Portofoliu lucrări relevante – in extenso

Conf. Dr. Daniel-Corneliu Leucuța

Journal of
Clinical Medicine

Review

Temporomandibular Joint Osteoarthritis Diagnosis Employing Artificial Intelligence: Systematic Review and Meta-Analysis

Oana Almășan [1], Daniel-Corneliu Leucuța [2,*], Mihaela Hedeșiu [3], Sorana Mureșanu [3] and Ștefan Lucian Popa [4]

[1] Department of Prosthetic Dentistry and Dental Materials, Iuliu Hațieganu University of Medicine and Pharmacy, 400006 Cluj-Napoca, Romania
[2] Department of Medical Informatics and Biostatistics, Iuliu Hațieganu University of Medicine and Pharmacy, 400349 Cluj-Napoca, Romania
[3] Department of Oral and Maxillofacial Surgery and Implantology, Iuliu Hațieganu University of Medicine and Pharmacy400029 Cluj-Napoca, Romania
[4] 2nd Medical Department, Iuliu Hațieganu University of Medicine and Pharmacy, 400006 Cluj-Napoca, Romania
* Correspondence: dleucuta@umfcluj.ro

Abstract: The aim was to systematically synthesize the current research and influence of artificial intelligence (AI) models on temporomandibular joint (TMJ) osteoarthritis (OA) diagnosis using cone-beam computed tomography (CBCT) or panoramic radiography. Seven databases (PubMed, Embase, Scopus, Web of Science, LILACS, ProQuest, and SpringerLink) were searched for TMJ OA and AI articles. We used QUADAS-2 to assess the risk of bias, while with MI-CLAIM we checked the minimum information about clinical artificial intelligence modeling. Two hundred and three records were identified, out of which seven were included, amounting to 10,077 TMJ images. Three studies focused on the diagnosis of TMJ OA using panoramic radiography with various transfer learning models (ResNet model) on which the meta-analysis was performed. The pooled sensitivity was 0.76 (95% CI 0.35–0.95) and the specificity was 0.79 (95% CI 0.75–0.83). The other studies investigated the 3D shape of the condyle and disease classification observed on CBCT images, as well as the numerous radiomics features that can be combined with clinical and proteomic data to investigate the most effective models and promising features for the diagnosis of TMJ OA. The accuracy of the methods was nearly equivalent; it was higher when the indeterminate diagnosis was excluded or when fine-tuning was used.

Keywords: temporomandibular joint; osteoarthritis; artificial intelligence; systematic review

Citation: Almășan, O.; Leucuța, D.-C.; Hedeșiu, M.; Mureșanu, S.; Popa,Ș. L. Temporomandibular Joint Osteoarthritis Diagnosis Employing Artificial Intelligence: Systematic Review and Meta-Analysis. *J. Clin. Med.* **2023**, *12*, 942. https://doi.org/10.3390/jcm12030942

Academic Editor: Eiji Tanaka

Received: 31 December 2022
Revised: 20 January 2023
Accepted: 23 January 2023
Published: 25 January 2023

1. Introduction

According to Cohen S., the term "artificial intelligence" (AI) is still a little confusing [1]. Artificial intelligence was initially described in 1956 by implementing specific learning algorithms in computers to effectively manage human issues [2]. Artificial intelligence applications are available in almost any medical and nonmedical area, increasing their presence in healthcare as a consequence of their broad use of big data and progressively changing the way practitioners approach disease [3].

Machine learning (ML) belongs to a class of computer algorithms that build models for characterizing and forecasting using previously known data [1].

In dentistry, AI is used in multiple areas; from determining the influence of dental aesthetics on facial attractiveness [4], intraoral scanning [5], forecasting post-operative skeletal changes in orthognathic surgical planning [6], maxillary sinus segmentation [7], early detection of oral cancer [8], alveolar bone segmentation from cone-beam computed tomography (CBCT) [9], obtaining fully automated cephalometric measurements from a web-based artificial intelligence-driven platform [10], assessing root position during orthodontic

treatment [11], introducing algorithms in dentomaxillofacial radiology [12], diagnosing an anteriorly displaced temporomandibular joint (TMJ) disk on magnetic resonance imaging (MRI) [13], and diagnosing TMJ disorders [14] or TMJ osteoarthritis [15,16].

Osteoarthritis (OA) is a major and severe disorder that has generally been accepted as a whole-organ disease or a combination of diseases [17]. It is described as the chronic destruction of the soft and hard tissues around joints, frequently associated with cartilage damage, bone remodeling, synovitis, and joint discomfort [18]. OA of the TMJ was found to affect 25% of the adult population (20 to 50 years) when clinical signs were sought along with MRI investigations [19], whereas in older patients, its prevalence increases drastically to 70% [20]. Osteoarthritis of the TMJ is one of the most frequent degenerative joint disorders [21,22] and is characterized by condyle flattening, resorption, osteophyte formation [23], and degenerative alterations of the articular eminence, such as erosion, sclerosis, or resorption [24–26].

The insufficiency of signs before severe joint destruction occurs renders the early diagnosis of TMJ OA difficult [22]. Therefore, diagnosing TMJ osteoarthritis efficiently and precisely is key to effective treatment planning. Furthermore, the significant prevalence of TMJ OA underlines the necessity for a comprehensive imagistic evaluation of this condition, especially using modern AI techniques.

To the best of our knowledge, we could not identify any systematic review assessing the use of AI in TMJ OA.

Thus, the aim of our paper was to systematically synthesize the current research and the influence of AI models on TMJ OA diagnosis using CBCT or panoramic radiography.

2. Materials and Methods

The systematic review was reported in accordance with the recommendations of the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA) Statement" [27]. The systematic review has been registered in the open science framework and can be found at the following address: https://osf.io/qnzd5/.

2.1. Eligibility Criteria

All publications on osteoarthritis of the temporomandibular joint that considered artificial intelligence as a diagnosis method were included. Exclusion criteria were considered case reports, systematic reviews, narrative or scoping reviews, abstracts, comments, communications, editorials, and letters to the editor.

2.2. Information Sources

In May 2022 we performed a structured electronic search in the following databases: PubMed, Embase, Scopus, Web of Science, LILACS, ProQuest, and SpringerLink. Where applicable, MeSH and Emtree terminology were employed. The last electronic search was performed on all databases on 28 May 2022.

2.3. Search Strategy

The following terms were used in the search strategy: "osteoarthritis", "degenerative joint disease", "temporomandibular joint", "temporomandibular joint disorders", "artificial intelligence", "machine intelligence", "machine learning", "deep learning", "supervised", "unsupervised", "support vector machines", "random forest", "classifier", "classification algorithm", "cross validation", "data mining", "feature detection", "feature extraction", feature learning", "feature selection", "k nearest neighbor", "pattern recognition", "KNN", "K-means", "principal component analysis" "XGBoost", "LightGBM", "neural network", "tensorflow", "PyTorch", "Keras", "ResNet". Search terms included synonyms, acronyms, and singular as well as plural form words. In Table 1, the full strategies adjusted for each database are shown.

Table 1. Search strategies for each database.

PubMed
("osteoarthritis" [MeSH Terms] OR osteoarthritis[All Fields] OR "Degenerative joint disease" OR ("degenerative" AND "joint" AND "disease")) AND ("temporomandibular joint"[MeSH Terms] OR ("temporomandibular"[All Fields] AND "joint"[All Fields]) OR "temporomandibular joint"[All Fields] OR "TMJ"[Title/Abstract] OR "temporomandibular joint disorders"[MeSH Terms] OR ("temporomandibular"[All Fields] AND "joint"[All Fields] AND "disorders"[All Fields]) OR "temporomandibular joint disorders"[All Fields] OR ("temporomandibular"[All Fields] AND "disorders"[All Fields]) OR "temporomandibular disorders"[All Fields] OR "TMD"[Title/Abstract]) AND ("Artificial intelligence"[MeSH Terms] OR "Artificial intelligence"[All Fields] OR "machine intelligence"[All Fields] OR "Machine Learning"[MeSH Terms] OR "Machine Learning"[All Fields] OR "Deep Learning"[MeSH Terms] OR "Deep Learning"[All Fields] OR ("Learning" AND ("supervised" OR "unsupervised")) OR "Support Vector Machines"[All Fields] OR "Random forest"[All Fields] OR "classifier"[All Fields] OR "classification algorithm"[All Fields] OR "cross validation"[All Fields] OR "data mining"[All Fields] OR "feature detection"[All Fields] OR "feature extraction"[All Fields] OR "feature learning"[All Fields] OR "feature selection"[All Fields] OR "k nearest neighbor"[All Fields] OR "pattern recognition"[All Fields] OR "KNN"[All Fields] OR "K-means"[All Fields] OR "Principal Component Analysis" OR "XGBoost"[All Fields] OR "LightGBM"[All Fields] OR "Neural Network"[All Fields] OR "Tensorflow"[All Fields] OR "PyTorch"[All Fields] OR "Keras"[All Fields] OR "ResNet"[All Fields])
EMBASE
('osteoarthritis'/exp OR osteoarthritis OR 'degenerative joint disease'/exp OR 'degenerative joint disease' OR ('degenerative AND ('joint'/exp OR 'joint') AND ('disease'/exp OR 'disease'))) AND ('temporomandibular' AND ('joint'/exp OR 'joint') OR 'temporomandibular joint'/exp OR 'temporomandibular joint' OR 'tmj' OR ('temporomandibular' AND ('joint'/exp OR 'joint') AND ('disorders'/exp OR 'disorders')) OR 'temporomandibular joint disorders'/exp OR 'temporomandibular joint disorders' OR ('temporomandibular' AND ('disorders'/exp OR 'disorders')) OR 'temporomandibular disorders' OR 'tmd') AND ('artificial intelligence'/exp OR 'artificial intelligence' OR 'machine learning'/exp OR 'machine learning' OR 'deep learning'/exp OR 'deep learning' OR 'deep neural network'/exp OR 'deep neural network' OR (('learning'/exp OR 'learning') AND ('supervised' OR 'unsupervised')) OR 'support vector machines'/exp OR 'support vector machines' OR 'random forest'/exp OR 'random forest' OR 'classifier'/exp OR 'classifier' OR 'knn' OR 'k-means' OR 'principal component analysis'/exp OR 'principal component analysis' OR 'xgboost'/exp OR 'xgboost' OR 'lightgbm' OR 'neural network'/exp OR 'neural network' OR 'tensorflow'/exp OR 'tensorflow' OR 'pytorch' OR 'keras' OR 'resnet'/exp OR 'resnet')
Scopus
ALL (("osteoarthritis" OR "degenerative joint disease" OR ("degenarative" AND "joint" AND "disease")) AND (("temporomandibular" AND "joint") OR "temporomandibular joint" OR "tmj" OR ("temporomandibular" AND "joint" AND "disorders") OR "temporomandibular joint disorders" OR ("temporomandibular" AND "disorders") OR "temporomandibular disorders" OR "tmd") AND ("artificial intelligence" OR "machine learning" OR "deep learning" OR "deep neural network" OR ("learning" AND ("supervised" OR "unsupervised")) OR "support vector machines" OR "random forest" OR "classifier" OR "knn" OR "k-means" OR "principal component analysis" OR "xgboost" OR "lightgbm" OR "neural network" OR "tensorflow" OR "pytorch" OR "keras" OR "resnet")) AND (LIMIT-TO (DOCTYPE , "ar") OR LIMIT-TO (DOCTYPE , "re")) AND (LIMIT-TO (SUBJAREA , "DENT"))
Web of Science
TS = (("osteoarthritis" OR "degenerative joint disease" OR ("degenerative" AND "joint" AND "disease")) AND (("temporomandibular" AND "joint") OR "temporomandibular joint" OR "tmj" OR ("temporomandibular" AND "joint" AND "disorders") OR "temporomandibular joint disorders" OR ("temporomandibular" AND "disorders") OR "temporomandibular disorders" OR "tmd") AND ("artificial intelligence" OR "machine learning" OR "deep learning" OR "deep neural network" OR ("learning" AND ("supervised" OR "unsupervised")) OR "support vector machines" OR "random forest" OR "classifier" OR "knn" OR "k-means" OR "principal component analysis" OR "xgboost" OR "lightgbm" OR "neural network" OR "tensorflow" OR "pytorch" OR "keras" OR "resnet"))
LILACS
tw:(("osteoarthritis" OR "degenerative joint disease" OR ("degenerative" AND "joint" AND "disease")) AND (("temporomandibular" AND "joint") OR "temporomandibular joint" OR "tmj" OR ("temporomandibular" AND "joint" AND "disorders") OR "temporomandibular joint disorders" OR ("temporomandibular" AND "disorders") OR "temporomandibular disorders" OR "tmd") AND ("artificial intelligence" OR "machine learning" OR "deep learning" OR "deep neural network" OR ("learning" AND ("supervised" OR "unsupervised")) OR "support vector machines" OR "random forest" OR "classifier" OR "knn" OR "k-means" OR "principal component analysis" OR "xgboost" OR "lightgbm" OR "neural network" OR "tensorflow" OR "pytorch" OR "keras" OR "resnet"))
Proquest

	("osteoarthritis" OR "degenerative joint disease" OR ("degenerative" AND "joint" AND "disease")) AND (("temporomandibular" AND "joint") OR "temporomandibular joint" OR "tmj" OR ("temporomandibular" AND "joint" AND "disorders") OR "temporomandibular joint disorders" OR ("temporomandibular" AND "disorders") OR "temporomandibular disorders" OR "tmd") AND ("artificial intelligence" OR "machine learning" OR "deep learning" OR "deep neural network" OR ("learning" AND ("supervised" OR "unsupervised")) OR "support vector machines" OR "random forest" OR "classifier" OR "knn" OR "k-means" OR "principal component analysis" OR "xgboost" OR "lightgbm" OR "neural network" OR "tensorflow" OR "pytorch" OR "keras" OR "resnet"); filters: article, peer-review, osteoarthritis
SpringerLink	
	"osteoarthritis" AND (("temporomandibular" AND "joint") OR ("temporomandibular" AND "disorders") OR "TMJ" OR "TMD") AND ("artificial intelligence" OR "machine learning" OR "deep learning" OR "neural network"); filters: article, Imaging/Radiology

2.4. Selection Process and Data Collection Process

The search had no time constraint, nor were there any search limits or filters. The online Endnote version was used to remove double entries [28], followed by manual removal. A Microsoft Excel file (Microsoft Office 365, MS, Redmond, WA, USA) [29] was used to organize the publications after all of the papers had been retrieved and to carry out an impartial, blind screening of the included studies. The selection was carried out independently by two researchers (O.A. and D.C.L.). When unsure whether to include a particular study, the researchers conferred with two more researchers to find their standpoint (S.M. and S.L.P.). The same authors independently evaluated the chosen articles for inclusion after accessing the full texts, with disagreements being settled through debate. Two reviewers (S.M. and S.L.P.) collected data from the articles in a predefined Excel form file [29]. Inadvertences were compared with the full-text article by a third and fourth author (M.H. and D.C.L.). The following data were acquired: (1) author and year of publication; (2) study population; (3) OA classification; (4) training, validation, and testing; (5) region of interest (ROI) extraction; (6) transfer learning models; (7) learning; (8) software; and (9) results. These data are presented in Supplementary Table S1. Version 6.0.6 of the Zotero software (Roy Rosenzweig Center for History and New Media, Fairfax, Virginia, USA) was used to manage all references [30].

2.5. Study Risk of Bias Assessment

Two reviewers (O.A. and D.C.L.) independently judged the methodological quality of each of the chosen articles; any discrepancies in their evaluations were then compared in order to reach a consensus. The QUADAS 2 risk of bias assessment (Table 2) [31] and the minimum information about clinical artificial intelligence modeling (MI-CLAIM) checklist (Table 3) [32] were used to study the risk of bias.

2.6. Effect Measures

The sensitivity and specificities of the AI classification of TMJ OA by human experts were computed for each study.

2.7. Synthesis Methods

OpenMeta {Analyst} software was used to perform the meta-analyses. We extracted the true positives, false positives, false negatives, and true negatives from each study. The sensitivity and specificity were computed using the random-effects model with the restricted maximum likelihood estimator and presented in forest plots. The heterogeneity of the meta-analysis results was assessed with I^2 and the $\chi 2$-based Q-test and qualified using the Cochrane Handbook recommendations [33]. For all results, the point estimator, 95% confidence intervals, and p-values were presented. A 0.05 level of significance was used for all statistical tests.

2.8. Reporting Bias Assessment

The publication bias assessment is inconsequential since there were few identified studies.

3. Results

3.1. Study Selection

A PRISMA flow diagram was used to portray the recruiting and selection process (Figure 1). A total of 203 records were identified from seven databases: PubMed, Embase, Scopus, Web of Science, LILACS, ProQuest, and SpringerLink. After removing duplicate records, 167 records were screened. Out of these, 150 were excluded from the screening process. Seventeen publications were sought for retrieval, but one was not retrieved, although it was requested by email from the corresponding author. Out of the articles assessed for eligibility, nine studies were excluded. Seven articles were included in the qualitative and quantitative synthesis, amounting to 10,077 TMJ images, of which, the meta-analysis included three studies, amounting to 5520 TMJ images.

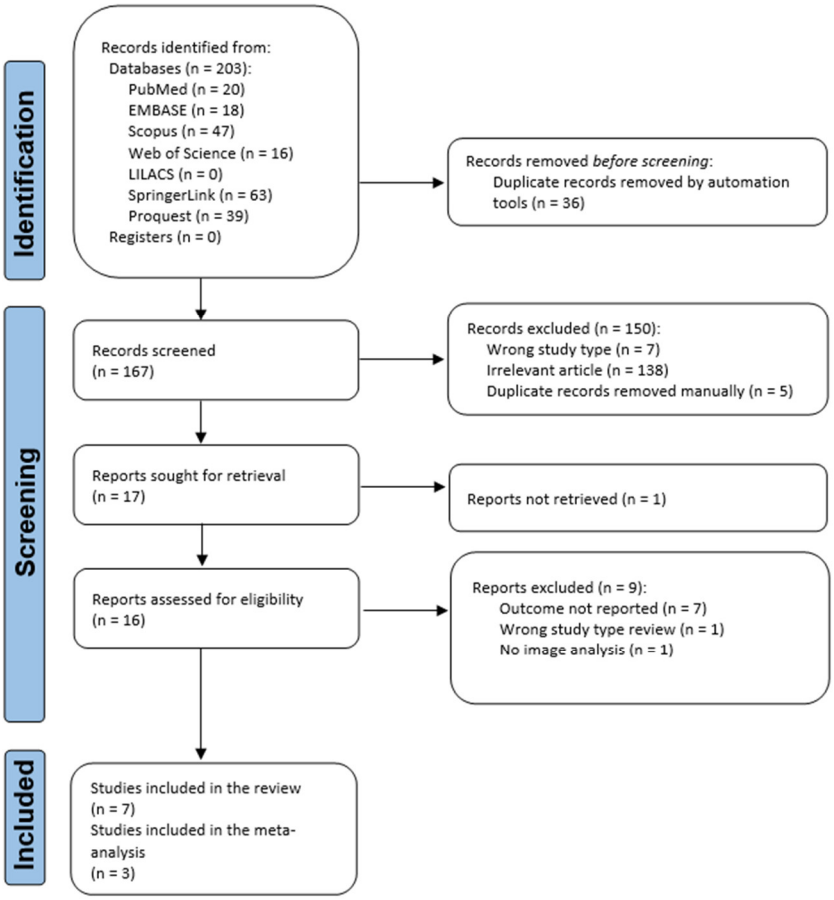

Figure 1. Flowchart of the identification, screening, and inclusion of articles in the systematic review.

3.2. Study Characteristics

The study characteristics are presented in Supplementary Table S1. We grouped the studies according to the imaging diagnosis techniques in CBCT [16,34–36] and panoramic radiography [15,37,38].

Bianchi J. [34] tested the diagnostic performance of four machine learning models: Logistic Regression, Random Forest, LightGBM, and XGBoost, trained on 52 features (clinical features (age, years of pain, vertical range unassisted and without pain, and others), 20 radiomics features (e.g., energy, entropy, bone volume, trabecular thickness, and others), and 14 serum and saliva biomarkers) and several interactions, finding that the XGBoost + LightGBM model achieved the highest accuracy of 0.823, AUC 0.870, and F1-score of 0.823 to diagnose the TMJ OA.

De Dumast P. [35] built a web-based system for storing, integrating, and computing biomedical data. They constructed 3D surface models from the CBCT and then applied a shape variation analyzer, a deep neural network classifier for osteoarthritis of the temporomandibular joint, to achieve a 91% agreement between the clinician and the SVA classifier.

Lee K.S. [36] constructed a diagnostic tool that uses artificial intelligence, a single-shot object detection model, to automatically identify normal, indeterminate TMJ OA, and TMJ OA in CBCT images. Their results, including indeterminate TMJ OA diagnosis vs. excluding them, were an average precision = 0.80 vs. 0.89, set average recall = 0.77 vs. 0.90, and F1 score = 0.78 vs. 0.89.

Zhang W. [16] used the same subjects as Bianchi J. [34] but used Learning using Privileged Information (LUPI) on 77 features (6 clinical, 46 imaging, and 25 protein) and interactions, finding that the LUPI method outperformed non-LUPI methods.

Choi E. [37] created an AI model and assessed the performance of the model using OPGs' TMJ OA diagnostics against an oromaxillofacial radiology (OMFR) specialist. Using a Karas' ResNet pre-trained model, an AI model was created and trained to divide panoramic radiography images into three groups: normal, uncertain OA, and OA. Results for the testing set including indeterminate TMJ OA diagnosis vs. excluding them were an accuracy = 0.51 vs. 0.78, weighted average precision = 0.55 vs. 0.78, weighted average recall = 0.51 vs. 0.78, and F1 score = 0.53 vs. 0.78.

Jung W. [15] created a diagnostic aid by categorizing panoramic images of TMJ into normal and osteoarthritis instances using pre-trained transfer learning models. ResNet-152 vs. EfficientNet-B7 accuracy, sensitivity, specificity, and area under the curve (AUC) values were 0.87, 0.94, 0.79, and 0.94, vs. 0.88, 0.86, 0.91, and 0.95.

Kim D. [38] used ResNet and Inception V3 pre-trained models and Visual Geometry Group-16 convolutional neural networks (CNNs) to suggest an algorithm that can extract the condylar area and assess its irregularity. The results concerning accuracy (ac.), sensitivity (Se), specificity (Sp), and AUC, without vs. with fine-tuning were: VGG16 ac. = 0.78 vs. 0.84, Se = 0.49 vs. 0.54, Sp = 0.86 vs. 0.94, AUC = 0.76 vs. 0.82; ResNet ac. = 0.77 vs. 0.81, Se = 0.41 vs. 0.47, Sp = 0.77 vs. 0.91, AUC = 0.57 vs. 0.79; Inception V3 ac. = 0.79 vs. 0.82, Se = 0.39 vs. 0.41, Sp = 0.82 vs. 0.94, and AUC = 0.51 vs. 0.83.

Concerning ROI identification, three studies used manual selection [16,34,36]. Jung W. [15] started with an automated tool, followed by manual selection of the ROI. De Dumast P. [35] segmented the CBCTs to create 3D surface models, and all condylar models were concurrently cropped to obtain the ROI. Choi E. [37] used a faster RCNN using the Inception V3 model to generate region proposals for the ROI. For each region, feature vectors were derived using Inception ResNet V2r, and an SVM predicted the class, followed by a bounding box regression for accurate object location. Kim D. [38] used an R-CNN to detect the TMJ and joint fossa and condyle, followed by a CNN to detect abnormalities based on the shape of the TMJ.

3.3. Results of Syntheses

From the studies that assessed panoramic radiography with AI, three studies presented the results of the ResNet classifications of TMJ OA; all studies excluded indeterminate TMJ OA diagnosis. We performed a meta-analysis of the test results without fine-tuning the models (Figure 2). The pooled sensitivity was 0.76 (95% CI 0.35—0.95), $p = 0.208$. The heterogeneity between the studies' results was considerable (I2 = 96.4%, $p < 0.001$).

The pooled specificity was 0.79 (95% CI 0.75–0.83), $p = 0.208$. Though the heterogeneity between the studies' results might not be important (I2 was 0%, $p = 0.464$).

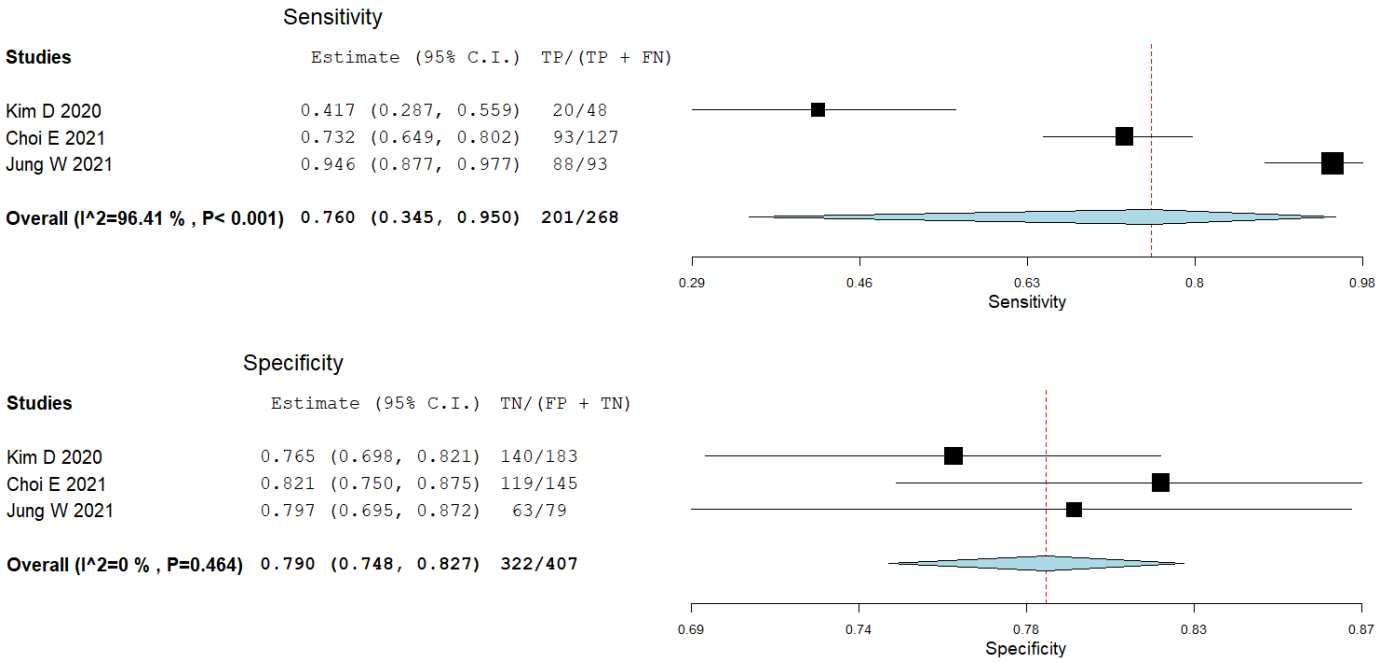

Figure 2. Forest plot for sensitivity and specificity of ResNet in classifying temporomandibular joint osteoarthritis (Choi [36], Jung, [16], Kim [37]).

3.4. Risk of Bias Assessment in Studies

The detailed QUADAS 2 risk of bias and applicability assessment is presented in Table 2 and Figure 3. We used two questions for this review that were assessed with the QUADAS 2 tool: for studies [15,37–39] of patients with TMD-related symptoms (without comorbidities that may influence the TMJ diagnosis) who are assessed with imagistic methods (panoramic radiography or CBCT), how accurate may an AI predict TMJ OA?; while for studies [16,34,35] of patients with TMD-related symptoms (without comorbidities that may influence the TMJ diagnosis) who are assessed with imagistic methods (any method) and other features (clinical and biomolecular), how accurate may an AI predict TMJ OA?

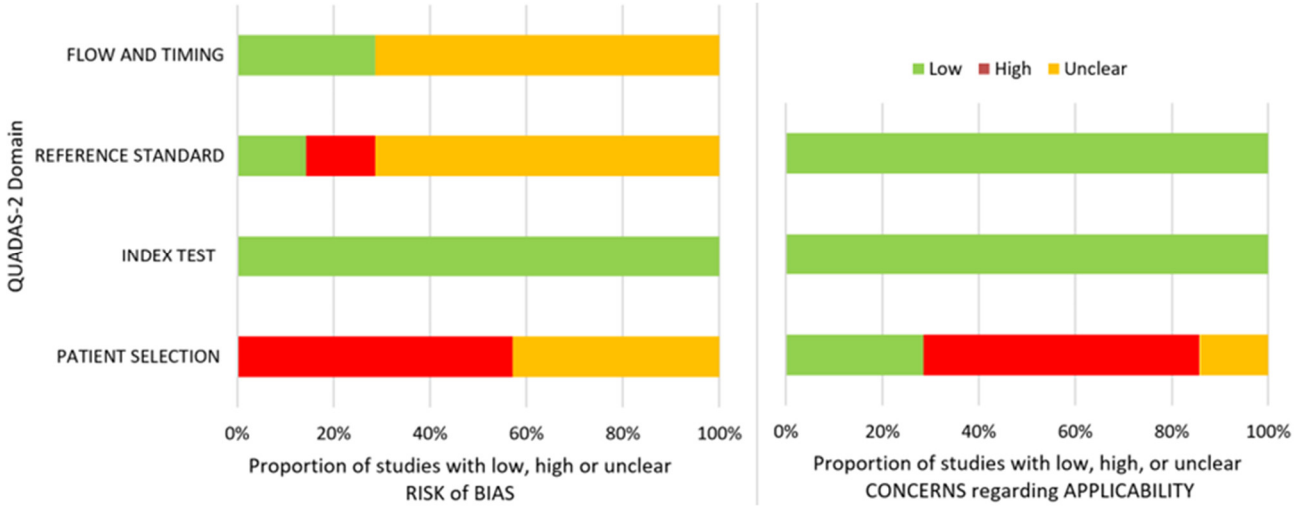

Figure 3. QUADAS 2 risk of bias and overall applicability assessment.

Table 2. QUADAS 2 risk of bias assessment.

Criteria	Choi * [36]	Jung * [16]	Kim * [37]	Lee * [34]	Bianchi # [32]	De Dumast # [33]	Zhang # [35]
Patient selection							
Signaling questions							
Was a consecutive or random sample of patients enrolled?	unclear [a]	unclear	unclear	unclear	unclear	unclear	unclear
Was a case-control design avoided?	yes	yes	yes	yes	no	unclear	no
Did the study avoid inappropriate exclusions?	yes	yes	yes	yes	yes	unclear	yes
Risk of bias assessment	unclear	high [d]	high [d]	unclear	high	unclear	high
Applicability	low	low	high [e]	high [f]	high [g]	unclear	high [h]
Index test							
Signaling questions							
Were the index test results interpreted without knowledge of the results of the reference standard?	yes	yes	yes	yes	yes	yes	yes
If a threshold was used, was it pre-specified?	NA	NA	NA	NA	NA	NA	NA
Risk of bias assessment	low	low	low	low	low	low	low
Applicability	low	low	low	low	low	low	low
Reference standard							
Signaling questions							
Is the reference standard likely to correctly classify the target condition?	unclear [b]	unclear [b]	no [i]	yes	unclear [b]	unclear [b]	unclear [b]
Were the reference standard results interpreted without knowledge of the results of the index test?	yes	yes	yes	yes	yes	yes	yes
Risk of bias assessment	unclear	unclear	high	low	unclear	unclear	unclear
Applicability	low	low	low	low	low	low	low
Flow and timing							
Signaling questions							
Was there an appropriate interval between index test(s) and reference standard?	yes	yes	yes	yes	yes	yes	yes
Did all patients receive a reference standard?	yes	yes	yes	yes	yes	yes	yes
Did patients receive the same reference standard?	unclear [c]	unclear [c]	unclear	yes	unclear	yes	unclear
Were all patients included in the analysis?	yes	yes	yes	yes	yes	yes	yes
Risk of bias assessment	unclear	unclear	unclear	low	unclear	low	unclear

*, Risk of bias assessment question: For patients with TMD-related symptoms (without comorbidities that may influence the TMJ diagnosis) who are assessed with imagistic methods (panoramic radiography or CBCT), how accurate may an AI predict TMJ-OA? # For patients with TMD-related symptoms (without comorbidities that may influence the TMJ diagnosis) who are assessed with imagistic methods (any method) and other features (clinical and biomolecular), how accurate may an AI predict TMJ-OA? [a], Symptoms + OPG + CBCT; [b], no information about experience, reliability; [c], multiple specialists; [d], excluded indeterminate diagnosis; [e], dental treated patients; [f], all diagnosed with TMD and TMJOA on CBCT; [g], excluded symptoms >= 10 years or important destruction; [h], used resubstitution validation; [i], CBCT not used for diagnosis but orthopantomography; NA, not applicable; TMJ, temporomandibular joint; TMD, temporomandibular disorder; AI, artificial

intelligence; CBCT, cone beam computed tomography; OA, osteoarthrosis; and QUADAS 2, quality assessment of diagnostic accuracy of studies.

Regarding the patient selection domain, four studies had a high risk of bias due to the exclusion of indeterminate diagnoses or due to the use of a case-control design; the other three studies had an unclear risk of bias. The index test domain was assessed to have a low risk of bias for all the studies. In connection with the reference standard, one of the studies had a low risk of bias, one had a high risk of bias, and five had an unclear risk of bias because the authors did not provide information about the experience of the image evaluators, and the reliability of the assessments. With reference to the flow and timing, five of the studies had an unclear risk of bias since multiple specialists may have performed the assessments of the TMJ diagnosis, and two had a low risk of bias.

In respect of applicability in the patient selection domain, four studies had a high risk of bias since some studies included treated patients, had all patients with TMD, or excluded patients with important destruction or long TMJ symptomatology. Next, one study had an unclear risk, and the other one had a low risk. Concerning the index test and reference standard, the risk was low.

The minimum information about the clinical artificial intelligence modeling (MI-CLAIM) checklist was used to assess the selected articles, and its results are presented in Table 3.

Table 3. The minimum information about the clinical artificial intelligence modeling (MI-CLAIM) checklist.

Study Design (Part 1)	Choi [36]	Jung [16]	Kim [37]	Lee [34]	Bianchi [32]	De Dumast [33]	Zhang [35]
The clinical problem in which the model will be employed is clearly detailed in the paper.	yes	yes	yes	yes	yes	yes	yes
The research question is clearly stated.	yes	yes	yes	yes	yes	yes	yes
The characteristics of the cohorts (training and test sets) are detailed in the text.	yes	yes	no	yes	yes	no	yes
The cohorts (training and test sets) are shown to be representative of real-world clinical settings.	yes	no	no	no	no	no	no
The state-of-the-art solution used as a baseline for comparison has been identified and detailed.	yes	unclear	unclear	yes	unclear	unclear	unclear
Data and optimization (Parts 2, 3)							
The origin of the data is described, and the original format is detailed in the paper.	yes	yes	no	yes	yes	no	yes
Transformations of the data before it is applied to the proposed model are described.	no	no	no	no	yes	yes	yes
The independence between the training and test sets has been proven in the paper.	yes	yes	yes	yes	yes	yes	yes
Details on the models that were evaluated, and the code developed to select the best model are provided.	yes *	yes *	yes *	yes *	yes *	yes *	yes *

Is the input data type structured or unstructured?	uns	uns	uns	uns	both	both	both
Model performance (Part 4)							
The primary metric selected to evaluate algorithm performance (e.g., AUC, F-score, etc.), including the justification for selection, has been clearly stated.	yes [a]	yes [a]	yes [a]	yes [a]	yes [a]	no	yes
The primary metric selected to evaluate the clinical utility of the model (e.g., PPV, NNT, etc.), including the justification for selection, has been clearly stated.	yes [a]	yes [a]	yes [a]	yes [a]	yes [a]	no	yes
The performance comparison between the baseline and the proposed model is presented with the appropriate statistical significance.	yes	yes [b]	yes [b]	yes [b]	yes [b]	yes [b]	yes
Model examination (Part 5)							
Examination technique 1a	no	no	no	no	no	no	no
Examination technique 2a	no	no	no	no	no	no	no
A discussion of the relevance of the examination results with respect to model/algorithm performance is presented.	yes	yes	yes	yes	yes	no	yes
A discussion of the feasibility and significance of model interpretability at the case level if examination methods are uninterpretable is presented.	NA	NA	NA	NA	NA	NA	NA
A discussion of the reliability and robustness of the model as the underlying data distribution shifts is included.	no	no	no	no	no	no	no
Reproducibility (Part 6): Choose the appropriate tier of transparency							
Tier 1: Complete sharing of the code.	no	no	no	no	yes	yes	no
Tier 2: Allow a third party to evaluate the code for accuracy/fairness; share the results of this evaluation.	no	no	no	no	no	no	no
Tier 3: Release of a virtual machine (binary) for running the code on new data without sharing its details.	no	no	no	no	no	no	no
Tier 4: No sharing.	yes	yes	yes	yes	no	no	yes

NA, not applicable; *, no code for automatic selection of the models—they were chosen by the authors; uns, unstructured; [a], no justification; and [b], no statistical test.

Study design: The clinical problem and research question were clearly stated in all the papers. The characteristics of the cohorts were not clearly detailed in two articles. The cohorts were not clearly representative of real-world clinical settings in six articles. State-of-the-art being used as a baseline for comparison was unclear with respect to the experience of the image evaluators.

Data and optimization: The origin of the data was not clearly described in two articles. Three articles performed transformations of the data before applying the model. All the papers described the independence between the training and the test sets and they gave the details on the models that were employed. Four studies used unstructured data

(images), while three used both structured (clinical and biological data) and unstructured data (images). One study did not clearly present the primary metric to assess the algorithm performance and clinical utility; nevertheless, they presented the confusion matrix. All the papers provided a performance comparison between the baseline and the proposed model.

Model examination: No study showed sensitivity analyses nor a discussion of the reliability and robustness of the model as the underlying data distribution shifts are included. Only one study did not discuss the relevance of the examination results with respect to model performance.

Reproducibility: Only two studies shared their code.

4. Discussion

Our exhaustive research of the literature identified several articles concerning TMJ OA classification with AI that were described and assessed for methodological quality. A meta-analysis was then applied to the studies that used ResNet for panoramic radiography assessment.

Two studies checked the diagnostic performance of several machine learning models on a large number of features (clinical, radiomics on CBCT, and proteomics from serum and saliva) [16,34] in an exploratory approach, with XGBoost + LightGBM being the most accurate, as well as LUPI methods, outperforming by a small margin the non-LUPI methods. One study used a single-shot detector deep learning framework designed for object detection on CBCT [36]. Another study reconstructed the 3D shape of condyles and used a shape variation analyzer to classify TMJ OA in five different morphological degeneration groups [35]. Three other studies assessed the pre-trained transfer learning models (ResNet, EfficientNet, VGG, and Inception V3) on panoramic radiographs [15,37,38], with the fine-tuned VGG model being the most accurate in the head-to-head comparison (on 2584 images [38]), but yielded small differences between them. Since all three studies assessed the ResNet model, we performed a meta-analysis to synthesize their results (on 5520 images). The pooled sensitivity was 0.76 (95% CI 0.35–0.95) with marked heterogeneity. The outlier study here was that of Kim D. [38], with a sensitivity of 0.42. This value was for a model without fine-tuning and could explain the difference. It is possible the other studies did not specify if they did or did not fine-tune their results. The pooled specificity was 0.79 (95% CI 0.75–0.83) with low heterogeneity. The overall accuracies or sensitivities and specificities are not very impressive, being clinically moderate. We must keep in mind that panoramic radiography is not the primary intention diagnosis test when it comes to TMJ imaging. CBCT, on the other hand, is more accurate in diagnosing the bone pathology of TMJ; however, in the selected studies, we could not identify similar studies using this imaging technique to perform a meta-analysis. It is difficult to compare the accuracies of AI classifying on panoramic radiography and CBCT since they were not trained on the same images, but the expectancy would be that AI trained on CBCT would outperform those trained on panoramic radiography.

Several studies excluded indeterminate TMJ OA diagnoses. This exclusion artificially increases the accuracies, as can be seen in the results of several studies. The use of AI in real-life scenarios would have lower diagnostic accuracies.

As expected, fine-tuned models outperformed the models without hyperparameter tweaks.

The selection of the ROI influences the accuracy of the training since a poorly chosen ROI cannot offer good discriminant information for the AI. Almost half of the studies used manual ROI selection that can offer high-quality training data, but this suffers from the pipelining of AI in real-life scenarios. One study combined an automated tool with manual selection. Three studies applied CNNs to generate, and another CNN to predict, the ROI, with the most sophisticated approach being the one used by Choi E. [37].

4.1. Limitations

The number of images used in several studies was low, nevertheless, the models had important accuracies (possibly due to the use of pre-trained models and data augmentation methods). The exclusion of indeterminate diagnoses or illegible and blurry images artificially increased the model accuracies in several studies. The exclusion of subjects with a history of orthognathic surgery, craniofacial trauma, and systemic diseases that could affect the TMJ limits AI usability in specific real-life scenarios. Moreover, the applicability of many studies is potentially limited since the typical scenario in which an AI system might be used is for subjects presenting with the symptomatology of TMD, however, several studies did not specify how they assessed such groups. In addition, the use of a case-control design in one study could have induced a selection bias. Concerning the reference standard, although the majority of the studies used good reference tests such as CBCT (with one exception that used orthopantomography, which is known to have reduced accuracy), they usually did not specify the observer experience and how many different observers assessed the images, nor their intra- and inter-rater reliability, thus potentially reducing the confidence in the standard test. One study used human intervention in confirming the region of interest, which precludes the creation of complete functional pipelines but helps accuracy; however, the other studies used automated methods. In addition, the studies did not perform sensitivity analyses and only a few studies had an appropriate tier of transparency by sharing their code.

4.2. Study Strengths

Finding new non-invasive approaches to diagnose TMJ OA accurately, forecast illness severity, devise treatment plans, assess prognosis, and track disease progression is an important result that can be built upon this work. However, our study exposes significant gaps in the data that need to be investigated further in follow-up research while providing a neutral summary of the available literature. A key advantage of our study is the comprehensive search strategy combined with seven different databases. Furthermore, we used two instruments to assess the quality of the included papers. The first one, the QUADAS 2 tool, is endorsed by the Cochrane Collaboration, which is regarded as providing the highest level of evidence-based medicine worldwide. The second one, while not a quality assessment tool, is the only instrument that assesses the reporting information on clinical artificial intelligence modeling. Finally, since several studies used the same methods, we performed a meta-analysis to obtain their pooled results.

5. Conclusions

Our extensive literature search identified a rather diverse spectrum of AI applications on TMJ OA classification. Some studies focused on the diagnosis of TMJ OA using panoramic radiography with different transfer learning models, on which we performed a meta-analysis regarding the ResNet model. The other studies focused on CBCT images concerning its 3D shape or disease classification or combined the numerous radiomics features with clinical and proteomic data to explore the best models and promising features for TMJ OA diagnosis. The accuracies of the methods were similar overall and varied between moderate to good, being higher when excluding indeterminate diagnoses or when using fine-tuning. Future studies should employ better methods to amend the current literature papers' limits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm12030942/s1, Supplementary Table S1: Study characteristics.

Author Contributions: Conceptualization, O.A. and D.-C.L.; methodology, D.-C.L., Ș.L.P., and O.A.; software, D.-C.L.; validation, O.A., Ș.L.P., S.M., and D.-C.L.; formal analysis, D.-C.L.; investigation, O.A., Ș.L.P., S.M., M.H., and D.-C.L.; data curation, O.A. and D.-C.L.; writing—original draft preparation, O.A. and D.-C.L.; writing—review and editing, O.A., D.-C.L., Ș.L.P., S.M., and M.H.;

visualization, O.A., D.-C.L., Ș.L.P., and M.H.; supervision, Ș.L.P. and M.H.; project administration, O.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available on request from the corresponding author, upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cohen, S. The Basics of Machine Learning: Strategies and Techniques. In *Artificial Intelligence and Deep Learning in Pathology*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 13–40; ISBN 978-0-323-67538-3.
2. Bianchi, J.; Ruellas, A.; Prieto, J.C.; Li, T.; Soroushmehr, R.; Najarian, K.; Gryak, J.; Deleat-Besson, R.; Le, C.; Yatabe, M.; et al. Decision Support Systems in Temporomandibular Joint Osteoarthritis: A Review of Data Science and Artificial Intelligence Applications. *Semin. Orthod.* **2021**, *27*, 78–86. https://doi.org/10.1053/j.sodo.2021.05.004.
3. Yu, K.-H.; Beam, A.L.; Kohane, I.S. Artificial Intelligence in Healthcare. *Nat. Biomed. Eng.* **2018**, *2*, 719–731. https://doi.org/10.1038/s41551-018-0305-z.
4. Obwegeser, D.; Timofte, R.; Mayer, C.; Eliades, T.; Bornstein, M.M.; Schätzle, M.A.; Patcas, R. Using Artificial Intelligence to Determine the Influence of Dental Aesthetics on Facial Attractiveness in Comparison to Other Facial Modifications. *Eur. J. Orthod.* **2022**, *44*, 445–451. https://doi.org/10.1093/ejo/cjac016.
5. Kim, S.-H.; Kim, K.B.; Choo, H. New Frontier in Advanced Dentistry: CBCT, Intraoral Scanner, Sensors, and Artificial Intelligence in Dentistry. *Sensors* **2022**, *22*, 2942. https://doi.org/10.3390/s22082942.
6. Ma, Q.; Kobayashi, E.; Fan, B.; Hara, K.; Nakagawa, K.; Masamune, K.; Sakuma, I.; Suenaga, H. Machine-learning-based Approach for Predicting Postoperative Skeletal Changes for Orthognathic Surgical Planning. *Robot. Comput. Surg.* **2022**, *18*, e2379. https://doi.org/10.1002/rcs.2379.
7. Morgan, N.; Van Gerven, A.; Smolders, A.; de Faria Vasconcelos, K.; Willems, H.; Jacobs, R. Convolutional Neural Network for Automatic Maxillary Sinus Segmentation on Cone-Beam Computed Tomographic Images. *Sci. Rep.* **2022**, *12*, 7523. https://doi.org/10.1038/s41598-022-11483-3.
8. Jubair, F.; Al-Karadsheh, O.; Malamos, D.; Al Mahdi, S.; Saad, Y.; Hassona, Y. A Novel Lightweight Deep Convolutional Neural Network for Early Detection of Oral Cancer. *Oral. Dis.* **2022**, *28*, 1123–1130. https://doi.org/10.1111/odi.13825.
9. Cui, Z.; Fang, Y.; Mei, L.; Zhang, B.; Yu, B.; Liu, J.; Jiang, C.; Sun, Y.; Ma, L.; Huang, J.; et al. A Fully Automatic AI System for Tooth and Alveolar Bone Segmentation from Cone-Beam CT Images. *Nat. Commun.* **2022**, *13*, 2096. https://doi.org/10.1038/s41467-022-29637-2.
10. Mahto, R.K.; Kafle, D.; Giri, A.; Luintel, S.; Karki, A. Evaluation of Fully Automated Cephalometric Measurements Obtained from Web-Based Artificial Intelligence Driven Platform. *BMC Oral Health* **2022**, *22*, 132. https://doi.org/10.1186/s12903-022-02170-w.
11. Lee, S.-C.; Hwang, H.-S.; Lee, K.C. Accuracy of Deep Learning-Based Integrated Tooth Models by Merging Intraoral Scans and CBCT Scans for 3D Evaluation of Root Position during Orthodontic Treatment. *Prog. Orthod.* **2022**, *23*, 15. https://doi.org/10.1186/s40510-022-00410-x.
12. Hung, K.F.; Ai, Q.Y.H.; Leung, Y.Y.; Yeung, A.W.K. Potential and Impact of Artificial Intelligence Algorithms in Dento-Maxillofacial Radiology. *Clin. Oral Investig.* **2022**, *26*, 5535–5555. https://doi.org/10.1007/s00784-022-04477-y.
13. Lin, B.; Cheng, M.; Wang, S.; Li, F.; Zhou, Q. Automatic Detection of Anteriorly Displaced Temporomandibular Joint Discs on Magnetic Resonance Images Using a Deep Learning Algorithm. *Dentomaxillofacal Radiol.* **2022**, *51*, 20210341. https://doi.org/10.1259/dmfr.20210341.
14. De Lima, E.D.; Paulino, J.A.S.; Freitas, A.P.L.D.F.; Ferreira, J.E.V.; Barbosa, J.D.S.; Silva, D.F.B.; Bento, P.M.; Amorim, A.M.A.M.; Melo, D.P. Artificial Intelligence and Infrared Thermography as Auxiliary Tools in the Diagnosis of Temporomandibular Disorder. *Dentomaxillofacal Radiol.* **2022**, *51*, 20210318. https://doi.org/10.1259/dmfr.20210318.
15. Jung, W.; Lee, K.-E.; Suh, B.-J.; Seok, H.; Lee, D.-W. Deep Learning for Osteoarthritis Classification in Temporomandibular Joint. *Oral Dis.* **2021,** 00, 1– 10 . https://doi.org/10.1111/odi.14056.
16. Zhang, W.; Bianchi, J.; Turkestani, N.A.; Le, C.; Deleat-Besson, R.; Ruellas, A.; Cevidanes, L.; Yatabe, M.; Goncalves, J.; Benavides, E.; et al. Temporomandibular Joint Osteoarthritis Diagnosis Using Privileged Learning of Protein Markers. *Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.* **2021**, *2021*, 1810–1813. https://doi.org/10.1109/EMBC46164.2021.9629990.
17. Deveza, L.A.; Loeser, R.F. Is Osteoarthritis One Disease or a Collection of Many? *Rheumatology* **2018**, *57*, iv34–iv42. https://doi.org/10.1093/rheumatology/kex417.
18. Alzahrani, A.; Yadav, S.; Gandhi, V.; Lurie, A.G.; Tadinada, A. Incidental Findings of Temporomandibular Joint Osteoarthritis and Its Variability Based on Age and Sex. *Imaging Sci. Dent* **2020**, *50*, 245–253. https://doi.org/10.5624/isd.2020.50.3.245.

