, the expression level of housekeeping genes varies extensively in the general population[38] between participants with different SMA phenotypes[22,23] and in response to drug treatment.[39] If endogenous controls are used in future clinical trials, it should be confirmed that the investigational drug does not modify the expression level of these controls. An alternative approach is used by Tiziano who developed an absolute real-time PCR essay, based on construction of standard curves and quantification of *SMN* mRNA molecules per total RNA,[26] avoiding the use of endogenous housekeeping genes.

All selected studies tested investigated putative peripheral blood biomarkers, except for Vezain, and Wadman studies including *SMN* transcript measurements in muscle, and fibroblasts, respectively. The extent to which *SMN* mRNA and protein levels differ among different tissues is still largely unknown, but selected studies show no correlation between *SMN* mRNA in blood cells compared with fibroblasts,[27] and skeletal muscle,[24] which suggest important expression differences between tissues. Moreover, a study found that the highest expression of *SMN* transcripts in fetuses was in the spinal cord compared with other tissues.[40] Taken together, it is uncertain whether SMA drug therapies will have the same effect in peripheral blood cells vs spinal motor neurons and whether putative blood biomarkers will be able to detect a treatment effect.

Age is an important confounding variable to be considered for future clinical trials. Studies suggest inverse relationship between age and SMN protein levels in peripheral blood in both patients with SMA and healthy controls,[23,27] confirming previous studies in mice[41] and humans[42] suggesting age-specific differences in *SMN* expression.[41,42] Reduced *SMN* expression may be a characteristic of normal aging because *SMN* expression has been found to be the highest during embryonic development, which declines after birth.[43] Future clinical trials must consider age-matched control groups for each SMA phenotype.

Methodological limitations and conflicting results of selected studies prevent to recommend any blood-derived measures as biomarkers for SMA. Overall, there is no strong evidence suggesting that SMN-related putative biomarkers can predict disease severity or pharmacoresponse. Among non–SMN-related biomarkers, pNF-H seems a promising biomarker of disease activity and treatment effect in infants with SMA. Additional studies are needed with longitudinal assessment of patients with SMA across functional groups and comparisons with age-matched controls to evaluate the stability of these putative biomarkers over time and in response to SMA therapeutics.

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Angela Navarrete-Opazo, MD, PhD	Aurora Research Institute, Advocate Aurora Health	Study design, literature search, selection of eligible studies, data collection, and writing of the manuscript.
Sheldon Garrison, PhD	Aurora Research Institute, Advocate Aurora Health	Literature search, selection of eligible studies, and revision of the manuscript for intellectual content.
Mindy Waite, PhD	Aurora Research Institute, Advocate Aurora Health	Revision of the manuscript for intellectual content.

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