19. Bernhardt, O.; Biffar, R.; Kocher, T.; Meyer, G. Prevalence and Clinical Signs of Degenerative Temporomandibular Joint Changes Validated by Magnetic Resonance Imaging in a Non-Patient Group. *Ann. Anat.* **2007**, *189*, 342–346. https://doi.org/10.1016/j.aanat.2007.02.008.
20. Schmitter, M.; Essig, M.; Seneadza, V.; Balke, Z.; Schröder, J.; Rammelsberg, P. Prevalence of Clinical and Radiographic Signs of Osteoarthrosis of the Temporomandibular Joint in an Older Persons Community. *Dentomaxillofacal Radiol.* **2010**, *39*, 231–234. https://doi.org/10.1259/dmfr/16270943.
21. Tanaka, E.; Detamore, M.S.; Mercuri, L.G. Degenerative Disorders of the Temporomandibular Joint: Etiology, Diagnosis, and Treatment. *J. Dent. Res.* **2008**, *87*, 296–307. https://doi.org/10.1177/154405910808700406.
22. Kalladka, M.; Quek, S.; Heir, G.; Eliav, E.; Mupparapu, M.; Viswanath, A. Temporomandibular Joint Osteoarthritis: Diagnosis and Long-Term Conservative Management: A Topic Review. *J. Indian Prosthodont. Soc.* **2014**, *14*, 6–15. https://doi.org/10.1007/s13191-013-0321-3.
23. Song, H.; Lee, J.Y.; Huh, K.-H.; Park, J.W. Long-Term Changes of Temporomandibular Joint Osteoarthritis on Computed Tomography. *Sci. Rep.* **2020**, *10*, 6731. https://doi.org/10.1038/s41598-020-63493-8.
24. Larheim, T.A.; Abrahamsson, A.-K.; Kristensen, M.; Arvidsson, L.Z. Temporomandibular Joint Diagnostics Using CBCT. *Dentomaxillofacal Radiol.* **2015**, *44*, 20140235. https://doi.org/10.1259/dmfr.20140235.
25. Boeddinghaus, R.; Whyte, A. Computed Tomography of the Temporomandibular Joint. *J. Med. Imaging Radiat. Oncol.* **2013**, *57*, 448–454. https://doi.org/10.1111/1754-9485.12021.
26. Delpachitra, S.N.; Dimitroulis, G. Osteoarthritis of the Temporomandibular Joint: A Review of Aetiology and Pathogenesis. *Br J. Oral. Maxillofac. Surg.* **2021**, *60*, 387–396. https://doi.org/10.1016/j.bjoms.2021.06.017.
27. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews. *BMJ* **2021**, *372*, n71. https://doi.org/10.1136/bmj.n71.
28. EndNote. Available online: https://access.clarivate.com/login?app=endnote (accessed on 12 July 2022).
29. *Microsoft Excel* (version 365); Microsoft: Redmond, WA, USA, 2019. Available online: https://office.microsoft.com/excel (accessed on 15 July 2022).
30. *Zotero*; Corporation for Digital Scholarship: Vienna, Va, USA, https://www.zotero.org/ (accessed on 15 July 2022)..
31. Whiting, P.F. QUADAS-2: A Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies. *Ann. Intern Med.* **2011**, *155*, 529. https://doi.org/10.7326/0003-4819-155-8-201110180-00009.
32. Norgeot, B.; Quer, G.; Beaulieu-Jones, B.K.; Torkamani, A.; Dias, R.; Gianfrancesco, M.; Arnaout, R.; Kohane, I.S.; Saria, S.; Topol, E.; et al. Minimum Information about Clinical Artificial Intelligence Modeling: The MI-CLAIM Checklist. *Nat Med* **2020**, *26*, 1320–1324. https://doi.org/10.1038/s41591-020-1041-y.
33. Higgins, J.; Thomas, J.; Chandler, J.; Cumpston, M.; Li, T.; Page, M.; Welch, V. *Cochrane Handbook for Systematic Reviews of Interventions*, 2nd ed.; John Wiley & Sons: Chichester, UK, 2019.
34. Bianchi, J.; de Oliveira Ruellas, A.C.; Gonçalves, J.R.; Paniagua, B.; Prieto, J.C.; Styner, M.; Li, T.; Zhu, H.; Sugai, J.; Giannobile, W.; et al. Osteoarthritis of the Temporomandibular Joint Can Be Diagnosed Earlier Using Biomarkers and Machine Learning. *Sci. Rep.* **2020**, *10*, 8012. https://doi.org/10.1038/s41598-020-64942-0.
35. De Dumast, P.; Mirabel, C.; Cevidanes, L.; Ruellas, A.; Yatabe, M.; Ioshida, M.; Ribera, N.T.; Michoud, L.; Gomes, L.; Huang, C.; et al. A Web-Based System for Neural Network Based Classification in Temporomandibular Joint Osteoarthritis. *Comput. Med. Imaging Graph.* **2018**, *67*, 45–54. https://doi.org/10.1016/j.compmedimag.2018.04.009.
36. Lee, K.S.; Kwak, H.J.; Oh, J.M.; Jha, N.; Kim, Y.J.; Kim, W.; Baik, U.B.; Ryu, J.J. Automated Detection of TMJ Osteoarthritis Based on Artificial Intelligence. *J. Dent. Res.* **2020**, *99*, 1363–1367. https://doi.org/10.1177/0022034520936950.
37. Choi, E.; Kim, D.; Lee, J.-Y.; Park, H.-K. Artificial Intelligence in Detecting Temporomandibular Joint Osteoarthritis on Orthopantomogram. *Sci. Rep.* **2021**, *11*, 10246. https://doi.org/10.1038/s41598-021-89742-y.
38. Kim, D.; Choi, E.; Jeong, H.G.; Chang, J.; Youm, S. Expert System for Mandibular Condyle Detection and Osteoarthritis Classification in Panoramic Imaging Using R-CNN and CNN. *Appl. Sci.* **2020**, *10*, 7464. https://doi.org/10.3390/app10217464.
39. Jeon, S.; Lee, K.C. Comparison of Cephalometric Measurements between Conventional and Automatic Cephalometric Analysis Using Convolutional Neural Network. *Prog. Orthod.* **2021**, *22*, 14. https://doi.org/10.1186/s40510-021-00358-4.

antioxidants

MDPI

Systematic Review

Walnut Intake Interventions Targeting Biomarkers of Metabolic Syndrome and Inflammation in Middle-Aged and Older Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

Letiția Mateș [1], Daniela-Saveta Popa [1,*], Marius Emil Rusu [2,*], Ionel Fizeșan [1] and Daniel Leucuța [3]

[1] Department of Toxicology, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, 8 Victor Babes, 400012 Cluj-Napoca, Romania; micu.letitia@umfcluj.ro (L.M.); ionel.fizesan@umfcluj.ro (I.F.)
[2] Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, 8 Victor Babes, 400012 Cluj-Napoca, Romania
[3] Department of Medical Informatics and Biostatistics, Faculty of Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, 8 Victor Babes, 400012 Cluj-Napoca, Romania; dleucuta@umfcluj.ro (D.L.)
* Correspondence: dpopa@umfcluj.ro (D.-S.P.); rusu.marius@umfcluj.ro (M.E.R.); Tel.: +40-264-450-555 (D.-S.P.)

Abstract: Biomarkers of metabolic syndrome and inflammation are pathophysiological predictors and factors of senescence and age-related diseases. Recent evidence showed that particular diet components, such as walnuts rich in antioxidant bioactive compounds and with a balanced lipid profile, could have positive outcomes on human health. A systematic search in PubMed, EMBASE, Cochrane Library, Scopus, and ClinicalTrials.gov databases was performed to retrieve randomized controlled trials published from the beginning of each database through November 2021, reporting on the outcomes of walnut consumption over 22 metabolic syndrome and inflammatory markers in middle-aged and older adults. The search strategy rendered 17 studies in the final selection, including 11 crossover and 6 parallel trials. The study revealed that walnut-enriched diets had statistically significant decreasing effects for triglyceride, total cholesterol, and LDL cholesterol concentrations on some inflammatory markers and presented no consequences on anthropometric and glycemic parameters. Although further studies and better-designed ones are needed to strengthen these findings, the results emphasize the benefits of including walnuts in the dietary plans of this age group.

Keywords: nuts; tree nuts; nut consumption; aging; age-related diseases; cardiometabolic markers; antioxidants; inflammation; lipid profile; diabetes

Citation: Mateș, L.; Popa, D.-S.; Rusu, M.E.; Fizeșan, I.; Leucuța, D. Walnut Intake Interventions Targeting Biomarkers of Metabolic Syndrome and Inflammation in Middle-Aged and Older Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Antioxidants* **2022**, *11*, 1412. https://doi.org/10.3390/antiox11071412

Academic Editor: Stanley Omaye

Received: 10 June 2022
Accepted: 19 July 2022
Published: 21 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Metabolic syndrome (MetS) conditions, chronic, low-grade inflammation, and oxidative stress are significant risk factors for morbidity and mortality with higher prevalence in the aging population [1]. These pathophysiological components increase the probability of age-associated diseases, including cardiovascular disease (CVD), type 2 diabetes (T2D), cognitive impairment, neurodegenerative disorders, or cancer [2,3]. Compelling evidence demonstrates that inflammatory markers, such as serum C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), are predictors and factors in cellular senescence and chronic inflammatory conditions [4].

Human and animal examinations suggested that plant matrices rich in antioxidant and anti-inflammatory compounds could prove efficient in protecting against oxidative stress and excessive inflammation [5–8]. Extensive research examined the effects of plant-based diets on various health outcomes [9,10]. Tree nuts, important plant nutrient sources, are rich

in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), tocols, phytosterols, and polyphenols, essential bioactive phytochemicals with demonstrated antioxidant properties [11]. Several studies consistently showed the antioxidant activity and anti-inflammation potential of the active compounds from tree nut kernels or by-products and their association with a reduced risk for CVD, T2D, cancer, and all-cause mortality [12–15]. Of the different types of nuts, walnuts are especially rich in linoleic acid (18:2n–6), α-linolenic acid (ALA) (18:3n–3), polyphenols, L-arginine, and magnesium [16], a unique phytochemical profile responsible for many beneficial effects. It was suggested that walnuts might modulate neuroplasticity, neuroprotection, and vasodilation of brain arteries [17] or decrease cancer growth, reduce metastasis, and increase cancer cell death via altering tumor gene expression [18].

Several studies have previously linked walnut intake with lipid profile beneficial effects and lowering of reactive oxygen species (ROS) and inflammatory markers in different age groups [19–21].

Contrary to the above results, a recent meta-analysis found no associations between walnut consumption and glucose homeostasis as well as inflammation [22]. Moreover, increasing dietary ALA intake did not affect inflammatory markers [23].

Based on these conflicting conclusions, we aimed to perform a systematic review and meta-analysis of randomized controlled trials (RCTs) to thoroughly assess the data concerning the effects of walnut intake on selected markers of inflammation and metabolic syndrome in mature adults. As the exact etiology of chronic inflammation and its potential causal function in unfavorable health outcomes are mostly unknown, research on markers of inflammation and the identification of pathways to control age-associated inflammation is of great relevance for the prevention of inflammation and management of age-associated diseases. To the best of our knowledge, this is the first meta-analysis conducted on the impact of walnut consumption on markers of inflammation and metabolic syndrome in middle-aged and older adults.

2. Materials and Methods

The current meta-analysis was performed following the PRISMA criteria guidelines [24]. The registration code is INPLASY202260058, with DOI 10.37766/inplasy2022.6.0058, https://inplasy.com/inplasy-2022-6-0058/ (accessed on 13 June 2022).

2.1. Eligibility Criteria

Our systematic review included (1) randomized controlled parallel or crossover trial studies that compared the effect of (2) walnuts consumption, (3) with a minimum 3-week intervention period in (4) middle-aged and older adults (≥40 years of age or mean age ≥ 50 years), (5.a) on MetS biomarkers, including waist circumference (WC), body weight (BW), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol (HDL-C), low-density lipoprotein (LDL) cholesterol (LDL-C), fasting blood glucose (FBG), and glycosylated hemoglobin A1c (HbA1c), as well as on the insulin resistance index (homeostatic model assessment for insulin resistance (HOMA-IR) and insulin), and on (5.b) inflammatory biomarkers, including C-reactive protein (CRP), high-sensitivity C-reactive protein (hs-CRP), interferon gamma (IFN-γ), E-selectin, VCAM-1, ICAM-1, TNF-α, and interleukins (IL-6 and IL-1β), as primary or secondary outcomes. We excluded: (1) abstracts, narrative reviews, comments, opinions, methodological papers, editorials, letters, observational studies, conference abstracts, case studies, in vitro studies, non-human, with a mechanistic, non-stochastic modeling, or any other publications lacking primary data and/or explicit method explanations; (2) irrelevant interventions (walnuts oil, walnut extract, nut mix); (3) irrelevant comparisons (compulsory comparison); (4) publications with full text not available; (5) duplicate studies or databases; and (6) publications in languages that were not known.

2.2. Information Sources

We performed a systematic literature search in PubMed, EMBASE, Cochrane Library, Scopus, and ClinicalTrials.gov databases for controlled trials describing the effects of walnut consumption on metabolic syndrome and inflammatory biomarkers in mature adults from the inception of each database through November 2021. The literature search had no language constraint. To ensure thorough research, the bibliographies of the included studies and current reviews were also screened.

2.3. Search Strategy

To search the databases, we used a combination of free-text words, along with their synonyms, singular and plural forms, thesaurus words (Medical Subject Headings for PubMed, and Emtree for EMBASE), and abbreviations concerning the following concepts: (1) walnuts; (2) inflammatory biomarkers, C-reactive protein, interleukins, tumor necrosis factor, vascular cell adhesion molecule, intercellular adhesion molecule, selectin, adiponectin, adhesion molecules; (3) metabolic syndrome, waist circumference, weight, body mass index, systolic and diastolic blood pressure, triglycerides, total, HDL-C and LDL-C, glycemia, HbA1c, insulin resistance, HOMA-IR, insulin; and (4) randomized controlled trial. The entire search strategy for each database is presented in Supplementary Table S1.

2.4. Selection Process

Three investigators (D.L., L.M., and D.-S.P.) independently checked the titles and abstracts for relevant articles. Following that, the full texts of those that looked to satisfy the selection criteria were retrieved for further selection. The same investigators independently checked each full text. In the event of a disagreement, the studies were debated until a consensus was reached. In the instance of multiple publications from the same trial, only the most recent or informative article was selected.

2.5. Data Items

Data regarding the outcomes were extracted in a spreadsheet Microsoft (Microsoft Office 365, MS, Redmond, WA, USA) Excel file: (1) inflammatory biomarkers, C-reactive protein, interleukins, tumor necrosis factor, vascular cell adhesion molecule, intercellular adhesion molecule, selectin, adiponectin, adhesion molecules; (2) metabolic syndrome, waist circumference, weight, body mass index, systolic and diastolic blood pressure, triglycerides, total, HDL and LDL cholesterol, glycemia, HbA1c, insulin resistance, HOMA-IR, insulin. For each variable, the baseline, final, and differences between baseline and final observations were extracted, as well as the differences between the interventions regarding the final values or the differences between baseline and final observations.

Furthermore, data regarding study characteristics were extracted in a spreadsheet file: country, study design, exposure period, washout period, participants number in each group, health status, age, female percentage, walnut intervention quantity and type, control intervention, and the outcome of interest.

Other investigators than those who extracted the initial full-text articles rechecked the extracted data.

2.6. Study Risk of Bias Assessment

The risk of bias was assessed for each selected article using the Risk of Bias 2 Tool from Cochrane [25] in duplicate, and the disagreements were resolved by discussion.

2.7. Effect Measures

For all the outcomes, we used the standardized mean difference in the synthesis and presentation of results.

2.8. Synthesis Methods

We calculated the means and standard deviations for each variable utilized in the meta-analysis. When the standard deviation (SD) was not known, it was calculated using the standard error (SE) or mean, medians and interquartile ranges (IQRs), confidence intervals (CIs), or *p*-values, according to Cochrane Handbook recommendations [26]. The differences between the intervention groups in terms of changes (baseline–final values) were the preferred values in analyses. Otherwise, we computed the differences between the final values if these data were unavailable for the changes. We calculated the mean difference (between changes or between final values) and the SE for each trial, either parallel or crossover, in order to be able to pool the results from both designs, as recommended by Elbourne et al. [27]. The meta software was used to perform meta-analyses on these mean differences and SE [28]. The standardized mean difference along with 95% CI was computed for each variable, using the random effects model due to clinical heterogeneity between the trials. The Paule–Mandel estimator was used to estimate the between-study variance within the inverse variance method. The statistical heterogeneity between the studies was assessed with χ2-based Q-test and I^2. Next, high leverage studies were identified with the dmeta package [29]. Furthermore, subgroup analyses were performed for risk of bias, trial design, exposure duration, walnut quantity, health status, control group, and age, in case more than ten studies were available. To assess the robustness of the results, a leave-one-out sensitivity analysis was used. If the *p*-value was less than 0.05, statistical significance was assumed. For all analyses, the R environment for statistical computing and graphics (R Foundation for Statistical Computing, Vienna, Austria) version 4.1.2 [30] was used.

2.9. Quality Assessment

We used the Cochrane Collaboration's Risk of Bias Tool 2 to examine the selected studies: the parallel trial version for the parallel studies and the crossover trial version for the crossover studies.

2.10. Reporting Bias Assessment

In case there were more than ten studies available to analyze a variable of interest, a funnel plot and the Egger test were performed to assess the presence of publication bias.

3. Results

A total of 685 articles were considered from the systematic search and review of relevant reference lists. After applying exclusion criteria, 17 articles were included in the systematic review and meta-analysis. The procedure of study inclusion and exclusion is shown in Figure 1. The characteristics of the included studies are revealed in Table 1 and Supplementary Table S2.

3.1. Metabolic Syndrome Biomarkers

The effects of walnut-enriched diets on the biomarkers of MetS and inflammation are presented in Table 2.

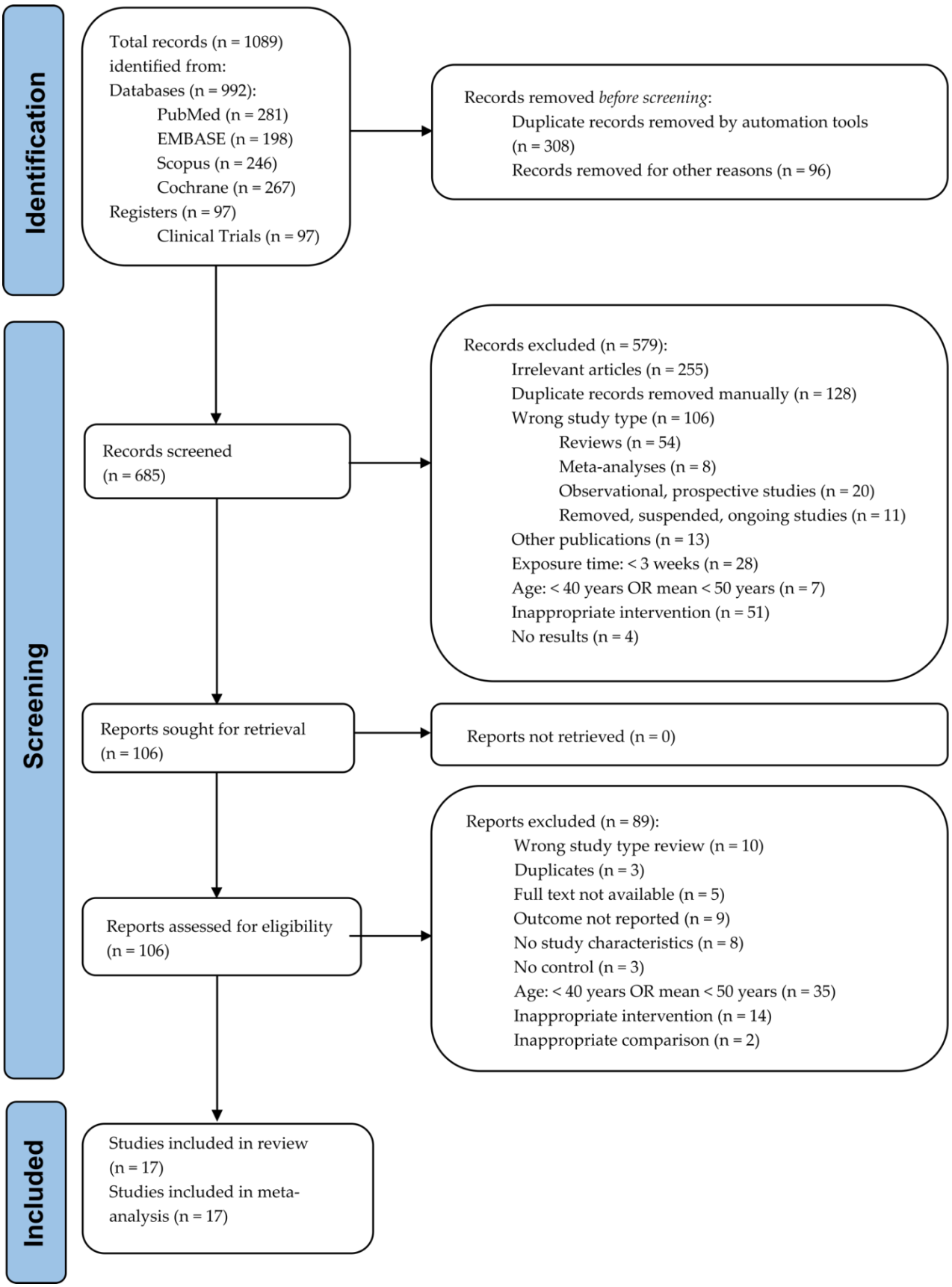

Figure 1. PRISMA flow diagram of study selection.

Table 1. Characteristics of the selected studies.

Reference	Country	Study (RCT) Design	Exposure Period	Washout Period	Participants (*n*), Health Status	Age (Years), (SD)/(IQR) (Range)	Female (%)	Walnut Intervention (g/d)	Control Intervention	Outcome of Interest
Zambón et al., 2000 [31]	Spain, USA	Crossover	6 weeks	0	49 polygenic hypercholesterolemia *	56 (±11)	47%	41–56 g/d (18% of the energy need)	MedD (no walnut)	BW, TC, LDL-C, HDL-C, TG
Ros et al., 2004 [32]	Spain	Crossover	4 weeks	0	20 healthy, non-smokers (hypercholesterolemia)	55 (±55.9)	60%	40–65 g/d (18% of energy need)	MedD (no walnut)	BW, SBP, DBP, TC, LDL-C, HDL-C, CRP,
Tapsell et al., 2004 [33]	Australia	Parallel	6 months	NA	58 T2D *	59.3 (±8.1)	41.37%	30 g/d—walnut-enriched modified low-fat diet	Modified low-fat diet (no walnuts)	BW, BMI, HbA1c, TC, LDL-C, HDL-C, TG
Olmedilla-Alonso et al., 2008 [34]	Spain	Crossover	5 weeks	1 month	25 CV risk, smokers	54.4 (±8.1)	40%	19.4 g/d (20% walnut-enriched meat products)	Restructured meat products (no walnut)	TC, HDL-C, LDL-C, TG, BW, SBP, DBP
Spaccarotella et al., 2008 [35]	USA	Crossover	8 weeks	2 weeks	21 healthy, non-smokers	65.9 (55–75)	0%	75 g/d (24% of energy need)	Western-type diet (no walnut)	SBP, DBP, TC, HDL-C, LDL-C
Tapsell et al., 2009 [36]	Australia	Parallel	1 year	NA	50 T2D *	54 (±8.7)	NI	30 g/d (walnut-enriched 2000 kcal diet, 30% fat)	2000 kcal diet, 30% fat (no walnut)	BW, FBG, TC, HDL-C, LDL-C, TG, HbA1c, insulin
Ma et al., 2010 [37]	USA	Crossover	8 weeks	8 weeks	21 T2D, non-smokers	58.1 (±9.2)	58.30%	56 g/d	Habitual diet (no walnut)	TC, HDL-C, LDL-C, TG, FPG, insulin, HOMA-IR, BW, BMI, WC, SBP, DBP
Torabian et al., 2010 [38]	USA	Crossover	6 months	0	87 healthy, non-smokers	54 (±10.2)	56%	28–64 g/d (12% of energy need)	Habitual diet (no walnut)	TC, LDL-C, HDL-C, TG
Canales et al., 2011 [39]	Spain	Crossover	5 weeks	4–6 weeks	22 CV risk, smokers	54.8 (±9.4)	40%	34–29 g/d (20% walnut-enriched meat)	Low-fat meat products (no walnut)	VCAM-1, ICAM-1, HDL-C
Katz et al., 2012 [40]	USA	Crossover	8 weeks	4 weeks	40 healthy, non-smokers (overweight, MetS risk)	57.4 (±11.9)	60.9%	56 g/d	Habitual diet (no walnut)	TC, HDL-C, LDL-C, TG, FPG, insulin, HOMA-IR, BW, BMI, WC, SBP, DBP
Wu et al., 2014 [41]	Germany, USA	Crossover	8 weeks	2 weeks	40 healthy *	60 (±6.32)	75%	43 g/d (replacing 30 g saturated fat in Western-type diet)	Western-type diet (no walnut)	TC, LDL-C, HDL-C, FBG, insulin, HOMA-IR, HbA1c, VCAM-1, ICAM-1
Bamberger et al., 2017 [42]	Germany	Crossover	8 weeks	4 weeks	194 healthy, non-smokers	63 (±7)	69%	43 g/d	Western-type diet (no walnut)	TC, LDL-C, HDL-C, TG
Bitok et al., 2018 [43]	USA, Spain	Parallel	2 years	NA	307 healthy *	69.4 (±3.9)	67%	28; 42; 56 g/d (15% of energy need)	Habitual diet (no walnut)	BW, WC
Domènech et al., 2019 [44]	USA, Spain	Parallel	2 years	NA	236 healthy * (60% mild hyper-tension)	68.8 (±3.3)	65%	30–60 g/d, (15% of energy need)	Habitual diet (no walnut)	SBP, DBP

Table 1. *Cont.*

Reference	Country	Study (RCT) Design	Exposure Period	Washout Period	Participants (*n*), Health Status	Age (Years), (SD)/(IQR) (Range)	Female (%)	Walnut Intervention (g/d)	Control Intervention	Outcome of Interest
Sanchis et al., 2019 [45]	Spain	Crossover	30 days	30 days	13 CKD *	71 (±10.11)	46.20%	30 g/d (walnut-enriched CKD diet)	CKD patients' diet (no walnut)	BMI, TC, HDL-C, LDL-C, TG, FBG, HbA1c, CRP
Abdrabalnabi et al., 2020 [46]	USA, Spain	Parallel	2 years	NA	625 healthy *	69.1 (±3.6)	67%	30; 45; 60 g/d (15% of energy need)	Habitual diet (no walnut)	BMI, SBP, DBP, TG, HDL-C, FBG
Cofán et al., 2020 [47]	USA, Spain	Parallel	2 years	NA	634 healthy *	69.1 (±3.6)	66%	30; 45; 60 g/d (15% of energy need)	Western-type diet (no walnut)	VCAM-1, ICAM-1, IL-6, IFN-γ, IL-1β, TNF-α, E-selectin, hs-CRP

*—non-specified smoking status; RCT—randomized controlled trials; NA—not applicable; BMI—body mass index; BW—body weight; CKD—chronic kidney disease; CV—cardiovascular; CRP—C-reactive protein; hs-CRP—high-sensitivity C-reactive protein; DBP—diastolic blood pressure; FBG—fasting blood glucose; HbA1c—glycosylated hemoglobin A1c; HDL-C—high-density lipoprotein cholesterol; HOMA-IR—homeostatic model assessment for insulin resistance; ICAM—intercellular adhesion molecule; IFN-γ—interferon gamma; IL-1β—interleukin-1β; IL-6—interleukin-6; IQR—interquartile range; LDL-C—low-density lipoprotein cholesterol; MedD—Mediterranean diet; MetS—metabolic syndrome; NI—no information; SBP—systolic blood pressure; SD—standard deviation; T2D—type 2 diabetes; TC—total cholesterol; TG—triglycerides; TNF-α—tumor necrosis factor-alpha; VCAM—the vascular cell adhesion molecule; WC—waist circumference.

Table 2. Effects of walnut-enriched diets on inflammatory and metabolic syndrome biomarkers.

Characteristic, Effect Size Type, SMD	Effect Size (95% CI)	*p*-Value	I^2 (95% CI)	*p*-Value	Egger Test	Studies
CRP (mg/L)	−0.37 (−1.39–0.65)	0.478	NC		NC	[32,45]
hs-CRP (mg/L)	−0.01 (−0.12–0.11)	0.903	NC		NC	[47]
IFN-γ (pg/mL)	−1.26 (−2.01–−0.51)	<0.001	NC		NC	[47]
IL-6 (pg/mL)	−0.18 (−0.33–−0.03)	0.021	NC		NC	[47]
IL-1β (pg/mL)	−0.1 (−0.16–−0.04)	<0.001	NC		NC	[47]
TNF-α (pg/mL)	−0.31 (−0.54–−0.08)	0.009	NC		NC	[47]
E-selectin (ng/mL)	−2.57 (−4.09–−1.05)	<0.001	NC		NC	[47]
ICAM-1 (ng/mL)	−0.02 (−0.11–0.07) ANC	0.672	-	-	-	[39,41,47]
VCAM-1 (ng/mL)	−0.11 (−0.32–0.1) ANC	0.305	-	-	-	[39,41,47]
WC (cm)	−0.14 (−0.8–0.51)	0.671	0 (0–89.6)	0.71	0.572	[37,40,43]
BMI (kg/m^2)	0.11 (−0.11–0.34)	0.326	63.1 (2.4–86)	0.028	0.683	[33,37,40,45,46]
BW (kg)	0 (−0.4–0.39)	0.987	22.2 (0–64.1)	0.253	0.537	[31–34,36,37,40,43]
SBP (mmHg)	−0.85 (−4.48–2.77)	0.644	64.4 (24–83.4)	0.006	0.699	[32,34,35,37,40,44–46]
DBP (mmHg)	−0.34 (−1.68–1)	0.62	35.3 (0–71.4)	0.146	0.551	[32,34,35,37,40,44–46]
FBG (mg/dL)	0.01 (0–0.02)	0.088	0 (0–74.6)	0.692	0.57	[36,37,40,41,45,46]
TG (mg/dL)	−7.41 (−10.89–−3.94)	<0.001	99.1 (99–99.3)	<0.001	0.264	[31–38,40–42,45,46]
TC (mg/dL)	−5.22 (−7.64–−2.8)	<0.001	97.4 (96.5–98.1)	<0.001	0.375	[31,32,34–38,40–42,45]
HDL-C (mg/dL)	−0.18 (−0.59–0.22)	0.375	47.4 (0–72.4)	0.029	0.507	[31–42,45,46]
LDL-C (mg/dL)	−5.93 (−7.77–−4.09)	<0.001	24.8 (0–61.8)	0.2	0.83	[31–38,40–42,45]
HbA1c (%)	0.08 (−0.04–0.2)	0.196	0 (0–84.7)	0.774	0.816	[33,36,41,45]
HOMA-IR	0.03 (−0.44–0.5)	0.891	57.1 (0–87.8)	0.097	0.95	[37,40,41]
Insulin (mIU/mL)	0.91 (−2.16–3.98)	0.561	65.4 (0–88.2)	0.034	0.505	[36,37,40,41]

ANC—algorithm did not converge (when study [39] was entered; thus, the result is based only on studies [41,47]); BMI—body mass index; BW—body weight; CI—confidence interval; CRP—C-reactive protein; hs-CRP—high-sensitivity C-reactive protein; DBP—diastolic blood pressure; FBG—fasting blood glucose; HbA1c—glycosylated hemoglobin A1c; HDL-C—high-density lipoprotein cholesterol; HOMA-IR—homeostatic model assessment for insulin resistance; ICAM—intercellular adhesion molecule; IFN-γ—interferon gamma; IL-1β—interleukin-1β; IL-6—interleukin-6; LDL-C—low-density lipoprotein cholesterol; NC—not computed for less than three studies; SBP—systolic blood pressure; SD—standard deviation; SMD—standardized mean change difference; TC—total cholesterol; TG—triglycerides; TNF-α—tumor necrosis factor-alpha; VCAM—vascular cell adhesion molecule; WC—waist circumference.

3.1.1. Triglycerides

From the selected studies, thirteen studies reported TG values. The meta-analysis found a higher reduction in TG values in the walnut group compared to the control group (SMD = −7.41 (95% CI: −10.89–−3.94), $p < 0.001$) (Figure 2). There was a significant heterogeneity between the studies, I^2 of 99.1% (95% CI: 99–99.3%), and the Q test for heterogeneity gave $p < 0.001$. The results remained statistically significant after performing a leave-one-out sensitivity analysis for each study. The studies that influenced the final result the most were Tapsell et al. (2009) [36] and Abdrabalnabi et al. [45]; their removal brought the I^2 to values lower than or equal to 7%. A subgroup analysis found that studies with a high risk of bias had higher reductions in TG values than studies with some concerns or low risk of bias, the pooled result remaining statistically significant only for high risk of bias studies (Figure 3). The subgroup analyses regarding treatment exposure duration, study population health, and diet showed statistically significant results for each subgroup (Supplementary Figures S1–S4). The subgroup exposed to walnut portions greater than 42 g/day had statistically significant results, but for those having lower portions, the pooled result lost its significance (Supplementary Figure S5). Finally, for the crossover studies subgroup, the final result was statistically significant, but not for the parallel studies subgroup (Supplementary Figure S6).

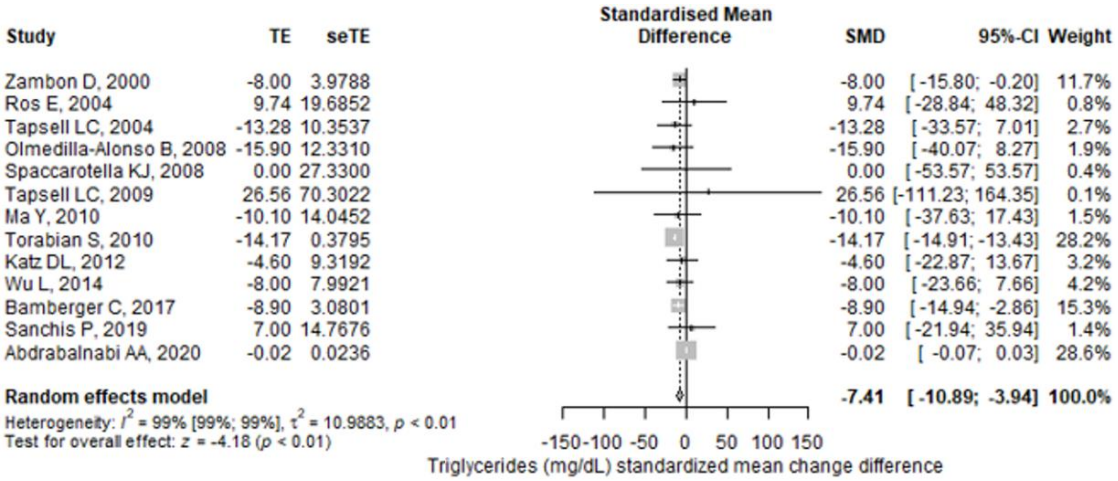

Figure 2. Forest plot for triglycerides (mg/dL) standardized mean change difference. TE—treatment effect; seTE—the standard error of the treatment effect; SMD—standardized mean difference; CI—confidence interval.

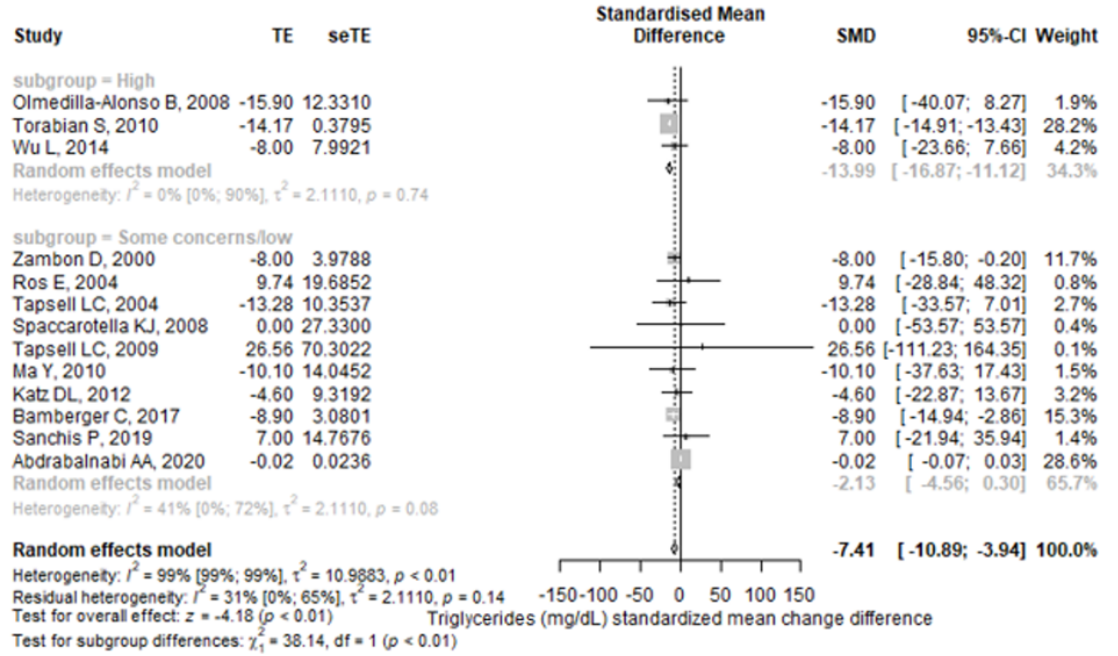

Figure 3. Forest plot for triglycerides (mg/dL) standardized mean change difference compared with subgroup analyses for risk of bias. TE—treatment effect; seTE—the standard error of the treatment effect; SMD—standardized mean difference; CI—confidence interval.

3.1.2. Total Cholesterol, LDL, and HDL Cholesterol

Results for mean differences in TC and LDL-C between intervention and control groups were reported in 12 trials (10 crossover and 2 parallel). We noticed significantly lower values for TC concentrations in walnut-enriched diets compared to control diets (SMD = −5.22, 95% CI: −7.64–−2.8), $p < 0.001$) (Figure 4), with significant heterogeneity between the experiments (I^2 = 99.1%; 95% CI: 99–99.3%, p-heterogeneity < 0.001).

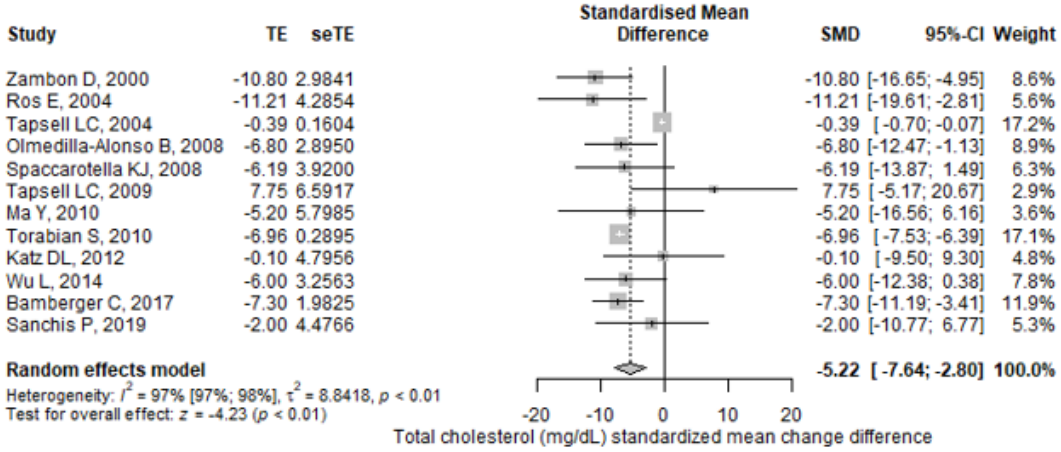

Figure 4. Forest plot for total cholesterol (mg/dL) standardized mean change difference. TE—treatment effect; seTE—the standard error of the treatment effect; SMD—standardized mean difference; CI—confidence interval.

Similarly, the meta-analyzed SMD displayed a significantly greater reduction in LDL-C concentrations with the walnut diets than with the control diets (SMD = −5.93; 95% CI: −7.77–−4.09, $p < 0.001$) (Figure 5), but without significant heterogeneity (I^2 = 24.8%; 95% CI: 0–61.8%, p-heterogeneity = 0.2).

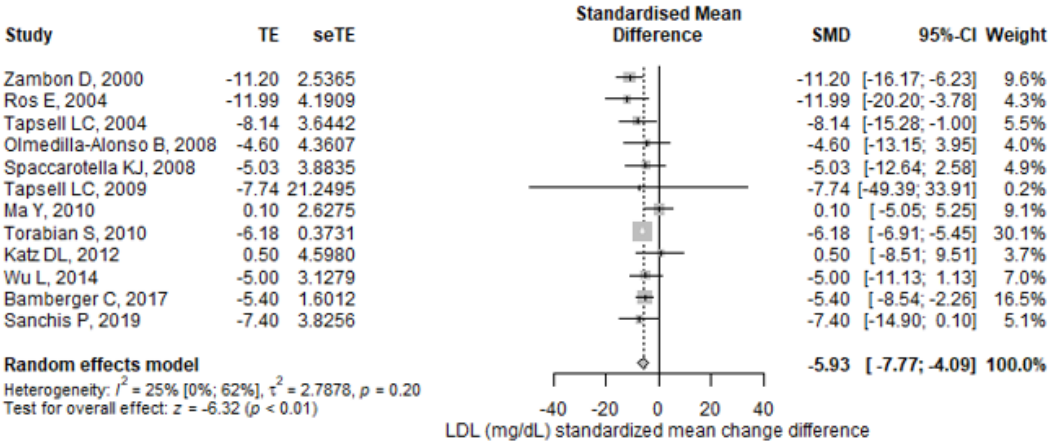

Figure 5. Forest plot for LDL cholesterol (mg/dL) standardized mean change difference. TE—treatment effect; seTE—the standard error of the treatment effect; SMD—standardized mean difference; CI—confidence interval.

Sensitivity analyses showed that the outcomes remained statistically significant after removing one study at a time for both parameters. The reports with the highest influence on the final effects were Tapsell et al. (2004) [33] and Tapsell et al. (2009) [36] for TC, while for LDL-C they were Torabian et al. [38] and Tapsell et al. (2009) [36].

In the subgroup analyses, for TC and LDL-C parameters, walnut diets had statistically significant effects in high risk and some concern studies for risk of bias, as well as for exposure duration, walnut quantity, population health, and diet in both study designs, parallel and crossover. The results remained significant for the participant subgroup with ages over 40 years (Supplementary Figures S7–S19).

Fourteen controlled trials documented results for HDL-C. There were no statistically significant changes in HDL-C concentrations between the walnut and the control diets (SMD = −0.18; 95% CI: −0.59–0.22, p = 0.375). However, a significant heterogeneity was reported (I^2 = 47.4%; 95% CI: 0–72.4%, p-heterogeneity = 0.029) (Supplementary Figure S20).

3.1.3. Anthropometric Markers

WC, BMI, and BW changes were reported in three, five, and eight trials, respectively. On these parameters, individual trials did not show significant differences compared to the control after following a walnut-enriched diet (WC SMD = −0.14; 95% CI: −0.8–0.51, $p = 0.671$; BMI SMD = 0.11; 95% CI: −0.11–0.34, $p = 0.326$; BW SMD = 0; 95% CI: −0.4–0.39, $p = 0.987$). A significant heterogeneity was observed only for BMI ($p = 0.028$) (Supplementary Figures S21–S23).

3.1.4. Blood Pressure

The effect on blood pressure was analyzed in eight studies (six crossover and two parallel). Walnut-enhanced diets did not significantly modify SBP (SMD = −0.85; 95% CI: −4.48–2.77, $p = 0.644$) or DBP (SMD = −0.34; 95% CI: −1.68–1, $p = 0.62$), with significant heterogeneity for SBP ($I^2 = 64.4\%$; 95% CI: 24–83.4%, p-heterogeneity = 0.006) (Supplementary Figures S24 and S25).

3.1.5. Glycemic Biomarkers

Similarly, no significant reductions were detected for FBG, HbA1c, HOMA-IR, and insulin assessed in six, four, three, and four studies, respectively. Compared with control diets, walnut-enriched diets accounted for a non-significant decrease of these glycemic markers (FBG SMD = 0.01; 95% CI: 0–0.02, $p = 0.088$; HbA1c SMD = 0.08; 95% CI: −0.04–0.2, $p = 0.196$; HOMA-IR SMD = 0.03; 95% CI: −0.44–0.5, $p = 0.891$; insulin SMD = 0.91; 95% CI: −2.16–3.98, $p = 0.561$). The Q test for heterogeneity gave a significant value only for insulin ($p = 0.034$) (Supplementary Figures S26–S29).

3.2. Inflammatory Biomarkers

In the meta-analysis of the inflammatory markers, the walnut consumption revealed no significant influence on CRP (SMD = −0.37; 95% CI: −1.39–0.65, $p = 0.478$) and hs-CRP (SMD = −0.01; 95% CI: −0.12–0.11, $p = 0.903$) (Supplementary Figures S30 and S31).

For the other studied inflammatory biomarkers, the walnut diet showed significant changes (IFN-γ SMD = −1.26; 95% CI: −2.01–−0.51, $p < 0.001$; IL-6 SMD = −0.18; 95% CI: −0.00–−0.03, $p < 0.001$; IL-1β SMD = −0.1; 95% CI: −0.16–−0.04, $p < 0.001$; TNF-α SMD = −0.31; 95% CI: −0.54–−0.08, $p = 0.009$; E-selectin SMD = −2.57; 95% CI: −4.09–−1.05, $p < 0.001$), but the publication bias test and the heterogeneity could not be calculated since there was only one assessed study (Supplementary Figures S32–S36).

The endothelial adhesion molecules, ICAM-1 and VCAM-1, could not be computed since the algorithm did not converge when the study of Canales et al. [39] was included in the analysis. When we excluded this analysis, the results based on the studies of Wu et al. and Cofan et al. [41,47] were not statistically significant.

3.3. Quality Assessment

The results obtained after quality (risk of bias) assessment for the six parallel and eleven crossover studies are presented in the Supplementary Materials (Supplementary Figures S37 and S38). Several papers [43,44,46,47] analyzed data from the same study, Walnuts and Healthy Aging (WAHA), and for the quality assessment they were considered as only one.

Concerning the randomization process domain, eleven studies (79%) had some concerns of bias, and three were at low risk of bias. The randomization generation method was presented in five studies. Only one study mentioned allocation concealment. Only two studies (14%) explained how randomization was undertaken. For crossover studies, seven had no information to assess the start of clinical study baseline differences, and three probably did not have differences, while for parallel trials, all four probably did not have differences.

For crossover trials, we assessed the risk of bias arising from period and carryover effects. Four studies had some concerns of bias, and seven were at low risk of bias. Five

studies had a similar number of subjects allocated to the interventions. Two studies probably did not have important differences in the number of subjects allocated to the interventions. The other studies reported no information. Five studies did not analyze whether the period effect was verified. All studies had sufficient time for the disappearance of any carryover effects before the outcome assessment in the second period.

Regarding deviations from the intended interventions, four studies were at high risk of bias, six had some concerns of bias, and four were at low risk of bias. Although not mentioned in all trials, participants, caregivers, and those administering interventions were likely all aware of the assigned intervention, except for one study where the investigator was blinded to the intervention. The studies did not mention whether deviations from the intended intervention arose due to trial context, except in one study where those deviations probably did not affect the outcome. Five studies mentioned or it could be deducted that they used an intention-to-treat analysis. Five studies gave no information about the use of an intention-to-treat analysis, and the other four stated or it could be deducted that they used a per-protocol analysis. Only one study was impacted by the lack of intention-to-treat analysis of the results.

Moreover, only one study had some concern of bias with respect to missing outcome data domain; the others had a low risk of bias. Seven studies probably had data for all or nearly all randomized participants. Six studies had important percentages of subjects that dropped out. One study did not report anything about missing data. No study provided missing data analysis or sensitivity analyses to demonstrate that missing data did not skew the results. In all the research, it is more likely to conclude that the missingness of the outcomes was unrelated to its genuine value.

Concerning the measuring of the outcome domain, all the studies were at low risk of bias. All of the studies used the same instruments and standard and exact measuring methods to test the outcomes at the same time points throughout their research (laboratory assays or anthropometric measurements). In the case of laboratory measurements, it is likely the measurement was blinded (only three studies reported it). It is unlikely that knowing the intervention would influence the measurement.

Considering the selection of the reported result domain, all the studies had a low risk of bias. Two studies had variables of interest for our review specified as the primary endpoint in the research protocol. Four studies had research protocols published before their study but with different primary endpoints compared to our review. Only one instrument and one statistical analysis approach were employed in all of the investigations for each variable of interest.

Overall, four studies were considered at high risk of bias, and the others showed some concerns of bias.

3.4. Reporting Bias Assessment

The Egger test yielded non-statistically significant findings for all of the outcomes of interest when it was used to examine the presence of publication bias. Moreover, funnel plots were not indicative of asymmetry either.

4. Discussion

To the best of our knowledge, the current study is the first systematic review and meta-analysis to focus on comprehensively analyzing the evidence to date regarding the effects of walnut-enriched diets on biomarkers of MetS and inflammation in middle-aged and older adults.

Walnut is considered a nutraceutical dietary source due to the high content of good fatty acids, such as MUFA and omega-3 PUFA, its nutritional value, the high antioxidant phytochemical content, and its beneficial effects on human health.

In the present meta-analysis, we assessed the results of seventeen randomized clinical trials that analyzed the impact of walnut-enriched diets. Our findings showed that walnut-enriched diets significantly decreased TG, TC, and LDL-C concentrations, while HDL-C

level was not significantly affected. No significant changes were noticed on anthropometric, cardiometabolic, and glycemic indices after higher walnut consumption. Moreover, the inflammatory biomarkers did not record statistically significant results.

Considering the evidence from recent meta-analyses, nut consumption [48–50] and walnut-enriched diets [22,51] are negatively associated with specific biomarkers of MetS and inflammation in different age groups.

Regarding the duration of exposure to treatment (in a range between 4 weeks and 2 years), the health of the studied population (healthy people, hypertensive, hypercholesterolemic, or T2D patients), and the diet (mostly Western-type or habitual diet, without walnuts), each subgroup presented statistically significant results. Different doses of walnut showed that the subgroup exposed to walnut portions greater than 42 g per day had statistically significant results. This result reinforces the Food and Drug Administration (FDA) [52] recommendation for the inclusion of 42 g (1.5 ounces) of walnuts in the daily diet and differs from the conclusions of another meta-analysis, which states that the TG lowering effects reach a plateau at doses higher than 20 g [53].

Our meta-analysis identified a statistically significant reduction of TG values ($p < 0.001$) in walnut consumption groups compared to control groups in the thirteen trials analyzed for this marker. Furthermore, it showed statistically significant decreases in terms of TC and LDL-C levels. Analyzing the twelve studies reporting results for mean differences in TC and LDL-C, we noticed significantly lower values for TC concentrations in walnut-enriched diets ($p < 0.001$) compared to control diets. Similarly, we registered a significantly greater reduction in LDL-C concentrations with the walnut diets ($p < 0.001$) than with the control diets. The statistically significant beneficial effects in the lipid profile noticed after walnut-enriched diets have the potential of decreasing the age-related disease risks for the age category targeted in this meta-analysis.

Several observational studies obtained the same answers. A cross-sectional study analyzing data from three large US prospective cohort studies concluded that an increase of 0.5 servings (~14 g) per day in walnut consumption was significantly associated with 17% lower CVD risk and 20% lower stroke risk [54]. After assessing the same data but with slightly different covariates, another study found that consuming at least one serving (~28 g) of walnuts per week was linked with 19% lower CVD risk and 17% lower stroke risk, in addition to a 21% decrease in the risk of CHD [55]. Similarly, a recent systematic review and meta-analysis of prospective studies revealed that higher walnut intake was associated with lower risks of CVD and CHD incidence [56]. Moreover, data from two large prospective cohort studies associated higher walnut consumption with a lower CVD risk and mortality and a greater life expectancy among U.S. older adults [57]. The PREDIMED study also disclosed a significantly lower risk of stroke in participants who consumed 30 g of mixed nuts (including 15 g of walnuts) per day compared with a no-nut consumer group [58].

Based on our results, the improvement of the lipid profile and decrease of oxidative stress and inflammation are primary mechanisms of walnut intake against CVD. Furthermore, bioactive compounds found in walnuts, both hydrophilic and lipophilic, could protect against MetS complications and CVD [15].

Thereby, polyphenols, hydrosoluble micronutrients found in walnuts such as quercetin and its glycosides, ellagic acid and ellagitannins, and cyanidin and proanthocyanidins [59] exert their antioxidant action through multiple mechanisms, including the activation of the Nrf2/ARE (nuclear factor erythroid 2-related factor 2/antioxidant response element) pathway. By this pathway, polyphenols increase the activity of some antioxidant and detoxifying enzymatic systems and down-regulate the nuclear factor kappa B (NF-κB) pathway that is directly implicated in the inflammatory response. Tocopherols and tocotrienols, as well as n-3 PUFAs and n-6 PUFAs and other lipophilic antioxidants from walnuts, can also inhibit the NF-κB pathway by activation of Nrf2/ARE. By preventing oxidation of LDL, antioxidants improve the lipid profile, preventing and reducing the formation of atherosclerotic plaques and the risks for CVD [11]. Melatonin, found in minute quantities in walnuts (3.5 ± 1.0 ng/g), holds antioxidant and anti-inflammatory properties, with CV

protection [16]. Moreover, phytosterols from walnuts can lower LDL-C levels. They are more hydrophobic than cholesterol and can dislocate cholesterol from intestinal micelles and reduce LDL-C absorption. In combination with n-3 PUFAs, phytosterols show both complementary and synergistic lipid-lowering effects [16].

In our study, the effect on blood pressure was analyzed in eight trials. Neither SBP nor DBP was significantly modified by walnut-enhanced diets, confirming the results of previous analyses [53,55,60]. Furthermore, our study did not show statistically significant changes in terms of glycemic markers, which also corroborated prior studies [22,51,53]. After following walnut-enriched diets, the anthropometric parameters did not show significant differences compared to the control. These results were consistent with those obtained in former works [49,61].

Low-grade chronic inflammation, referred to as inflammaging in the older population, plays a key role in atherosclerosis, while inflammation biomarker concentrations can predict future T2D or CVD events [62]. The results of our study showed no significant effects of walnut intake on inflammatory markers. These findings concur with recently published data showing that the hs-CRP level was not influenced by walnut consumption [53]. Moreover, our findings agree with a recently published meta-analysis of both interventional and observational studies, which established that walnut intake had no statistical significance on glucose homeostasis and inflammation [22]. In contrast, observational studies found that nut consumption was inversely associated with inflammatory markers [63]. These findings might point to other types of nuts being responsible for these positive effects. However, Cofán et al. (2020) [47] are the only researchers who have studied several biomarkers of inflammation in correlation with a walnut-diet and found statistically significant reduction for IL-6, IFN-γ, IL-1β, TNF-α, and E-selectin, but not for hs-CRP and adhesion molecules VCAM-1 and ICAM-1. These results are noteworthy, but further clinical trials are needed to confirm them.

The negative relationship between walnut intake and MetS pathophysiology may also be attributed to the antioxidant and anti-inflammatory activity of vitamin E [62] and other antioxidant phytochemicals found in walnuts [64].

Similar to other studies, our meta-analysis presents several limitations. The most important limitation concerns the risk of bias present in the selected studies. Blinding participants and personnel in the case of walnut eating is clearly challenging, particularly for participants, and was not performed in the studies. Nonetheless, because the majority of the outcomes of interest are objective laboratory measurements, this methodological shortcoming is less likely to impact the measurement of the findings. The absence of any declaration on allocation concealment (just one study mentioned it) and the randomization process (stated in five studies) is the most critical issue. The Cochrane Risk of Bias Tool 2 has a dose of subjectivity in the assessment, and we deemed most of the studies to have some concerns of bias. However, if there had been no allocation concealment, in reality, the trials would have been regarded as having a high risk of bias overall. This is more troublesome for parallel designs, although they only accounted for roughly a third of the total in our assessment. Nevertheless, we performed subgroup analyses for studies with high bias and some concerns of bias and the main results of our review remained statistically significant in both cases. Another disadvantage is the relatively small number of individuals per research; nevertheless, systematic inclusion of a large number of publications helps to increase overall power. We had a long list of potential outcomes, but only a few papers provided measurements for several of them. For some outcomes, there was an important heterogeneity, but after the sensitivity leave-one-out analysis, they seemed robust and remained statistically significant.

Additionally, our review has several strengths: (1) the publications' methodological flaws were assessed using the newest edition of the Cochrane Collaboration's Risk of Bias Tool, version 2, one of the most prestigious organizations that conducts systematic reviews and creates high-quality instruments for study validity evaluation; (2) a comprehensive search strategy was used; (3) many databases (PubMed, Embase, Scopus, Cochrane

Database) were searched; (4) only randomized controlled trials were included; (5) sensitivity and subgroup analyses were performed; and (6) twenty-two metabolic syndrome and inflammatory markers in middle-aged and older adults were assessed.

Future studies should focus more on inflammatory markers that were assessed in only a small number of studies, but with significant results. The value close to statistical significance level of fasting blood glucose suggests a need for further studies to check if this was a spurious result or a real useful signal. Furthermore, the quality of future randomized controlled trials on walnut diets should be improved, especially regarding allocation concealment, the randomization process, and intention to treat analyses.

5. Conclusions

In conclusion, despite some heterogeneity in the intervention outcomes, our meta-analysis found significant amelioration in the lipid profiles (TG, TC, and LDL-C levels) with walnut consumption compared with different control diets in the studied age category, middle-aged and older adults. Incipient data from a single study [47], which should be further investigated, suggest that long-term walnut consumption displayed potential benefits in lowering inflammation and indirectly on preventing several age-related diseases. Even though further and better-designed studies are needed to strengthen these findings, the results stress the importance of including walnuts in the dietary plans of middle-aged and older populations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox11071412/s1.

Author Contributions: Conceptualization, D.L., M.E.R., L.M. and D.-S.P.; methodology, D.L.; investigation, L.M., M.E.R., D.-S.P., D.L. and I.F.; writing—original draft preparation, D.L., M.E.R. and L.M.; writing—reviewing, and editing, L.M., M.E.R., D.L., D.-S.P. and I.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ALA	α-linolenic acid
apoB	apolipoprotein B
BMI	body mass index
BW	body weight
CI	confidence interval
CI	confidence interval
CRP	C-reactive protein
CVD	cardiovascular diseases
DBP	diastolic blood pressure
eTE	the standard error of the treatment effect
FBG	fasting blood glucose
HbA1c	glycosylated hemoglobin A1c
HDL-C	high density lipoprotein-cholesterol
HOMA-IR	homeostatic model assessment for insulin resistance
hs-CRP	high-sensitivity C-reactive protein
ICAM-1	intercellular adhesion molecule-1
IF	interferon gamma
IL-1β	interleukin-1β
IL-6	interleukin-6
IQR	interquartile ranges
LDL-C	low density lipoprotein-cholesterol
MedD	Mediterranean diet

MetS	metabolic syndrome
MUFAs	monounsaturated fatty acids
NF-κB	nuclear factor kappa B
Nrf2/ARE	nuclear factor erythroid 2-related factor 2/antioxidant response element
PUFAs	polyunsaturated fatty acids
RCT	randomized controlled trial
ROS	reactive oxygen species
SBP	systolic blood pressure
SD	standard deviation
SE	standard error
SMD	standardized mean difference
SMD	standardized mean change difference
T2D	type 2 diabetes
TC	total cholesterol
TE	treatment effect
TG	triglycerides
TNF-α	tumor necrosis factor-alpha
VCAM-1	vascular cell adhesion molecule-1
W	weight
WC	waist circumference

References

1. Rea, I.M.; Gibson, D.S.; McGilligan, V.; McNerlan, S.E.; Denis Alexander, H.; Ross, O.A. Age and Age-Related Diseases: Role of Inflammation Triggers and Cytokines. *Front. Immunol.* **2018**, *9*, 586. [CrossRef] [PubMed]
2. Franceschi, C.; Campisi, J. Chronic Inflammation (Inflammaging) and Its Potential Contribution to Age-Associated Diseases. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2014**, *69*, S4–S9. [CrossRef]
3. Furman, D.; Campisi, J.; Verdin, E.; Carrera-Bastos, P.; Targ, S.; Franceschi, C.; Ferrucci, L.; Gilroy, D.W.; Fasano, A.; Miller, G.W.; et al. Chronic Inflammation in the Etiology of Disease across the Life Span. *Nat. Med.* **2019**, *25*, 1822–1832. [CrossRef] [PubMed]
4. Kirkland, J.L.; Tchkonia, T. Cellular Senescence: A Translational Perspective. *EBioMedicine* **2017**, *21*, 21–28. [CrossRef] [PubMed]
5. Rapa, S.F.; di Iorio, B.R.; Campiglia, P.; Heidland, A.; Marzocco, S. Inflammation and Oxidative Stress in Chronic Kidney Disease—Potential Therapeutic Role of Minerals, Vitamins and Plant-Derived Metabolites. *Int. J. Mol. Sci.* **2020**, *21*, 263. [CrossRef]
6. Popa, D.S.; Bigman, G.; Rusu, M.E. The Role of Vitamin K in Humans: Implication in Aging and Age-Associated Diseases. *Antioxidants* **2021**, *10*, 566. [CrossRef]
7. Ajabnoor, S.M.; Thorpe, G.; Abdelhamid, A.; Hooper, L. Long-Term Effects of Increasing Omega-3, Omega-6 and Total Polyunsaturated Fats on Inflammatory Bowel Disease and Markers of Inflammation: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Eur. J. Nutr.* **2021**, *60*, 2293–2316. [CrossRef]
8. Kiss, B.; Popa, D.-S.; Crişan, G.; Bojiţă, M.; Loghin, F. The Evaluation of Antioxidant Potential of Veronica Officinalis and Rosmarinus Officinalis Extracts by Monitoring Malondialdehide and Glutathione Levels in Rats. *Farmacia* **2009**, *57*, 432–441.
9. Trautwein, E.A.; McKay, S. The Role of Specific Components of a Plant-Based Diet in Management of Dyslipidemia and the Impact on Cardiovascular Risk. *Nutrients* **2020**, *12*, 2671. [CrossRef]
10. Fizeșan, I.; Rusu, M.E.; Georgiu, C.; Pop, A.; Ștefan, M.G.; Muntean, D.M.; Mirel, S.; Vostinaru, O.; Kiss, B.; Popa, D.S. Antitussive, Antioxidant, and Anti-Inflammatory Effects of a Walnut (*Juglans Regia* L.) Septum Extract Rich in Bioactive Compounds. *Antioxidants* **2021**, *10*, 119. [CrossRef]
11. Rusu, M.E.; Simedrea, R.; Gheldiu, A.M.; Mocan, A.; Vlase, L.; Popa, D.S.; Ferreira, I.C.F.R. Benefits of Tree Nut Consumption on Aging and Age-Related Diseases: Mechanisms of Actions. *Trends Food Sci. Technol.* **2019**, *88*, 104–120. [CrossRef]
12. de Souza, R.G.M.; Schincaglia, R.M.; Pimente, G.D.; Mota, J.F. Nuts and Human Health Outcomes: A Systematic Review. *Nutrients* **2017**, *9*, 1311. [CrossRef] [PubMed]
13. Pop, A.; Fizesan, I.; Vlase, L.; Rusu, M.E.; Cherfan, J.; Babota, M.; Gheldiu, A.-M.; Tomuta, I.; Popa, D.-S. Enhanced Recovery of Phenolic and Tocopherolic Compounds from Walnut (*Juglans Regia* L.) Male Flowers Based on Process Optimization of Ultrasonic Assisted-Extraction: Phytochemical Profile and Biological Activities. *Antioxidants* **2021**, *10*, 607. [CrossRef] [PubMed]
14. Ros, E.; Singh, A.; O'Keefe, J.H. Nuts: Natural Pleiotropic Nutraceuticals. *Nutrients* **2021**, *13*, 3269. [CrossRef] [PubMed]
15. Rusu, M.E.; Mocan, A.; Ferreira, I.C.F.R.; Popa, D.S. Health Benefits of Nut Consumption in Middle-Aged and Elderly Population. *Antioxidants* **2019**, *8*, 302. [CrossRef]
16. Ros, E.; Izquierdo-Pulido, M.; Sala-Vila, A. Beneficial Effects of Walnut Consumption on Human Health: Role of Micronutrients. *Curr. Opin. Clin. Nutr. Metab. Care* **2018**, *21*, 498–504. [CrossRef] [PubMed]
17. Blondeau, N.; Lipsky, R.H.; Bourourou, M.; Duncan, M.W.; Gorelick, P.B.; Marini, A.M. Alpha-Linolenic Acid: An Omega-3 Fatty Acid with Neuroprotective Properties—Ready for Use in the Stroke Clinic? *BioMed Res. Int.* **2015**, *2015*, 519830. [CrossRef]

18. Hardman, W.E.; Primerano, D.A.; Legenza, M.T.; Morgan, J.; Fan, J.; Denvir, J. Dietary Walnut Altered Gene Expressions Related to Tumor Growth, Survival, and Metastasis in Breast Cancer Patients: A Pilot Clinical Trial. *Nutr. Res.* **2019**, *66*, 82–94. [CrossRef]
19. Borkowski, K.; Yim, S.J.; Holt, R.R.; Hackman, R.M.; Keen, C.L.; Newman, J.W.; Shearer, G.C. Walnuts Change Lipoprotein Composition Suppressing TNFα-Stimulated Cytokine Production by Diabetic Adipocyte. *J. Nutr. Biochem.* **2019**, *68*, 51–58. [CrossRef]
20. Hwang, H.J.; Liu, Y.; Kim, H.S.; Lee, H.; Lim, Y.; Park, H. Daily Walnut Intake Improves Metabolic Syndrome Status and Increases Circulating Adiponectin Levels: Randomized Controlled Crossover Trial. *Nutr. Res. Pract.* **2019**, *13*, 105–114. [CrossRef]
21. Arab, L.; Dhaliwal, S.K.; Martin, C.J.; Larios, A.D.; Jackson, N.J.; Elashoff, D. Association between Walnut Consumption and Diabetes Risk in NHANES. *Diabetes/Metab. Res. Rev.* **2018**, *34*, e3031. [CrossRef] [PubMed]
22. Cahoon, D.; Shertukde, S.P.; Avendano, E.E.; Tanprasertsuk, J.; Scott, T.M.; Johnson, E.J.; Chung, M.; Nirmala, N. Walnut Intake, Cognitive Outcomes and Risk Factors: A Systematic Review and Meta-Analysis. *Ann. Med.* **2021**, *53*, 971–997. [CrossRef] [PubMed]
23. Su, H.; Liu, R.; Chang, M.; Huang, J.; Jin, Q.; Wang, X. Effect of Dietary Alpha-Linolenic Acid on Blood Inflammatory Markers: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Eur. J. Nutr.* **2018**, *57*, 877–891. [CrossRef] [PubMed]
24. Page, M.J.; Moher, D.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. PRISMA 2020 Explanation and Elaboration: Updated Guidance and Exemplars for Reporting Systematic Reviews. *BMJ* **2021**, *372*, n160. [CrossRef]
25. Sterne, J.A.C.; Savović, J.; Page, M.J.; Elbers, R.G.; Blencowe, N.S.; Boutron, I.; Cates, C.J.; Cheng, H.Y.; Corbett, M.S.; Eldridge, S.M.; et al. RoB 2: A Revised Tool for Assessing Risk of Bias in Randomised Trials. *BMJ* **2019**, *366*, l4898. [CrossRef]
26. Higgins, J.P.T.; Thomas, J.; Chandler, J.; Cumpston, M.; Li, T.; Page, M.J.; Welch, V.A. (Eds.) *Cochrane Handbook for Systematic Reviews of Interventions*, 2nd ed.; John Wiley & Sons: Chichester, UK, 2019; ISBN 9781119536628.
27. Elbourne, D.R.; Altman, D.G.; Higgins, J.P.; Curtin, F.; Worthington, H.V.; Vail, A. Meta-Analyses Involving Cross-over Trials: Methodological Issues. *Int. J. Epidemiol.* **2002**, *31*, 140–149. [CrossRef]
28. Balduzzi, S.; Rücker, G.; Schwarzer, G. How to Perform a Meta-Analysis with R: A Practical Tutorial. *Evid. Based Ment. Health* **2019**, *22*, 153–160. [CrossRef]
29. Harrer, M.; Cuijpers, P.; Furukawa, T.A.; Ebert, D.D. *Doing Meta-Analysis with R: A Hands-On Guide*, 1st ed.; CRC Press: Boca Raton, FL, USA, 2021. [CrossRef]
30. R Core Team R: A Language and Environment for Statistical Computing. Available online: https://www.R_project.org/ (accessed on 4 June 2022).
31. Zambón, D.; Sabaté, J.; Muñoz, S.; Campero, B.; Casals, E.; Merlos, M.; Laguna, J.C.; Ros, E. Substituting Walnuts for Monounsaturated Fat Improves the Serum Lipid Profile of Hypercholesterolemic Men and Women A Randomized Crossover Trial. *Ann. Intern. Med.* **2000**, *132*, 538–546. [CrossRef]
32. Ros, E.; Núñez, I.; Pérez-Heras, A.; Serra, M.; Gilabert, R.; Casals, E.; Deulofeu, R. A Walnut Diet Improves Endothelial Function in Hypercholesterolemic Subjects: A Randomized Crossover Trial. *Circulation* **2004**, *109*, 1609–1614. [CrossRef]
33. Tapsell, L.C.; Gillen, L.J.; Patch, C.S.; Batterham, M.; Owen, A.; Baré, M.; Kennedy, M. Including Walnuts in a Low-Fat/Modified-Fat Diet Improves HDL Cholesterol-to-Total Cholesterol Ratios in Patients With Type 2 Diabetes. *Diabetes Care* **2004**, *27*, 2777–2783. [CrossRef]
34. Olmedilla-Alonso, B.; Granado-Lorencio, F.; Herrero-Barbudo, C.; Blanco-Navarro, I.; Blázquez-García, S.; Pérez-Sacristán, B. Consumption of Restructured Meat Products with Added Walnuts Has a Cholesterol-Lowering Effect in Subjects at High Cardiovascular Risk: A Randomised, Crossover, Placebo-Controlled Study. *J. Am. Coll. Nutr.* **2008**, *27*, 342–348. [CrossRef] [PubMed]
35. Spaccarotella, K.J.; Kris-Etherton, P.M.; Stone, W.L.; Bagshaw, D.M.; Fishell, V.K.; West, S.G.; Lawrence, F.R.; Hartman, T.J. The Effect of Walnut Intake on Factors Related to Prostate and Vascular Health in Older Men. *Nutr. J.* **2008**, *7*, 13. [CrossRef] [PubMed]
36. Tapsell, L.C.; Batterham, M.J.; Teuss, G.; Tan, S.Y.; Dalton, S.; Quick, C.J.; Gillen, L.J.; Charlton, K.E. Long-Term Effects of Increased Dietary Polyunsaturated Fat from Walnuts on Metabolic Parameters in Type II Diabetes. *Eur. J. Clin. Nutr.* **2009**, *63*, 1008–1015. [CrossRef] [PubMed]
37. Ma, Y.; Njike, V.Y.; Millet, J.; Dutta, S.; Doughty, K.; Treu, J.A.; Katz, D.L. Effects of Walnut Consumption on Endothelial Function in Type 2 Diabetic Subjects: A Randomized Controlled Crossover Trial. *Diabetes Care* **2010**, *33*, 227–232. [CrossRef] [PubMed]
38. Torabian, S.; Haddad, E.; Cordero-Macintyre, Z.; Tanzman, J.; Fernandez, M.L.; Sabate, J. Long-Term Walnut Supplementation without Dietary Advice Induces Favorable Serum Lipid Changes in Free-Living Individuals. *Eur. J. Clin. Nutr.* **2010**, *64*, 274–279. [CrossRef]
39. Canales, A.; Sánchez-Muniz, F.J.; Bastida, S.; Librelotto, J.; Nus, M.; Corella, D.; Guillen, M.; Benedi, J. Effect of Walnut-Enriched Meat on the Relationship between VCAM, ICAM, and LTB4 Levels and PON-1 Activity in ApoA4 360 and PON-1 Allele Carriers at Increased Cardiovascular Risk. *Eur. J. Clin. Nutr.* **2011**, *65*, 703–710. [CrossRef]
40. Katz, D.L.; Davidhi, A.; Ma, Y.; Kavak, Y.; Bifulco, L.; Njike, V.Y. Effects of Walnuts on Endothelial Function in Overweight Adults with Visceral Obesity: A Randomized, Controlled, Crossover Trial. *J. Am. Coll. Nutr.* **2012**, *6*, 415–423. [CrossRef]
41. Wu, L.; Piotrowski, K.; Rau, T.; Waldmann, E.; Broedl, U.C.; Demmelmair, H.; Koletzko, B.; Stark, R.G.; Nagel, J.M.; Mantzoros, C.S.; et al. Walnut-Enriched Diet Reduces Fasting Non-HDL-Cholesterol and Apolipoprotein B in Healthy Caucasian Subjects: A Randomized Controlled Cross-over Clinical Trial. *Metab. Clin. Exp.* **2014**, *63*, 382–391. [CrossRef]

42. Bamberger, C.; Rossmeier, A.; Lechner, K.; Wu, L.; Waldmann, E.; Stark, R.G.; Altenhofer, J.; Henze, K.; Parhofer, K.G. A Walnut-Enriched Diet Reduces Lipids in Healthy Caucasian Subjects, Independent of Recommended Macronutrient Replacement and Time Point of Consumption: A Prospective, Randomized, Controlled Trial. *Nutrients* **2017**, *9*, 1097. [CrossRef]
43. Bitok, E.; Rajaram, S.; Jaceldo-Siegl, K.; Oda, K.; Sala-Vila, A.; Serra-Mir, M.; Ros, E.; Sabaté, J. Effects of Long-Term Walnut Supplementation on Body Weight in Free-Living Elderly: Results of a Randomized Controlled Trial. *Nutrients* **2018**, *10*, 1317. [CrossRef]
44. Domènech, M.; Serra-Mir, M.; Roth, I.; Freitas-Simoes, T.; Valls-Pedret, C.; Cofán, M.; López, A.; Sala-Vila, A.; Calvo, C.; Rajaram, S.; et al. Effect of a Walnut Diet on Office and 24-Hour Ambulatory Blood Pressure in Elderly Individuals: Findings from the WAHA Randomized Trial. *Hypertension* **2019**, *73*, 1049–1057. [CrossRef] [PubMed]
45. Sanchis, P.; Molina, M.; Berga, F.; Muñoz, E.; Fortuny, R.; Costa-Bauzá, A.; Grases, F.; Buades, J.M. A Pilot Randomized Crossover Trial Assessing the Safety and Short-Term Effects of Walnut Consumption by Patients with Chronic Kidney Disease. *Nutrients* **2020**, *12*, 63. [CrossRef] [PubMed]
46. Abdrabalnabi, A.; Rajaram, S.; Bitok, E.; Oda, K.; Beeson, W.L.; Kaur, A.; Cofán, M.; Serra-Mir, M.; Roth, I.; Ros, E.; et al. Effects of Supplementing the Usual Diet with a Daily Dose of Walnuts for Two Years on Metabolic Syndrome and Its Components in an Elderly Cohort. *Nutrients* **2020**, *12*, 451. [CrossRef] [PubMed]
47. Cofán, M.; Rajaram, S.; Sala-Vila, A.; Valls-Pedret, C.; Serra-Mir, M.; Roth, I.; Freitas-Simoes, T.M.; Bitok, E.; Sabaté, J.; Ros, E. Effects of 2-Year Walnut-Supplemented Diet on Inflammatory Biomarkers. *J. Am. Coll. Cardiol.* **2020**, *76*, 2282–2284. [CrossRef]
48. Tindall, A.M.; Johnston, E.A.; Kris-Etherton, P.M.; Petersen, K.S. The Effect of Nuts on Markers of Glycemic Control: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Am. J. Clin. Nutr.* **2019**, *109*, 297–314. [CrossRef]
49. Fernández-Rodríguez, R.; Mesas, A.E.; Garrido-Miguel, M.; Martínez-Ortega, I.A.; Jiménez-López, E.; Martínez-Vizcaíno, V. The Relationship of Tree Nuts and Peanuts with Adiposity Parameters: A Systematic Review and Network Meta-Analysis. *Nutrients* **2021**, *13*, 2251. [CrossRef]
50. Fernández-Rodríguez, R.; Martínez-Vizcaíno, V.; Garrido-Miguel, M.; Martínez-Ortega, I.A.; Álvarez-Bueno, C.; Eumann Mesas, A. Nut Consumption, Body Weight, and Adiposity in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutr. Rev.* **2022**, *80*, 645–655. [CrossRef]
51. Neale, E.P.; Guan, V.; Tapsell, L.C.; Probst, Y.C. Effect of Walnut Consumption on Markers of Blood Glucose Control: A Systematic Review and Meta-Analysis. *Br. J. Nutr.* **2020**, *124*, 641–653. [CrossRef]
52. Tarantino, L.M. *Qualified Health Claims: Letter of Enforcement Discretion-Walnuts and Coronary Heart Disease*; (Docket No 02P-0292); U.S. Food and Drug Administration: Washington, DC, USA, 2004.
53. Arabi, S.M.; Bahrami, L.S.; Milkarizi, N.; Nematy, M.; Kalmykov, V.; Sahebkar, A. Impact of Walnut Consumption on Cardio Metabolic and Anthropometric Parameters in Metabolic Syndrome Patients: GRADE-Assessed Systematic Review and Dose-Response Meta-Analysis of Data from Randomized Controlled Trials. *Pharmacol. Res.* **2022**, *178*, 106190. [CrossRef]
54. Liu, X.; Guasch-Ferré, M.; Drouin-Chartier, J.P.; Tobias, D.K.; Bhupathiraju, S.N.; Rexrode, K.M.; Willett, W.C.; Sun, Q.; Li, Y. Changes in Nut Consumption and Subsequent Cardiovascular Disease Risk Among US Men and Women: 3 Large Prospective Cohort Studies. *J. Am. Heart Assoc.* **2020**, *9*, e013877. [CrossRef]
55. Guasch-Ferré, M.; Li, J.; Hu, F.B.; Salas-Salvadó, J.; Tobias, D.K. Effects of Walnut Consumption on Blood Lipids and Other Cardiovascular Risk Factors: An Updated Meta-Analysis and Systematic Review of Controlled Trials. *Am. J. Clin. Nutr.* **2018**, *108*, 174–187. [CrossRef] [PubMed]
56. Becerra-Tomás, N.; Paz-Graniel, I.; Kendall, C.; Kahleova, H.; Rahelić, D.; Sievenpiper, J.L.; Salas-Salvadó, J. Nut Consumption and Incidence of Cardiovascular Diseases and Cardiovascular Disease Mortality: A Meta-Analysis of Prospective Cohort Studies. *Nutr. Rev.* **2019**, *77*, 691–709. [CrossRef] [PubMed]
57. Liu, X.; Guasch-Ferré, M.; Tobias, D.K.; Li, Y. Association of Walnut Consumption with Total and Cause-Specific Mortality and Life Expectancy in U.S. Adults. *Nutrients* **2021**, *13*, 2699. [CrossRef] [PubMed]
58. Estruch, R.; Ros, E.; Salas-Salvadó, J.; Covas, M.-I.; Corella, D.; Arós, F.; Gómez-Gracia, E.; Ruiz-Gutiérrez, V.; Fiol, M.; Lapetra, J.; et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. *N. Engl. J. Med.* **2018**, *378*, e34. [CrossRef] [PubMed]
59. Rusu, M.E.; Gheldiu, A.M.; Mocan, A.; Vlase, L.; Popa, D.S. Anti-Aging Potential of Tree Nuts with a Focus on the Phytochemical Composition, Molecular Mechanisms and Thermal Stability of Major Bioactive Compounds. *Food Funct.* **2018**, *9*, 2554–2575. [CrossRef] [PubMed]
60. Li, J.; Jiang, B.; Santos, H.O.; Santos, D.; Singh, A.; Wang, L. Effects of Walnut Intake on Blood Pressure: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Phytother. Res.* **2020**, *34*, 2921–2931. [CrossRef]
61. Banel, D.K.; Hu, F.B. Effects of Walnut Consumption on Blood Lipids and Other Cardiovascular Risk Factors: A Meta-Analysis and Systematic Review. *Am. J. Clin. Nutr.* **2009**, *90*, 56–63. [CrossRef]
62. Lopez-Garcia, E.; Schulze, M.B.; Fung, T.T.; Meigs, J.B.; Rifai, N.; Manson, J.E.; Hu, F.B. Major Dietary Patterns Are Related to Plasma Concentrations of Markers of Inflammation and Endothelial Dysfunction 1-3. *Am. J. Clin. Nutr.* **2004**, *80*, 1029–1064. [CrossRef]

63. Yu, Z.; Malik, V.S.; Keum, N.N.; Hu, F.B.; Giovannucci, E.L.; Stampfer, M.J.; Willett, W.C.; Fuchs, C.S.; Bao, Y. Associations between Nut Consumption and Inflammatory Biomarkers. *Am. J. Clin. Nutr.* **2016**, *104*, 722–728. [CrossRef]
64. Rusu, M.E.; Fizesan, I.; Pop, A.; Mocan, A.; Gheldiu, A.M.; Babota, M.; Vodnar, D.C.; Jurj, A.; Berindan-Neagoe, I.; Vlase, L.; et al. Walnut (*Juglans Regia* L.) Septum: Assessment of Bioactive Molecules and in Vitro Biological Effects. *Molecules* **2020**, *25*, 2187. [CrossRef]

Received: 23 May 2021 | Revised: 7 August 2021 | Accepted: 16 August 2021

DOI: 10.1111/eci.13671

REVIEW

WILEY

Intravascular ultrasound insights into the unstable features of the coronary atherosclerotic plaques: A systematic review and meta-analysis

Calin Homorodean[1,2] | **Daniel-Corneliu Leucuta**[3] | **Mihai Ober**[2] | **Romana Homorodean**[2] | **Mihail Spinu**[1] | **Maria Olinic**[1,2] | **Dan Tataru**[1,2] | **Dan-Mircea Olinic**[1,2]

[1]Internal Medicine Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

[2]Emergency County Hospital Cluj Napoca, Cluj-Napoca, Romania

[3]Medical Informatics and Biostatistics Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Correspondence
Daniel-Corneliu Leucuta, Medical Informatics and Biostatistics Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania.
Email: dleucuta@umfcluj.ro

Funding information
Unitatea Executiva pentru Finantarea Invatamantului Superior, a Cercetarii, Dezvoltarii si Inovarii, Grant/Award Number: PN-III-P2-2.1-PED-2019-2404

Abstract

Background: There is a lack of a comprehensive picture of plaque geometry and composition of unstable atherosclerotic lesions as observed with intravascular ultrasound techniques.

We analysed through a systematic review with meta-analysis 39 characteristics of atherosclerotic plaques in three scenarios involving culprit and non-culprit lesions from acute coronary syndromes vs stable angina pectoris patients, and culprit vs non-culprit lesions in acute coronary syndromes patients.

Methods: A systematic search of PubMed and EMBASE, from inception to April 2020 was performed. The combined odds ratios or mean differences of all IVUS characteristics were calculated with random-effects models.

Results: Twenty-eight studies involving 5434 subjects, and 5618 lesions were included. Culprit lesions in acute coronary syndromes have larger plaque areas and remodeling indexes (MD = 0.13 [0.08; 0.17], $p < 0.001$) and contained larger necrotic cores (MD = 0.67 (95% CI 0.19;1.15), $p = 0.006$) that stable angina culprit lesions. In acute patients, culprit plaques were also more remodeled, had larger necrotic cores and had more frequently a Thin-Cap Fibroatheroma morphology (OR = 1.79 (95% CI 1.21; 2.65), $p = 0.004$) than non-culprit lesions. Non-culprit lesions in acute syndromes were more often ruptured (OR = 2.25 (95% CI:1.05; 4.82), $p = 0.037$) or Thin-Cap Fibroatheromas than in stable angina.

Conclusion: Culprit lesions from acute coronary patients are larger, more positively remodeled and contained more lipids as compared to stable angina lesions or non-culprit in acute patients. Non culprit lesions are also more often complicated or vulnerable in acute than stable patients.

KEYWORDS

acute coronary syndrome, intravascular ultrasound, non-culprit lesion, plaque morphology, vessel remodeling

1 | INTRODUCTION

Plaque rupture is responsible for the majority 'of acute coronary syndromes.[1] Historical data about plaque instability are provided by postmortem researches. Sudden cardiac deaths occurred most often in relation to massive fissuring of lipid-rich atheromatous plaques. Although thrombi are usually found at sites that had already significant stenosis, there was a minority that developed in lesions less than 50% stenosed.[2]

The disagreement between lesions severity and subsequent clinical events was also found by angiographic studies demonstrating non-significant stenosis at coronarography performed one year before the acute occlusion.[1]

On the other hand, coronary acute culprit sites harbor large atherosclerotic plaques[2] and not "early", small plaques as it could be assumed.[3]

The discrepancy between stenosis and plaque volume is due to the process of positive remodeling that permits vessel walls to accumulate large plaques, attenuating the encroachment of the plaque on the lumen and bulging to the exterior.

Regarding lesion vulnerability, lipid-reach core is more important than plaque size.[4] The risk of plaque rupture is linked to the size of the lipid-rich necrotic core, the thickness of the fibrous cap covering the core, and the degree of cap inflammation.[5] Libby et al. developed the concept that fibrous cap undergoes thinning before the onset of rupture.[6] In a series of sudden cardiac deaths, 60% of acute thrombi resulted from the rupture of thin cap fibroatheromas (TCFA).[7]

It seems that ruptured plaques arise from large plaques, not necessarily stenotic, developed by an adaptive remodeling of the vessel wall and containing large necrotic cores. It is the pathological studies too suggesting a closed relation between the necrotic core stimulating inflammation and both positive remodeling and fibrous cap thinning responsible for rupture.

These concepts have been studied in vivo by different intravascular imaging techniques.

Angioscopic complex lesions were predominantly found in compensatory enlarged coronary segments, whereas smooth lesions were predominantly found in shrunken segments.[8]

There is a lack of clear evidence or contradictory data regarding the characteristics of the unstable atherosclerotic lesions observed with intravascular ultrasound (IVUS) techniques. Thus, not all IVUS studies confirmed larger remodeling indexes with higher plaque burden and larger necrotic cores in culprit lesions from acute coronary syndromes (ACS) when compared to stable angina pectoris (SAP).[9–11] Furthermore, the data are also contradictory regarding the differences between culprit and non-culprit lesions in the same ACS patients.[12,13] Therefore, we performed a systematic review with meta-analysis of the studies addressing these issues.

The central question of our research was: which are the most relevant IVUS findings to the instability potential of coronary atherosclerotic plaques?

Our study had several targets: [1] to review the IVUS differences between atherosclerotic lesions in acute and stable coronary disease (both culprit and non-culprit lesions),[2] To underline IVUS insights into the vulnerability of coronary atherosclerotic plaques,[3] To assess IVUS findings about the multifocal character of plaque instability in patients with acute coronary syndromes (culprit vs non-culprit lesions in ACS patients).

2 | METHODS

2.1 | Eligibility criteria

We conducted a systematic search using the PICOS guidance, to identify studies involving patients with percutaneous coronary interventions (PCI), and grey-scale/virtual histology (Integrated Backscattered, Virtual Histology, iMap) IVUS performed at the level of culprit lesions. Studies have to include at least one IVUS comparison involving a specific type of lesion (culprit/non-culprit), between acute vs stable patients or two types of lesion in the ACS patients. We performed three analyses:

The first one included studies that compared patients with ACS, as exposure group, with SAP patients. Culprit lesions in both arms were imagined by an IVUS technique.

The second analysis included studies performed on ACS patients, comparing IVUS features of culprit lesions, as exposure, with non-culprit lesions.

The third analysis included patients with ACS in the exposure group and SAP in control group, and compared non-culprit lesions, imaged by IVUS.

We looked for studies regarding several outcomes: Grey-Scale IVUS analysis (remodeling index, EEM (external elastic membrane)) cross-sectional area (CSA)–vessel area, lumen area, plaque area (P&M–plaque and media area), plaque burden, and plaque rupture), and Virtual Histology-IVUS analysis (necrotic core (%, mm^2), fibrous tissue (%, mm^2), Fibrolipidic tissue (%, mm^2), dense calcium (%, mm^2), and TCFAs). We included all published observational studies (cohort studies, case-control studies, case series) till the index date, without language restriction. We excluded studies with irrelevant population, intervention, comparison, outcome, other study types, duplicate studies, cohorts already used (we analyzed the most inclusive one), full text not available.

2.2 | Information sources

The searches were performed in PubMed and EMBASE databases, on their websites, from inception to 30 April 2020.

2.3 | Search

The full search strategies were devised together by two investigators, and are presented in the Table S1.

2.4 | Study selection

First, we combined the results of the two searches and removed the duplicates. Next, two investigators independently screened the titles and abstracts to find eligible studies. For the articles that met the prespecified criteria, the full text was obtained. Finally, these articles were assessed for inclusion in the study. The disagreements were resolved by discussion. Furthermore, the references of the enrolled researches were also evaluated in order to find studies not found with the previous search strategy. When full text was unavailable authors were contacted by ResearchGate.com or emails when available.

2.5 | Data collection process

From each article the data was extracted separately in two spreadsheets by two investigators. Any difference found was verified with the full text of the paper. Articles in other languages than English were translated by authors or by Google Scholar.

2.6 | Data items

The standardized form for data extraction consisted in an Excel spreadsheet with 185 columns (items), to be filled in for every analysed study. Data was independently collected and recorded by two investigators and included information about:

study (title, authors, journal name, year of publication, uni/multicentric, origin countries, recruiting year, study type),

selection (exclusion criteria: irrelevant population/comparison/outcome, duplicate study, cohort already used, full text not available; quality assessment).

IVUS probe and technique (grey-scale, VH, iMAP, MHz),

patients and lesions included in exposed and control groups (diagnosis, baseline characteristics, number of patients/ lesions, type of lesions, comparison type, measuring type, coronary artery location, no of vessels examined).

IVUS features of the lesions in both arms (exposed and control). A detailed list of the recorded IVUS features are presented in Tables S5,S6 and S7 from Supplementary materials for each of the three sub-analyses.

For studies that didn't provide the mean and standard deviation we estimated them using median, interquartile ranges, and sample sizes.[14]

In case of studies where we need to combine two means and two standard deviations of two subgroups, we used the formulas from Cochrane Handbook.[15]

2.7 | Risk of bias in individual studies

Two investigators independently assessed each included article for risk of bias using the Newcastle Otawa Scale, for cohort studies.[16] In case of disagreement a decision was made through discussion. The items from the NOS that were not applicable to the assessed studies were removed. We added a few items to check comparability regarding several confounders.

2.8 | Summary measures

For continuous outcomes we computed the difference in means, while for binary outcomes we computed the odds ratios, along with 95% confidence intervals.

2.9 | Synthesis of results

The meta-analyses were performed in R environment for statistical computing and graphics, version 4.0.2 (R Foundation for Statistical Computing)[17], using the meta R package.[18] For all outcomes we computed the random effects estimates since we assumed clinical variability between the studies. For continuous outcomes we used restricted maximum likelihood to estimate the heterogeneity variance, while for binary outcomes we used the Mantel-Haenszel estimator. As a measure of inconsistency, we calculated the I2 with a 95% confidence interval and associated *p*-value. When I2 was >50% we performed sensitivity analyses, to assess the robustness of the results, by excluding high leverage studies, or outliers, identified with dmetar package. For meta-analyses with more than 10 studies a random effects metare-gression was performed, using Knapp-Hartung method to compute confidence intervals and *p*-values, with explanatory variables the study design, the study quality, and the study year.

Reporting of the study conforms to broad EQUATOR guidelines.[19] PRISMA and MOOSE statement guidance were followed during the reporting of the review.[20]

3 | RESULTS

Initially, 661 studies were appraised at abstract level and 48 full text articles were assessed. Twenty studies were excluded due to irrelevant comparison, unavailable full text or duplicate data, leaving 28 studies involving 5434 subjects,

and 5618 lesions that met the inclusion criteria for our review (Figure 1).

3.1 | Study characteristics

The details of the analysed studies (including baseline clinical characteristics) are presented in Table S2.

3.2 | Results of syntheses

3.2.1 | Culprit lesions in ACS vs SAP (22 studies)[3,8–12,21–36]

There were no differences in the distribution of lesions on LM (left main), LAD (left anterior descending), LCx (left circumflex) or marginal ramus lesions between ACS and SAP patients. However, RCA (right coronary artery) lesions were more frequent in ACS patients (OR:1.73 95% CI: 1.13–2.64).

As compared to SAP lesions, ACS culprit lesions have larger remodeling indexes (MD: 0.13 [0.08; 0.17], $p < 0.001$) (Figure 2A) and external elastic membrane area (CSA) (MD: 2.32 (95% CI 1.58; 3.05), $p < 0.001$) while lumen areas were similar. Positive remodeling was thus more frequent among ACS culprit lesions (OR: 4.23 [1.78; 10.08], $p = 0.001$). Also, plaques areas (MD = 2.18 (95% CI 1.54;2.81), $p < 0.001$) and plaque burden were larger in ACS culprit lesions (MD = 4.79 [2.97; 6.62], $p < 0.001$) (Figure 2B). Although there was heterogeneity between the studies results, after performing sensitivity analyses the results proved to be robust for remodeling index and plaque burden area (Table S7). Regarding positive remodeling we performed a metaregression, with study year and study design as explanatory variables, but they didn't yeld significant results. Nevertheless, the prospective design favoured increased positive remodeling difference ($p = 0.119$), while reducing heterogeneity with 4.63% (Table S8).

ACS lesions contain larger necrotic cores at minimal lumen are (MLA) site both in area (MD = 0.67 (95% CI 0.19; 1.15), $p = 0.006$) (Figure 3A) and as a percentage of the plaque (MD = 2.89 [0.02; 5.77], $p = 0.049$) (Figure 3B) but failing in reaching significance when considering volume percentage (MD = 2.4 (95% CI -0.02;4.82), $p = 0.051$) (Figure 3C). In all three cases, the studies results were heterogenous.

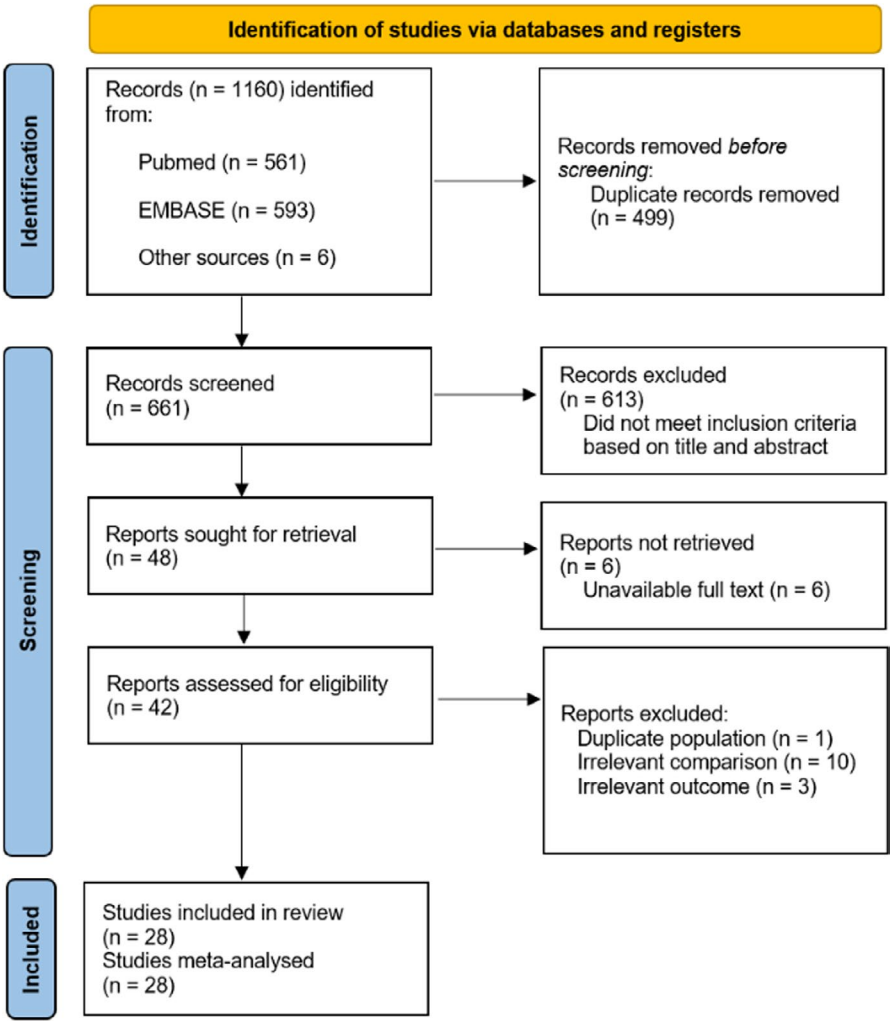

FIGURE 1 Flow diagram

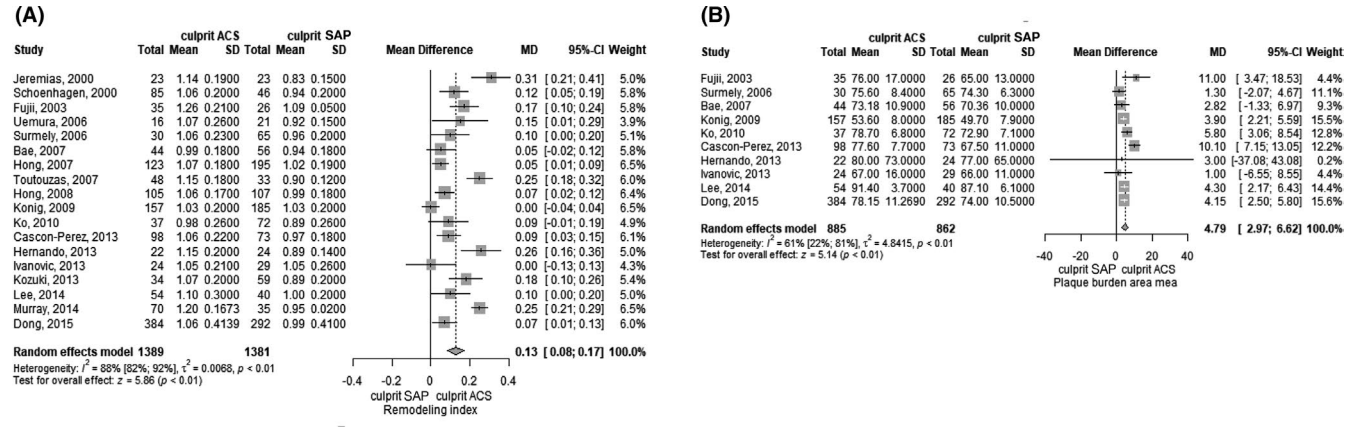

FIGURE 2 Grey-scale IVUS imaging of culprit lesions in ACS patients vs. target lesions in SAP patients: An analysis of the remodeling index and plaque burden area are shown in panels A and B. ACS, Acute coronary syndromes; CI, Confidence interval; IVUS, Intravascular ultrasound; MD, Mean difference; SAP, Stable angina pectoris; SD, Standard deviation

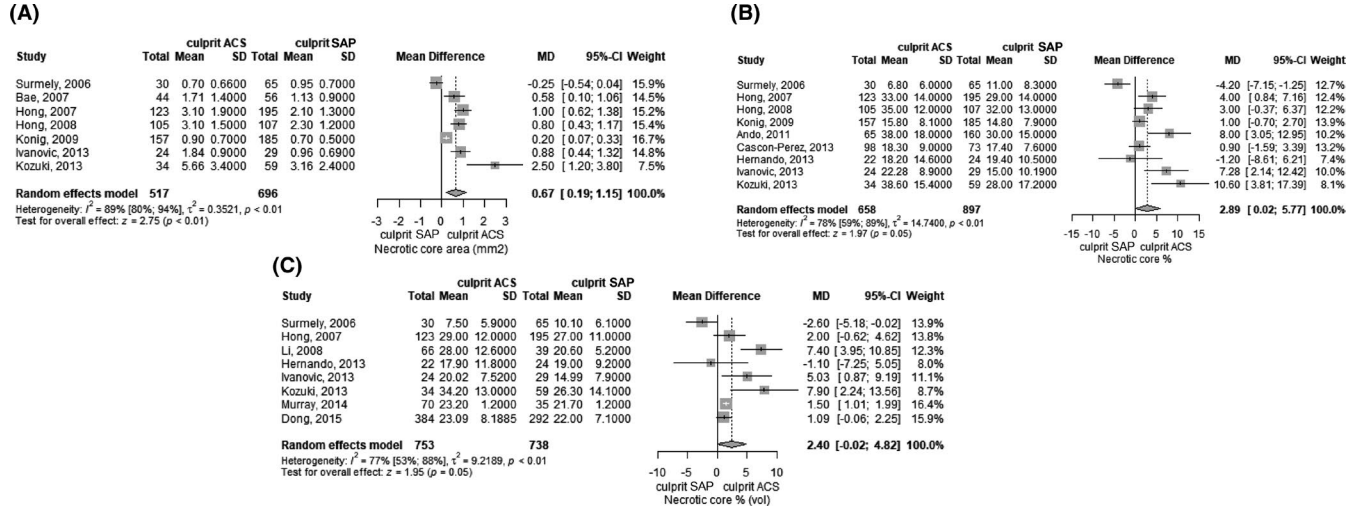

FIGURE 3 IVUS–VH imaging of culprit lesions in ACS vs SAP patients: Necrotic cores were compared with respect to mean area (A), percent of the plaque area at MLA (B), percent of the plaque volume (C). ACS, Acute coronary syndrome; CI, Confidence interval; IVUS, Intravascular ultrasound; MD, Mean difference; MLA, Minimal lumen area; SAP, Stable angina pectoris; SD, Standard deviation; VH, Virtual histology

Sensitivity analyses shown robustness of the result for necrotic core area, but yielded unclear results for necrotic core percentage, and necrotic core volume percentage.

Dense calcium area proportions were similar at the level of lesions MLA between ACS and SAP patients (MD = −0.9 (95% CI −2.07; 0.27)) but when plaque volumes were compared, ACS lesions were less calcified (MD = −1,59 (95% CI −3.01; −0.16), $p = 0.029$).

Plaque ruptures (OR = 5.07 [3.02; 8.51], $p < 0.001$) (Figure 4A) and TCFAs (OR = 2.12 [1.24; 3.64], $p = 0.006$) (Figure 4B), were significantly more frequent among ACS culprit lesions. Heterogeneity was found between the study results. Nevertheless, the results were consistent even after sensitivity analyses, concerning plaque rupture and TCFA.

3.2.2 | Culprit lesions vs non-culprit lesions in in ACS patients (6 studies included in analysis)[9,12,13,29,36,37]

There were no differences in LAD, LCx, RCA location of culprit vs non-culprit lesions. However, as expected, LM lesions were less frequent culprit (OR:0.02, 95% CI: 0–0.41).

In ACS patients, when comparing culprit to non-culprit lesions, the former ones were larger with a higher plaque burden and had a higher remodeling index (Figure 5A), being more positively remodeled (OR = 3.5 (95% CI 1.2–10.17), $p = 0.021$). Culprit lesions were more stenosed, but the vessel EEM area was not different (Figure 5B). The studies' results regarding remodeling index were homogenous. On the other

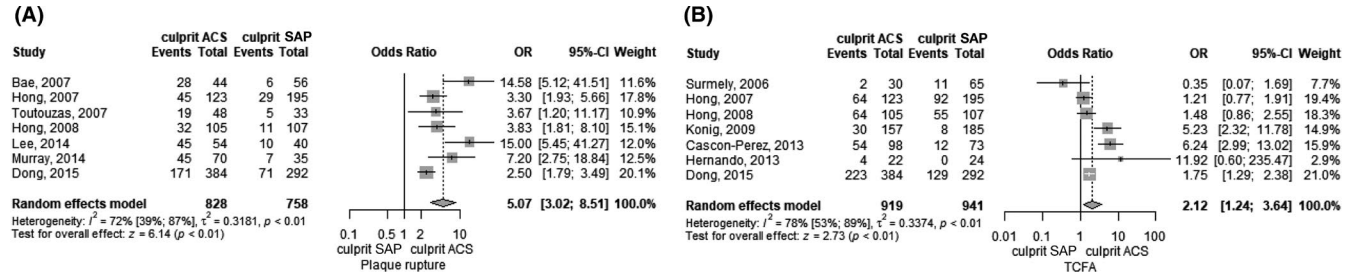

FIGURE 4 IVUS imaging in culprit ACS vs target SAP lesions regarding instability: An analysis of the odds of plaque rupture and TCFA morphology are depicted in panels A and B. ACS, Acute coronary syndromes; CI, Confidence interval; IVUS, Intravascular ultrasound; OR, Odds ratio; SAP, Stable angina pectoris; TCFA, Thin-cap fibroatheroma

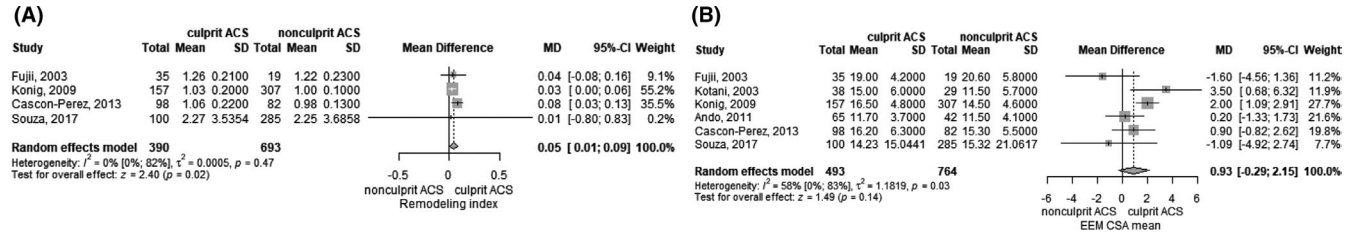

FIGURE 5 IVUS imaging of culprit vs non-culprit lesions in ACS patients: An analysis of the remodeling index and EEM CSA are shown in panels A and B. ACS, Acute coronary syndroms; CI, Confidence interval; EEM CSA, External elastic membrane cross-sectional area; IVUS, Intravascular ultrasound; MD, Mean difference; SAP, Stable angina pectoris; SD, Standard deviation

hand EEM CSA mean results were heterogenous and unlikely to find significant differences.

Concerning composition, necrotic cores were larger in culprit lesions (MD = 0.4, [95% CI: 0.28;0,52]) but plaque composition did not differ (The necrotic core percentage: MD = 0.48 (95% CI −1.74;2.7)). Dense calcium percent of the plaque area was similar in culprit vs non-culprit ACS lesions (MD = −0.03 (−1.81;1.75)).

TCFAs were more common in culprit plaques (OR = 1.79 (95% CI 1.21–2.65), p = 0.004) (Figure 6A) while ruptured plaque frequencies were not different in culprit and non-culprit lesions (OR = 3.4 (95% CI 0.51–22.62), p = 0.205) (Figure 6B). Plaque rupture results were heterogenous.

3.2.3 | Non culprit lesions in ACS vs SAP patients (7 studies)[9,25,36,38–41]

In the analysis of non-culprit lesions in ACS vs SAP only two studies reported the location of plaques, LAD lesions being more frequent in the SAP group.

Regarding non-culprit lesions in ACS vs SAP patients, our analysis did not find significant differences regarding remodeling index (MD = 0.28 (95% CI −0.27; 0.83), p = 0.322), EEM CSA (MD = 0.18 (95% CI −0.58;0.94), p = 0.641), plaque area (MD = −0.07 (95% CI −0.45;0.32), p = 0.737), or plaque composition (necrotic core percentage: MD = 6.03 (95% CI −1.62;13.69), p = 0.122). Necrotic core volume was larger in ACS patients (MD = 1.2 (95% CI 0.94;1.46), p < 0.001) although a single study reported that item. Dense calcium percent of the plaque was similar in culprit vs non-culprit ACS lesions (MD = −0.03 (−1.81;1.75)).

ACS non-culprit lesions were more frequently ruptured (OR = 2.25 (95% CI 1.05;4.82), p = 0.037) and had more often a TCFA morphology (OR = 3.34 (95% CI 2.08;5.36), p < 0.001) (Figure 7A,B). The studies' results were homogenous regarding TCFA.

3.3 | Risk of bias

The studies were of good methodological quality according to the Newcastle Ottawa risk of bias scale, in respect of selection criteria, and outcome assessment (Table S3, Supplementary Figure 1). A limited number of studies reached good comparability by using matching for several criteria, or undertaking measurements on the same cohort. Nevertheless, the compared groups were generally similar on several important confounding characteristics.

We checked for evidences of publication bias, and we couldn't find them, except a statistically significant p-value for the Egger test regarding plaque rupture in the comparison between culprit ACS and culprit SAP (Table S4).

4 | DISCUSSION

Our pooled analysis of IVUS data has shown that in unstable patients as compared to stable ones, culprit lesions have

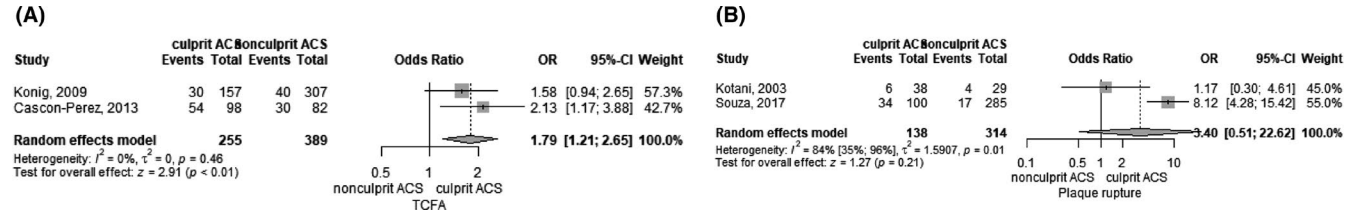

FIGURE 6 IVUS imaging in culprit vs nonculprit ACS lesions. An analysis of the odds of plaque rupture and TCFA morphology are shown in panels A and B. ACS, Acute coronary syndromes; CI, Confidence interval; IVUS, Intravascular ultrasound; OR, Odds ratio; SAP, Stable angina pectoris; TCFA, Thin-cap fibroatheroma

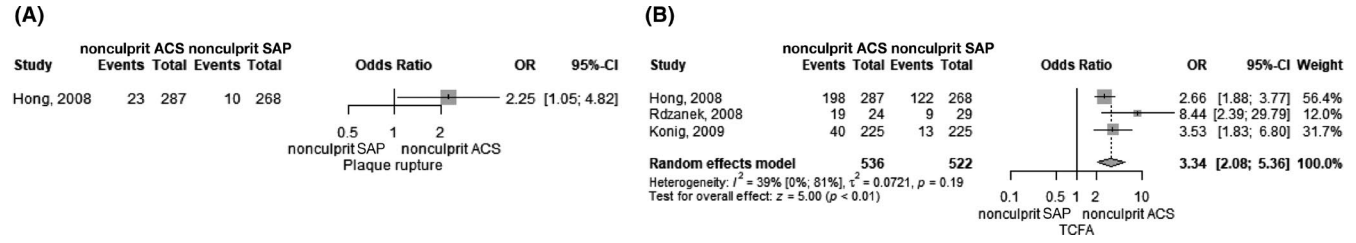

FIGURE 7 IVUS imaging of non-culprit lesion in ACS vs SAP patients: The odds of appearance of ruptured plaques and TCFAs are analysed in panels A and B. ACS, Acute coronary syndroms; CI, Confidence interval; IVUS, Intravascular ultrasound; OR, Odds ratio; SAP, Stable angina pectoris; TCFA, Thin-cap fibroatheroma

larger plaque burdens although producing similar lumen area, as they are more positively remodeled. Moreover, ACS culprit lesions have larger necrotic cores and were more frequently TCFAs or ruptured than SAP culprit lesions.

Positive remodeling is the main process permitting vessel wall to accumulate large plaque. In fact, it has been shown that only when more than 30%–40% of the area bordered by the arterial external elastic membrane is occupied by the plaque, does a sharp decline in lumen area and percent stenosis become evident.[42]

Regarding plaque vulnerability, necropsy studies found that the presence of the lipid-reach core is more important than the size of plaque area.[4,43] Davis et al reported that in intact aortic plaques, a core occupying more than 40% of the plaque area was the critical threshold for vulnerability.[43]

The close relation between adaptive remodeling and plaques vulnerability, centered on the necrotic core and inflammation emerges from both pathological and in vivo studies.

Post-mortem studies reported that compensatory enlarged lesions with large plaques contain significantly more histopathologic markers of vulnerability than shrinkaged lesions with small plaques.[44] Pasterkamp et al found significantly more atheroma, more macrophages (CD68 positive) and more memory T cells (CD45RO positive) while less collagen and less smooth muscle cells in the arterial cross-sections with the largest plaque area.[44] A double hit theory has been proposed, meaning that the inflammatory response emerged in the necrotic core, release metalloproteinase that degrades matrix components beneath the medial layer at the base of the plaque, so that vessel wall suffers an outward stretch as well as beneath the fibrous cap promoting plaque instability[44]. The findings from our IVUS analysis are in line with these data as the acute culprit lesions were both more positive remodeled and have larger necrotic cores than the stable lesions.

Lee et al examined the differences between ST elevation myocardial infarction (STEMI) and stable angina culprit plaques both on IVUS and histology, as the plaques were removed with atherectomy.[33] IVUS found larger, more positive remodeled plaques in STEMI lesions, while histologically, they showed more immunostaining for macrophage markers.[33] In such STEMI culprit lesions inflammatory cells may reside also in older parts of the thrombus that was linked to microemboli both before and after PCI.[45]

Other data supporting the above-mentioned "double hit" theory is provided by OCTstudies.[46] Over a follow-up period of 6 month the percent change in fibrous cap thickness by OCT correlated negatively with the percent variation of the external elastic membrane (EEM) area.[46]

Thus, adaptive remodeling is associated with an increased risk of plaque rupture, although chronic luminal narrowing caused by plaque formation is retarded.[21]

Another mechanism responsible of positive remodeling include biomechanical forces and shear stress. High blood velocity acts on the uninvolved segments of the arterial wall opposite to the plaque, leading to enlargement until a normal level of wall shear stress is restored.[42] Conversely, low velocities and low shear stress favour atherogenesis leading to vessel narrowing, which increases velocity and reestablishes again normal shear stress.[8,42,47]

The PREDICTION study showed that low endothelial shear stress at baseline was a predictor of plaque progression at follow-up.[48]

The only study of our analysis with data about arterial compliance[21] found a relation between increased vascular distensibility and positive vessel remodeling.

We also compared culprit to non-culprit lesions in the same ACS patients. Thus, general risk factors and genetics affected equally the compared lesions. Our pooled analysis showed that the culprit ones were larger, more positively remodeled, more stenosed but with similar external elastic membrane areas.

Regarding composition at MLA site, in culprit lesions, necrotic cores are larger as absolute areas but similar as a proportion of the total plaque area. This finding is not surprising as plaque areas are also larger in culprit than non-culprit lesion. TCFA morphology was more common among culprit lesions, while ruptured plaque were similarly distributed in culprit and non-culprit lesions.

There are studies showing that the culprit site of the lesion is not necessarily the MLA site. Landmark pathological studies involving computer modelling of stress distribution across the vessel wall show that lesions concentrate stress on the plaque cap especially at its junction with the adjacent intima.[49] Therefore, lateral margins of the lipid pool are the most frequent site of intimal tear.[49]

IVUS studies showed that rupture occurs in 63% of cases at the shoulders of the plaque, at sites different from the most stenosed one.[50] Furthermore, rupture site had a worse phenotype than MLA sites with larger plaque area and more positive remodeling[50] and also larger necrotic cores.[51] On the other hand, regarding non-ruptured plaques, a higher percentage of TCFAs were demonstrated at maximal necrotic core site as compared to MLA site.[52,53]

Unstable lesions are also associated with focal calcium deposits that may be related to fibrous cap disruption.[54] Calcium in a spotty distribution (small calcium deposits within an arc of less than 90 degrees) has previously been observed, pathologically, in sudden coronary death victims[7]. While spotty calcification was more commonly associated with unstable plaques, extensive calcification was more common with stable plaques.[7] VH-IVUS also showed that coronary plaques with a smaller arc of spotty calcifications have lipid-rich characteristics.[55]

Additionally, calcified nodules are the underlying mechanism of acute coronary events in 2%–7% of coronary artery thrombosis[56] being one of the morphologies of vulnerable plaque.[54]

Microcalcifications may arise inside lipid pool following the apoptosis of smooth muscle cells or macrophages. They coalesce into larger mases over time to form speckles, further progressing to calcified sheets or plates. Fragmentation of these sheets lead to nodules that may extend to the lumen and become protuberant with discontinuation of the endothelium.[56]

Only two studies[12,37] included in our pooled analysis reported the extension of the arc of calcium by grey-scale IVUS at the level of culprit lesions in ACS vs SAP patients, founding no differences between them.

More data are provided by VH-IVUS about the extension of dense calcium inside plaques. Our pooled analysis showed smaller calcium proportions of the culprit plaque volumes but similar percent of the plaque area at MLA site, in ACS patients as compared to SAP patients. Murray et all reported that high NC/DC ratio (Necrotic Core/Dense Calcium) was associated with an unstable lesion phenotype.[34]

Concerning calcium nodules, two studies compared their presence inside culprit lesions from ACS and SAP patients[29,35] and other two culprit vs non-culprit lesions[13,29] without revealing any differences.

As expected, our metanalysis found more ruptured plaques in ACS culprit lesions as compared to SAP ones. Moreover, non-culprit lesions in ACS patients are also more frequently ruptured and have more often a TCFA morphology than SAP patients. However, in ACS patients, there were not significant differences regarding plaque rupture between culprit and non-culprit lesions.

Our findings are in line with the studies establishing that a multifocal instability process is present in ACS[57], with multiple plaques ruptures, beside the culprit one.[9,36,50,58–60] Maehara et al reported multiple ruptured lesions in 15% of the patients. Goldstein et al suggested that plaque instability may not represent a random accident, but perhaps may reflect a "pan-coronary" process.[61]

It appears that plaque rupture may be a frequent event that only occasionally leads to ACS.[12] It is not clear why some plaque ruptures lead to clinical manifestations, whereas others remain asymptomatic and heal, perhaps leading to disease progression.[12] In an IVUS study, Fuji et al demonstrated that ulcerated ruptured plaques leading to ACS have smaller lumen CSA, greater plaque burden in vessels with greater positive remodeling.[12] Therefore, the clinical picture may be dependent on the original severity of stenosis or thrombus formation.[50])

Asymptomatic ruptured plaques may be associated with lesion progression when they heal. These data are in line with pathological studies showing that ulceration, thrombosis and healing of the plaque is associated with plaque progression.[62] Levin et al. showed that 80% of angiographic complex lesions had pathologic evidence of plaque rupture, plaque haemorrhage, or thrombus.[63]

Intraplaque haemorrhage from leaky vasa vasorum infiltrating the plaque from adventitia, is considered as an important factor for necrotic core enlargement and the progression of coronary atheroma[64]. Cholesterol derived from erythrocyte membranes may exceed a critical level, forming

an immiscible cholesterol phase and ultimately crystallizing. Furthermore, the percentage of cholesterol clefts is greater in lesions that have ruptured than in fibrocalcific plaques.[64] Cholesterol clefs are detected especially by high resolution imaging techniques as OCT.[65]

4.1 | Limitations and strengths

The inherent limitations of observational studies included in the analysis is the main limitation. Although the selection and assessment of the outcome were of good quality, with low risk of bias, the comparison suffered, since few studies used matching or same subject comparisons. Nevertheless, several important confounders were similarly distributed within the compared groups, but, this is not enough to limit the confounding bias possibility.

In patients with multivessel disease, identification of the culprit lesions that would be imaged by IVUS may be difficult. Generally, it was performed on the basis of the combination of the angiographic lesion appearance, electrocardiographic and echocardiography changes.[36]

Also, the use of IVUS to identify high-risk plaques and distinguish the culprit lesion present some drawbacks and challenges.

Thrombus formation is often not detected with gray scale ultrasound. [60]

Because of the limited IVUS resolution, some ruptured plaques with small cavities might be missed, especially when the cavity is filled by thrombus. [60]

The difficulties of IVUS-VH in detecting the fibrous cap could lead to an overestimation of TCFA. Conversely, the incidence of VH-TCFA in lesions with plaque rupture, may be underestimate, because the necrotic core may have embolized after plaque rupture [35]

TCFAs are better identified by OCT imaging technique. [65] Nevertheless, plaque burden and remodeling analysis require the high imaging penetration capacity of IVUS, especially in large vessels. OCT fails to identify external elastic membrane when plaques with necrotic cores are present due to attenuation of the light by the lipids.[65]

As unstable plaques are dynamic in nature, their morphology may change in time, until the imaging moment.[66]

The composition of the plaques was generally reported at the level of MLA although it is known that this is not the worse level regarding necrotic cores and vulnerability. Only several studies reported composition at both levels.[53]

IVUS catheters used were different among the studies included in our analysis. Different spectral analysis algorithms of the IVUS radiofrequency data were used (VH, iMAP etc).

The majority of the analyses showed heterogeneity between the studies' results. But the results were concordant regarding the direction of the association, with rare studies stating the opposite. Therefore, this similarity argues for having confidence in the results.

For only one comparison we found a statistically significant *p*-value for the Egger test, that assesses publication bias, but since all the other comparison were not statistically significant, it is possible that this observation might be due to hazard.

Our study is the only systematic review with metanalysis on the topic, it includes the largest amount of studies assessing plaques using IVUS in the most important comparison scenarios, it has a robust methodology, including a systematic and extensive literature search in several databases, without language restriction. An exhaustive IVUS plaque characterization emerged as we included in our analysis all greyscale and VH parameters.

4.2 | Practice implications

There are high-risk features of atherosclerotic plaques imaged by IVUS that have been shown to correlate with future MACE in previous studies.[67] However, plaques with characteristics of vulnerability have a highly dynamic nature preventing an efficient implementation of interventional treatment strategies towards the plaques whose risk of future events is greater than interventional treatment. Therefore, a better understanding of vulnerable plaque behaviour is needed. From this perspective, our study represents a step forward, as we have analysed a large number of IVUS features in culprit lesions from both unstable and stable patients and underlined the differences between them. Therefore, the present analysis results into a better understanding of the vessel wall distribution and composition of plaques in ACS patients compared to stable ones.

It also provides some insights into the interaction with coronary interventions. PCI (especially with large diameter stents and high pressures) against such large, lipidic loaded plaques might be associated with more microembolies, microvascular obstruction and therefore with no-reflow/slow flow phenomenon.[45] Actually, it is known that ACS patients with no-reflow phenomenon after stent deployment have larger necrotic cores lesions.[68]

4.3 | Future research

An extension and completion of the findings about plaque composition and instability with data revealed by imaging methods with higher resolution as OCT. Nevertheless, due to its lower tissue penetration OCT is less suited than IVUS for evaluation of remodeling and plaque burden.

5 | CONCLUSIONS

Our pooled analysis on coronary IVUS imaging showed that culprit lesions in ACS patients are more positively remodeled with larger plaque burdens and had larger necrotic cores than both culprit lesions from SAP patients and non-culprit lesions from the same ACS. Non-culprit lesions from ACS patients were more frequently ruptured or had a TCFA morphology than non-culprit lesions from SAP patients. The studies had generally a low risk of bias. Heterogenous results were observed, but the majority of studies agreed in respect to the direction of the association.

AKNOWLEDGEMENT

This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2019-2404, within PNCDI III.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ORCID

Daniel-Corneliu Leucuta https://orcid.org/0000-0003-4218-8622

REFERENCES

1. Ambrose JA, Tannenbaum MA, Alexopoulos D, et al. Angiographic progression of coronary artery disease and the development of myocardial infarction. *J Am Coll Cardiol*. 1988;12:56-62.
2. Davies MJ, Thomas A. Thrombosis and acute coronary -artery lesions in sudden cardiac ischemic death. *N Engl J Med*. 1984;3101137-3101140.
3. Schoenhagen P, Zaida KM, Kapadia SR, Crowe TD, Nissen SE, Tuzcu EM. Extent and direction of arterial remodeling in stable versus unstable coronary syndromes. *Circulation*. 2000;101:598-603.
4. Fernandez-Ortiz A, Badimon JJ, Fark E, et al. Characterization of the relative thrombogenicity of atherosclerotic plaque components: implications for consequences of plaque rupture. *J Am Coll Cardiol*. 1994;23:1562-1569.
5. Falk E, Wilensky RL. Prediction of coronary events by intravascular imaging. *J Am Coll Cardiol*. 2012;5(Suppl. 5):S38-S41.
6. Libby P, Schoenbeck U, Mach F, Selwyn AP, Ganz P. Current concepts in cardiovascular pathology: the role of LDL cholesterol in plaque rupture and stabilization. *Am J Med*. 1998;104(2A):14S-18S.
7. Virmani R, Kolodgie FD, Burke AP, et al. Lesson from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol*. 2000;20:1262-1275.
8. Smits PC, Pasterkamp G, de Jaegere PP, et al. Angioscopic complex lesions are predominantly compensatory enlarged: an angioscopy and intracoronary ultrasound study. *Cardiovasc Res*. 1999;41:458-464.
9. Konig A, Bleie O, Dudek D, Marso S, Rogers JH, et al. Coronary plaque dimensions and composition by intravascular ultrasound radio frequency lesion segment analysis in stable and unstable angina patients. *Coron Artery Dis*. 2009;20(5):309-316.
10. Hernando L, Corros C, Gonzalo N, et al. Morphological characteristics of culprit coronary lesions according to clinical presentation: insights from a multimodality imaging approach. *Int J Cardiovasc Imaging*. 2013;29(1):13-21.
11. Surmely JF, Nasu K, Fujita H, et al. Coronary plaque composition of culprit/target lesions according to the clinical presentation: a virtual histology intravascular ultrasound analysis. *Eur Heart J*. 2006;27(24):2939-2944.
12. Fujii K, Kobayashi Y, Minz GS, et al. Intravascular ultrasound assessment of ulcerated ruptured plaques. a comparison of culprit and nonculprit lesions of patients with acute coronary syndromes and lesions in patients without acute coronary syndromes. *Circulation*. 2003;108:2473-2478.
13. Souza CF, Maehara A, Mintz GS, et al. Tissue characterization and phenotype classification in patients presenting with acute myocardial infarction: Insights from the iWonder study. *Catheter Cardiovasc Interv*. 2017;90(7):1107-1114.
14. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol*. 2014;14:135.
15. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (Editors). *Cochrane Handbook for Systematic Reviews of Interventions* (2nd ed). John Wiley & Sons: Chichester (UK). 2019:167–168.
16. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality in Nonrandomized Studies in Meta-Analyses*. 2012. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
17. R Core Team. *R: A Language and Environment for Statistical Computing*. Viena, Austria: R Foundation for Statistical Computing. 2020. https://www.R-project.org/
18. Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: a practical tutorial. *Evidence-Based Mental Health*. 2019;153-160.
19. Simera I, Moher D, Hoey J, Schulz KF, Altman DG. A catalogue of reporting guidelines for health research. *Eur J Clin Invest*. 2010;40(1):35-53.
20. Moher D, Liberati A, Tetzlaff J, Altman DG. PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009;151:264-269.
21. Jeremias A, Spies C, Herity NA, et al. Coronary artery compliance and adaptive vessel remodelling in patients with stable and unstable coronary artery disease. *Heart*. 2000;84:314-319.
22. Bae JH, Kwon TG, Kim KH, Hyun DW, Kim KY, Kim DS. In-vivo coronary plaque composition in patients with acute coronary syndrome: a virtual histology intravascular ultrasound study. *Korean Circ J*. 2007;37(9):437-442.
23. Uemura R, Tanabe J, Yokoyama H, Ohaki M. Impact of histological plaque characteristics on intravascular ultrasound parameters at culprit lesions in coronary artery disease. *Int Heart J*. 2006;47(5):683-693.
24. Hong MK, Minz GS, Lee CW, et al. Comparison of virtual histology to intravascular ultrasound of culprit coronary lesions in acute coronary syndrome and target coronary lesions in stable angina pectoris. *Am J Cardiol*. 2007;100:953-959.
25. Hong MK, Minz GS, Lee CW, et al. A three-vessel virtual histology intravascular ultrasound analysis of frequency and distribution of thin-cap fibroatheromas in patients with acute coronary syndrome or stable angina pectoris. *Am J Cardiol*. 2008;101:568-572.

26. Hong MK, Minz GM, Lee CW, et al. Comparison of coronary plaque rupture between stable angina and acute myocardial infarction a three-vessel intravascular ultrasound study in 235 patients. *Circulation*. 2004;110:928-933.
27. Toutouzas K, Synetos A, Stefanadi E, et al. Correlation between morphologic characteristics and local temperature differences in culprit lesions of patients with symptomatic coronary artery disease. *J Am Coll Cardiol*. 2007;49(23):2264-2271.
28. Ko YG, Son JW, Park SM, et al. Effect of vessel size on lipid content of coronary plaques assessed by integrated backscatter intravascular ultrasound. *Circ J*. 2010;74(4):754-759.
29. Cascón-Pérez JD, de la Torre-Hernández JM, Ruiz-Abellón MC, et al. Characteristics of culprit atheromatous plaques obtained in vivo by intravascular ultrasound radiofrequency analysis: results from the CULPLAC study. *Am Heart J*. 2013;165(3):400-407.
30. Ivanovíc M, Rancić M, Rdzanek A, Filipjak KJ, Opolski G, Cvetanović J. Virtual histology study of atherosclerotic plaque composition in patients with stable angina and acute phase of acute coronary syndromes without ST segment elevation. *Srp Arh Celok Lek*. 2013;141:308-314.
31. Li XM, Huang CX, Wang T, Zhuang SW, Zhou H, Tian B. Comparison of coronary plaque composition among patients with acute coronary syndrome and stable coronary artery diseases. *Chin Med J*. 2008;121:534-539.
32. Kozuki A, Shinke T, Otake H, et al. Feasibility of a novel radiofrequency signal analysis for in-vivo plaque characterization in humans: comparison of plaque components between patients with and without acute coronary syndrome. *Int J Cardiol*. 2013;167:1591-1596.
33. Lee CW, Hwang I, Park CS, et al. Differences in intravascular ultrasound and histological findings in culprit coronary plaques between ST-segment elevation myocardial infarction and stable angina. *J Thromb Thrombolysis*. 2014;37(4):443-449.
34. Murray SW, Stables RH, Garcia-Garcia HM, et al. Construction and validation of a plaque discrimination score from the anatomical and histological differences in coronary atherosclerosis: the Liverpool IVUS-V-HEART (intra vascular ultrasound-virtual-histology evaluation of atherosclerosis requiring treatment) study. *EuroIntervention*. 2014;10(7):815-823.
35. Dong L, Mintz GS, Witzenbichler B, et al. Comparison of plaque characteristics in narrowings with ST-elevation myocardial infarction (STEMI), non-STEMI/unstable angina pectoris and stable coronary artery disease (from the ADAPT-DES IVUS Substudy). *Am J Cardiol*. 2015;115(7):860-866.
36. Ando H, Amano T, Matsubara T, et al. Comparison of tissue characteristics between acute coronary syndrome and stable angina pectoris–an integrated backscatter intravascular ultrasound analysis of culprit and non-culprit lesions. *Circ J*. 2011;75:383-390.
37. Kotani J, Mintz GS, Castagna MT, et al. Intravascular ultrasound analysis of infarct-related and non–infarct-related arteries in patients who presented with an acute myocardial infarction. *Circulation*. 2003;107:2889-2893.
38. Rodriguez-Granillo GA, Garcia-Garcia HM, McFadden EP, et al. In vivo intravascular ultrasound-derived thin-cap fibroatheroma detection using ultrasound radiofrequency data analysis. *J Am Coll Cardiol*. 2005;46:2038-2042.
39. Nakamura T, Kubo N, Funayama H, Sugawara Y, Ako J, Momomura S. Plaque characteristics of the coronary segment proximal to the culprit lesion in stable and unstable patients. *Clin Cardiol*. 2009;32(8):E9-E12.
40. Rdzanek A, Kochman J, Pietrasik A, Wilczynska J, Rancic M, Opolski G. The prevalence of potentially unstable coronary lesions in patients with coronary artery disease–virtual histology study. *Kardiol Pol*. 2008;66(3):244-250.
41. Liu HL, Zhang J, Ma DX, et al. Coronary plaque characterization of nonculprit or nontarget lesions assessed by analysis of in vivo intracoronary ultrasound radio-frequency data. *Chin Med J (Engl)*. 2009;122(6):622-626.
42. Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. *N Engl J Med*. 1987;316:1371-1375.
43. Davies MJ, Richardson PD, Woolf N, Katz D r, Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. *Br Heart J*. 1993;69:377-381.
44. Pasterkamp G, Schoneveld AH, van der Wal AC, et al. Relation of arterial geometry to luminal narrowing and histologic markers for plaque vulnerability: the remodeling paradox. *J Am Coll Cardiol*. 1998;32:655-662.
45. Marc MC, Iancu AC, Ober CD, et al. Pre-revascularization coronary wedge pressure as marker of adverse long-term left ventricular remodelling in patients with acute ST-segment elevation myocardial infarction. *Sci Rep*. 2018;8(1):1897.
46. Yamada R, Okura H, Kume T, et al. Relationship between arterial and fibrous cap remodeling: a serial three-vessel intravascular ultrasound and optical coherence tomography study. *Circ Cardiovasc Interv*. 2010;3(5):484-490.
47. Langille BL, O'Donnell F. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science*. 1986;231(4736):405-407. 10.1126/science.3941904
48. Stone PH, Saito S, Takahashi S, et al. Prediction of progression of coronary artery disease and clinical outcomes using vascular profiling of endothelial shear stress and arterial plaque characteristics: the PREDICTION Study. *Circulation*. 2012;126(2):172-181. 10.1161/CIRCULATIONAHA.112.096438
49. Richardson PD, Davies MJ, Born GV. Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. *Lancet*. 1989;2(8669):941-944. 10.1016/s0140-6736(89)90953-7
50. Maehara A, Mintz GS, Bui AB, et al. Morphologic and angiographic features of coronary plaque rupture detected by intravascular ultrasound. *J Am Coll Cardiol*. 2002;40:904-910.
51. Rodriguez-Granillo GA, Garcia-Garcia HM, Valgimigli M, et al. Global characterization of coronary plaque rupture phenotype using three-vessel intravascular ultrasound radiofrequency data analysis. *Eur Heart J*. 2006;27:1921-1927.
52. de Graaf MA, van Velzen JE, de Graaf FR, et al. The maximum necrotic core area is most often located proximally to the site of most severe narrowing: a virtual histology intravascular ultrasound study. *Heart Vessels*. 2013;28(2):166-172.
53. Konig A, Bleie O, Rieber J, et al. Intravascular ultrasound radiofrequency analysis of the lesion segment profile in ACS patients. *Clin Res Cardiol*. 2010;99:83-91.
54. Mintz GS. Intravascular imaging of coronary calcification and its clinical implications. *JACC Cardiovasc Imaging*. 2015;8(4):461-471. 10.1016/j.jcmg.2015.02.003
55. Inaba S, Okayama H, Funada JI, et al. Relationship between smaller calcifications and lipid-rich plaques on integrated backscatter-intravascular ultrasound. *Int J Cardiol*. 2010;145(2):347-348. 10.1016/j.ijcard.2009.12.011

56. Mori H, Torii S, Kutyna M, Sakamoto A, Finn AV, Virmani R. Coronary artery calcification and its progression: what does it really mean? *JACC Cardiovasc Imaging*. 2018;11(1):127-142. 10.1016/j.jcmg.2017.10.012
57. Asakura M, Ueda Y, Yamaguchi O, et al. Extensive development of vulnerable plaques as a pan-coronary process in patients with myocardial infarction: an angioscopic study. *J Am Coll Cardiol*. 2001;37:1284-1288.
58. Tanaka A, Shimada K, Sano T, et al. Multiple plaque rupture and C-reactive protein in acute myocardial infarction. *J Am Coll Cardiol*. 2005;45:1594-1599.
59. Rioufol G, Finet G, Andre-Fouet X, et al. Multiple ruptures of atherosclerotic plaques in acute coronary syndrome. endocoronary ultrasonography study of three arteries. *Arch Mal Coeur Vaiss*. 2002;95:157-165.
60. von Birgelen C, Klinkhart W, Minz GS, et al. Plaque Distribution and Vascular Remodeling of Ruptured and Nonruptured Coronary Plaques in the Same Vessel: An Intravascular Ultrasound Study In Vivo. *J Am Coll Cardiol*. 2001;37:1864-1870.
61. Goldstein JA, Demetriou D, Grines CL, Pica M, Shoukfeh M, O'Neill WW. Multiple complex coronary plaques in patients with acute myocardial infarction. *N Engl J Med*. 2000;28:915-922.
62. Mann J, Davies MJ. Mechanisms of progression in native coronary artery disease: role of healed plaque disruption. *Heart*. 1999;82:265-268.
63. Levin DC, Fallon JT. Significance of the angiographic morphology of localized coronary stenoses: histopathologic correlations. *Circulation*. 1982;66:316-320.
64. Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R. Concept of vulnerable/unstable plaque. *Arterioscler Thromb Vasc Biol*. 2010;30(7):1282-1292. 10.1161/ATVBAHA.108.179739
65. Spinu M, Olinic DM, Olinic M, Homorodean C. In vivo imaging of complicated atherosclerotic plaque-role of optical coherence tomography (OCT). *Rom J Morphol Embryol*. 2018;59(2):469-478.
66. Kubo T, Maehara A, Mintz GS, et al. The dynamic nature of coronary artery lesion morphology assessed by serial virtual histology intravascular ultrasound tissue characterization. *J Am Coll Cardiol*. 2010;55(15):1590-1597. 10.1016/j.jacc.2009.07.078
67. Stone GW, Maehara A, Lansky AJ. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med*. 2011;364(3):226-235. 10.1056/NEJMoa1002358
68. Hong YJ, Jeong MH, Choi YH, et al. Impact of plaque components on no-reflow phenomenon after stent deployment in patients with acute coronary syndrome: a virtual histology-intravascular ultrasound analysis. *Eur Heart J*. 2011;32(16):2059-2066.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Homorodean C, Leucuta D-C, Ober M, et al. Intravascular ultrasound insights into the unstable features of the coronary atherosclerotic plaques: A systematic review and meta-analysis. *Eur J Clin Invest*. 2021;00:e13671. https://doi.org/10.1111/eci.13671

Received: 5 May 2021 | Revised: 27 September 2021 | Accepted: 28 September 2021

DOI: 10.1111/jep.13635

Journal of Evaluation in Clinical Practice
International Journal of Public Health Policy and Health Services Research

WILEY

ORIGINAL PAPER

Concordance of pharmacist versus patient responses regarding counselling in community pharmacy

Camelia Bucsa PhD[1] | Andreea Farcas PhD[1] | Mihaela Udrea PhD[1] | Marius Bojita PhD[1] | Cristina Mogosan PhD[1] | Daniel Leucuta PhD[2]

[1]Drug Information Research Center, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

[2]Department of Medical Informatics and Biostatistics, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Correspondence
Andreea Farcas, Drug Information Research Center, "Iuliu Hatieganu" University of Medicine and Pharmacy, 6A Pasteur Street, Cluj-Napoca 400349, Romania.
Email: afarcas@umfcluj.ro

Abstract

Rationale and objectives: Patient counselling on medication is one of the activities that can and should be performed in community pharmacy. Patient counselling was proved to have a positive effect on clinical outcomes, quality of life, drug/disease knowledge, satisfaction and reduced health-service utilization. Our objective was to assess the degree of concordance between the responses of patient and pharmacist on the same questions regarding provided counselling.

Methods: Data from two questionnaires containing a common block of 14 questions regarding the counselling provided on the medications use, safety concerns, storage, validity term, disposal and disease monitoring was paired and analysed. Questionnaires were paired based on a code and Kappa Cohen coefficient (KCc) and the prevalence adjusted biased adjusted kappa (PABAK) were calculated to evaluate the degree of concordance between pharmacist versus patient responses. The values of the KCc and PABAK were interpreted as per Altman.

Results: For the 14 questions, data from 2047 to 2378 questionnaires collected from 520 community pharmacies in 10 of Romania's counties were analysed. The highest level of concordance ('very good') was achieved on the items regarding the counselling on the medicines' route of administration (PABAK = 0.88), time of administration (PABAK = 0.80) and dosage (PABAK = 0.82). The highest disagreement (week concordance) was found on the question regarding the counselling on the medicines adverse effects (PABAK = 0.01), where 44.8% of patients responded that they received counselling as compared to 93.1% of the pharmacists who responded that they offered counselling. For the rest of the questions, moderate concordance was found.

Conclusion: Overall a moderate level of concordance between patient and pharmacist responses was found on the majority of the questions, with the highest level found for drug use (dosage, route and time of administration). The highest discrepancy was found for the counselling on the medicines adverse effects.

KEYWORDS
agreement, community pharmacy, concordance, counselling, pharmacist patient communication

1 | INTRODUCTION

Over the past decades, the pharmacist profession has undergone a change in practice, with additional emphasis on counselling and providing pharmaceutical care to the patient. Studies carried out worldwide on the impact of the pharmacist-led counselling underline the positive effect of this change on clinical outcomes, quality of life, drug/ disease knowledge, satisfaction and reduced health service utilization among patients.[1–9]

However, in everyday pharmaceutical practice, the provision of pharmaceutical care and counselling was showed to be limited. In a study from 2017 in Saudi Arabia that included 11 community pharmacies, counselling was evaluated by observers. They found that only 20.1% of the counselling contents of the pharmacist–patient interaction were performed adequately.[10] A 2015 study, conducted in Basel, Switzerland, in 20 community pharmacies, concludes that only half of the total (1476) surveyed patients received counselling on treatment adherence.[11] This study also mentions differences in perception of counselling from the pharmacist and patient perspective. This difference may come from deficient understanding of the counselling provided (poor communication).

Several organizations have defined specific items of counselling that should be considered during the pharmacist–patient interaction. The United States Pharmacopoeia (USP) issued a 35-items guideline for pharmacist counselling[12] and the American Society of Health-System Pharmacists (ASHP) issued the Guidelines on Pharmacist-Conducted Patient Education and Counselling[13] with specific items to be followed during the process of counselling. In Europe, The European Directorate for the Quality of Medicines & HealthCare (EDQM) recommends to all governments to promote and implement pharmaceutical care, of which pharmacist counselling is part.[14,15]

In Romania, patient counselling activities and monitoring the patient therapy for drug–drug interactions, adherence, efficacy and adverse drug reactions (ADRs) are activities covered by the Good Pharmacy Practice (GPP) regulations which were implemented in 2010 and are still valid and in line with the international recommendations.[16] The counselling sessions are not reimbursed in Romania and there are no incentives for the pharmacist to perform the counselling sessions apart from the professional and ethical obligations. Professional pride and work-related satisfaction might also contribute to providing patient counselling. However, a recent survey that has been conducted on Romanian patients has outlined that counselling is not optimal and that the communication process with the patient needs to be improved through multi-question inquiries and drug-related advice, such as drug administration methods, dosage and common adverse reactions.[17]

Based on the GPP regulations, pharmacists and patients were inquired on the counselling activities performed in the community pharmacies in a wider survey. Results on the pharmacists and patients responses regarding different aspects of the pharmacy visit were already published.[18,19] As different recent studies have highlighted gaps in pharmacist–patient communication and counselling,[20–23] we aimed to additionally assess in a post-hoc analysis the concordance between the patients' and pharmacists' responses on the counselling provided during a pharmacy visit, by matching a common block of questions regarding counselling. While, there is a developing body of research using audio and video recordings to describe the content of pharmacist–patient communication,[20,24,25] the concordance between the patient and pharmacist perceived counselling has not been studied yet. Stimulated recall of counselling information provided in pharmacist–patient interaction, with crosschecking the two parties' answers, may provide additional, comprehensive evidence on the type of counselling offered and on what kind of information ultimately reaches the patient. To our knowledge, applying concordance statistics methods have not been used so far in order to test counselling impact on a large sample of pharmacists and patients.

2 | METHODS

2.1 | Study design and setting

For this concordance analysis, data collected in a previous survey were used.[18,19] The survey was performed in 520 community pharmacies, between February and August 2012. Pharmacies have been selected based on convenience-based sampling. Pharmacists were invited to join the study, and those that provided their consent were subsequently included in the study. The pharmacies involved in the study represented 30% of the community pharmacies in the included counties, respectively, 9.8% of the pharmacies in Romania at that time.

Each rural pharmacy received via email 10 questionnaires (less than the urban pharmacies due to the lower number of patients) and each urban pharmacy received 20 questionnaires to be filled-in on the following day by the pharmacists and patients, after each pharmacist–patient interaction, for the first 10/20 patients of the pharmacy that day. Pharmacists were informed on the methodology (filling-in the questionnaires for the first 10/20 patients that enter the pharmacy that respective day, after each patient visit) and briefly on the scope of the research (to evaluate the patient counselling in the community pharmacy). The questionnaires were self-administered, anonymous and based on voluntary participation, thus implying consent to participate.

2.2 | Variables and measurement

The two questionnaires were developed based on the GPP rules from Romania and were tailored in terms of language for pharmacists or patients.[16] For each patient that filled the questionnaire, the pharmacist completed a corresponding one. The questionnaires were anonymous and contained a common block of 14 yes/no questions about the counselling provided during the pharmacy visit that just ended (Table 1). Answering 'yes' to the questions regarding provided counselling was considered a positive answer (i.e., counselling was provided), while answering 'no' was considered a negative answer

TABLE 1 Concordance of pharmacist and patient responses regarding counselling in community pharmacy

Counselling item	No. of questionnaires	+Pa/+Ph % (*n*)	+Pa/−Ph (*n*)	−Pa/+Ph (*n*)	−Pa/−Ph (*n*)	Cohen's kappa coefficient (95% CI)[a]	PABAK	Positive agreement (95% CI)	Negative agreement (95% CI)
Route of administration	2378	93.2 (2217)	0.4 (9)	5.4 (129)	1.0 (23)	0.23 (0.15-0.31)	0.88	0.97 (0.96-0.97)	0.25 (0.17-0.33)
Administration in relation to meals	2355	89.1 (2099)	1.1 (25)	7.9 (187)	1.9 (44)	0.33 (0.27-0.39)	0.80	0.95 (0.95-0.96)	0.29 (0.23-0.36)
Dose, time interval between doses, length of treatment	2331	87.1 (2031)	1.5 (36)	8.4 (195)	3.0 (69)	0.26 (0.19-0.33)	0.82	0.95 (0.94-0.95)	0.37 (0.31-0.44)
Treatment scheme	2257	72.4 (1634)	1.7 (39)	19.8 (447)	6.10 (137)	0.27 (0.23-0.32)	0.57	0.87 (0.86-0.88)	0.36 (0.32-0.40)
Contraindications and precautions for use	2317	68.3 (1582)	2.2 (52)	23.1 (535)	6.4 (148)	0.23 (0.19-0.27)	0.49	0.84 (0.83-0.86)	0.34 (0.30-0.38)
Drug–drug and drug–food interactions	2273	64.4 (1464)	3.0 (68)	22.1 (503)	10.5 (238)	0.33 (0.29-0.37)	0.50	0.84 (0.82-0.85)	0.45 0.42-0.49)
Adverse drug reactions	2294	44.2 (1013)	0.7 (17)	49.0 (1123)	6.1 (141)	0.09 (0.07-0.10)	0.01	0.64 (0.62-0.66)	0.20 (0.17-0.23)
Effect of drug on laboratory analyses	2047	31.6 (646)	3.5 (71)	24.2 (495)	40.8 (835)	0.47 (0.43-0.50)	0.45	0.70 (0.67-0.72)	0.75 (0.73-0.77)
Drug storage conditions	2262	61.3 (1386)	2.7 (61)	18.6 (421)	17.4 (349)	0.49 (0.45-0.53)	0.57	0.85 (0.84-0.87)	0.62 (0.59-0.65)
Drug validity period	2245	49.9 (1120)	2.7 (60)	26.4 (592)	21.1 (473)	0.40 (0.37-0.44)	0.41	0.77 (0.76-0.79)	0.59 (0.56-0.62)
Lifestyle and diet	2246	64.2 (1441)	2.6 (59)	23.6 (530)	9.6 (216)	0.30 (0.26-0.34)	0.48	0.83 (0.82-0.84)	0.42 (0.39-0.46)
Consulting pharmacist/ physician in case of adverse drug reactions	2282	62.8 (1433)	1.8 (42)	26.5 (605)	8.9 (202)	0.26 (0.23-0.30)	0.43	0.82 (0.80-0.83)	0.38 (0.35-0.42)
Encouraging pharmacy monitoring	2187	62.4 (1365)	3.1 (67)	21.0 (4959)	13.5 (296)	0.39 (0.35-0.43)	0.52	0.84 (0.82–0.85)	0.53 (0.49-0.57)
Obligation to return to the pharmacy the unused psychotropic/narcotic drugs	1704	23.2 (395)	1.8 (30)	32.5 (554)	42.5 (725)	0.35 (0.32-0.39)	0.31	0.58 (0.54-0.60)	0.71 (0.69-0.74)

Abbreviations: CI, confidence interval; +Pa, positive for patient (the patient stated that the counselling was provided); +Pa/+Ph % (*n*) (both the patient and the pharmacist stated that the counselling was provided), the percentage of positive answers for both patient and pharmacist, out of total answers, and the absolute number; +Ph, positive for pharmacist (the pharmacist stated that the counselling was provided); −Pa, negative for patient (the patient stated that the counselling was not provided); −Ph, negative for pharmacist (the pharmacist stated that the counselling was not provided); PABAK, prevalence and biased adjusted kappa.

[a]All the Cohen's Kappa results are statistically significant with the *P*-value of <0.001.

(i.e., counselling was not provided). The questionnaires were then paired based on a unique code and the concordance analysis was performed.

2.3 | Statistical analyses

Categorical data were presented as counts and percentages. The concordance statistics between pharmacists' and patients' answers were computed using a spreadsheet for the calculation of comprehensive statistics for the assessment of diagnostic tests and inter-rater agreement.[26] Cohen's kappa coefficient with 95% confidence intervals was calculated and also a test for its significance. Kappa coefficient measures the normed difference between the observed rate of agreement and the expected purely by chance rate of agreement and takes values between 0 (less than chance agreement) and 1 (almost perfect agreement). Altman's interpretation for the strength of agreement of Kappa coefficient (≤0.20—poor; 0.21–0.40—fair; 0.41–0.60—moderate; 0.61–0.80—good; 0.81–1.00—very good) was used.[27] However, kappa coefficient does not take into account the bias between observers (the extent of disagreement) or the distribution of data across the categories (prevalence). The interpretation of kappa alone without indication on the prevalence or bias can be inaccurate so the prevalence adjusted biased adjusted kappa (PABAK) was also calculated. The interpretation for PABAK is the same as for Cohen's kappa. The positive and negative agreement with 95% confidence intervals is also provided. These represent agreement regarding the positive and the negative answer to the question. The positive agreement estimates the conditional probability, given that one of the raters (patient or pharmacist), randomly selected, makes a positive rating, the other rater (patient or physician) will also do so. For all the tests the two-tailed *P*-value was computed, and a 0.05 level of significance was used.

3 | RESULTS

The total number of pharmacists participating in the survey was 994 and they filled in 3155 questionnaires (3.17 questionnaires/pharmacist and 6.07 questionnaires/pharmacy). The total number of questionnaires from patients was 3303 (6.35 questionnaires/pharmacy). The concordance analysis was performed on a pair of 2047–2378 patient–pharmacist questionnaires (pairing based on the unique questionnaire code), depending on how many of the 14 questions were answered (pairing performed at question level). Figure 1 is summarizing this data.

The concordance (Cohen's kappa coefficient, PABAK, positive and negative agreement) between patient's and pharmacist's responses are depicted in Table 1. The percentage of answers stating whether counselling was provided or not, seen from different perspectives, as well as the prevalence of answers stating that counselling was provided are presented in Table 2.

The information about the 'route of administration', the 'administration in relation to meals', the 'dose, time interval between doses and the length of treatment', had very high positive agreement (0.95–0.97),

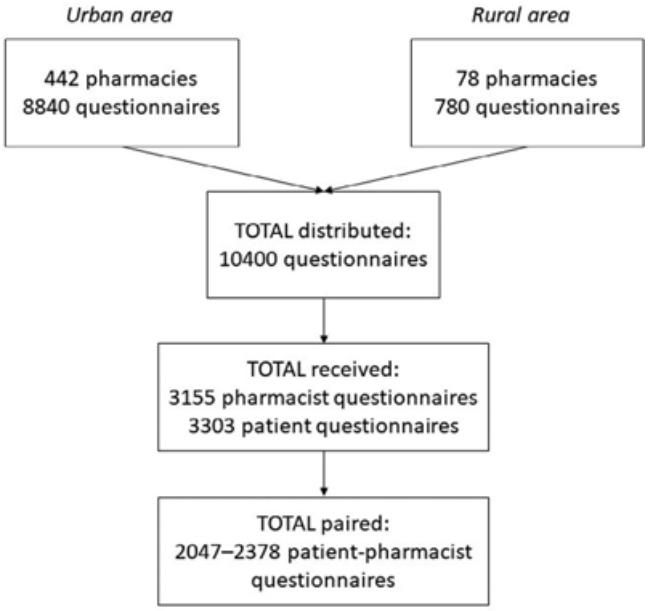

FIGURE 1 Flowchart on distributed and analysed questionnaires

relatively low negative agreement (0.25–0.37), very good PABAK and fair statistically significant Cohen's Kappa coefficient. The percentage of patients stating that the counselling was provided if pharmacists stated they provided counselling was very high (91.2%–94.5%). Also, the percentage of patients stating that the counselling was not provided if pharmacists stated they did not provided counselling was above average (65.7%–71.9%). The prevalence of pharmacists stating they provided counselling was very high (95.5%–98.7%).

The majority of the questions, regarding 'treatment scheme', 'contraindications and precautions for use', 'drug-drug and drug-food interactions', 'drug storage conditions', 'lifestyle and diet', 'consulting pharmacist/physician in case of adverse drug reactions', 'encouraging pharmacy monitoring', had high (0.82–0.87) positive agreement, low to moderate negative agreement (0.34–0.62) and moderate PABAK. The percentage of patients stating that the counselling was provided if pharmacists stated they provided counselling was above average (70.3%–78.5%). Also, the percentage of the patients stating that the counselling was not provided if pharmacists stated they did not provided counselling was above average (74.0%–86.6%). The prevalence of pharmacists stating that they provided counselling was moderate to high (79.9%–92.2%). 'Drug validity period' was close to this group of questions too.

The ADRs item had the pharmacist positive prevalence very high (93.1%), the PABAK of 0.01, the second lowest positive agreement (0.64) and the lowest negative agreement (0.20). The percentage of patients stating that counselling was provided if pharmacists stated they provided counselling was the second lowest between questions (47.4%). The percentage of patients stating that counselling was not provided if pharmacists stated they did not provided counselling was high (89.2%).

The 'effect of drug on laboratory analyses', and the 'obligation to return to the pharmacy the unused psychotropic/ narcotic drugs',

TABLE 2 The percentage of positive and negative answers, seen from different perspectives, as well as the positive answers' prevalence

Item	No. of questionnaires	+Pa/+Ph % sensitivity	+Ph/+Pa % positive predictive value	−Pa/−Ph % specificity	−Ph/−Pa % negative predictive value	+Ph prevalence %	+Pa prevalence %
Route of administration	2378	94.5	99.6	71.9	15.1	98.7	93.6
Administration in relation to meals	2355	91.8	98.8	63.8	19.0	97.1	90.1
Dose, time interval between doses, length of treatment	2331	91.2	98.3	65.7	26.1	95.5	88.7
Treatment scheme	2257	78.5	97.7	77.8	23.5	92.2	74.1
Contraindications and Precautions for use	2317	74.7	96.8	74.0	21.7	91.4	70.5
Drug–drug and drug–food interactions	2273	74.4	95.6	77.8	32.1	86.5	67.4
Adverse drug reactions	2294	47.4	98.3	89.2	11.2	93.1	44.8
Effect of drug on laboratory analyses	2047	56.6	90.1	92.2	62.8	55.7	35.0
Drug storage conditions	2262	76.7	95.9	86.6	48.3	79.9	63.9
Drug validity period	2245	65.4	94.9	88.7	44.4	76.3	52.6
Lifestyle and diet	2246	73.1	96.1	78.5	29.0	87.8	66.8
Consulting pharmacist/ physician in case of adverse drug reactions	2282	70.3	97.2	82.8	25.0	89.3	64.6
Encouraging pharmacy monitoring	2187	74.8	95.3	81.5	39.2	83.4	65.5
Obligation to return to the pharmacy the unused psychotropic/narcotic drugs	1704	41.6	92.9	96.0	56.7	55.7	24.9

Abbreviations: +Pa prevalence, the percentage of positive patients' answers; +Pa, positive for patient (the patient stated that the counselling was provided); +Pa/+Ph %, % of positive patient answer if pharmacist answered positive (the percentage of the patients that stated that the counselling was provided in the subgroup of interactions where pharmacists stated they provided counselling); +Ph prevalence, the percentage of positive pharmacists' answers; +Ph, positive for pharmacist (the pharmacist stated that the counselling was provided); +Ph/+Pa %, % of positive pharmacist answers if patient answered positive (the percentage of the pharmacists that stated that the counselling was provided in the subgroup of interactions where patients stated they received counselling); −Pa/−Ph %, % of negative patient answers if pharmacist answered negative (the percentage of the patients that stated that the counselling was not provided in the subgroup of interactions where pharmacists stated they did not provide counselling); −Ph/−Pa %, % of negative pharmacist answers if patients answered negative (the percentage of the pharmacists that stated that the counselling was not provided in the subgroup of interactions where patients stated they did not receive counselling).

were similar to the ADRs question, but their prevalence was the lowest between all the questions (55.7% for both). Also, the PABAK, was moderate (0.45) and fair (0.31), respectively.

4 | DISCUSSION

The effect of the community pharmacist counselling in different chronic or acute diseases has been well documented so far in different researches.[1,3,4] To what extent pharmacist counselling is happening in everyday life is more difficult to assess. Mystery shopper is one of the most used research methods in this regard,[28,29] aside videotaped encounters in the pharmacy.[20,25] As an alternative method, both pharmacist and patient counselling perspectives were assessed and then tested for the level of concordance between the two in this present analysis. A crosscheck between the information contained in both the pharmacist and the patient questionnaires increases the chances that the responses that are in high concordance are as close to reality as possible. To our knowledge, this is the first study to analyse concordance between pharmacists' and patients' answers on counselling, using the Cohen kappa method, and adding new insight on what kind of information reaches the patient in the counselling process.

In this study, we found that pharmacists counselling on the drug use does take place, with high concordance between pharmacists' and patients' answers for some items related to medication use. However, for all items, the prevalence of answers indicating that counselling provided was higher for pharmacists, as compared to patients. This is an important finding outlining that, discrepancies between what pharmacists think they are communicating and what patients ultimately understand do exist, implying that pharmacists and patients might not be sharing similar views on what constitutes effective communication. One explanation for this would be that the pharmacist counselled the

patient on a given item, but was not perceived as such by the patient (no effective communication between the two), due to different reasons, or the patients might simply not have been receptive to all counselling given. Effective communication with patients improves health status.[30] The pharmacist is seen as a communicator by the World Health Organization (WHO); this is also reiterated in their report 'The role of the pharmacist in the health care system: preparing the future pharmacist'.[31] Pharmacists must therefore adapt their communication to the wide variety of patient needs and achieve patient-centred communication.[32] Another explanation would be the recall bias. Pharmacists may have recalled that they provided counselling, especially on common day-to-day aspects which are more difficult to keep track of, while in fact they did not. In this regard, it may be useful to implement a system that allows tracking of the counselling activities and that can be quantified when needed. This type of system allowed Montgomery et al. team to follow-up the patients receiving a pharmaceutical care service in Sweden using the Swedish national patient medication records database. They found that one-third of the drug-related problems that needed pharmaceutical care service were side effects of drugs.[33]

The highest level of concordance between the two raters was registered for the items regarding counselling on the drug use. This is in line with the published literature which shows that information on how the medication should be used, is usually provided during pharmacist–patient interaction.[10,11] For these items, not only that the prevalence of pharmacists' positive answers was very high (95.5%–98.7%), but also the percentage of positive patients' answers if the pharmacists gave a positive answer is very high (91.2%–94.5%). This enables the conclusion that the counselling on these items was provided and it was perceived as such by both raters. Based on these results, it can also be concluded that effective communication on these items was in place for the high majority of the patients.

However, counselling on the drug use is not sufficient for a successful therapy. Other aspects, like patient safety, have a major impact on the effectiveness of treatment and should be prioritized in pharmacy counselling. In our study, the counselling on ADRs had the lowest level of concordance (PABAK = 0.01). This could be due to miscommunication between pharmacist and patient to some extent. By contrast, Shah and Chewning[34] found a strong positive correlation when comparing independent observer's answer to patient's answers regarding the provision of counselling on side effects in community pharmacies from Wisconsin, The USA. Also, in The USA, The U.S. Agency for Healthcare Research and Quality's 11-composite, validated Pharmacy Survey on Patient Safety Culture questionnaire applied to staff members who provide dispensing, clinical and support services within an integrated health delivery system showed that the concept of patient safety was the highest-scoring composite across all staff members.[35] Counselling on ADRs is recommended by the GPP regulations in Romania.[16] The ASHP guidelines also recommends counselling on potential common and severe adverse effects that may occur, actions to prevent or minimize their occurrence, and actions to take if they occur.[13] Therefore, counselling patients on potential adverse effects is important as it could allow for detection of ADRs when experienced by the patients and for timely and appropriate management and future prevention. Moreover, it would allow for subsequent reporting if these ADRs are serious or unknown, thus contributing to the drug safety monitoring and pharmacovigilance.

The counselling on 'effect of drug on laboratory analyses' and 'the obligation to return the unused psychotropic/narcotic drugs to the pharmacy' was the lowest among all the questions (55.7%), as reported by pharmacists. This is expected to happen, as this kind of information is only needed in specific occasions of the pharmacist–patient interaction.

4.1 | Advantages

To the best of our knowledge, this is the first study to analyse concordance between pharmacists' and patients' answers on counselling, using the Cohen kappa method. The study has a large sample of over 2000 questionnaires collected from 520 community pharmacies from 10 Romanian counties. Furthermore, these pharmacies represented at the time of the survey 30% of the community pharmacies in the included counties and 9.8% of the pharmacies in Romania. Therefore, the study has greater generalizability than single centre studies.

4.2 | Limitations

The main limitation of the study is represented by the fact the questionnaires were self-administered and related to a well establish sample of patients (the first 10/20 patients of the study day). Pharmacists might have counselled these patients more thoroughly than on a regular basis (the Hawthorne effect), thus increasing the prevalence of positive answers to the information given to the patients. However, the pharmacists in community pharmacies in Romania are usually overwhelmed with administrative work and they repeatedly express their wish to perform more counselling and this research may have been perceived also as an opportunity to highlight this issue. We could not measure these opposite effects with our methodology, that are difficult to avoid even with other study methodologies, except perhaps the mystery shopper methodology. The other limitations have a rather small impact on the study results interpretation. Using questionnaires addresses respondents' memory (both pharmacists and patients) and thus implies that a memory bias might be present. However, since the questionnaire was answered shortly after the discussion with the pharmacist, this bias is rather limited. Its impact can be in two directions, diminishing the overall concordance between the patient's and pharmacist answers, but more specifically, diminishing the positive agreement. Depending on individuals' perception, some patients might falsely say they were counselled on a specific item, even if they were not, or on the contrary—some patients might falsely say they were not counselled on a specific item, even if they were, depending on personal affinities. The first type would decrease the false negatives, and increase the positive agreement, while the second type would increase the false negatives and

decrease the positive agreement. However, this bias is likely to be small. The fact that the questionnaires were handed by the pharmacists to the patients, might induce a selection bias, depending on which cases they chose for the study, even though they were specifically asked to hand the questionnaires to the first 10/20 patients of the pharmacy. As with any survey, the non-responders cannot be characterized in the study.

5 | CONCLUSIONS

This study provides many insights into the specific aspects of GPP-recommended pharmacist counselling by analysing the agreement between pharmacist and patient on data reported by each of the two parties after their interaction. Very good concordance was found regarding counselling on the 'route of administration', 'the administration in relation to meals' and 'the dose, time interval between doses and the length of treatment'. A low concordance was found for counselling on ADRs, that was stated as performed by the majority of pharmacists, but it was confirmed by only half of the patients.

CONFLICT OF INTEREST

None to declare.

AUTHOR CONTRIBUTIONS

Camelia Bucsa: Conceptualization; data curation; methodology; project administration; supervision; roles/writing—original draft; Writing—review and editing. **Andreea Farcas**: Conceptualization; methodology; roles/writing—original draft; writing—review and editing. **Mihaela Udrea**: Methodology; project administration; resources; writing—review and editing. **Marius Bojita**: Resources; supervision; writing—review and editing. **Cristina Mogosan**: Conceptualization; methodology; writing—review and editing. **Daniel Leucuta**: Conceptualization; data curation; formal analysis; methodology; software; validation; writing—review and editing.

DATA AVAILABILITY STATEMENT

Data sharing not applicable - no new data generated.

ORCID

Andreea Farcas https://orcid.org/0000-0003-2109-8409

REFERENCES

1. Krass I, Dhippayom T. Pharmaceutical care—impact on quality of life in patients with type 2 diabetes: a review. *Clin Audit*. 2013;5:17–32. doi:10.2147/ca.s30589
2. Sakthong P, Sangthonganotai T. A randomized controlled trial of the impact of pharmacist-led patient-centered pharmaceutical care on patients' medicine therapy-related quality of life. *Res Soc Adm Pharm*. 2018;14(4):332-339. doi:10.1016/j.sapharm.2017.05.001
3. Kheir N, Emmerton L, Shaw J. Can pharmacists influence the health-related quality of life of patients with asthma?: the New Zealand pharmaceutical care experience. *Sultan Qaboos Univ Med J*. 2001;3(2):69-75.
4. Okumura LM, Rotta I, Correr CJ. Assessment of pharmacist-led patient counseling in randomized controlled trials: a systematic review. *Int J Clin Pharmacol*. 2014;36(5):882-891. doi:10.1007/s11096-014-9982-1
5. Baratta F, Allais G, Rolando S, et al. Prevention, education and counselling: the worldwide role of the community pharmacist as an epidemiological sentinel of headaches. *Neurol Sci*. 2019;40(1):15-21. doi:10.1007/s10072-019-03794-7
6. Alomar MJ, Qandil S, Al-Hilwani HMA, Malkat DM, Caroline C. Evaluation of the community pharmacist's behavior towards a prescription of antidiabetic and antiasthma drugs. *Pharm Pract*. 2011;9(1):37-43. doi:10.4321/S1886-36552011000100006
7. Reeves L, Robinson K, McClelland T, Adedoyin CA, Broeseker A, Adunlin G. Pharmacist interventions in the management of blood pressure control and adherence to antihypertensive medications: a systematic review of randomized controlled trials. *J Pharm Pract*. 2020;2020:089719002090357. doi:10.1177/0897190020903573
8. Rehman A, Amin F, Sadeeqa S. Prevalence of asthma and its management: a review. *J Pak Med Assoc*. 2018;68(12):1823-1827.
9. Santos AP, Mesquita AR, Oliveira KS, Lyra DP Jr. Assessment of community pharmacists´ counselling skills on headache management by using the simulated patient approach: a pilot study. *Pharm Pract*. 2013;11(1):3-7. doi:10.4321/s1886-36552013000100002
10. Alfadl AA, Alrasheedy AA, Alhassun MS. Evaluation of medication counseling practice at community pharmacies in Qassim region, Saudi Arabia. *Saudi Pharm J*. 2018;26(2):258-262. doi:10.1016/j.jsps.2017.12.002
11. Boeni F, Arnet I, Hersberger KE. Adherence counseling during patient contacts in Swiss community pharmacies. *Patient Prefer Adherence*. 2015;9:597-505. doi:10.2147/PPA.S76027
12. Puumalainen I, Halonen P, Enlund H, Johnson K, Airaksinen M. Validation of the United States Pharmacopeia (USP) medication counselling behaviour guidelines. *Pharm Edu*. 2005;5:87-96. doi:10.1080/15602210500141085
13. ASHP. ASHP guidelines on pharmacist-conducted patient education and counseling. *Am J Heal Pharm*. 1997;54(4):431-434. doi:10.1093/ajhp/54.4.431
14. Van Mil JWF. Pharmaceutical care, European developments in concepts, implementation, teaching, and research: a review. *Pharm World Sci*. 2004;26:303-311.
15. European Directorate for the Quality of Medicines. Pharmaceutical Care Policies and Prac Tices for a Safer, more Responsible and Cost-Effective Health System PHARMACEUTICAL CARE Policies and Practices for a Safer, more Responsible and Cost-Effective Health System. www.edqm.eu. Accessed February 24, 2020.
16. Regulile de Bună Practică Farmaceutică, Aprobate Prin Ordinul Ministerului Sănătăţii Nr. 75/2010 (Anexa I).
17. Rusu A, Vari CE, Hancu G, et al. Brief assessment of pharmacist-patient communication efficiency in Romanian pharmacies. *Farmacia*. 2018;66(6):1091-1096. doi:10.31925/FARMACIA.2018.6.25
18. Iancu M, Bucsa C, Farcas A, et al. Patient's counselling and management of adverse drug reactions and drug interactions in the community pharmacy. *Farmacia*. 2015;63(1):80-85.
19. Iancu ME, Bucsa C, Farcas AM, Leucuta D-C, Dincu A, Bojita MT. Counseling provided by the pharmacist in Romanian community pharmacies: the patients' perspective. *Clujul Med*. 2014;87(2):113-118. doi:10.15386/cjmed-257
20. Murad MS, Chatterley T, Guirguis LM. A meta-narrative review of recorded patient-pharmacist interactions: exploring biomedical or patient-centered communication? *Res Soc Adm Pharm*. 2014;10(1):1-20. doi:10.1016/j.sapharm.2013.03.002
21. Chong WW, Aslani P, Chen TF. Pharmacist-patient communication on use of antidepressants: a simulated patient study in community pharmacy. *Res Soc Adm Pharm*. 2014;10(2):419-437. doi:10.1016/j.sapharm.2013.05.006
22. Maes KA, Ruppanner JA, Imfeld-Isenegger TL, Hersberger KE, Lampert ML, Boeni F. Dispensing of prescribed medicines in Swiss

community pharmacies-observed counselling activities. *Pharmacy*. 2018;7(1):1. doi:10.3390/pharmacy7010001
23. Paravattil B, Kheir N, Yousif A. Utilization of simulated patients to assess diabetes and asthma counseling practices among community pharmacists in Qatar. *Int J Clin Pharmacol*. 2017;39(4):759-768. doi:10.1007/s11096-017-0469-8
24. Olsson E, Ingman P, Ahmed B, Kälvemark SS. Pharmacist-patient communication in Swedish community pharmacies. *Res Soc Adm Pharm*. 2014;10(1):149-155. doi:10.1016/j.sapharm.2013.03.001
25. Van Dijk M, Blom L, Koopman L, et al. Patient-provider communication about medication use at the community pharmacy counter. *Int J Pharm Pract*. 2016;24(1):13-21. doi:10.1111/ijpp.12198
26. Mackinnon A. A spreadsheet for the calculation of comprehensive statistics for the assessment of diagnostic tests and inter-rater agreement. *Comput Biol Med*. 2000;30(3):127-134. doi:10.1016/S0010-4825(00)00006-8
27. Altman D. *Practical Statistics for Medical Research*. 1st ed. Oxford: Chapman and Hall; 1991.
28. Alte D, Weitschies W, Ritter CA. Evaluation of consultation in community pharmacies with mystery shoppers. *Ann Pharmacother*. 2007;41(6):1023-1030. doi:10.1345/aph.1H565
29. Watson M, Norris P, Granas A. A systematic review of the use of simulated patients and pharmacy practice research. *Int J Pharm Pract*. 2006;14(2):83-93. doi:10.1211/ijpp.14.2.0002
30. Stewart M, Brown JB, Donner A, et al. The impact of patient-centered care on outcomes. *J Fam Pract*. 2000;49(9):796-704.
31. WHO (World Health Organization). The Role of the Pharmacist in the Health Care System: Preparing the Future Pharmacist. Vancouver; 1997.
32. Kerr A, Strawbridge J, Kelleher C, et al. How can pharmacists develop patient-pharmacist communication skills? A realist review protocol. *Syst Rev*. 2017;6(1):1-7. doi:10.1186/s13643-016-0396-0
33. Montgomery AT, Sporrong SK, Tully MP, Lindblad ÅK. Follow-up of patients receiving a pharmaceutical care service in Sweden. *J Clin Pharm Ther*. 2008;33(6):653-662. doi:10.1111/j.1365-2710.2008.00965.x
34. Shah B, Chewning B. Conceptualizing and measuring pharmacist-patient communication: a review of published studies. *Res Social Adm Pharm*. 2006;2(2):153-185. doi:10.1016/j.sapharm.2006.05.001
35. Herner SJ, Rawlings JE, Swartzendruber K, Delate T. Pharmacy survey on patient safety culture: benchmarking results. *J Patient Saf*. 2017;13(1):37-42. doi:10.1097/PTS.0000000000000102

How to cite this article: Bucsa C, Farcas A, Udrea M, Bojita M, Mogosan C, Leucuta D. Concordance of pharmacist versus patient responses regarding counselling in community pharmacy. *J Eval Clin Pract*. 2022;28:558–565. doi:10.1111/jep.13635

Online version at http://www.minervamedica.it

Panminerva Medica 2021 December;63(4):508-18
DOI: 10.23736/S0031-0808.20.04171-3

REVIEW

Noninvasive biomarkers in predicting nonalcoholic steatohepatitis and assessing liver fibrosis: systematic review and meta-analysis

Abdulrahman ISMAIEL [1], Daniel-Corneliu LEUCUTA [2] *, Stefan-Lucian POPA [1], Sharmila FAGOONEE [3], Rinaldo PELLICANO [4], Ludovico ABENAVOLI [5], Dan L. DUMITRASCU [1]

[1]2nd Department of Internal Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2]Department of Medical Informatics and Biostatistics, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; [3]Institute for Biostructure and Bioimaging, National Research Council, Molecular Biotechnology Center, Turin, Italy; [4]Unit of Gastroenterology, Molinette Hospital, Turin, Italy; [5]Department of Health Sciences, Magna Graecia University, Catanzaro, Italy

*Corresponding author: Daniel-Corneliu Leucuta, Department of Medical Informatics and Biostatistics, Iuliu Hatieganu University of Medicine and Pharmacy, 400349 Cluj-Napoca, Romania. E-mail: dleucuta@umfcluj.ro

ABSTRACT

INTRODUCTION: Nonalcoholic steatohepatitis (NASH) is characterized by hepatic steatosis with inflammation, ballooned hepatocytes and possible fibrosis, which may progress to liver cirrhosis. Although liver biopsy, remains the diagnostic gold standard of NASH, several noninvasive biomarkers have been studied, to avoid the need for this invasive procedure. We performed a systematic review with meta-analysis to evaluate the accuracy of several noninvasive biomarkers in predicting NASH and assessing liver fibrosis in NASH patients.

EVIDENCE ACQUISITION: An electronic search on PubMed and Embase was systematically performed. The principal summary outcome was the area under the curve (AUC), assessing the accuracy of NashTest, BARD (Body Mass Index, AST/ALT ratio, diabetes) score, NAFLD fibrosis score (NFS), APRI (aspartate aminotransferase-to-Platelet Ratio Index), and Fibrosis-4 (FIB-4) Index in predicting NASH and assessing liver fibrosis.

EVIDENCE SYNTHESIS: Thirteen studies involving 6557 adult patients were included in the qualitative assessment of this review, out of which, six studies were included in the quantitative assessment. Prediction of NASH was evaluated better using NFS (AUC of 0.687) and FIB-4 (AUC of 0.729). Fibrosis stages 0 *vs*. 1-4 was diagnosed better using NFS (AUC of 0.718) and FIB-4 (AUC of 0.723). Advanced fibrosis was assessed better by BARD (AUC of 0.673), APRI (AUC of 0.762), NFS (AUC of 0.787) and FIB-4 (AUC of 0.821).

CONCLUSIONS: FIB-4 predicted NASH and quantified liver fibrosis, stages 0 *vs*. 1-4 more precisely compared to NFS, APRI, and BARD. However, considering that methodological quality of the assessed studies is limited, the results should be considered with caution.

(*Cite this article as:* Ismaiel A, Leucuta DC, Popa SL, Fagoonee S, Pellicano R, Abenavoli L, *et al.* Noninvasive biomarkers in predicting nonalcoholic steatohepatitis and assessing liver fibrosis: systematic review and meta-analysis. Panminerva Med 2021;63:508-18. DOI: 10.23736/S0031-0808.20.04171-3)

KEY WORDS: Liver diseases; Biomarkers; Systematic review.

Introduction

Nonalcoholic fatty liver disease (NAFLD), a common condition intensely studied with increased morbidity and mortality as well as several intrahepatic and extrahepatic complications,[1-5] is defined by excessive hepatic fat accumulation in the absence of significant alcohol consumption or other causes of secondary hepatic steatosis.[6-8] Currently, no approved therapies are present for treating NAFLD.[9-13] Several risk factors, including metabolic syndrome, obesity, diabetes, high cholesterol levels, sedentary lifestyle, and genetic predisposition, can increase the susceptibility of developing NAFLD.[14-16]

NAFLD represents a spectrum of conditions ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH) and cirrhosis.[7, 17] Unlike NAFL,

which is usually associated with a benign clinical course and low mortality rates, NASH is associated with a more progressive course leading to liver cirrhosis in about 10-15% of patients, which may complicate with liver failure and hepatocellular carcinoma.[18] Moreover, NASH is associated with lower survival rates, as showed in long-term longitudinal studies.[18, 19] Therefore, it is crucial to identify NASH in order to provide risk stratification, preventing disease progression, and further complications.

Histopathological evaluation through liver biopsy remains the gold standard for diagnosing NASH. Nonetheless, liver biopsy is considered an invasive procedure that can lead to rare but important potential complications.[20] Moreover, liver biopsy can also be associated with sampling errors and interobserver variability, which may hinder its performance. Therefore, it is clearly not a suitable method for screening purposes in a pathology such as NAFLD, which affects almost a quarter of the world's population.[21] Since noninvasive biomarkers have several potential benefits being a rapid method of diagnosis with lower risks and costs, they have been recently studied for predicting NASH and assessing liver fibrosis. These biomarkers can be based on the evaluation of a single substance such as cytokeratin-18 fragments (CK18-Fs) or the calculation of specific scores based on multiple parameters such as NashTest (Biopredictive; Paris, France), BARD score (Body Mass Index, AST/ALT ratio, diabetes), NAFLD fibrosis score (NFS), aspartate aminotransferase (AST)-to-Platelet Ratio Index (APRI) and Fibrosis-4 (FIB-4) Index.

To our knowledge, there is no systematic review focusing on accuracy of the noninvasive biomarkers in the context of NASH. Therefore, we conducted a systematic literature search with the aim of evaluating the diagnostic accuracy of several noninvasive biomarkers in NASH, including NashTest, BARD score, NFS, APRI, and FIB-4 Index. Furthermore, we conducted a meta-analysis assessing the accuracy of these scores in predicting the diagnosis of NASH and quantifying liver fibrosis.

Evidence acquisition

This systematic review and meta-analysis was written according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines.

Data sources and search strategy

A computerized search in two electronic databases, PubMed and Embase, was conducted in order to identify observational studies evaluating noninvasive biomarkers in NASH. We used the following search terms and combinations of terms: "nonalcoholic steatohepatitis" (all fields) OR "non-alcoholic steatohepatitis" (all fields) OR "non-alcoholic steatohepatitis" (all fields) OR "NASH" (all fields) AND "APRI" (all fields) OR "AST to Platelet Ratio Index" (all fields) OR "BARD score" (all fields) OR "FIB-4 score" (all fields) OR "fibrosis-4 score" (all fields) OR "NFS" (all fields) OR "NAFLD fibrosis score" (all fields) OR "NashTest" (all fields). The literature search was performed from inception unitl November 6, 2019 using human filters, while excluding conference abstracts and conference papers. No duration, country, or language restrictions were used during the search. Titles and abstracts were then screened for eligibility, followed by the evaluation of full texts of the articles that fulfilled the inclusion and exclusion criteria was conducted. Eligible studies were assessed with data extraction performed. Two reviewers (AI and SLP) conducted an independent extraction for the data from eligible studies. Any discrepancies in extracted data were resolved by mutual consensus. The extracted data included author names, publication year, country of origin, design of the study, total sample size, Body Mass Index (BMI), mean age, method used to diagnose NASH, evaluated noninvasive biomarkers, gender ratio, NASH percentage from the study population, the interval between biopsy and the noninvasive biomarker or score calculation, description of the liver biopsy technique, histopathological classification system used, ALT and AST levels as well as the main study findings. Moreover, positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, and Area under Receiver Operating Characteristic (AUROC) were also extracted.

Eligibility criteria

Inclusion criteria of original articles in this systematic review were as follows: 1) observational cohort population-based or hospital-based and case-control studies, examining the association between NASH and noninvasive biomarkers; 2) histopathological confirmation of NASH through liver biopsy by the detection of hepatic steatosis, ballooning, and lobular inflammation, in the absence of other secondary causes of hepatic steatosis, significant alcohol consumption based on each study definition and other causes of chronic liver disease (CLD); 3) the evaluation of the following noninvasive biomarkers: NashTest, BARD score, NFS, APRI and FIB-4 Index; and 4) adult individuals (aged ≥18 years) without restrictions to gender, race or ethnicity.

Exclusion criteria included the following: 1) studies that used methods other than liver biopsy for diagnosing

NASH; 2) studies that evaluated noninvasive biomarkers other than NashTest, BARD score, NFS, APRI, and FIB-4 Index; 3) studies published in languages other than English, Italian, German or Romanian languages; 4) case reports, reviews, practice guidelines, commentaries, conference abstracts, conference papers, articles in press, editorials, short surveys, letters; 5) studies including participants with other causes of secondary hepatic steatosis, significant alcohol consumption or other known causes of CLD; and 6) studies including participants with confirmed liver cirrhosis regardless of the etiology or end-stage liver disease awaiting liver transplantation.

Risk of bias assessment in individual studies

Quality assessment evaluating the risk of bias and internal validity of all included studies was performed in a similar manner using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS).[22] Two authors (AI and DCL) applied this tool independently by evaluating the items included in the assessment and answering them by either "yes," "no," or "unclear." In the presence of disagreement between the evaluation of the two authors, a consensus was reached through a discussion. The methodological quality assessment results did not affect the eligibility of the studies.

Summary measures and synthesis of results

The principal summary outcome was the area under the curve (AUC), assessing the accuracy of several noninvasive biomarkers, including NashTest, BARD score, NFS, APRI, and FIB-4 Index in evaluating NASH and liver fibrosis. R with Metafor package (OpenMeta [Analyst]) was used to conduct the data analyses of the systematic review and meta-analysis.[23, 24] We used the χ^2 based Q-test and I^2 for assessing between-study heterogeneity. The Q test and I^2 statistics were utilized to evaluate the heterogeneities among the studies. As per the recommendations of the Cochrane Handbook for identifying and measuring heterogeneity, we estimated I^2 values of 0% to 40% as not important; 30% to 60% as moderate heterogeneity; 50% to 90% as substantial heterogeneity; and 75% to 100% as considerable heterogeneity. We rounded the upper and lower confidence intervals (CI) with 0.1 in studies that reported an AUC with the same upper and lower CI. Standard error of AUC was calculated based on CI and the point estimate. We used random-effects model as the analysis method and restricted maximum likelihood (REML) with a CI of 95% and 3 digits of precision. Data from each study were reported as the estimated AUC with 95% CI, lower bound, upper bound, standard error, and P value. The statistical tests results were considered statistically significant if the P value <0.05. The analyses were conducted if two or more studies reported the same outcome and AUC.

Evidence synthesis

The initial search yielded one thousand eight hundred and sixty-two studies, out of which one hundred and sixty-four studies were on PubMed, and one thousand six hundred and ninety-eight studies were on Embase. After using human filters on PubMed, the search was left with one hundred and eight studies. After applying all filters (human filters, while excluding conference abstracts and conference papers) on Embase, five hundred and fifty-nine studies remained. Finally, a total of six hundred and sixty-seven studies remained after using all filters to both electronic databases. From those, a total of one hundred and twelve duplicate studies were discovered and excluded. The total number of studies that were assessed by their title and abstract for eligibility according to the inclusion and exclusion criteria were five hundred fifty-five studies, as shown in Figure 1. The results of the five hundred fifty-five screened articles were as follows: 1) two hundred and seventy articles with titles and abstracts irrelevant to this

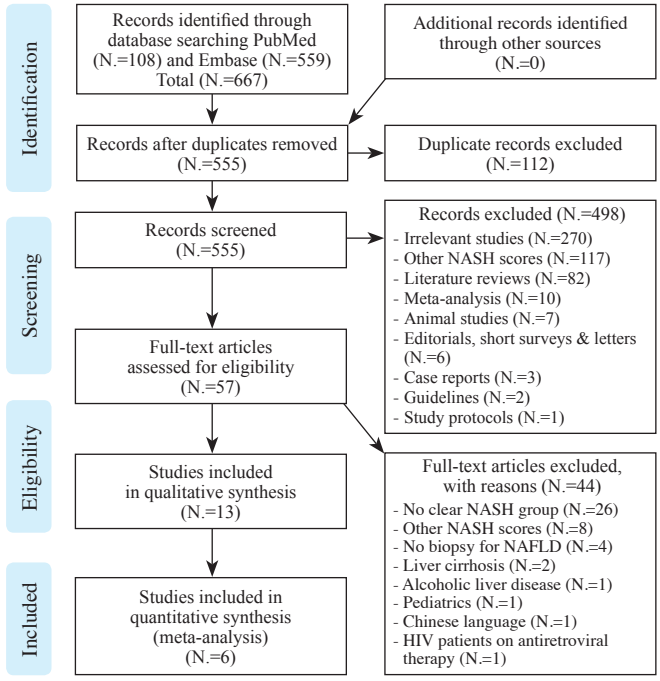

Figure 1.—The PRISMA flow diagram for the search and selection processes of this systematic review.

review topic; 2) one hundred and seventeen studies that assessed the accuracy of noninvasive biomarkers other than NashTest, BARD score, NFS, APRI, and FIB-4 Index; 3) eighty two literature reviews; 4) ten systematic reviews and/or meta-analyses; 5) seven studies conducted on animals; 6) six editorials, short surveys and letters to the editor; 7) three case-reports; 8) two guidelines; 9) one study protocol; and 10) fifty-seven article abstracts that met the primary criteria. We excluded four hundred and ninety-eight studies after the first screening process. The remaining fifty-seven articles underwent further evaluation of their full text out of which we excluded forty-four articles due to the following reasons: 1) twenty six articles with no clear NASH group; 2) eight studies assessing other noninvasive biomarkers in NASH; 3) four studies without histopathological confirmation for diagnosing NAFLD; 4) two studies with liver cirrhosis; 5) one study involving alcoholic liver disease; 6) one study conducted on pediatric population; 7) one study involving *Human immunodeficiency virus* (HIV) patients on antiretroviral therapy; and 8) one article in Chinese language. A total of thirteen articles fulfilled our inclusion and exclusion criteria and were included in the qualitative synthesis for analysis.[25-37]

The main characteristics of the included studies are mentioned in Supplementary Digital Material 1: Supplementary Table I.[25-37] A total of 6557 subjects were included in this review. The gender distribution was higher for females, with 3751 participants (57.2%) and 2806 males (42.8%). NASH was present in 5,254 individuals, about 80% of the total study population. Ten articles had retrospective study design.[26-31, 33, 35-37] Three studies had a prospective study design.[25, 32, 34] Six studies were conducted in Asia (Japan N.=5, Malaysia N.=1), three in Europe (France N.=1, Germany N.=1, Greece N.=1), three in the Americas (USA N.=2) and one study was conducted in 26 countries (North and South America, Europe, Australia, New Zealand, and Asia).

Liver biopsy description

All included studies performed histopathological evaluation of samples obtained by liver biopsy. The description of the liver biopsy with the minimum required length for evaluation was reported in only two studies.[26, 36]

Histological scoring systems for grading and staging NASH

Several histological scoring systems were used to evaluate NASH. The most commonly used classification for grading and staging the lesions of NAFLD was the NAFLD activity score (NAS), which was used in a total of eight studies.[25, 31-37] Kawamura *et al*. graded and staged NASH using the Brunt criteria.[26] NAFLD was classified as NAFL or NASH using the Matteoni's classification in a study conducted by Tada *et al*.[30] Furthermore, three studies used both NAS and Brunt criteria.[27-29]

Noninvasive biomarkers in NASH

We included only studies evaluating he accuracy of NashTest, BARD score, NFS, APRI, and FIB-4 Index. In ten studies, the FIB-4 score was used,[26-31, 33, 35-37] followed by seven studies evaluating APRI[26-29, 35-37] and NFS[27, 31, 33-37] each, three studies evaluating BARD,[26, 27, 33] two studies evaluating NashTest,[25, 32] one ActiTest[32] and NashTest-2[32] each.

Noninvasive biomarkers in predicting NASH

In predicting the presence of NASH, three studies evaluated FIB-4,[30, 33, 35] two evaluated NFS,[33, 35] two evaluated NashTest,[25, 32] and one evaluated BARD,[33] APRI,[35] and ActiTest[32] each. Figure 2[30, 33, 35] summarizes the results obtained in the meta-analysis evaluating noninvasive biomarkers in predicting NASH. The pooled studies for the analysis evaluating NFS in predicting NASH demonstrated an overall AUC of 0.687 with 95% CI: 0.612-0.762, P value <0.001 and I^2=0. FIB-4 predicted NASH with an overall AUC of 0.729 with 95% CI: 0.678-0.780, P value <0.001 and I^2=0.001.

Noninvasive biomarkers in predicting liver fibrosis in NASH

Eight studies evaluated FIB-4 in quantifying liver fibrosis in NASH,[26-29, 31, 33, 35, 37] followed by six studies evaluating APRI,[26-29, 35, 37] five evaluating NFS[27, 31, 33, 35, 37] and three evaluating BARD.[26, 27, 33] Figure 3[27, 31, 33, 35, 37] summarizes the results obtained in the meta-analysis evaluating noninvasive biomarkers in assessing liver fibrosis in NASH. Fibrosis stages 0 *vs*. 1-4 was evaluated using NFS and FIB-4. NFS demonstrated an AUC=0.718 with 95% CI: 0.651-0.785, P value <0.001, and I^2=69.188 suggesting substantial heterogeneity. Moreover, FIB-4 also was evaluated reporting an AUC=0.723 with 95% CI: 0.696-0.751, P value <0.001, and I^2=0 in quantifying fibrosis stages 0 *vs*. 1-4.

According to METAVIR, fibrosis stages, F3 and F4 were considered as advanced fibrosis. Several studies evaluated

TABLE I.—*Recommended quality items derived from QUADAS tool.*[22]

N.		Poynard *et al.*[25]	Kawamura *et al.* [26]	Kawamura *et al.* [27]	Nishikawa *et al.* [28]
Evaluation Criteria					
1.	Was the spectrum of patients representative of the patients who will receive the test in practice?	Yes	Yes	Yes	Yes
2.	Were selection criteria clearly described?	Yes	Yes	Yes	Yes
3.	Is the reference standard likely to correctly classify the target condition?	Yes	Yes	Yes	Yes
4.	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Yes	Unclear	Unclear	Unclear
5.	Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	Yes	Yes	Yes	Yes
6.	Did patients receive the same reference standard regardless of the index test result?	Yes	Yes	Yes	Yes
7.	Was the reference standard independent of the index test (i.e., the index test did not form part of the reference standard)?	Yes	Yes	Yes	Yes
8.	Was the execution of the index test described in sufficient detail to permit replication of the test?	Yes (in the literature)	Yes	Yes	Yes
9.	Was the execution of the reference standard described in sufficient detail to permit its replication?	Partially	Partially	Partially	No
10.	Were the index test results interpreted without knowledge of the results of the reference standard?	No influence	No influence	No influence	No influence
11.	Were the reference standard results interpreted without knowledge of the results of the index test?	Unclear	Unclear	Unclear	Unclear
12.	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	No influence	No influence	No influence	No influence
13.	Were uninterpretable/ intermediate test results reported?	Not applicable	Not applicable	Not applicable	Not applicable
14.	Were withdrawals from the study explained?	Not applicable	Not applicable	Not applicable	Not applicable
Potential additional quality items					
1.	Were cut-off values established before the study was started?	Yes	Yes	Yes	Yes
2.	Is the technology of the index test unchanged since the study was carried out?	Yes	Yes	Yes	Yes
3.	Did the study provide a clear definition of what was considered to be a 'positive' result?	Yes	Yes	Yes	Yes
4.	Had test operators had appropriate training?	Unclear	Unclear	Unclear	Yes
5.	Was treatment withheld until both the index test and reference standard were performed?	Unclear	Unclear	Unclear	Unclear
6.	Were data on observer variation reported and within an acceptable range?	No	No	No	No
7.	Were data on instrument variation reported and within an acceptable range?	No	No	No	No
8.	Were objectives prespecified?	Yes	Yes	Yes	Yes
9.	Was the study free of commercial funding?	No	Yes	Unclear	No

whether noninvasive markers could differentiate advanced fibrosis (F0-2 *vs*. F3-4). The pooled studies for evaluating whether BARD can detect advanced fibrosis reported an overall AUC=0.673 with 95% CI: 0.592-0.753, P value <0.001, and I^2=0. APRI predicting advanced fibrosis reported an AUC=0.762 with 95% CI: 0.733-0.790, P value <0.001, and I^2=0. NFS demonstrated an overall AUC: 0.787 with 95% CI: 0.733 – 0.840, P value <0.001 and I^2=92.864 suggesting considerable heterogeneity among included studies. Furthermore, FIB-4 demonstrated an overall AUC=0.821 with 95% CI: 0.773-0.870, P value <0.001 and I^2=92.06 suggesting considerable heterogeneity among included studies.

Bias evaluation

Risk of bias in individual studies was evaluated using the QUADAS tool as demonstrated in Table I.[22, 25-37] There were several issues regarding bias presence in the reviewed studies. Only five studies reported the time between the two diagnostic tests. Two studies did not use the reference

Kakisaka *et al.*[29]	Tada *et al.*[30]	Anstee *et al.*[31]	Bril et al *et al.*[32]	Chuah *et al.*[33]	Liebig *et al.*[34]	Polyzos *et al.*[35]	Reddy *et al.*[36]	Siddiqui *et al.*[37]
Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Unclear	Yes	Yes	Unclear	Unclear	Unclear	Yes	No	Yes
Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes
Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yes (in the literature)	Yes (in the literature)	Yes (in the literature)	Yes (in the literature)	Yes (in the literature)	Yes	Yes	Yes	Yes
Partially	Partially	Partially	Partially	Partially	No	Partially	No	Partially
No influence	No influence	No influence	No influence	No influence	No influence	No influence	No influence	No influence
Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
No influence	No influence	No influence	No influence	No influence	No influence	No influence	No influence	No influence
Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Yes	Not applicable	Not applicable	Not applicable	Not applicable	Yes	Not applicable	Not applicable	Not applicable
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Unclear	Unclear	Unclear	Unclear	Yes	Unclear	Yes	Yes	Unclear
Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
No	No	No	No	No	No	No	No	No
No	No	No	No	No	No	No	No	No
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yes	Yes	Unclear	No	Yes	Yes	No	No	Yes

test for all the subjects in the study. The majority of the studies did not have a complete description of how the reference test was performed, the information regarding the biopsy sample position being missing, and sometimes the histopathological examination was not completely described. It was not clear if the reference standard results were interpreted without knowledge of the results of the index test, in all the assessed studies. The test operators training and also the withholding of an eventual treatment during the diagnostic tests were not specified in the majority of studies. No study reported observer or instrument variation. Half of the studies had commercial funding or a lack of transparence regarding funding. We did not check for publication bias due to the small number of studies used in analyses.

Discussion

Although several published literature reviews, systematic reviews, and meta-analyses discussed and evaluated noninvasive biomarkers in predicting NAFLD and quan-

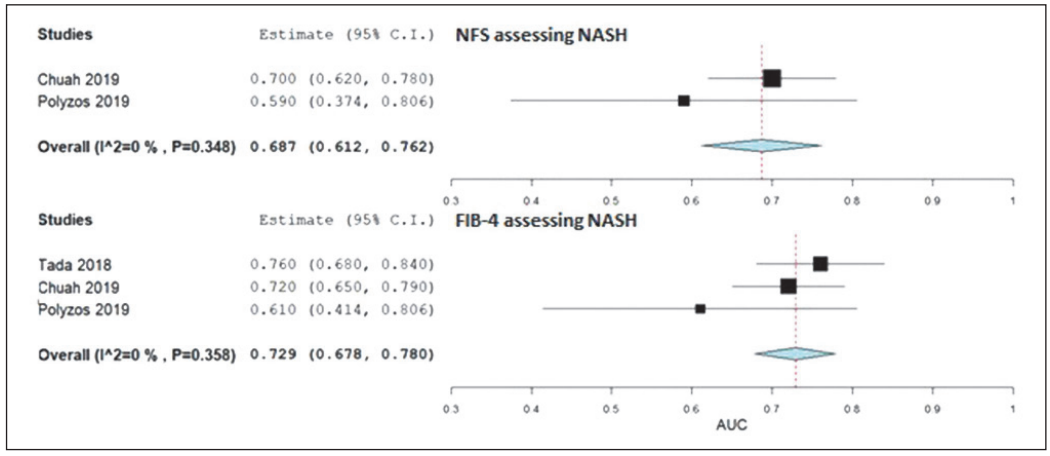

Figure 2.—Studies evaluating NFS and FIB-4 in the prediction of NASH.
AUC: area under the curve; NASH: nonalcoholic steatohepatitis; NFS: NAFLD fibrosis score; FIB-4: fibrosis-4.

tifying liver fibrosis,[38-42] none evaluated these findings in patients with NASH. To the best of our knowledge, this is the first systematic review and meta-analysis to evaluate the diagnostic accuracy of several noninvasive biomarkers, NashTest, BARD score, NFS, APRI, and FIB-4 Index, in predicting NASH and quantifying liver fibrosis severity in presence of NASH. In our systematic review and meta-analysis, a total number of thirteen articles were included in our qualitative synthesis, out of which, ten articles were retrospective, and three were prospective, with a total number of 6557 subjects. Moreover, six articles were included in our quantitative synthesis. The present review demonstrated that FIB-4 predicted the presence of NASH slightly better than NFS. Moreover, FIB-4 quantified liver fibrosis stages 0 *vs*. 1-4 more accurately compared to NFS. Furthermore, advanced fibrosis was predicted best using FIB-4, followed by NFS, APRI, and BARD in order of accuracy.

Several points need to be further discussed. Firstly, we noticed that the prevalence of NASH in the included sample was approximately 80%, which might be explained by the fact that several studies did not have a control group, while most other studies included a bigger sample size of NASH patients compared to controls.

Secondly, we included studies that performed liver biopsy with histopathological confirmation as it is the current gold standard in differentiating NAFLD from NASH as well as measuring liver fibrosis.[43] Moreover, recommendations of clinical practice guidelines mention that liver biopsy must be performed in order to accurately diagnose NASH.[44] The following criteria is usually used for the diagnosis of NASH including the presence of >5% macrovesicular steatosis, inflammation, and liver cell ballooning, typically with a predominant centrilobular distribution.[45]

Thirdly, the current literature is very limited in data evaluating noninvasive biomarkers in predicting NASH. According to our study, FIB-4 demonstrated a better AUC in predicting NASH compared to NFS with low heterogeneity between included studies that evaluated this association. Several studies investigated noninvasive biomarkers in NAFLD, but they were not discriminant enough for distinguishing between NASH and NAFLD.[40-42] A systematic review performed by Miller *et al*. investigated the relationship between NASH and hepatocyte apoptosis, demonstrating that cytokeratin 18 (CK-18), tissue polypeptide-specific antigen, and keratin 18 were the most studied noninvasive biomarkers of hepatocyte apoptosis in the prediction of the presence of NASH. Moreover, the authors of the study concluded that these noninvasive biomarkers are not able to replace liver biopsy, but they could have an essential role in triaging patients for liver biopsy, reducing the economic burden.[39]

Fourthly, the evaluation of stage 0 *vs*. 1-4 of liver fibrosis in NASH was shown to be predicted better using FIB-4. Moreover, advanced fibrosis was better evaluated using FIB-4, followed by NFS, APRI, and BARD. A meta-analysis, performed by Sun *et al*., analyzed the accuracy of FIB-4 Index, NFS, and BARD score in predicting advanced liver fibrosis in a total of 1038 NAFLD adult patients. The authors reported that FIB-4 Index group had a cut-off of 1.30, pooled sensitivity and specificity with 95% CI and the Spearman's rank correlation coefficient were 0.800, and the P value was 0.200 without threshold effect. Moreover, a poor diagnostic accuracy of NFS with a cut-off value of 1.455, 95% pooled sensitivity and

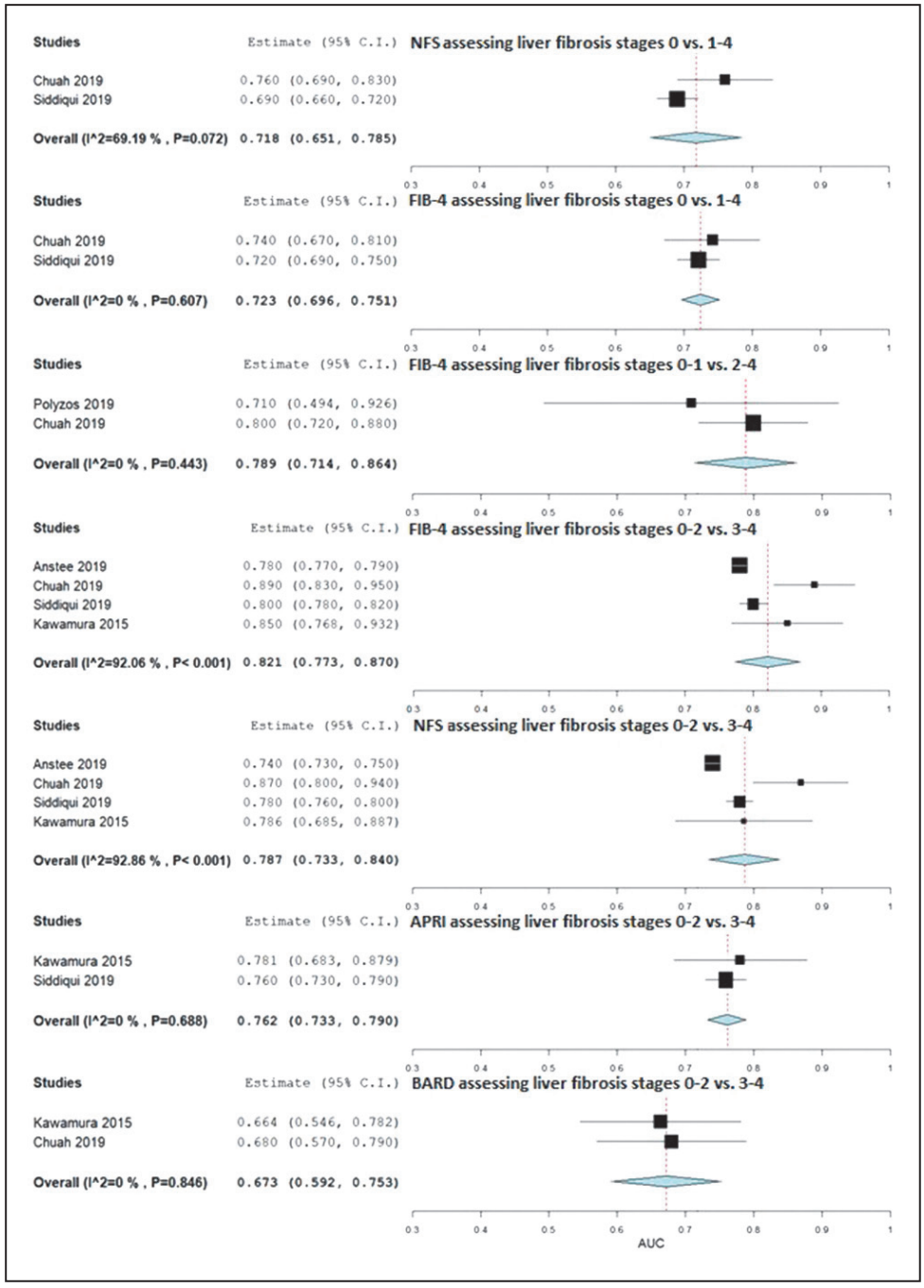

Figure 3.—Studies evaluating NFS, FIB-4, APRI, BARD in assessing liver fibrosis stages.
AUC: area under the curve; NFS: NAFLD fibrosis score; FIB-4: fibrosis 4; APRI: AST to Platelet Ratio Index.

specificity and the 0.600 Spearman's rank correlation coefficient and a P value of 0.400, indicating the absence of a threshold effect, as well as BARD score, with a two score cut-off levels, 95% pooled sensitivity and specificity and Spearman's rank correlation coefficient was 1.000 with a P value of 0.000 with a threshold effect that was found. The authors concluded that these noninvasive biomarkers are impossible to apply in clinical practice, and only the FIB-4 Index may be used as an efficient method for predicting advanced fibrosis in NAFLD.[38]

Limitations of the study

Some important potential limitations of our systematic review and meta-analysis should be mentioned. We analyzed only five noninvasive biomarkers in predicting NASH and quantifying liver fibrosis in NASH patients. Other noninvasive biomarkers[46, 47] were not included in our analysis. We chose the most commonly used scoring systems in estimating liver fibrosis.[48] The included noninvasive biomarkers used parameters that do not directly reflect the processes involved in liver fibrosis or NASH, which might mean that these noninvasive biomarkers do not directly indicate liver fibrosis changes or NASH. The limited published studies evaluating noninvasive biomarkers did not allow us to assess except two scores in predicting NASH. Therefore, more studies evaluating other noninvasive biomarkers in predicting NASH are necessary. The number of studies included in our qualitative and quantitative synthesis is small due to the limited published data. Further research with larger sample size populations can be realized through international collaborations that allow the collection of large samples of NASH patients and the standardization of investigative tools. Finally, due to heterogeneity between studies, results should be interpreted cautiously. The analyzed studies were predominantly performed in tertiary centers, and thus having more probability to include cases with advanced NAFLD. Therefore, these results are less applicable in other settings. Nevertheless, diagnostic studies in primary and secondary care, using liver biopsy are difficult to be conducted. The liver biopsy comes with the problems of sample biopsy, and observer variability. Very few studies presented information regarding the length and quality of liver biopsy, how biopsy was performed, and no study presented information on observer variability, and very few studies presented the experience of the pathologists. Thus, the accuracy of the reference test might be reduced. The lack of transparency regarding funding, or the commercial funding of the included studies, can influence the results of the studies.

Nevertheless, our systematic review and meta-analysis have also important strengths. The main strength is that we concentrated mainly on comparing the diagnostic accuracy of five routinely feasible noninvasive biomarkers that can be easily performed in the clinical setting. Moreover, we included only studies that compared the diagnostic accuracy of noninvasive biomarkers involving NASH patients diagnosed using the gold standard, liver biopsy and histopathological grading, providing consistency of methods and subjects in the included study of our qualitative and quantitative assessment.

Conclusions

In conclusion, our systematic review and meta-analysis found that FIB-4 predicted the presence of NASH, as well as quantified liver fibrosis, stages 0 *vs*. 1-4 more precisely compared to NFS. Moreover, advanced liver fibrosis considered as stages 3-4 was predicted in the following order being evaluated best by FIB-4, followed by NFS, APRI, and BARD. Nevertheless, the results should be used with caution since the methodological quality of the assessed studies is imperfect. Currently, noninvasive biomarkers are not able to replace liver biopsy and histopathological evaluation. However, they could present a critical and vital role in triaging patients for liver biopsy, therefore decreasing the associated economic burden. The literature is limited in terms of data evaluating noninvasive biomarkers in NASH, and the published studies suffer from methodological flaws as presented above. Therefore, future better designed and conducted research is necessary in order to identify high accuracy and cost-efficient noninvasive biomarkers[49, 50] for NASH diagnosis and liver fibrosis assessment in NASH patients, reducing the associated complications as well as the overall morbidity and mortality.

References

1. Armstrong MJ, Adams LA, Canbay A, Syn WK. Extrahepatic complications of nonalcoholic fatty liver disease. Hepatology 2014;59:1174–97.

2. Ismaiel A, Colosi HA, Rusu F, Dumitrașcu DL. Cardiac Arrhythmias and Electrocardiogram Modifications in Non-Alcoholic Fatty Liver Disease. A Systematic Review. J Gastrointestin Liver Dis 2019;28:483–93.

3. Chacko KR, Reinus J. Extrahepatic Complications of Nonalcoholic Fatty Liver Disease. Clin Liver Dis 2016;20:387–401.

4. Correale M, Tricarico L, Leopizzi A, Mallardi A, Mazzeo P, Tucci S, *et al.* Liver disease and heart failure. Panminerva Med 2020;62:26–37.

5. Ismaiel A, Al Srouji N. Subclinical Left Ventricular Systolic Dysfunction Assessed Using Myocardial Strain Measured by Speckle Tracking in Nonalcoholic Fatty Liver Disease – Systematic Review. Glob J Med Therap. 2020;2:1–8.

6. Sanyal AJ; American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002;123:1705–25.

7. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221–31.

8. Charlton M. Nonalcoholic fatty liver disease: a review of current understanding and future impact. Clin Gastroenterol Hepatol 2004;2:1048–58.

9. Ismaiel A, Dumitrascu DL. How to Reduce Cardiovascular Risk in Nonalcoholic Fatty Liver Disease. Am J Ther 2020. [Epub ahead of print]

10. Ratziu V, Goodman Z, Sanyal A. Current efforts and trends in the treatment of NASH. J Hepatol 2015;62:S65–75.

11. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, *et al.* The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012;55:2005–23.

12. Ismaiel A, Al Srouji N. Effects of Acetylsalicylic Acid on Nonalcoholic Fatty Liver Disease - Systematic Review. Glob J Med Therap. 2019;1:1–7.

13. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388–402.

14. Iqbal U, Perumpail BJ, Akhtar D, Kim D, Ahmed A. The Epidemiology, Risk Profiling and Diagnostic Challenges of Nonalcoholic Fatty Liver Disease. Medicines (Basel) 2019;6:41.

15. Ismaiel A, Dumitrascu DL. Genetic predisposition in metabolic-dysfunction-associated fatty liver disease and cardiovascular outcomes-Systematic review. Eur J Clin Invest 2020;50:e13331.

16. Suciu A, Abenavoli L, Pellicano R, Luzza F, Dumitrascu DL. Transaminases: oldies but goldies. A narrative review. Minerva Gastroenterol Dietol 2020;66:246–51.

17. Ismaiel A, Dumitraşcu DL. Cardiovascular Risk in Fatty Liver Disease: The Liver-Heart Axis-Literature Review. Front Med (Lausanne) 2019;6:202.

18. Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, *et al.* Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865–73.

19. Söderberg C, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, *et al.* Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology 2010;51:595–602.

20. Actis GC, Olivero A, Lagget M, Pellicano R, Smedile A, Rizzetto M. The practice of percutaneous liver biopsy in a gastrohepatology day hospital: a retrospective study on 835 biopsies. Dig Dis Sci 2007;52:2576–9.

21. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73–84.

22. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol 2003;3:25.

23. Wallace BC, Dahabreh IJ, Trikalinos TA, Lau J, Trow P, Schmid CH. Closing the Gap between Methodologists and End-Users: R as a Computational Back-End. JSTAT 2012;49:15.

24. Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package. JSTAT 2010;36:48.

25. Poynard T, Ratziu V, Charlotte F, Messous D, Munteanu M, Imbert-Bismut F, *et al.*; LIDO Study Group; CYTOL study group. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcoholo steato hepatitis in patients with non-alcoholic fatty liver disease. BMC Gastroenterol 2006;6:34.

26. Kawamura Y, Saitoh S, Arase Y, Ikeda K, Fukushima T, Hara T, *et al.* Three-dimensional magnetic resonance imaging for stringent diagnosis of advanced fibrosis associated with nonalcoholic steatohepatitis. Hepatol Int 2013;7:850–8.

27. Kawamura Y, Ikeda K, Arase Y, Sorin Y, Fukushima T, Kunimoto H, *et al.* New discriminant score to predict the fibrotic stage of non-alcoholic steatohepatitis in Japan. Hepatol Int 2015;9:269–77.

28. Nishikawa H, Enomoto H, Iwata Y, Kishino K, Shimono Y, Hasegawa K, *et al.* Clinical significance of serum Wisteria floribunda agglutinin positive Mac-2-binding protein level in non-alcoholic steatohepatitis. Hepatol Res 2016;46:1194–202.

29. Kakisaka K, Suzuki Y, Fujiwara Y, Abe T, Yonezawa M, Kuroda H, *et al.* Evaluation of ballooned hepatocytes as a risk factor for future progression of fibrosis in patients with non-alcoholic fatty liver disease. J Gastroenterol 2018;53:1285–91.

30. Tada T, Kumada T, Toyoda H, Saibara T, Ono M, Kage M. New scoring system combining the FIB-4 index and cytokeratin-18 fragments for predicting steatohepatitis and liver fibrosis in patients with nonalcoholic fatty liver disease. Biomarkers 2018;23:328–34.

31. Anstee QM, Lawitz EJ, Alkhouri N, Wong VW, Romero-Gomez M, Okanoue T, *et al.* Noninvasive Tests Accurately Identify Advanced Fibrosis due to NASH: Baseline Data From the STELLAR Trials. Hepatology 2019;70:1521–30.

32. Bril F, McPhaul MJ, Caulfield MP, Castille JM, Poynard T, Soldevila-Pico C, *et al.* Performance of the SteatoTest, ActiTest, NashTest and FibroTest in a multiethnic cohort of patients with type 2 diabetes mellitus. J Investig Med 2019;67:303–11.

33. Chuah KH, Wan Yusoff WN, Sthaneshwar P, Nik Mustapha NR, Mahadeva S, Chan WK. MACK-3 (combination of hoMa, Ast and CK18): A promising novel biomarker for fibrotic non-alcoholic steatohepatitis. Liver Int 2019;39:1315–24.

34. Liebig S, Stoeckmann N, Geier A, Rau M, Schattenberg JM, Bahr MJ, *et al.* Multicenter Validation Study of a Diagnostic Algorithm to Detect NASH and Fibrosis in NAFLD Patients With Low NAFLD Fibrosis Score or Liver Stiffness. Clin Transl Gastroenterol 2019;10:e00066.

35. Polyzos SA, Slavakis A, Koumerkeridis G, Katsinelos P, Kountouras J. Noninvasive Liver Fibrosis Tests in Patients with Nonalcoholic Fatty Liver Disease: An External Validation Cohort. Horm Metab Res 2019;51:134–40.

36. Reddy YK, Marella HK, Jiang Y, Ganguli S, Snell P, Podila PS, *et al.* Natural History of Non-Alcoholic Fatty Liver Disease: A Study With Paired Liver Biopsies. J Clin Exp Hepatol 2020;10:245–54.

37. Siddiqui MS, Yamada G, Vuppalanchi R, Van Natta M, Loomba R, Guy C, *et al.*; NASH Clinical Research Network. Diagnostic Accuracy of Noninvasive Fibrosis Models to Detect Change in Fibrosis Stage. Clin Gastroenterol Hepatol 2019;17:1877–1885.e5.

38. Sun W, Cui H, Li N, Wei Y, Lai S, Yang Y, *et al.* Comparison of FIB-4 index, NAFLD fibrosis score and BARD score for prediction of advanced fibrosis in adult patients with non-alcoholic fatty liver disease: A meta-analysis study. Hepatol Res 2016;46:862–70.

39. Miller MH, Ferguson MA, Dillon JF. Systematic review of performance of non-invasive biomarkers in the evaluation of non-alcoholic fatty liver disease. Liver Int 2011;31:461–73.

40. Pascale A, Pais R, Ratziu V. An overview of nonalcoholic steatohepatitis: past, present and future directions. J Gastrointestin Liver Dis 2010;19:415–23.

41. Grigorescu M. Noninvasive biochemical markers of liver fibrosis. J Gastrointestin Liver Dis 2006;15:149–59.

42. Motola DL, Caravan P, Chung RT, Fuchs BC. Noninvasive Biomarkers of Liver Fibrosis: Clinical Applications and Future Directions. Curr Pathobiol Rep 2014;2:245–56.

43. Berger D, Desai V, Janardhan S. Con: Liver Biopsy Remains the Gold Standard to Evaluate Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. Clin Liver Dis (Hoboken) 2019;13:114–6.

44. Sporea I, Popescu A, Dumitraşcu D, Brisc C, Nedelcu L, Trifan A, *et al.* Nonalcoholic Fatty Liver Disease: status Quo. J Gastrointestin Liver Dis 2018;27:439–48.

45. Dumitrascu DL, Neuman MG. Non-alcoholic fatty liver disease: an update on diagnosis. Clujul Med 2018;91:147–50.

46. Durazzo M, Marzari L, Bonetto S, Ferro A, Ghigo MC, Belci P, *et al.* Noninvasive diagnosis of fibrosis in non-alcoholic fatty liver disease: diagnostic accuracy of different scores. Minerva Gastroenterol Dietol 2020. [Epub ahead of print]

47. Lardi LL, Lul RM, Port GZ, Coral GP, Peres A, Dorneles GP, *et al.* Fibromax and inflamatory markers cannot replace liver biopsy in the evaluation of non-alcoholic fatty liver disease. Minerva Gastroenterol Dietol 2020. [Epub ahead of print]

48. Zhou JH, Cai JJ, She ZG, Li HL. Noninvasive evaluation of nonalcoholic fatty liver disease: current evidence and practice. World J Gastroenterol 2019;25:1307–26.

49. Rosso C, Caviglia GP, Younes R, Ribaldone DG, Fagoonee S, Pellicano R, *et al.* Molecular mechanisms of hepatic fibrosis in chronic liver diseases. Minerva Biotecnol 2020;32:121–7.

50. Federico A, Dallio M. Liver fibrosis: which are independent predictors? Minerva Med 2019;110:183–4.

Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions.—Abdulrahman Ismaiel, Ludovico Abenavoli and Dan L. Dumitrascu have given substantial contributions to manuscript conception, Abdulrahman Ismaiel and Daniel-Corneliu Leucuta to search strategy, study selection, risk of bias assessment and data analysis, Abdulrahman Ismaiel and Stefan-Lucian Popa to data extraction, Abdulrahman Ismaiel, Stefan-Lucian Popa, Sharmila Fagoonee, Rinaldo Pellicano, Daniel-Corneliu Leucuta and Stefan-Lucian Popa to manuscript writing, Dan L.Dumitrascu and Ludovico Abenavoli to manuscript conception and critical revision for important intellectual content. All authors read and approved the final version of the manuscript.

History.—Article first published online: November 9, 2020. - Manuscript accepted: October 27, 2020. - Manuscript received: October 9, 2020.

Supplementary data.—For supplementary materials, please see the HTML version of this article at www.minervamedica.it

Observational Study

Medicine®

OPEN

Intrajejunal vs oral levodopa-carbidopa therapy in Parkinson disease

A retrospective cohort study

Luminita C. Popa, MD[a], Daniel-Corneliu Leucuta, MD, PhD[b,*], Nicoleta Tohanean, MD, PhD[a], Stefan-Lucian Popa, MD, PhD[c], Lacramioara Perju-Dumbrava, MD, PhD[a]

Abstract

Levodopa-carbidopa intestinal gel (LCIG) is a method of continuous administration of levodopa – the standard treatment in Parkinson disease (PD, a neurodegenerative disorder characterized by resting tremor, rigidity, gait impairment, and bradykinesia), thought to reduce the short-life and pulsatile problems of oral administration. We aimed to study the effects of Levodopa-Carbidopa therapy in 2 separate groups: one with intrajejunal administration of Levodopa-Carbidopa gel and the second with oral therapy.

We performed an observational retrospective Romanian cohort study on 61 patients diagnosed with PD patients, with Hoehn and Jahr 3 and 4 stages, recruited from a single regional tertiary center in Cluj-Napoca, Romania, between 2009 and 2019.

The mean adjusted UPDRS III (and similarly for UPDRS II) improved in the LCIG compared to the oral therapy group with 15.6 (95% CI 12.0–19.2, $P < .001$), and with 18.4 (95% CI 13.8–22.9, $P < .001$), stratified for the Hoehn and Jahr stages 3 and 4. There was a 41.7% (10) reduction in dyskinesia, and 29.2% reduction in wearing off/on-off at 1 year in the LCIG group compared to 0% (0) dyskinesia reduction, and 2.7% reduction in wearing off/on-off in the oral therapy group.

Continuous intrajejunal infusion of LCIG ensures a significant and clinical reduction in motor fluctuations compared to oral therapy in advanced PD, even after adjustment for important confounders.

Abbreviations: CI = confidence interval, COMT = catechol-O-methyltransferase inhibitor, IQR = interquartile range, LCIG = levodopa-carbidopa intestinal gel, MAO-B = monoamine oxidase-B, OMT = oral medical therapy, PD = Parkinson disease, PEG-J = percutaneous endoscopic transgastric jejunostomy, SD = standard deviation, STN-DBS = subthalamic nucleus deep brain stimulation, UPDRS = the unified Parkinson disease rating scale.

Keywords: LCIG, levodopa-carbidopa intestional gel, Parkinson disease, PD

1. Introduction

Parkinson disease (PD) is a neurodegenerative disorder characterized by resting tremor, rigidity, gait impairment, bradykinesia, sleep dysfunction, mood disorders, cognitive impairment, and dementia.[1] The underlying pathogenesis PD is not yet fully understood. It is thought to consist of the interaction between many genetic and environmental factors. This lack of knowledge explains the inability to make a precise diagnosis in the early stages and the limitations of treatment success in the later stages.

Levodopa is the amino-acid precursor of dopamine and has the function of recharging the depleted dopamine. For more than 4 decades, levodopa was described as the most efficient treatment in PD. Because of its short plasma half-life, oral levodopa may cause pulsatile striatal receptor stimulation, which leads to dyskinesias and a wide range of complications.[2–4] To diminish these types of complications, researchers developed levodopa-carbidopa intestinal gel (LCIG). It is delivered by using a percutaneous pump, set in place through an endoscopic intervention. This way, it leads to a constant plasma level of levodopa, therefore delivering a continuous dopaminergic stimulation.[4]

The intrajejunal administration of LCIG is one of the most efficient and frequently recommended pharmacological combination in PD. Nevertheless, studies found a wide range of motor and non-motor complications with the treatment.[4]

Because relevant studies engaged in the comparison between new therapeutical methods are still limited, we aimed to study the effects of levodopa-carbidopa in 2 separate groups: one with oral therapy and the second with intrajejunal administration of levodopa-carbidopa gel.

Editor: Hansen Chen.

No funding was received for this study.

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

The authors have no conflicts of interests to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] *Neurology Department,* [b] *Department of Medical Informatics and Biostatistics,* [c] *2nd Medical Department, "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania.*

[*] *Correspondence: Daniel-Corneliu Leucuta, Department of Medical Informatics and Biostatistics, Universitatea de Medicină si Farmacie Iuliu Hatieganu, 6 Pasteur Street, Cluj-Napoca, Cluj 400349, Romania (e-mail: dleucuta@umfcluj.ro).*

How to cite this article: Popa LC, Leucuta DC, Tohanean N, Popa SL, Perju-Dumbrava L. Intrajejunal vs oral levodopa-carbidopa therapy in Parkinson disease: a retrospective cohort study. Medicine 2020;99:46(e23249).

Received: 21 March 2020 / Received in final form: 10 September 2020 / Accepted: 22 September 2020

http://dx.doi.org/10.1097/MD.0000000000023249

2. Methods

2.1. Study design and setting

We performed an observational retrospective Romanian cohort study on 61 patients diagnosed with PD recruited from a single regional tertiary center in Cluj-Napoca, Romania, between 2009 and 2019.

2.2. Patients

We included PD patients with Hoehn and Jahr 3 and 4 stages, receiving oral administration of levodopa-carbidopa, or levodopa-carbidopa intrajejunal treatment. We excluded patients with an unclear diagnosis of PD, other Parkinsonian syndromes, neurodegenerative diseases, concomitant narrow-angle glaucoma, having contraindications for the placement of a nasogastric sonde or jejunal tube and oncological diseases.

2.3. Variables

We gathered the data from medical files and the hospital database. We set our outcome of interest the unified Parkinson disease rating scale (UPDRS) II and III reductions, and secondary the improvement in dyskinesia and wearing off/on-off in 1 year follow-up. Our exposure variable was the intrajejunal treatment compared to oral therapy. Besides these variables, we collected predictors and potential confounders, as well as variables to describe the sample better: demographic data (age, gender, place of residence), PD symptoms and evolution (disease duration, treatment duration, Hoehn and Yahr at baseline, UPDRS II and III, dyskinesia, Wearing off/on-off at baseline and 1 year follow-up), hallucinations, drug-induced psychosis, PD connex problems (mixed anxiety-depressive disorder, mild cognitive impairment, Parkinson dementia), oral treatment, additional treatments (deep brain stimulation), death, comorbidities (hypertension, atrial fibrillation, ischaemic stroke/cerebral lacunarism, type 2 diabetes, dyslipidemia, polyneuropathy), anemia related data (iron deficiency anemia, folate-deficiency anemia, B12 vitamin deficiency). UPDRS is one of the most frequently used questionnaires that follows the longitudinal course of Parkinson disease, but also the most commonly used scale in the clinical study of Parkinson disease and provides insight into the patients disease in a more objective manner.[5,6]

All subjects in both groups were assessed with the same scales that are commonly used in the hospital practice.

To minimize selection bias, we included subjects from the same hospital and excluding similar medical entities to PD. To minimize confounding, we performed adjustments in multiple regression analyses for important potential confounder variables, and stratified analyses.

2.4. Statistical analysis

Categorical data were presented as counts and percentages. Continuous data were presented as means and standard deviations (for normally distributed data) or medians and quartiles (1 and 3, for non-normally distributed data). Comparisons between the 2 groups for categorical data were made with the Chi-Squared test or with Fisher exact test, while for continuous data were made with *t* test for independent samples (for normally distributed data), or with Wilcoxon rank-sum test (for non-normally distributed data). To further assess the relationship between intrajejunal treatment compared to oral one, we used multivariate linear regression models, adjusted for age, Parkinson disease duration, treatment duration, Hoehn, and Yahr stage at the beginning. Since the Hoehn and Yahr stage appears to be a confounder, we also performed the same multivariate analysis, stratified by its 2 stages, 3 and 4. For all models, we checked the assumptions of residuals normality, heteroskedasticity (using the Breusch Pagan test of heteroskedasticity), linearity (using component residual plots), outliers and leverage points (Cooks D distance, studentized residuals). For multivariate models, we checked the assumptions of multicollinearity (using variance inflation factors), confounding (checking for a marked change in models coefficients when adding new variables to the model).

We removed 2 outliers/leverage points to correct for homoskedasticity – although the models were similar. Missing data was not imputed. For all statistical tests, the significance level was 0.05, and the two-tailed *P* value was calculated. All statistical analyses were performed with the R environment for statistical computing and graphics (R Foundation for Statistical Computing, Vienna, Austria), version 3.6.1.[7]

2.5. Ethics statement

The study was performed in agreement with the Declaration of Helsinki and was approved by the "Iuliu Hatieganu" University of Medicine and Pharmacy Ethics Committee.

3. Results

A total of 61 subjects with a mean age of 70.4 years (8.5 - standard deviation, ranging from 55 to 85 years), were enrolled in the study.

The characteristics of the intrajejunal therapy group and oral therapy group are compared in Table 1. Demographically the subjects were similar except for a higher frequency of female subjects in the intrajejunal group. The intrajejunal therapy group had a statistically significant longer PD history (6 years median difference), while the duration from the initiation of treatment was not significantly different.

The baseline clinical manifestations (Hoehn and Yahr stage; UPDRS II, and III; dyskinesia; Wearing off/On-Off) of PD disease were significantly worse in the intrajejunal group compared to the oral therapy group. The observed cognitive impairment and anxiety-depressive disorder were more frequent in the intrajejunal group than the oral therapy group, but only the latter reached a statistically significant level.

Regarding comorbidities, the cardiovascular, metabolic, were not significantly different between groups, although observed values were higher in the oral therapy group, except hypertension that was significantly different. Anemia-wise, the observed deficits were higher in the intrajejunal group, but not significantly different (except for iron deficiency anemia).

3.1. Intrajejunal therapy group-specific characteristics

Hallucinations and drug-induced psychosis were exceptional before the therapy, but a quarter of the subjects developed them after the therapy (see Table 2).

Table 1

Comparative analysis of Parkinson disease subjects receiving intrajejunal therapy and oral therapy group.

	Intrajejunal therapy (n = 24)	Oral therapy (n = 37)	*P* value
Age (years), mean (SD)	70.12 (7.66)	70.59 (9.11)	.835
Gender (female), n (%)	11 (45.83)	8 (21.62)	.046
Place of residence (rural), n (%)	3 (12.5)	2 (5.41)	.373
Duration of PD (years), median (IQR)	15 (13.75–20.25)	9 (6–13)	<.001
Duration from initiation of therapy (years), median (IQR)	5 (3.75–7)	4 (3–6)	.461
Hoehn şi Yahr stages la baseline			
3	2 (9.09)	29 (78.38)	<.001
4	20 (90.91)	8 (21.62)	
UPDRS II at baseline, median (IQR)	37 (33–39)	17 (13–24)	< .001
UPDRS II at 1 year, median (IQR)	27 (21–31.5)	24 (19–31)	.27
UDPRS II Diference at 1 year -baseline, median (IQR)	10 (6–12.5)	-7 (-8–-5)	<.001
UPDRS III at baseline, median (IQR)	41.5 (38–45)	24 (18–27)	<.001
UPDRS III at 1 year, median (IQR)	30 (28.5–35.5)	30 (26–34)	.307
UDPRS III Diference at 1 year-baseline, median (IQR)	11 (8.5–13)	−7 (−10–5)	<.001
Dyskinesia at baseline, n (%)	17 (70.83)	5 (13.51)	<.001
Dyskinesia at 1 year, n (%)	8 (33.33)	6 (16.22)	.12
Dyskinesia evolution in 1 year, n (%)			
dissapearing:	10 (41.67)	0 (0)	<.001
absent:	6 (25)	31 (83.78)	
persistent:	7 (29.17)	5 (13.51)	
newly occured:	1 (4.17)	1 (2.7)	
Dyskinesia evolution at 12 months (improvement vs. same or worsening), n (%)	10 (41.67)	0 (0)	<.001
Wearing off/On-Off at baseline, n (%)	24 (100)	10 (27.03)	<.001
Wearing off/On-Off at 1 year, n (%)	17 (70.83)	14 (37.84)	.012
Wearing off/On-Off evolution in 1 year, n (%)			
dissapearing:	7 (29.17)	1 (2.7)	<.001
absent:	0 (0)	22 (59.46)	
persistent:	17 (70.83)	9 (24.32)	
newly occured:	0 (0)	5 (13.51)	
Wearing off/On-Off evolution at 12 months (improvement vs. same or worsening), n (%)	7 (29.17)	1 (2.7)	.005
Deep brain stimulation, n (%)	1 (4.17)	0 (0)	.393
Decease, n (%)	6 (25)	3 (8.11)	.136
Mixed anxiety–depressive disorder, n (%)	13 (54.17)	8 (21.62)	.009
Mild cognitive impairment, n (%)	10 (41.67)	14 (37.84)	.765
Parkinson Dementia, n (%)	4 (16.67)	4 (10.81)	.7
Drug-induced psychosis, n (%)	5 (20.83)	0 (0)	.007
Hypertension, n (%)	6 (25)	21 (56.76)	.015
Permanent atrial fibrilation, n (%)	1 (4.17)	4 (10.81)	.64
Ischaemic Stroke / cerebral lacunarism, n (%)	5 (20.83)	16 (44.44)	.06
Diabetes type II, n (%)	1 (4.17)	8 (21.62)	.076
Polyneuropathy, n (%)	16 (66.67)	22 (61.11)	.662
Dyslipidemia, n (%)	3 (12.5)	7 (18.92)	.726
Iron deficiency anemia, n (%)	5 (20.83)	1 (2.78)	.033
Folate-deficiency anemia, n (%)	5 (20.83)	6 (16.67)	.741
Vitamin B12 deficiency, n (%)	2 (8.33)	4 (11.11)	1

SD = standard deviation, IQR = interquartile range, PD = Parkinson disease, UPDRS = the unified Parkinson disease rating scale.

3.2. Description of the oral therapy group specifics

The oral therapy group received in majority levodopa with carbidopa, about half of them received monoamine oxidase-B inhibitors and dopaminergic agonist, followed by amantadine, and the least frequent anticholinergic agents or catechol-O-methyltransferase inhibitors (see Table 3).

3.3. Comparative disease evolution under treatment

The evolution of PD clinical manifestations was statistically significant and clinically clearly better in the intrajejunal therapy group compared to the oral therapy regarding UPDRS II and III improvement, dyskinesia, and wearing off/On-Off at 1 year (see Table 1). Moreover, the oral therapy group had a diminishing of all the previously stated clinical manifestations at a year follow-up compared to the baseline evaluation. The difference in dyskinesia improvement in favor of intrajejunal therapy was of 47.47%, and statistically significant. While the difference in wearing off/On-Off improvement in favor intrajejunal therapy was of 26.47%, and statistically significant.

In order to check if the UPDRS II and III improvement, in 1 year, in the intrajejunal group, compared to oral therapy, was not due to other variables, we performed an adjustment in a multiple linear regression adjusting for age, Parkinson disease duration, treatment duration, Hoehn and Yahr stage at the beginning (see Table 4 and Table 5). In all the models the intrajejunal treatment had the most important effect in improving UPDRS II and III compared to all the other variables. Its effect was both important

Table 2

Characteristics of the intrajejunal therapy group.

Characteristic	Number (%) (n = 24)
Administration of Levodopa-Carbidopa on the nasogastric tube(test phase) but without administration of intrajejunal Levodopa-Carbidopa	4/24 (16.67)
Hallucinations	
Hallucinations before PEG-J	1/24 (4.17)
Number of years of hallucinations before PEG-J	0: 23/24 (95.83); 3: 1/24 (4.17)
Hallucinations after PEG-J	6/24 (25)
Hallucinations after PEG-J number of months, median (IQR)	0 (0–0.75)
Drug-induced psychosis	
Drug-induced psychosis before PEG-J	0/24 (0)
Drug-induced psychosis after PEG-J	6/24 (25)

IQR = interquartile range, PEG-J = percutaneous endoscopic transgastric jejunostomy.

Table 3

Drugs used for the patients under oral therapy.

Characteristic	Number (%) (n = 37)
Levodopa-Carbidopa	36 (100.00)
MAO-B Inhibitors	20 (55.56)
Dopaminergic Agonists	15 (41.67)
Anticholinergic Agents	2 (5.56)
Amantadine	7 (19.44)
COMT inhibitor (Entcapone)	4/35 (11.43)

MAO-B = monoamine oxidase-B, COMT = catechol-O-methyltransferase inhibitor.

and statistically significant. The determination coefficient for the univariate and for the multivariate models containing the intrajejunal treatment was important (above 0.74). All multivariate models were statistically significant. The relation between intrajejunal treatment and UPDRS II and III remained similar even after adjustment, and even on stratified analyses regarding the Hoehn and Yahr stage.

4. Discussions

Using the data collected during a decade in a tertiary clinical center, this analysis compared the clinical outcomes, side effects and complications between 2 separate groups of PD patients treated with LCIG and oral therapy. We found that UPDRS II and III scores statistically and clinically improved in the LCIG group compared to the oral therapy group, and the results stayed stable even after adjusting for age, disease duration, treatment duration, and stratified for Hoehn and Yahr stage at the beginning of the therapy. Dyskinesia, and wearing Off/On-Off diminished statistically and clinically in the LCIG group compared to the oral therapy group.

A study performed by Nyholm et al on 24 patients with advanced PD, compared daytime intraduodenal levodopa-carbidopa gel infusion as monotherapy with oral conventional combination therapies.[8] The median total UPDRS score at the end of each treatment arm was 53 with Conventional and 35 with Infusion ($P < .05$) and infusion provided lower median scores in all parts of the UPDRS, a result similar to ours.

A study on 11 patients with advanced PD analyzed the efficacy and safety of LCIG delivered continuously through an intrajejunal percutaneous tube (PEG-J).[9] LCIG contained a water-based suspension with micronized levodopa (20 mg/ml) and carbidopa (5 mg/ml) in methylcellulose (Duodopa) and was administered by continuous jejunal infusion for 12 hour/day using a portable pump (CADD-Legacy) by PEG-J.[9] The efficacy and safety outcomes were assessed by using the UPDRS parts II, III, and IV and were performed at baseline (T0) before LCIG initiation, and after 3 (T3) and 6 (T6) months of therapy.[9] The result was that patients showed statistically significant ($P < .05$) higher performances in activities of daily living, statistically significant ($P < .001$) lower incidence and severity of motor fluctuations, as rating by UPDRS part IV, compared to their best oral therapy and the success rate for PEG-J placement was 100%.[9] Previous research found that continuous intrajejunal infusion of LCIG provide a significant clinical improvement and improves UPDRS,[10–13] a result similar to ours. However, device and procedural complications, while generally of mild severity,

Table 4

The unified Parkinson disease rating scale II improvement (UPDRS II at therapy initiation minus UPDRS II at 12 months follow-up) assessment by univariate analyses, then in relation with therapy in multivariate regression, adjusted for age, Parkinson disease duration, treatment duration, Hoehn and Yahr stage at the beginning, and in stratified multivariate analysis by Hoehn and Yahr stage.

	Unstratified analyses							Stratified by Hoehn & Yahr = 3			Stratified by Hoehn & Yahr = 4		
	B	(95% CI)	P value	R2	B adjusted*	(95% CI)	P value	B adjusted**	(95% CI)	P value	B adjusted**	(95% CI)	P value
Age (years)	−0.17	(−0.42–0.08)	.183	0.03	−0.12	(−0.24–0)	.042	0.003	(−0.08–0.09)	.941	−0.33	(−0.58–-0.08)	.011
Parkinson's disease duration (years)	0.52	(0.2–0.84)	.002	0.16	−0.02	(−0.22–0.18)	.845	−0.08	(−0.25–0.09)	.342	0.06	(−0.30–0.41)	.741
Treatment duration (years)	0.24	(−0.71–1.19)	.614	0.005	−0.21	(−0.72–0.3)	.422	−0.09	(−0.58–0.41)	.724	−0.21	(−1.04–0.62)	.605
Hoehn and Yahr stage at the beginning	9.29	(5.7–12.88)	<.001	0.32	0.33	(−2.46–3.11)	.815		–	–	–	–	–
Therapy (intrajejunal vs. oral)	15.17	(13.17–17.18)	<.001	0.80	15.19	(12.37–18.01)	<.001	15.4	(12.46–18.34)	<.001	14.72	(10.25–19.19)	<.001
Adjusted R2					0.81			0.8			0.74		

* model containing all the variables in the table.

** model containing all the variables in the model, excepting the stratifying variable (Hoehn and Yahr stage); R^2 – coefficient of determination.

CI = confidence interval.

Table 5

The unified Parkinson disease rating scale III improvement (UPDRS III at therapy initiation minus UPDRS III at 12 months follow-up) assessment by univariate analyses, then in relation with therapy in multivariate regression, adjusted for age, Parkinson disease duration, treatment duration, Hoehn and Yahr stage at the beginning, and in stratified multivariate analysis by Hoehn and Yahr stage.

	Unstratified analyses							Stratified by Hoehn & Yahr = 3			Stratified by Hoehn & Yahr = 4		
	B	(95% CI)	P value	R2	B adjusted*	(95% CI)	P value	B adjusted**	(95% CI)	P value	B adjusted**	(95% CI)	P value
Age (years)	−0.22	(−0.51–0.07)	.138	0.04	-0.2	(−0.31–-0.08)	.001	−0.14	(−0.24–0.03)	.010	−0.33	(−0.58–0.07)	.014
Parkinson's disease duration (years)	0.68	(0.35–1.02)	<.001	0.23	0.12	(−0.07–0.3)	.209	0.07	(−0.14–0.27)	.500	0.14	(−0.19–0.46)	.385
Treatment duration (years)	0.12	(−0.98–1.23)	.824	0.001	-0.26	(−0.74–0.22)	.285	−0.06	(−0.66–0.54)	.827	−0.28	(−1.06–0.49)	.461
Hoehn and Yahr stage at the beginning	10.79	(6.57–15.02)	<.001	0.32	−0.54	(−3.33–2.25)	−.54	–	–	–	–	–	–
Therapy (intrajejunal vs oral)	18.14	(15.99–20.29)	<.001	0.84	17.76	(14.92–20.6)	17.76	15.6	(12.01–19.18)	<.001	18.35	(13.84–22.87)	<.001
Adjusted R^2					0.86			0.78			0.81		

* model containing all the variables in the table.
** model containing all the variables in the model, excepting the stratifying variable (Hoehn and Yahr stage); R^2 – coefficient of determination.
CI = confidence interval.

were present and were explained by the severity and progression of the disease.[10–13]

A long-term retrospective study analyzing advanced therapies in PD including oral medical therapy (OMT), LCIG and subthalamic nucleus deep brain stimulation (STN-DBS), found that OFF time improved to the same extent in STN-DBS and LCIG (−62% vs −54.5%; $P = .830$) and worsened with OMT (+78.6%; $P < .001$). Our study similarly found improvement in Wearing off/On-Off in LCIG compared to OMT. STN-DBS and LCIG yielded greater improvement on dyskinesia compared to OMT (dyskinesia duration: −66.1% vs −9.0% vs +24.2% [$P = .001$][14] similar to our study were dyskinesia at 1 year improved in the LCIG group compared to OMT group. The vast majority of studies have reported positive outcomes in motor complications with reduced duration of OFF time, increased ON time and plasma drug levels were maintained relatively stable in patients with LCIG therapy.[15–22]

4.1. Study limitations

The study has several limitations. Even though the sample size was not that large, the results are highly statistically significant, and the adjusted coefficients and confidence intervals for the main results are distant from the value of 0, thus suggesting a strong force of association. As with any observational study designs, residual confounding cannot be excluded even if we adjusted for several variables in the multivariate analysis. More extensive studies with more confounder adjusted models are warranted. Nevertheless, the large determination coefficient and the large adjusted coefficients suggest that this association is more likely to withstand adjustment for other confounders. Unknown confounders may diminish the association between intrajejunal treatment and disease progression.

The fact that the clinical status of PD patients was poorer in the intrajejunal group compared to the control group is normal since intrajejunal therapy is initiated in more advanced stages. Moreover, we tried to have subjects in both groups as homogenous as possible, thus limiting them to having only stage 3 and 4 for the Hoehn and Yahr stage. However, even with this initial difference, the improvement in outcomes in the intrajejunal group is important.

Since the cohort of PD patients has characteristics similar to patients from regional tertiary centers, the results are generalizable to this type of population. The reduced set of exclusion criteria helps to this generalizability.

Having taken into account the statistically significant and clinically important relation between intrajejunal treatment and clinical manifestations of PD, after adjustment for important confounders, for a cohort of similar subjects with advanced stage of PD, and also the similar findings of other studies, we have good arguments sustaining this relationship.

5. Conclusions

Continuous intrajejunal infusion of LCIG ensures a statistically significant and clinical important reduction of UPDRS II and III, compared to oral therapy in advanced PD patients, and the results stayed stable even after adjusting for age, disease duration, treatment duration, and stratified for Hoehn and Yahr stage at the beginning of the therapy. The same differences were found also for dyskinesia and wearing Off/On-Off that were diminished in the LCIG group.

Author contributions

Luminita Celia Popa carried out the study, analyzed the data, and wrote the paper; Daniel Corneliu Leucuta made substantial contributions to the analysis of the data, interpretation, and revised the drafts; Nicoleta Tohanean analyzed the data; Stefan-Lucian Popa made contributions to the conception of the manuscript; L. Perju-Dumbrava analyzed the data, supervised the work and critically revised the manuscript. All authors read and approved the final manuscript.

Conceptualization: Luminita Celia Popa, Lacrimioara Perju-Dumbrava.
Data curation: Daniel Corneliu Leucuta, Nicoleta Tohanean, Stefan Lucian Popa.
Formal analysis: Daniel Corneliu Leucuta.

Investigation: Luminita Celia Popa, Nicoleta Tohanean, Stefan Lucian Popa.
Methodology: Luminita Celia Popa, Daniel Corneliu Leucuta, Lacrimioara Perju-Dumbrava.
Project administration: Luminita Celia Popa.
Supervision: Lacrimioara Perju-Dumbrava.
Writing – original draft: Luminita Celia Popa.
Writing – review & editing: Luminita Celia Popa, Daniel Corneliu Leucuta, Nicoleta Tohanean, Stefan Lucian Popa, Lacrimioara Perju-Dumbrava.

References

[1] Schapira AHV, Chaudhuri KR, Jenner P. Non-motor features of Parkinson disease. Nat Rev Neurosci 2017;18:435–50.
[2] Reichmann H. Premotor diagnosis of Parkinson's disease. Neurosci Bull 2017;33:526–34.
[3] GBD 2015 Mortality and Causes of Death Collaborators.Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016;388:1459–544.
[4] Wang L, Li J, Chen J. Levodopa-carbidopa intestinal gel in Parkinson's disease: a systematic review and meta-analysis. Front Neurol 2018;9:620.
[5] Ramaker C, Marinus J, Stiggelbout AM, et al. Systematic evaluation of rating scales for impairment and disability in Parkinson's disease. Mov Disord 2002;17:867–76.
[6] Cruse B, Morales-Briceño H, Chang FCF, et al. 24-hour levodopa-carbidopa intestinal gel may reduce troublesome dyskinesia in advanced Parkinson's disease. NPJ Parkinsons Dis 2018;4:34.
[7] R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2019. Available from: http://www.r-project.org.
[8] Nyholm D, Nilsson Remahl AI, Dizdar N, et al. Duodenal levodopa infusion monotherapy vs oral polypharmacy in advanced Parkinson disease. Neurology 2005;64:216–23.
[9] Zulli C, Sica M, De Micco R, et al. Continuous intra jejunal infusion of levodopa-carbidopa intestinal gel by jejunal extension tube placement through percutaneous endoscopic gastrostomy for patients with advanced Parkinson's disease: a preliminary study. Eur Rev Med Pharmacol Sci 2016;20:2413–7.
[10] Catalán MJ, Antonini A, Calopa M, et al. Can suitable candidates for levodopa/carbidopa intestinal gel therapy be identified using current evidence? eNeurologicalSci 2017;8:44–53.
[11] Fernandez HH, Vanagunas A, Odin P, et al. Levodopa-carbidopa intestinal gel in advanced Parkinson's disease open-label study: interim results. Parkinsonism Relat Disord 2013;19:339–45.
[12] Slevin JT, Fernandez HH, Zadikoff C, et al. Long-term safety and maintenance of efficacy of levodopa-carbidopa intestinal gel: an open-label extension of the double-blind pivotal study in advanced Parkinson's disease patients. J Parkinsons Dis 2015;5:165–74.
[13] Jost WH. Unwanted effects and interaction of intrajejunal levodopa/carbidopa administration. Expert Opin Drug Saf 2014;13:447–58.
[14] Merola A, Espay AJ, Romagnolo A, et al. Advanced therapies in Parkinson's disease: long-term retrospective study. Parkinsonism Relat Disord 2016;29:104–8.
[15] Pickut BA, van der Linden C, Dethy S. Intestinal levodopa infusion: the Belgian experience. Neurol Sci 2014;35:861–6.
[16] Olanow CW, Kieburtz K, Odin P. Continuous intrajejunal infusion of levodopa-carbidopa intestinal gel for patients with advanced Parkinson's disease: a randomised, controlled, double-blind, double-dummy study. Lancet Neurol 2014;13:141–9.
[17] Isacson D, Bingefors K, Kristiansen IS. Fluctuating functions related to quality of life in advanced Parkinson disease: effects of duodenal levodopa infusion. Acta Neurol Scand 2008;118:379–86.
[18] Fernandez HH, Standaert DG, Hauser RA. Levodopa-carbidopa intestinal gel in advanced Parkinson's disease: final 12-month, open-label results. Mov Disord 2015;30:500–9.
[19] Clarke CE, Worth P, Grosset D. Systematic review of apomorphine infusion, levodopa infusion and deep brain stimulation in advanced Parkinson's disease. Parkinsonism Relat Disord 2009;15:728–41.
[20] Nilsson D, Nyholm D, Aquilonius SM. Duodenal levodopa infusion in Parkinson's disease—long-term experience. Acta Neurol Scand 2001;104:343–8.
[21] Antonini A, Isaias IU, Canesi M. Duodenal levodopa infusion for advanced Parkinson's disease: 12-month treatment outcome. Mov Disord 2007;22:1145–9.
[22] Antonini A, Mancini F, Canesi M. Duodenal levodopa infusion improves quality of life in advanced Parkinson's disease. Neurodegener Dis 2008;5:244–6.

MEDIAN NERVE ULTRASONOGRAPHY DIAGNOSTIC ACCURACY AND ELECTRODIAGNOSTIC EVALUATION OF CARPAL TUNNEL SYNDROME IN TYPE 2 DIABETES MELLITUS

Nicu Cătălin Drăghici[1, 2, 3], Daniel-Corneliu Leucuța[4], Maria Magdalena Tămaș[5], Tudor Dimitrie Lupescu[1, 3], Ștefan Strilciuc[1, 3], Simona Rednic[5], Dafin Fior Mureşanu[1, 3]

[1]Department of Clinical Neurosciences, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania - [2]"IMOGEN" Institute, Centre of Advanced Research Studies, Cluj-Napoca, Romania -[3]"RoNeuro" Institute, Centre for Neurological Research and Diagnostic, Cluj-Napoca, Romania - [4]Department of Medical Informatics and Biostatistics, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania - [5]Department of Rheumatology, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

ABSTRACT

Introduction*: Carpal tunnel syndrome (CTS) and type 2 diabetes mellitus (DM) are two prevalent pathologies. However, CTS is sometimes challenging to diagnose and can be easily overlooked in diabetic patients. The study aimed to evaluate the diagnostic accuracy of ultrasound (US) to identify CTS in patients with type 2 DM, by using clinical diagnosis and electroneuronography (ENG) testing as a reference standard.*

Material and methods*: We conducted a prospective diagnostic accuracy cross-sectional study and we analyzed clinically and performed through US and ENG, 106 wrists of 53 consecutive patients known with type 2 DM.*

Results*: We have obtained good results regarding the cross-sectional area (CSA) at the wrist, with an area under the receiver operating characteristics (AUROC) = 0.854, 95% CI (0.776 - 0.92) and AUROC = 0.833 95% CI (0.749 - 0.906) for wrist - forearm difference (WFD), respectively. Moreover, the US sensitivity for CTS was 86.1 % at the wrist level for a cut-off at 10.5 mm². Likewise, the WFD had a sensitivity of 83.3 % for a cut-off of 3 mm².*

Conclusion*: Median nerve US at the wrist had a moderately good diagnostic accuracy in CTS diagnosis in type 2 DM.*

Keywords: *carpal tunnel syndrome, diabetes mellitus type 2, median nerve ultrasonography, electromyography.*

DOI: 10.19193/0393-6384_2020_6_539

Received *March 27 2020;* ***Accepted*** *September 14, 2020*

Introduction

Carpal tunnel syndrome (CTS) is the most common peripheral focal mononeuropathy and occurs as a result of median nerve compression at the level of the wrist[(1)]. It is clinically manifested by a constellation of signs and symptoms, consequence of motor, sensory, and autonomic nerve impairment: numbness and tingling in the first three fingers and 1/2 of the IV finger, pain in the median nerve territory and hypotrophy of the thenar muscles[(2,3)].

The CTS pathogenicity is multifactorial and unclear. The most common theories are: a) increased intracarpal pressure; b) decreased median nerve mobility; c) flexor tendon thickening and tightening during activity; d) median nerve deformation, and e) increased stiffness of the synovium and flexor retinaculum[(4)]. The incidence of CTS increases with age, affecting obese people and women in particular. Likewise, the risk of developing CTS is increased in occupational factors involving repetitive work or vibrating tools[(5)].

Moreover, type 2 diabetes mellitus (DM) is a risk factor for CTS (6). The increased prevalence of CTS in patients with type 2 DM has been intensively studied in previous decades, but some studies show that type 2 DM and its duration do not seem to be an independent risk factor for CTS[7,8]. However, in clinical practice, the median nerve entrapment in diabetic patients may sometimes be overlooked. Currently, the gold standard for the diagnosis of CTS in the general population, is the clinical diagnosis confirmed by nerve conduction studies (NCS)[9]. Electroneurography (ENG) adds value in the diagnosis of CTS because a) it can confirm or deny suspected diagnosis; b) it considers other causes of mimics of CTS; c) it brings various and fast methods of CTS evaluation; d) it appreciates the prognosis[10]. In the meantime, non-invasive techniques are increasingly being studied in the diagnosis of CTS. Ultrasonography (US) and power Doppler US, microvascular imaging and shear-wave elastography are methods comprehensively studied in the diagnosis of CTS in the general population, being correlated with its severity[11-13]. However in diabetics' patients these methods are not evaluated. The aim of the study was to evaluate the diagnostic accuracy of US to identify CTS in patients with type 2 DM, using the wrist and forearm cross-sectional area, having clinical diagnosis and ENG as a reference standard.

Material and methods

Study population

In this prospective diagnostic accuracy study, we included 53 consecutive patients with type 2 DM, who were evaluated for ENG in the Neurophysiology Laboratory of the IMOGEN Institute. The screening of patients with possible peripheral nervous system involvement was performed by the diabetologist in the outpatient clinic based on clinical criteria. All patients were clinically evaluated, after that they were US and ENG tested.

Test methods

The reference standard test consisted of a clinical diagnosis combined with ENG testing. To determine the clinical diagnosis of CTS, the following symptoms have been reported: numbness, tingling, or pain in the least 2 of digits I, II, III, and ½ of IV for at least a month[14].

ENG recording was performed by a physician with four years of experience using a Viking CareFusion device. During the investigations, the skin temperature variability was between 32°C - 34°C. Using the bar electrode for nerve stimulation and surface electrodes for response recording, we performed NCS for both median and ulnar nerves[15]. The ENG diagnosis of CTS was established based on the recommendation of the American Association of Neuromuscular & Electrodiagnostic Medicine (AANEM), meaning a median compound muscle action potential (CMAP) latency > 4.2 ms or a median antidromic sensitive velocity (digit II) < 40 ms[16]. The ENG evaluator was blinded for the US results and unblinded for clinical information.

The index test - US evaluation - was performed by a physician with nine years of experience in the musculoskeletal US, unblinded for clinical information, using a Siemens Acuson S 2000 ultrasonography device. The upper limb was held in a comfortable position, and an 18 MHz transducer spotted the median nerve in the axial plane. The cross-sectional area (CSA) was calculated above the mid flexor retinaculum and 5 cm proximal to this point at the mid-forearm level. Two US determinations were performed for each site, and the average of the two measurements was calculated to establish the median CSA. Subjects with bifid median nerve were also included in the study[17], and the examiner was blinded from ENG results. The reliability of intra-observer measurements was measured with the intraclass correlation coefficient for wrist CSA and forearm CSA, obtaining 0.979 (95% CI 0.947-0.992, $p<0.001$), respectively 0.891 (95% CI 0.744-0.956, $p<0.001$). There were no side effects following the two examinations.

Statistical analysis

Continuous data were described as means and standard deviations for normally distributed data and as medians and interquartile range for data that did not follow a normal distribution. Categorical data were expressed as counts and percentages. Comparisons between the two groups regarding skewed continuous data were performed using the Wilcoxon rank-sum test. Partial Spearman correlation coefficient, along with its 95% confidence interval, adjusted for age and diabetes duration, was used to assess the correlation between body mass index

(BMI, weight in kg/height in m^2) and US and ENG tests. We computed the area under the receiver operating characteristic (AUROC) along with its 95% confidence interval (found using bootstrapping) and plotted a receiver operating characteristic chart for different US measurements, compared to the reference standard (clinical diagnosis and ENG testing). The best sensitivity (Se) and specificity (Sp) cut-off was identified as having the highest Youden value (Se + Sp - 1) for the US measurements with the highest AUROCs. Then, by visual inspection of the ROC plot, a cut-off favoring sensitivity over specificity was identified for the same US measurements with the highest AUROCs. No pre-specified cut-offs were used for the diagnosis for the index test. For all statistical analyses, we set a 0.05 confidence level, and the two-tailed p-value was computed. The intraclass correlation coefficient with a 95% confidence interval and a statistical test for its significance was calculated to assess intra-ratter reliability of US measurements in wrist and forearm (on 20 measures). All statistical analyses were computed in R environment for statistical computing and graphics, version 3.6.0.

Statement of human and animal rights. All the patients agreed to and signed the informed consent, and the local Ethics Committee approved the study no.26/2016. The research was in accordance with the ethical standards of the committee and with the Helsinki Declaration.

Results

A total of 53 consecutive diabetic patients with a mean (SD) age of 62 ± 6.25 years were included. The patients' characteristics of the study population can be consulted in Table 1.

For CTS diagnosis, 106 wrists were clinically evaluated, followed by US and ENG testing. The US measurements were performed on the same week after the clinical and ENG evaluation. Based on clinical findings, 70 wrists meet the diagnosis criteria for possible or probable CTS, from which 36 wrists were ENG confirmed for CTS diagnosis. The measurements of the two tests are summarized in Table 2. All US and ENG parameters, except for the CSA at the forearm, show a statistically significant difference between the two groups.

To check whether body weight was linked to CTS, we assessed the partial correlation coefficient between BMI vs. US and ENG measurements. As can be seen in Table 3, we have found no correlation between them among our patients.

In Figure 1 and Table 4 we summarized the

Characteristic	Statistics
Age (years), mean (SD)	62.38 (6.25)
Sex (female), n (%)	30 (56.6)
Diabetes duration (months), median (IQR)	120 (72 - 168)
Glycaemia, mean (SD)	128.79 (20.03)
1 - Hb A1c (%), median (IQR)	6.9 (6.2 - 7.7)
Waist circumference (cm), mean (SD)	111.57 (15.62)
BMI, mean (SD)	31.72 (4.75)

SD – standard deviation, IQR – interquartile range, BMI – body mass index (weight in kg/height in m^2)

Table 1: Baseline demographic, clinical, and paraclinical patients' characteristics.

Characteristic median (IQR)	CTS present (n=36)	CTS absent (n=70)	P - value *
CSA - wrist (mm^2)	12.75 (11 - 14.88)	9 (7.62 - 10.38)	< 0.001
CSA - forearm (mm^2)	7.75 (6.5 - 9)	7 (6 - 8)	0.048
wrist/forearm Ratio	1.83 (1.42 - 2)	1.32 (1.15 - 1.5)	< 0.001
wrist - forearm difference (mm^2)	5 (3.5 - 7)	2 (1 - 3)	< 0.001
median CMAP Latency (ms)	4.95 (4.4 - 5.6)	3.6 (3.4 - 4)	< 0.001
antidromic median NCS (digit II) (ms)	33 (26.5 - 36.25)	46 (42 - 51.75)	< 0.001

CTS – carpal tunnel syndrome, CSA – cross-sectional area, CMAP – compound muscle action potential, NCS – Nerve conduction study, IQR – interquartile range, Wilcoxon rank-sum test *

Table 2: Ultrasonographic and electroneurographic tests measurements in type 2 diabetes mellitus patients.

Diagnostic method	Correlation coefficient (95% CI)	P-value*
CSA - wrist (mm^2)	0.14 (-0.07 - 0.29)	0.161
wrist to forearm difference (mm^2)	0.11 (-0.09 - 0.26)	0.258
wrist to forearm ratio	0.08 (-0.12 - 0.2)	0.411
median CMAP latency (ms)	0.14 (-0.06 - 0.25)	0.145
antidromic median NCS (digit II) (ms)	-0.14 (-0.23 - 0.14)	0.157

The values were adjusted for age and diabetes duration.

CSA – cross-sectional area, CMAP – compound motor unit action potential, NCS – nerve conduction study Spearman partial correlation coefficient *, CI – confidence interval

Table 3: Partial Spearman correlation coefficient between body mass index vs. ultrasonographic and electroneurographic tests in type 2 diabetes mellitus patients.

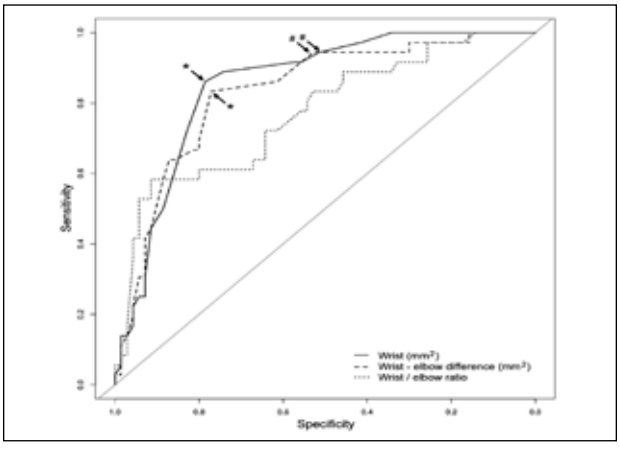

Fig. 1: Receiver Operating Characteristic plot classifying carpal tunnel syndrome with clinical diagnosis combined with electrodiagnostic testing as the standard test, and ultrasonographic measurements as index tests in type 2 diabetes mellitus patients (* best Sensitivity and Specificity cut-off, # cut-off favouring sensitivity over specificity).

AUROC for US, having the clinical evaluation and ENG confirmation as gold standard. At the level of the wrist, the US has an AUROC = 0.854, 95% CI (0.776 - 0.92), with a CSA cut-off at 10.5 mm² for the best Se and Sp.

With a cut-off at 8.25 mm², the Se of the US increases up to 94.4% to the impairment of the Sp. For the wrist to forearm difference (WFD), the AUROC was 0.833, 95% CI (0.749 - 0.906) for the best Se and Sp, with the best cut-off at 3 mm². The Se can be increased up to 94.4% with a cut-off of around 2.25 mm². Finally, the wrist to forearm ratio (WFR) showed a poor AUROC for US.

Discussions

	AUROC (95%CI)	Cut-off	Sensitivity %	Specificity %
Wrist (mm²)	0.854 (0.776 - 0.92)	10.5 *	86.1	78.8
		8.25 #	94.4	51.4
WFD (mm²)	0.833 (0.749 - 0.906)	3.0 *	83.3	77.1
		2.25 #	94.4	52.9
WFR	0.771 (0.669 - 0.86)	1.67	58.3	91.4
WFD – wrist to forearm difference, WFR – wrist to forearm ratio, CI – confidence interval, * best Sensitivity and Specificity cut-off, # cut-off favouring sensitivity over specificity				

Table 4: The area under Receiver Operating Characteristic (AUROC) classifying carpal tunnel syndrome with clinical diagnosis combined with electroneurographic testing as the standard test, and ultrasonographic measurements as index tests, in type 2 diabetes mellitus patients.

The purpose of this study was to determine the US accuracy in the diagnosis of CTS in type 2 DM. The importance of US in the diagnosis of entrapment neuropathies is more and more studied, with important practical applications in the diagnosis of early stages of CTS[18-20]. Currently, there is no consensus regarding the normal CSA values, but Cartwright proposes an agreement and, in a recent review, presents a series of normal values used in clinical practice[21]. The variability of US standard values from previous research can be explained by the body anthropometric measures that seem to influence the occurrence and severity of CTS[22]. Thus, in a recent study, Kleermaeker et al. demonstrate that the CSA of the median nerve depends on the wrist circumference and is strongly correlated with this parameter[23]. Obesity seems to be involved in the rising incidence of CTS, but in our study, enlarging the wrist CSA was not associated with a BMI increase. In this case, we assumed that the US performance could be altered due to adipose cellular tissue in diabetic patients. Still, recent studies have demonstrated that BMI and diabetes do not change echogenicity and US visibility of peripheral nerves[24]. Moreover, the ENG parameters: reducing the sensitive nerve conduction velocity and prolonging the CMAP latency were not associated with a BMI increase. Other studies have explored the relationship between age and median CSA and showed that the median nerve US has good Se and Sp in elderly patients and seems to be less influenced by age[25].

Following other research, our study demonstrates a good Se and Sp of US in CTS diagnosis[26-28]. Likewise, in the diabetic population, a recent study shows good accuracy of the US in the diagnosis of CTS in diabetic patients with and without diabetic neuropathy[29]. Other studies propose additional criteria - US evaluation of proximal, inlet, outlet and distal to the carpal tunnel - to extend US accuracy in CTS diagnosis[30]. Furthermore, Hobson et al. introduces the notion of WFR and shows that a ratio between wrist and forearm > 1.4 has a 100% sensitivity in CTS diagnosis in the general population[31]. This is in contrast to our study conducted on diabetic patients, in which the WFR had a low sensitivity at 58.3% with a cut-off at 1.67. However, our results are similar to recent studies demonstrating that the WFR is not indicated and should be used with prudence in the diagnosis of CTS in diabetic patients[32].

We believe that the present study is relevant and has some important practical implications. It demonstrates that the median nerve US evaluation has a moderately good diagnostic accuracy in patients with CTS and diabetes. Moreover, in this category of subjects where the symptoms of CTS may clinically be confused with diabetic neuropathy, an increase in the number of diagnostic tools for CTS diagnosis leads to a higher number of patients treated as a result of conservative or surgical treatment[33]. The study has a rather low statistical uncertainty, especially for its main findings, having relatively narrow confidence intervals, and statistically significant results. By using a prospective approach and a consecutive group of patients, this study has a good generalizability of type 2 DM.

This study has several shortcomings. The number of patients was limited, although it achieved its aim, and had statistically significant results. The two examiners were not blinded to the patient's symptoms, although they were blinded to the other diagnostic method.

Conclusion

Median nerve US - specifically wrist CSA and WFD - had a moderately good diagnostic accuracy in the diagnosis of CTS in patients with type 2 DM.

Our viewpoint is that this fast, non-invasive, and cost-effective method could be used as a screening tool for CTS in diabetic patients.

References

1) Lee EY, Lim AYT. Nerve Compression in the Upper Limb. Clin Plast Surg. 2019; 46: 285-93.
2) Roll SC, Case-Smith J, Evans KD. Diagnostic Accuracy of Ultrasonography VS. Electromyography in Carpal Tunnel Syndrome: A Systematic Review of Literature. Ultrasound Med Biol 2011; 37: 1539-53.
3) Doughty CT, Bowley MP. Entrapment Neuropathies of the Upper Extremity. Med Clin North Am 2019; 103: 357-70.
4) Sucher BM, Schreiber AL. Carpal Tunnel Syndrome Diagnosis. Phys Med Rehabil Clin N Am 2014; 25: 229-47.
5) Leung D. Carpal Tunnel Syndrome. Encycl Neurol Sci 2014; 77: 602-5.
6) Pourmemari MH, Shiri R. Diabetes as a risk factor for carpal tunnel syndrome: A systematic review and meta-analysis. Diabet Med 2016; 33: 10-16.
7) Comi G, Lozza L, Galardi G, Ghilardi MF, Medaglini S, Canal N. Presence of carpal tunnel syndrome in diabetics: effect of age, sex, diabetes duration and polyneuropathy. Acta Diabetol Lat 1985; 22: 259-62.
8) Hendriks SH, Van Dijk PR, Groenier KH, Houpt P, Bilo HJG, Kleefstra N. Type 2 diabetes seems not to be a risk factor for the carpal tunnel syndrome: A case control study. BMC Musculoskelet Disord 2014; 15: 1-5.
9) Tatar IG, Kurt A, Yavasoglu NG, Hekimoglu B. Carpal tunnel syndrome: Elastosonographic strain ratio and cross-sectional area evaluation for the diagnosis and disease severity. Med Ultrason 2016; 18: 305-11.
10) Wang L. Guiding Treatment for Carpal Tunnel Syndrome. Phys Med Rehabil Clin N Am 2018; 29: 751-60.
11) Cingoz M, Kandemirli SG, Alis DC, Samanci C, Kandemirli GC, Adatepe NU. Evaluation of median nerve by shear wave elastography and diffusion tensor imaging in carpal tunnel syndrome. Eur J Radiol 2018; 101: 59-64.
12) Karahan AY, Arslan S, Ordahan B, Bakdik S, Ekiz T. Superb microvascular imaging of the median nerve in carpal tunnel syndrome: An electrodiagnostic and ultrasonographic study. J Ultrasound Med 2018; 37: 2855-61.
13) Ting BL, Blazar PE, Collins JE, Mora AN, Salajegheh MK, Amato AA, et al. Median Nerve Ultrasonography Measurements Correlate With Electrodiagnostic Carpal Tunnel Syndrome Severity. J Am Acad Orthop Surg. 2019; 27(1): e17–23.
14) Rempel D, Evanoff B, Amadio PC, de Krom M, Franklin G, Franzblau A, et al. Consensus criteria for the classification of carpal tunnel syndrome in epidemiologic studies. Am J Public Health 1998; 88: 1447-51.
15) Werner RA, Andary M. Electrodiagnostic evaluation of carpal tunnel syndrome. Muscle and Nerve 2011; 44: 597-607.
16) Jablecki CK, Andary MT, Floeter MK, Miller RG, Quartly CA, Vennix MJ, et al. Practice parameter for electrodiagnostic studies in carpal tunnel syndrome: Summary statement. Muscle and Nerve 2002; 25: 918-22.
17) Kasius KM, Claes F, Meulstee J, Verhagen WI. Bifid median nerve in carpal tunnel syndrome: do we need to know? Muscle Nerve 2014; 50: 835-43.
18) Chari B, McNally E. Nerve Entrapment in Ankle and Foot: Ultrasound Imaging. Semin Musculoskelet Radiol 2018; 22: 354-63.
19) Klauser AS, Buzzegoli T, Taljanovic MS, Strobl S, Rauch S, Teh J, et al. Nerve Entrapment Syndromes at the Wrist and Elbow by Sonography. Semin Musculoskelet Radiol 2018; 22: 344-53.
20) Bang M, Kim JM, Kim HS. The usefulness of ultrasonography to diagnose the early stage of carpal tunnel syndrome in proximal to the carpal tunnel inlet. Medicine 2019; 98: e16039.
21) Cartwright MS, Hobson-Webb LD, Boon AL, Alter KE, Hunt CH, Flores VH, et al. Evidence-based guideline: neuromuscular ultrasound for the diagnosis of carpal tunnel syndrome. Muscle Nerve 2012; 46: 287-93.
22) Mondelli M, Curti S, Mattioli S, Aretini A, Ginanneschi F, Greco G, et al. Associations Between Body Anthropometric Measures and Severity of Carpal Tunnel Syndrome. Arch Phys Med Rehabil 2016; 97: 1456-64.
23) De Kleermaeker FGCM, Meulstee J, Verhagen WIM. The controversy of the normal values of ultrasonography in carpal tunnel syndrome: diagnostic accuracy of wrist-dependent CSA revisited. Neurol Sci 2019; 40: 1041-7.
24) Stolz LA, Acuña JG, Gaskin K, Murphy AM, Friedman L, Stears-Ellis S, et al. Echogenicity and ultrasound visibility of peripheral nerves of the upper extremity. Med Ultrason 2018; 20: 199-204.
25) Roghani RS, Lokk J, Hashemi SE, Holisaz MT, Gohari F, Delbari A. The diagnostic accuracy of median nerve ultrasonography in elderly patients with carpal tunnel syndrome: Sensitivity and specificity assessment. Clin Interv Aging 2018; 13: 1953-62.
26) Torres-Costoso A, Martínez-Vizcaíno V, Álvarez-Bueno C, Ferri-Morales A, Cavero-Redondo I. Accuracy of Ultrasonography for the Diagnosis of Carpal Tunnel Syndrome: A Systematic Review and Meta-Analysis. Arch Phys Med Rehabil 2018; 99: 758-765.
27) Ažman D, Hrabač P, Demarin V. Use of Multiple Ultrasonographic Parameters in Confirmation of Carpal Tunnel Syndrome. J Ultrasound Med 2018; 37: 879-89.
28) Park GY, Kwon DR, Seok JI, Park DS, Cho HK. Usefulness of ultrasound assessment of median nerve mobility in carpal tunnel syndrome. Acta radiol 2018; 59: 1494-9.
29) Kotb MA, Bedewi MA, Aldossary NM, Mahmoud G, Naguib F. Sonographic assessment of carpal tunnel syndrome in diabetic patients with and without polyneuropathy. Medicine 2018; 24: 0-4.
30) Ng AWH, Griffith JF, Lee RKL, Tse WL, Wong CWY, Ho PC. Ultrasound carpal tunnel syndrome: additional criteria for diagnosis. Clin Radiol 2018; 73: 214.
31) Hobson-Webb LD, Massey JM, Juel VC, Sanders DB. The ultrasonographic wrist-to-forearm median nerve area ratio in carpal tunnel syndrome. Clin Neurophysiol 2008; 119: 1353-7.
32) Steinkohl F, Loizides A, Gruber L, Karpf M, Mörsdorf G, Gruber I, et al. Ultrasonography for the Diagnosis of Carpal Tunnel Syndrome in Diabetic Patients: Missing the Mark? RoFo 2019; 191: 117-21.

33) Velázquez-Rueda ML, Hernández-Méndez-Villamil E, Mendoza-Muñoz M, Rivas-Montero JA, Espinosa-Gutiérrez A.Strength and function of hand before and after release of carpal tunnel in patients with type 2 diabetes mellitus by open and endoscopic approach. Case-control study. Acta Ortop Mex 2018; 32: 22-27.

Corresponding Author:
Daniel-Corneliu Leucuța, M.D., Senior Lecturer
Str. Pasteur 6, 400012, Cluj-Napoca, Romania
Phone: +40-264-597256
E-mail: dleucuta@umfcluj.ro
(Romania)

Cancer Management and Research

Dovepress

open access to scientific and medical research

Open Access Full Text Article

REVIEW

High dose vs low dose irradiation of the subventricular zone in patients with glioblastoma—a systematic review and meta-analysis

This article was published in the following Dove Press journal:
Cancer Management and Research

Sergiu Șușman[1,2,*]
Daniel-Corneliu Leucuța[3]
Gabriel Kacso[4,5,*]
Ștefan Ioan Florian[6,7]

[1]Department of Morphological Sciences, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; [2]Department of Neuropathology-Imogen Research Center, Emergency County Hospital, Cluj-Napoca, Romania; [3]Department of Medical Informatics and Biostatistics, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; [4]Department of Oncology and Radiotherapy, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; [5]Amethyst Radiotherapy Center, Cluj-Napoca, Romania; [6]Department of Neurosciences, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; [7]Department of Neurosurgery, Emergency County Hospital, Cluj-Napoca, Romania

*These authors contributed equally to this work

Purpose: The published data indicate that the irradiation of the subventricular zone (SVZ) might play a role in the treatment of patients with glioblastoma (GBM). We aimed to determine whether radiation treatment doses (high vs low) applied to the SVZ can lead to an increase in progression free survival (PFS) and overall survival (OS).

Patients and methods: We undertook a systematic review and meta-analysis according to the PICOS research criteria of patients with glioblastoma which received high doses compared to low doses in order to determine if they have a better survival in observational and experimental studies.

Results: Our survey of the literature yielded 2573 unique records. After screening, 17 were assessed for eligibility, and in the end 8 were included in the qualitative and 4 in the quantitative analysis. Subjects who received higher doses of ipsilateral SVZ (iSVZ) irradiation had a statistically significant better PFS than those receiving lower doses (HR 0.58 [95% CI 0.42–0.82], p=0.002). Subjects receiving higher doses of contralateral SVZ (cSVZ) irradiation did not have a statistically significant better PFS than those receiving lower doses (HR =0.89 [95% CI 0.35–2.26], p=0.81). Also for OS the subjects receiving higher doses to the iSVZ did not have a statistically significant better survival than those receiving lower doses (HR =0.75 [95% CI 0.51–1.11], p=0.15).

Conclusion: The data indicate a possible involvement of the SVZ in the onset and progression of the GBM, as well as a possible role of the SVZ in radiation therapy.

Keywords: glioblastoma, radiation therapy, neural stem cells, survival

Introduction

The WHO 2016 classification ranks GBM as a grade IV tumor, survival at 5 years being extremely rare despite the multimodal treatment administered nowadays.[28] The histopathological criteria for GBM are a diffuse growth pattern accompanied by brisk mitotic activity, necrosis, and microvascular proliferation.[43] Alongside the intrinsic features of the tumor, the interaction between the GBM and the surrounding normal brain structures, such as the SVZ, is currently taken into consideration in the attempt to understand the onset, progression, and resistance to treatment.[13,30,41]

The SVZ is an area 3–5 mm thick covering the wall of the lateral ventricles. Especially important during the development of the brain, in adulthood it contains NSC (neural stem cells) and provides a particular environment (the niche) which these cells need in order to preserve their biological properties.[26,35,45]

Correspondence: Daniel-Corneliu Leucuța
Department of Medical Informatics and Biostatistics, Iuliu Hațieganu University of Medicine and Pharmacy, 6 Pasteur Street, Cluj-Napoca 400012, Romania
Tel +4 059 7256 int 2502
Email dleucuta@umfcluj.ro

http://doi.org/10.2147/CMAR.S206033

The importance of the interaction between GBM and the SVZ has been highlighted, as the tumors coming into contact with the area that contains NSC have a worse prognosis than those located at a distance.[2,3,11,17,25,36] The subject needs more research because there are also studies that could not confirm the prognostic role of SVZ invasion by GBM.[15,16]

Given the diffuse extension of tumor cells in GBM, the significant migration potential of the NSC from the SVZ to the pathological areas (including tumor lesions), and the possibility of communication via the cerebrospinal fluid secreted by the choroid plexus (CP), questions have been asked about a possible long-distance interaction between GBM and the ipsi- and the contralateral SVZ.[4,14,23,27]

Thus, a number of studies have ascertained the existence of a correlation between the irradiation of the SVZ (ipsi- and contralateral), the administered doses, and survival.[6,7,11,19] Nevertheless, not all of the published studies have reached the same conclusions, as other authors failed to identify an increase in survival in the patients subjected to a radiotherapy protocol that also targeted the SVZ.[8,20]

Considering the importance of the topic and the divergent conclusions in the literature, in the present study we performed a systematic review with meta-analysis of the observational and experimental studies done on patient populations histopathologically diagnosed with GBM, which compare the doses of radiotherapy administered to the iSVZ and cSVZ and their effect upon survival.

Materials and methods

We undertook a systematic review and meta-analysis taking into account the PRISMA guideline.[34]

Eligibility criteria

The PICO research question was: In patients with glioblastoma (Population), do high doses of radiotherapy (Intervention) compared to low doses of radiotherapy (Comparison intervention) offer better progression-free survival or overall survival (Outcome), in observational and experimental studies (Study design)? We considered all published original articles published till December 2018, in any language.

Information sources

We undertook a comprehensive research in seven bibliographic databases (Pubmed, EMBASE, Cochrane CENTRAL, Web of Science (Science Citation Index Expanded (SCI-EXPANDED) −1975-present, Emerging Sources Citation Index (ESCI) –2015-present, Conference Proceedings Citation Index- Science (CPCI-S) - 1990-present, SCOPUS, Proquest, and LILACS). A further search was done in the WHO International Clinical Trials Registry Platform, and Clinical Trials.

Search

We used customized search strategies for each search engine. The search strategy included: glioblastoma, radiotherapy, radiation, cerebral/lateral ventricles, periventricular, subventricular, subependimal, stem cells, progenitor cells. The full search strategy for the Pubmed database is presented in the supplementary file. We limited the results to human subjects, articles, letters to the editor, meeting abstracts, proceedings papers, and reviews. The reference lists of the useful articles were screened to find further papers to include in the research. The search results were combined in EndNote online (Clarivate Analytics, Philadelphia, United States), and duplicates were removed. The selection of eligible articles and their quality assessment were performed in Mendeley (Elsevier, Amsterdam, Netherlands).

Study selection

Two authors independently proceeded to read all the titles and abstracts resulted after the search endeavor to select includible papers. Differences in opinion were solved by discussion.

Data collection process

Two authors independently extracted data from papers using a form in Microsoft Excel. The forms were confronted and differences were corrected by rechecking the papers.

Data items

The collected data were: study design, number of subjects, radiotherapy dose cut-off, mean SVZ volume and dose (ipsilateral, contralateral, and bilateral), progression free survival, overall survival (follow-up, hazard ratio in univariate and multivariate analysis, CI, p-value), confounders that were adjusted in multivariate analyses.

Risk of bias in individual studies

All the included studies were assessed for the presence of bias using the Newcastle - Ottawa Quality Assessment Scale (NOS), independently by two authors.[44] Divergences in assessment were solved by discussion.

Summary measures

The principal summary measure was hazard ratio obtained in multivariate analysis, comparing the high with the low dose radiation regimes, for progression free survival and for overall survival.

Synthesis of results

We performed a meta-analysis using the fixed and random effects models (the last model was chosen in case of important heterogeneity) to obtain the final pooled results in case we found at least two studies offering the principal summary measure. The corresponding summary measure with a 95% confidence interval and p-value was computed. The heterogeneity of the results was assessed using the inconsistency index (I^2), and the Q test was performed for heterogeneity.

Risk of bias across studies

A screening for publication bias was not performed using a funnel plot or formal tests, since the number of studies was too small for an adequate assessment.

Additional analyses

For all statistical tests used, the significance level alpha was 0.05, and the two tailed p value was computed. All statistical analyses were performed in R environment for statistical computing and graphics (R Foundation for Statistical Computing, Vienna, Austria), version 3.4.3 [R Core team. Vienna, Austria].

Results

Study selection

The search for papers yielded 2573 unique records. During screening we excluded papers for the following reasons: studies not on the topic, studies that didn't compare high dose with low dose irradiation, studies that didn't target the SVZ zone, studies that didn't use irradiation, other gliomas, case reports, abstracts of congress presentations, reviews. After screening 17 were assessed for eligibility, and in the end 8 were included in the qualitative and 4 in the quantitative analysis (Figure 1). Four studies were excluded from the quantitative analysis for the following reasons: Gupta et al,[33] 2012 – was excluded for having continuous dose, not in the binary form, high dose vs. low dose, while all the other studies had it in binary format. Kusumawidjaja et al,[20] 2014 – was excluded for having irradiation doses that were not comparable with the other studies (70 dose escalated vs 60 conventional, and no dose below 60 – while all the others compared high doses with doses below 60). Evers et al,[10] 2010, and Khalifa et al,[19] 2016 – were excluded due to the fact that they didn't provide the HR needed for the analysis.

Study characteristics and risk of bias

The study characteristics and the NOS score assessment for their risk of bias are presented in Table 1. The total number of subjects included in the meta-analysis (from the four selected studies) was 328. The high dose group had 97 (29.6%) of the subjects, while the low-dose group had 231 (70.4%) of the subjects.

Results of individual studies

The results of the studies are presented in Table 2 (multivariate analyses) and in Figures 2–4 (Forest plot)

Synthesis of results

Progression free survival

Ipsilateral SVZ irradiation

The pooled HR for progression free survival of high versus low irradiation dose of ipsilateral SVZ was 0.58 (95% CI 0.42–0.82), p=0.002, using the fixed effects model (test for heterogeneity p-value=0.41; I^2=~0%) (Figure 2). Subjects receiving higher doses of ipsilateral SVZ irradiation had statistically significant better PFS than those receiving lower doses.

Contralateral SVZ irradiation

The pooled HR for progression free survival of high versus low irradiation dose of contralateral SVZ was 0.89 (95% CI 0.35–2.26), p=0.81, using the random effects model (test for heterogeneity p-value=0.02, I^2=65.7%) (Figure 3). Subjects receiving higher doses of contralateral SVZ irradiation did not have statistically significant better PFS than those receiving lower doses.

Overall survival

Ipsilateral SVZ irradiation

The pooled HR for overall survival of high versus low irradiation dose of ipsilateral SVZ was 0.75 (95% CI 0.51–1.11), p=0.15, using the fixed effects model (test for heterogeneity p-value=0.55; I^2=~0%). (Figure 4). Subjects receiving higher doses of ipsilateral SVZ irradiation did not have statistically significant better overall survival than those receiving lower doses.

Contralateral SVZ irradiation

We couldn't find at least two studies that presented the contralateral SVZ irradiation HR, so a meta-analysis for

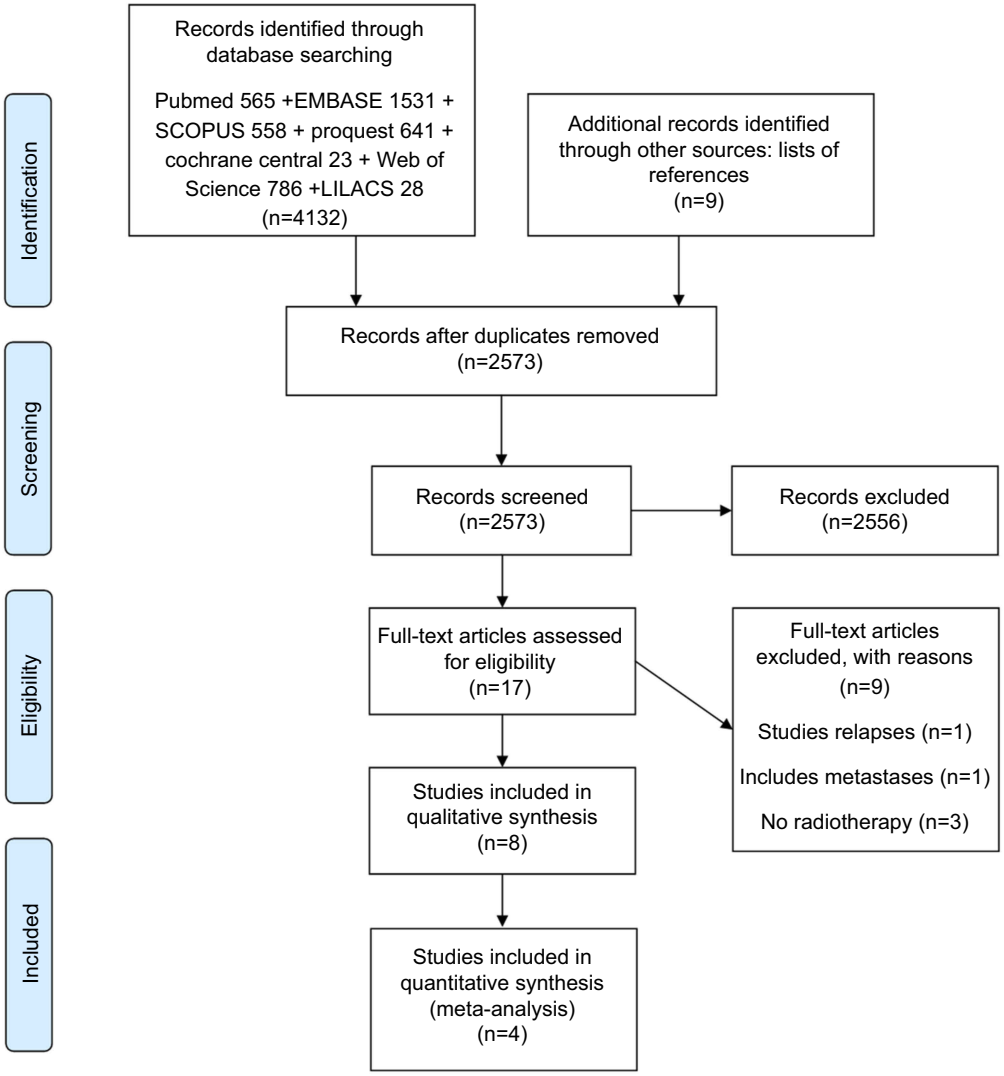

Figure 1 Flowchart of the results of the literature search.

this outcome was not possible. The only study that presented such a result was Gupta 2012, but it was for the continuous variable indicating the dosage, not for the comparison between high and low dosage.

Risk of bias across studies

The quality of the studies assessed using the NOS was adequate. Some studies had a lower NOS score due to the fact they lacked confounder adjustment, or the source of the patients in the two groups was not always the same hospital.

Clinical trials

The search in all databases provided only observational studies. We found two ongoing clinical trials, NCT02177578 and NCT02039778, in the experimental studies databases, but no finished ones.

Discussion

In the present study we undertook a systematic review and meta-analysis on a population of patients with GBM treated by multimodal treatment. We focused on RT treatment and compared high doses to low doses administered on iSVZ and cSVZ in order to determine if the patients with high doses have a better survival.

Studies description

According to Lee et al, a dose >59.4 Gy administered on the iSVZ led to increased PFS, but without any increase in OS, in patients with GBM in both univariate and multivariate models. Although a certain improvement in OS has been identified, this trend is not statistically significant.[22]

The study by Evers et al. draws on previously published data which indicate an increase in PFS in patients

Table 1 The study characteristics and the NOS score assessment for their risk of bias

			Mean SVZ volume cc			Mean SVZ dose (Gy)			Progression free survival			Overall survival			Study quality
Study (Author, year)	Number of patients	High dose group	IL	CL	BL	IL	CL	BL	Median (IQR)[range], months	HR univariate (95% CI)	p-value	Median (IQR) [range] {95%CI}, months	HR univariate (95% CI)	p-value	NOS
Lee et al., 2013 [2222]	173	iSVZ >59.4 vs. <59.4 (n:21/152)	4.3±1.3	5.0 ±1.6		49.2	35.2	60.1	10.4 [0.1–71.3]	0.56 (0.32–0.98)	0.042	19.6 [4.4–104.0]	0.67 (0.38–1.19)	0.173	9
		bSVZ >43 vs <43											0.74 (0.51–1.06)	0.103	
Kusumawidjaja et al., 2014 [2020]	72	iSVZ - 70 dose escalated vs 60 conventional				60.6 (33.4–69.8)	39.5 (19.4–61.2)	49.1 (28.3–64.3)	7.1 {5.6–9.6} vs. 11.1 (6.0–24.6)	0.95 (0.9–1)	0.052	15.2 {11–18.6} vs. 18.4 {12.5–31.4}	1.03 (0.97–1.10)	0.352	5.5
		cSVZ, bSVZ									>0.05			>0.05	
Evers et al., 2010 [1111]	55	>43	5.05			46±15.5	41 ±16.1		15 vs. 7.2		0.03				7
Khalifa et al., 2016 [1919]	43	bSVZ >40Gy	5 (3.4–11)	5.5 (3.4–9.6)	10.6 (6.8–20.6)	51.3 (17.9–61.4)	15.4 (1.4–48.7) bSVZ	35 (10.8–51.8)	6.5 {4.4–9.3}			22.7 {14.5–26.2}			8
Elicin et al., 2014 [88]	60	cSVZ>59.2 (n:14/46)	5.2±2.4	6.4 ±2.3	11.6 ±4.2	58.8±6.5	44.9 ±15.9	51.9 ±10.4	all 9.5 (95 % CI 7.7–11.1), 10.37 {95 % CI 8.37–13.53} vs 7.1 {95 % CI 3.5–8.97}	2.42 (1.18–4.71)	0.018	19.27 (95 % CI 12.77–25.23)	4.83 (1.71–13.97)	0.004	7

(*Continued*)

Table 1 (Continued).

			Mean SVZ volume cc			Mean SVZ dose (Gy)			Progression free survival			Overall survival			Study quality
Adeberg et al., 2014b [33]	65	iSVZ>40 (n: 31/23)	14.05mL [8.41–22.80 mL]	14.50mL [8.68–23.80 mL]		40.67Gy [14.84–56.87Gy]	20.86Gy [4.10–45.07Gy]		7.1 [1.6–52.4]	0.40 (0.24–0.78)	0.043	20.8 [4.3–53.8]	0.65 (0.34–1.24)	0.1	9
		cSVZ>30								0.44 (0.21–0.92)	0.03		1.53 (0.36–6.43)	0.56	
Gupta et al., 2012 [3333]	40	iSVZ≥58	5.6±2.5	6.4±3		58.7	53.6	56.2	11 {8.9–13.0}, 10 vs. 11		0.92	17 {11.6–22.4}, 17 vs. 15		0.95	9
		iSVZ continuous													
		cSVZ≥58							10 vs. NR		0.02	14 vs. NR		0.05	
		cSVZ continuous													
		bSVZ continuous													
		bSVZ≥58							10 vs. 14		0.06	14 vs. NR		0.22	
		i,c, bSVZ>43									>0.05			>0.05	
		i,c, bSVZ>50									>0.05			>0.05	
Chen et al., 2013 [77]	116	iSVZ≥40 (n:31/10)	7.05 [2.99–14.2]	7.91 [4.18–14.6]	14.76 [5.37–28.3]	48.7 [1.96–60]	34.4 [1.59–60]	41.5 [1.77–60]		0.824 (0.506–1.34)	0.434		0.926 (0.570–1.50)	0.754	9
		iSVZ≥40 gross total resection only								0.471 (0.209–1.06)	0.07	17.5 vs. 15.6	0.607 (0.280–1.32)	0.207	
		c,bSVZ≥40													

Notes: N. – number of subjects per study; n – number of subjects per group; iSVZ, cSVZ, bSVZ – ipsilateral, contralateral, bilateral subventricular zone; IL – ipsilateral; CL – contralateral; BL – bilateral; continuous data is presented as mean ± one standard deviation, or median (IQR – interquartile range), or [range], or {95% CI – confidence interval}; NOS - Newcastle-Ottawa Scale (article quality assessment scale); NR – non reported; HR – hazard ratio.

Table 2 Multivariate analyses results found in the studies

Study	High dose group	Multivariate analysis				Confounder analysis							
Author, year		PFS HR (95% CI)	p-value	OS HR (95% CI)	p-value	Age	Gender	Extent of resection	Tumor location	SVZ dose	RPA class	KPS	MGMT promoter status
Lee et al., 2013 [2222]	iSVZ >59.4 vs. <59.4	0.45 (0.25–0.82)	0.009	0.65 (0.35–1.21)	0.177	yes		yes		yes			
Elicin et al., 2014 [88]	cSVZ>59.2	1.72 (0.80–3.53)	0.161	1.49 (0.72–2.88)	0.268		yes						
Adeberg et al., 2014b [33]	iSVZ>40	0.52 (0.26–1.03)	0.06				yes		yes			yes	yes
	cSVZ>30	0.45 (0.20–0.98)	0.04							yes			
Gupta et al., 2012 [3333]	iSVZ continuous	0.91 (0.80–1.03)	0.116	0.87 (0.77–0.98)	0.025	yes	yes	yes		yes	yes	yes	
	cSVZ continuous	0.96 (0.71–1.30)	0.797	0.95 (0.72–1.26)	0.736								
	bSVZ continuous	1.06 (0.97–1.15)	0.187	1.08 (0.97–1.19)	0.162								
Chen et al., 2013 [77]	iSVZ≥40	0.749 (0.453–1.24)	0.259	0.827 (0.502–1.36)	0.455	yes		yes				yes	
		0.385 (0.165–0.901)	0.028	0.385 (0.165–0.895)	0.027								
			>0.05		>0.05								

Abbreviations: GBM, glioblastoma multiforme; SVZ, subvnetricular zone; iSVZ, cSVZ, bSVZ, ipsilateral, contralateral, bilateral subventricular zone; IL, ipsilateral; CL, contralateral; BL, bilateral; NSC, neural stem cells; CP, choroid plexus; GTR, gross total resection; NOS, Newcastle-Ottawa Scale (article quality assessment scale); PFS, progression free survival; OS, overall survival; HR, hazard ratio; RPA class, recursive partitioning analysis class; KPS, Karnofsky performance score; MGMT, O(6)-methylguanine-DNA methyltransferase; IQR, interquartile range; CI, confidence interval; NR, non reported.

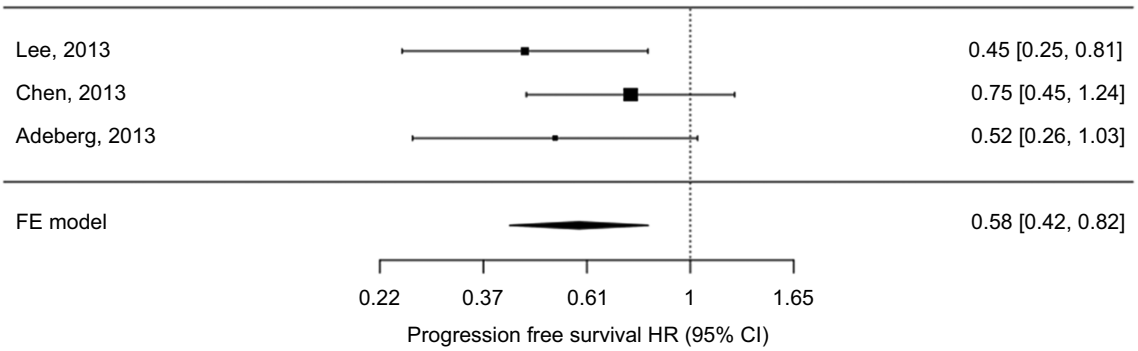

Figure 2 Fixed effects meta-analysis of progression free survival hazard ratio (HR) comparing high v low ipsilateral SVZ irradiation doses.

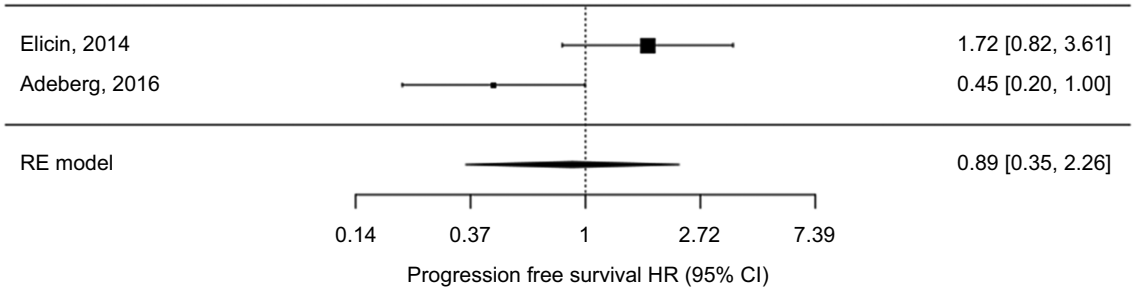

Figure 3 Random effects meta-analysis of progression free survival hazard ratio (HR) comparing high vs low contralateral SVZ irradiation doses.

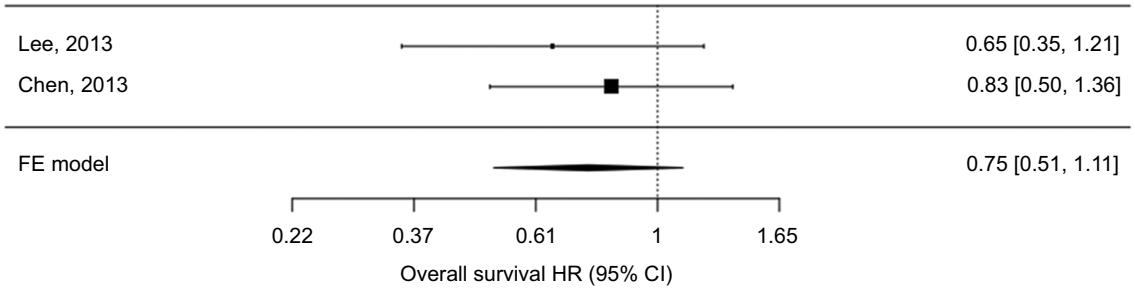

Figure 4 Fixed effects meta-analysis of overall survival hazard ratio (HR) comparing high vs low ipsilateral SVZ irradiation doses.

with grade III and IV gliomas who received a dose of 43 Gy in the bilateral SVZ.[11] This dose is likely to increase the PFS in patients with grade III tumors, but not in those with grade IV tumors. This demonstrates the importance of dosage in the treatment of GBM. Chen et al. identified an increase in both PFS and OS in GBM patients who received a >40Gy dose on the iSVZ. However, this is an independent predictor only for patients with gross total resection (GTR).[6,7] Consequently, the residual tumor mass can be more important in patient relapse than the NSC located in the SVZ.[30]

Adeberg found an increase in PFS in patients who received a >40Gy dose on the iSVZ and >30Gy on the cSVZ, respectively. These results could also be ascribed to the differences in contoured SVZ volume, as Adeberg took into account the anterior, superior, and inferior aspects, as opposed to other authors, who only looked at the anterior aspects of the SVZ. Another observation of this study is that higher doses (>30 Gy) administered on the cSVZ entail higher total bilateral doses, with a life-limiting side effect. This could also account for the results of our own study, which highlighted an improved PFS only for the iSVZ. No relation could be identified between the OS and the dose volume parameters. Furthermore, the irradiation of the subgranular layer of the dentate gyrus, a secondary location that could offer an suitable micro-environment to stem cells, had no effect on both PFS and OS.[3,38]

The study by Elicin included in this meta-analysis provides data that come to contradict those in the literature, showing diminished survival, albeit non statistically significant.[8] Given the heterogeneous nature of the data published in the literature, it is difficult to find studies with similar results.

Strengths and weaknesses

The studies included in our meta-analysis sought to mitigate the inherent deficiencies of the analyses conducted on retrospective series of patients. The selected populations were relatively homogeneous, with lengthy follow-ups, and the multivariate analyses factored in the extent of resection, age, and the KPS score. The variables chosen to be adjusted in the multivariate analyses were also heterogeneous between studies. As with all observational studies, residual confounding remains a possible source of bias. The risk of bias across individual studies assessed using the NOS was low, their quality being adequate. The systematic approach to the search of the literature, in numerous databases, and with a rigorous search strategy, limits the risk of incomplete retrieval of relevant literature. Since the number of retrieved studies was small, the assessment of publication bias through any method is not reliable.

However, there are differences between studies and between the patients included in the same study, when it comes to the doses applied on the SVZ and to the variation in shape/volume of the contoured SVZ. The cutoff used for identifying the high-dose and low-dose groups was different between the analyzed studies, some using 59.4 Gy (Lee et al), others 40 Gy (Adeberg et al and Chen et al). This induces an overlap, increases the heterogeneity of the studies and limits the conclusions. However it is interesting to see that regarding ipsilateral SVZ irradiation progression free survival, if the cutoff is high (59.4, for Lee et al), their result is larger than in studies where the cutoff is lower (40 Gy for Adeberg et al and Chen et al) - Figure 2. This seems to suggest that higher cutoffs increase the survival more than lower cutoffs. Moreover this overlap would more likely decrease the likelihood of finding statistically significant results, and our study found a statistically significant difference even with this overlap, which seems to sustain our conclusion for progression free survival. The same tendency might be seen regarding the overall survival where the higher-dose group (Lee et al) was better placed than the lower-dose group (Chen at al), but here the results were not statistically significant. For the contralateral SVZ irradiation both studies used the lower cutoff value, thus the comparison is not influenced.

Tumor volume might also play a role in survival for GBM patients, but our meta-analysis could not take this into account since individual studies didn't control for it in multivariable analyses.

It is important to note that only the study of Chen at al[7] used intensity modulated radiotherapy (IMRT), whereas all others tridimesional conformal radiotherapy (3DCRT). IMRT provides dosimetric advantages in both target volumes coverage and sparing of healthy neighboring structures. Considering complex shape of the SVZ, its' very small volume (4–5 cc), IMRT would be preferable. The accuracy of delineating the ipsi and contralateral SVZ can induce significant biases if not rigorously predefined (for example 4 mm, not 3 to 5 mm next to the lateral ventricles) and not centrally reviewed by an experienced neuro-radiologist, generating – at such small volumes- significant differences in dose-volume histograms. As target structures, we would favor a PTV expansion of 3 mm of both iSVZ and cSVZ and - derived from this systematic review - a "prescription dose" of 43 Gy/30fr. Hippocampus delineation would be also of interest, as well as its' dosimetric analysis correlated with neuro-cognitive function for potential longer-term survivors.

As with any systematic review, the data should be interpreted with care. Since we have only secondary data, and not individual patient data, it is difficult to conclude that longer progression free survival is independent of all important prognostic factors. The heterogeneity of the treatment regimen and even molecular biology also take a toll on the results. The multivariate analyses of each study included in the meta-analysis had a different selection of confounders and methods. Thus confounders in the meta-analysis are taken into account in a heterogeneous way.

Scientifically, to the best of our knowledge this is the first review with meta-analysis on the topic. The present work represents an evidence-based improvement in knowledge regarding the implications of subventricular zone irradiation in patients with glioblastoma. Future work can be built upon this current state of knowledge, in order to shed more light on the topic. The possible role of the subventricular zone in the progression of GBM might change the clinical treatment paradigm for this deadly disease. This will be a shift from the focus only on the lesion towards a more complex approach that takes into account the biology of other brain structures (SVZ and CP) and their interaction with GBM.

Study outcome

Our systematic review and meta-analysis indicate that there is a statistically significant difference in PFS between the GBM patients who received high vs. low radiotherapy doses in iSVZ. Normally, this gain should mean a higher OS, but this could not be confirmed by the present study. For cSVZ, no difference in PFS between high dose patients and low dose patients could be identified. This could be explained by the fact that in the patients whose cSVZ was also irradiated, the tumor was in a more advanced stage at the time of the radiotherapy, and the irradiation of this zone came as a consequence of the adequate coverage of the entire volume.

The location of the tumor is also to be taken into account. Studies conducted on patients whose tumors come into contact with the SVZ have indicated decreased OS and PFS.[15,32] This could be explained by the fact that the NSC located in the SVZ can increase the aggressiveness of the tumor.[24,31] It would be interesting to determine whether there is a "dialog" between the SVZ and the tumors located at a distance from it, and to identify the cellular and molecular mechanisms involved.[21,37] Animal models have indicated that the NSC show tropism for gliomas, but in humans the role of NSC remains to be determined.[1] Considering the dependence of stem cells on their microenvironment, one element than could be taken into consideration is the interaction between NCS and tumor stem cells and SVZ structures, such as the CP.[9,18,23,42] According to our own observations, CPs show changes in volume and aspect in GBM patients, suggesting a possible change in their activity during the oncogenesis. A number of studies have indicated the importance of the CP in the morphogenesis and the onset of neurodegenerative diseases.[10,18,29] Given the microenvironment they create as part of the SVZ, the CPs may play a part in the biology of the NSC and in their interaction with the tumor processes of the central nervous system.[5,12,39,40] An investigation of the normal and the pathological biology of the SVZ, and of the CP as part of the latter, could provide new information likely to increase the effectiveness of the current therapeutic methods, and even lead to new treatments confirming our preliminary observations on CP morphology.

Insight for future research

Finally, until fundamental research and the ongoing clinical trials (NCT02177578 and NCT02039778) provide us with new data that could make possible the application of these preliminary findings in the actual clinical practice, a randomized prospective trial remains necessary. This trial should include as uniform a population as possible, receiving the same doses (iSVZ, cSVZ,), on the same tumor volumes. The location of the tumor, the GTR, the age, the status of the MGMT gene promoter, and the administered medication should also be factored in.

Conclusion

Our systematic review and meta-analysis indicate that there was a statistically significant difference in PFS between the GBM patients who received high vs. low radiotherapy doses in iSVZ, but we couldn't find a similar result regarding overall survival. Although the data published so far do not lead to a firm conclusion, they nevertheless open new perspectives on the mechanisms involved in the onset and progression of GBM, regarding a possible involvement of the SVZ in the progression of the GBM, as well as a possible role of the SVZ in radiation therapy. Integrating functional MRI, PET and/or fluorescence imaging with tracers coupled to monoclonal antibodies against NSC (like CD133) are the most promising modalities for clinical application of CSCs detection and accurate target delineation and dose-histogram analysis.

Acknowledgment

No funding was received for this work.

Authors' contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Aboody KS, Brown A, Rainov NG, et al. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc Natl Acad Sci*. 2000;97:12846–12851. doi:10.1073/pnas.97.23.12846
2. Adeberg S, Bostel T, König L, Welzel T, Debus J, Combs SE. A comparison of long-term survivors and short-term survivors with glioblastoma, subventricular zone involvement: a predictive factor for survival? *Radiat Oncol*. 2014;9:95. doi:10.1186/1748-717X-9-95
3. Adeberg S, König L, Bostel T, et al. Glioblastoma recurrence patterns after radiation therapy with regard to the subventricular zone. *Int J Radiat Oncol Biol Phys*. 2014;90:886–893. doi:10.1016/j.ijrobp.2014.07.027
4. Bagó JR, Sheets KT, Hingtgen SD. Neural stem cell therapy for cancer. *Methods*. 2016;99:37–43. doi:10.1016/j.ymeth.2015.08.013

5. Baruch K, Deczkowska A, David E, et al. Aging-induced type I interferon signaling at the choroid plexus negatively affects brain function. *Science*. 2014;346:89–93. doi:10.1126/science.1255826
6. Chen L, Quinones-Hinojosa A, Ford E, et al. Increased radiation dose to the SVZ improves survival in patients with GBM. *Int J Radiat Oncol Biol Phys*. 2012;84:S8. doi:10.1016/j.ijrobp.2012.07.027
7. Chen L, Guerrero-Cazares H, Ye X, et al. Increased subventricular zone radiation dose correlates with survival in glioblastoma patients after gross total resection. *Int J Radiat Oncol Biol Phys*. 2013;15:616–622. doi:10.1016/j.ijrobp.2013.02.014
8. Elicin O, Inac E, Uzel EK, Karacam S, Uzel OE. Relationship between survival and increased radiation dose to subventricular zone in glioblastoma is controversial. *J Neurooncol*. 2014;118:413–419. doi:10.1007/s11060-014-1424-3
9. Emerich DF, Skinner SJM, Borlongan CV, Vasconcellos AV, Thanos CG. The choroid plexus in the rise, fall and repair of the brain. *BioEssays*. 2005;27:262–274. doi:10.1002/(ISSN)1521-1878
10. Emerich DF, Schneider P, Bintz B, Hudak J, Thanos CG. Aging reduces the neuroprotective capacity, VEGF secretion, and metabolic activity of rat choroid plexus epithelial cells. *Cell Transplant*. 2007;16:697–705. doi:10.3727/000000007783465145
11. Evers P, Lee PP, DeMarco J, et al. Irradiation of the potential cancer stem cell niches in the adult brain improves progression-free survival of patients with malignant glioma. *BMC Cancer*. 2010;10:384. doi:10.1186/1471-2407-10-663
12. Falcão AM. The path from the choroid plexus to the subventricular zone: go with the flow! *Front Cell Neurosci*. 2012;6:1–8. doi:10.3389/fncel.2012.00034
13. Gollapalli K, Ghantasala S, Kumar S, et al. Subventricular zone involvement in glioblastoma-a proteomic evaluation and clinicoradiological correlation. *Sci Rep*. 2017;7:1–13. doi:10.1038/s41598-017-01202-8
14. Grégoire C-A, Goldenstein BL, Floriddia EM, Barnabé-Heider F, Fernandes KJL. Endogenous neural stem cell responses to stroke and spinal cord injury. *Glia*. 2015;63:1469–1482. doi:10.1002/glia.22851
15. Han S, Li X, Qiu B, et al. Can lateral ventricle contact predict the ontogeny and prognosis of glioblastoma? *J Neurooncol*. 2015;124:45–55. doi:10.1007/s11060-015-1858-2
16. Ho J, Ondos J, Ning H, et al. Chemoirradiation for glioblastoma multiforme: the national cancer institute experience. *PLoS One*. 2013;8(8):e70745. doi:10.1371/journal.pone.0070745
17. Jafri NF, Clarke JL, Weinberg V, Barani IJ, Cha S. Relationship of glioblastoma multiforme to the subventricular zone is associated with survival. *Neuro Oncol*. 2013;15:91–96. doi:10.1093/neuonc/nos268
18. Kaur C, Rathnasamy G, Ling EA. The choroid plexus in healthy and diseased brain. *J Neuropathol Exp Neurol*. 2016;75:198–213. doi:10.1093/jnen/nlv030
19. Khalifa J, Tensaouti F, Lusque A, et al. Subventricular zones: new key targets for glioblastoma treatment. *Radiother Oncol*. 2016;119:S302–S303. doi:10.1016/S0167-8140(16)31897-7
20. Kusumawidjaja G, Gan P, Tan D, et al. Dose-escalated intensity modulated radiation therapy and increased radiation doses to subventricular zones in treatment outcomes of patients with glioblastoma multiforme. *Int J Radiat Oncol Biol Phys*. 2014;90:S289–S290. doi:10.1016/j.ijrobp.2014.05.980
21. Lee C, Hu J, Ralls S, et al. The molecular profiles of neural stem cell niche in the adult subventricular zone. *PLoS One*. 2012;7:e50501. doi:10.1371/journal.pone.0050501
22. Lee P, Eppinga W, Lagerwaard F, et al. Evaluation of high ipsilateral subventricular zone radiation therapy dose in glioblastoma: A pooled analysis. *Int J Radiat Oncol Biol Phys*. 2013;86:609–615. doi:10.1016/j.ijrobp.2013.01.009
23. Lehtinen MK, Bjornsson CS, Dymecki SM, et al. The choroid plexus and cerebrospinal fluid: emerging roles in development, disease, and therapy. *J Neurosci*. 2013;33:17553–17559. doi:10.1523/JNEUROSCI.3846-13.2013
24. Liang THK, Kuo SH, Wang CW, et al. Adverse prognosis and distinct progression patterns after concurrent chemoradiotherapy for glioblastoma with synchronous subventricular zone and corpus callosum invasion. *Radiother Oncol*. 2016;118:16–23. doi:10.1016/j.radonc.2016.01.001
25. Lim DA, Cha S, Mayo MC, et al. Relationship of glioblastoma multiforme to neural stem cell regions predicts invasive and multifocal tumor phenotype. *Neuro Oncol*. 2007;9:424–429. doi:10.1215/15228517-2007-023
26. Lim DA, Alvarez-Buylla A. The adult Ventricular – Subventricular Zone (V-SVZ) and Olfactory Bulb (OB) neurogenesis. *Cold Spring Harb Perspect Biol*. 2016;8:a01882. doi:10.1101/cshperspect.a018820
27. Lin R, Iacovitti L. Classic and novel stem cell niches in brain homeostasis and repair. *Brain Res*. 2015;1628:327–342. doi:10.1016/j.brainres.2015.04.029
28. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol*. 2016;131:803–820. doi:10.1007/s00401-016-1545-1
29. Lun MP, Monuki ES, Lehtinen MK. Development and functions of the choroid plexus-cerebrospinal fluid system. *Nat Rev Neurosci*. 2015;16:445–457. doi:10.1038/nrn3921
30. Marques-Torrejon MA, Gangoso E, Pollard SM. Modelling glioblastoma tumour-host cell interactions using adult brain organotypic slice co-culture. *Dis Model Mech*. 2018;11:dmm031435. doi:10.1242/dmm.031435
31. Mistry AM, Dewan MC, White-Dzuro, et al. survival in glioblastomas is specific to contact with the ventricular-subventricular zone, not subgranular zone or corpus callosum. *J Neurooncol*. 2017;132:341–349. doi:10.1007/s11060-017-2374-3
32. Mistry AM, Hale AT, Chambless LB, Weaver KD, Thompson RC, Ihrie RA. Influence of glioblastoma contact with the lateral ventricle on survival: a meta-analysis. *J Neurooncol*. 2017;131:125–133. doi:10.1007/s11060-016-2278-7
33. Gupta T, Nair V, Paul SN, et al. Can irradiation of potential cancer stem-cell niche in the subventricular zone influence survival in patients with newly diagnosed glioblastoma? *J Neurooncol*. 2012;109:195–203. doi:10.1007/s11060-012-0887-3
34. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6:e1000097. doi:10.1371/journal.pmed.1000097
35. Ortega JA, Memi F, Radonjic N, et al. The subventricular zone: a key player in human neocortical development. *Neuroscientist*. 2018;24:156–170. doi:10.1177/1073858417691009
36. Parsa AT, Wachhorst S, Lamborn KR, et al. significance of intracranial dissemination of glioblastoma multiforme in adults. *J Neurosurg*. 2005;102:622–628. doi:10.3171/jns.2005.102.4.0622
37. Qin EY, Cooper DD, Abbott KL, et al. Neural precursor-derived pleiotrophin mediates subventricular zone invasion by glioma. *Cell*. 2017;170:845–859. doi:10.1016/j.cell.2017.07.016
38. Rizzo AE, Yu J, Suh J, et al. Investigating the relationship between radiation dose to neural stem cell niches and survival in GBM. *Int J Radiat Oncol Biol Phys*. 2014;90:S283–S284. doi:10.1016/j.ijrobp.2014.05.965
39. Silva-Vargas V, Maldonado-Soto AR, Mizrak D, Codega P, Doetsch F. Age-dependent niche signals from the choroid plexus regulate adult neural stem cells. *Cell Stem Cell*. 2016;19:643–652. doi:10.1016/j.stem.2016.06.013
40. Strominger I, Elyahu Y, Berner O, et al. The choroid plexus functions as a niche for T-cell stimulation within the central nervous system. *Front Immunol*. 2018;9:1066. doi:10.3389/fimmu.2018.01066
41. Vallard A, Espenel S, Guy J-B, et al. Targeting stem cells by radiation: from the biological angle to clinical aspects. *World J Stem Cells*. 2016;8:243–250. doi:10.4252/wjsc.v8.i8.243
42. Vandenbroucke RE. A hidden epithelial barrier in the brain with a central role in regulating brain homeostasis: implications for aging. *Ann Am Thorac Soc*. 2016;13:S407–S410. doi:10.1513/AnnalsATS.201609-676AW

43. Weller M, van Den Bent M, Tonn JC, et al. European Association for Neuro-Oncology (EANO) guideline on the diagnosis and treatment of adult astrocytic and oligodendroglial gliomas. *Lancet Oncol*. 2017;18:e315–e329. doi:10.1016/S1470-2045(17)30072-4
44. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. *Ottawa Hosp Res Inst*. 2018. Available at http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.
45. Xu W, Lakshman N, Morshead CM. Building a central nervous system: the neural stem cell lineage revealed. *Neurogenesis*. 2017;4:e1300037. doi:10.1080/23262133.2017.1300037

Supplementary material

The Pubmed search strategy was: (Glioblastoma[MeSH Terms] OR Glioblastoma[All Fields] OR Glioblastomas[All Fields] OR GBM[All Fields] OR ((glioma[MeSH Terms] OR glioma[All Fields] OR gliomas[All Fields] OR Astrocytoma [MeSH Terms] OR Astrocytoma [All Fields] OR Astrocytomas [All Fields]) AND ("Grade IV"[All Fields]))) AND (radiotherapy[MeSH Terms] OR radiotherapy[All Fields] OR radiotherapies[All Fields] OR Radiation[All Fields] OR Radiations[All Fields] OR Irradiation[All Fields] OR Irradiations[All Fields] OR irradiate[All Fields] OR irradiated[All Fields]) AND (Cerebral Ventricles[MeSH Terms] OR ((Cerebrum[MeSH Terms] OR Cerebral[All Fields] OR Brain[MeSH Terms] OR brain[All Fields]) AND (Ventricle [All Fields] OR Ventricles[All Fields] OR peri-ventricular[All Fields] OR periventricular[All Fields] OR lateral ventricle[All Fields] OR lateral ventricles[All Fields] OR subventricular [All Fields] OR subventricular zone[All Fields] OR subventricular zones[All Fields] OR SVZ OR ependyma[MeSH Terms] OR ependyma[All Fields] OR ependymas[All Fields] OR subependymal[All Fields] OR Stem Cells[MeSH Terms] OR Stem Cell[All Fields] OR Stem Cells[All Fields] OR Progenitor Cell[All Fields] OR Progenitor Cells[All Fields]))) AND "humans"[MeSH Terms].

OVERLAPPING OF FUNCTIONAL ESOPHAGEAL DISORDERS AND IRRITABLE BOWEL SYNDROME, IN MUSICIANS AND ATHLETES

ORIGINAL ARTICLE
ARTIGO ORIGINAL
ARTÍCULO ORIGINAL

SOBREPOSIÇÃO DE DISTÚRBIOS ESOFÁGICOS FUNCIONAIS E SÍNDROME DO INTESTINO IRRITÁVEL EM MÚSICOS E ESPORTISTAS

SOBREPOSICIÓN DE TRASTORNOS ESOFÁGICOS FUNCIONALES Y SÍNDROME DEL INTESTINO IRRITABLE EN MÚSICOS Y DEPORTISTAS

Sebastian Nedelcut[1] (Doctor)
Daniel-Corneliu Leucuta[2] (Doctor)
Dan Lucian Dumitrascu[1] (Doctor)

1. Iuliu Hatieganu University of Medicine and Pharmacy, Medical Department, Cluj-Napoca, Cluj, Romania.
2. Iuliu Hatieganu University of Medicine and Pharmacy, Medical Informatics and Biostatistics Department, Cluj-Napoca, Cluj, Romania.

Correspondence:
Daniel-Corneliu Leucuța
6 Pasteur Street, 400349, Cluj-Napoca, Romania.
dleucuta@umfcluj.ro

ABSTRACT

Introduction: Functional gastrointestinal disorders (FGIDs) are the most common disorders in the general population. These disorders can overlap, decreasing the quality of life. Objective: We analyzed the prevalence of functional esophageal disorders (FED) and irritable bowel disease (IBS), and their overlapping and associated factors in musicians and athletes. Methods: A cross-sectional study was conducted using FGID and associated factors questionnaires administered to four groups: instrumentalists, singers, athletes, and a control group of healthy volunteers. Results: Of the 161 subjects, 62 (38.51%) had only FED, 76 (47.2%) had only IBS, and 23 (14.29%) had FED-IBS overlap. Subjects with FED-IBS overlap had more severe symptoms of IBS, especially hard and lumpy stools and constipation, compared to those with IBS alone. IBS subtype was more frequent in the overlap group, while not specified IBS type was less frequent. Regarding FED, we found that subjects with FED-IBS overlap had more functional heartburn and less functional dysphagia symptoms. There was a higher risk of overlap in instrumentalists and smokers. Conclusions: FED and IBS are frequently encountered in musicians and athletes. Subjects with FED-IBS overlap presented more frequent and severe symptoms. Instrumentalists and smokers are at higher risk of overlap. ***Level of Evidence IV; Case series.***

Keywords: Esophageal diseases; Irritable bowel syndrome; Comorbidity; Music; Athletic performance.

RESUMO

Introdução: Os distúrbios gastrintestinais funcionais (DGIF) são os mais comuns na população em geral. Esses distúrbios podem se sobrepor, diminuindo a qualidade de vida. Objetivo: Analisamos a prevalência dos distúrbios funcionais esofágicos (DFE) e da síndrome do intestino irritável (SII), sua sobreposição e os fatores associados em músicos e esportistas. Métodos: Realizou-se um estudo transversal por meio de questionários sobre DGIF e fatores associados, administrados a quatro grupos: instrumentistas, cantores, esportistas e um grupo controle de voluntários saudáveis. Resultados: Dos 161 indivíduos, 62 (38,51%) tinham só DFE, 76 (47,2%) tinham só SII e 23 (14,29%) tinham sobreposição de DFE e SII. Os indivíduos com sobreposição de DFE e SII tinham sintomas mais intensos de SII, especialmente fezes duras e encaroçadas e constipação em comparação com os que tinham só SII. O subtipo SII foi mais frequente no grupo de sobreposição, enquanto o tipo SII indefinido foi menos frequente. Quanto ao DFE, verificamos que os indivíduos com sobreposição DFE-SII tinham mais sintomas de azia funcional e menos de disfagia funcional. Houve maior risco de sobreposição em instrumentistas e fumantes. Conclusões: DFE e SII são frequentes em músicos e esportistas. Os indivíduos com sobreposição de DFE e SII apresentaram sintomas mais frequentes e mais severos. Os instrumentistas e os fumantes têm maior risco de sobreposição. ***Nível de Evidência IV; Série de casos.***

Descritores: *Doenças do esôfago; Síndrome do intestino irritável; Comorbidade; Música; Desempenho atlético.*

RESUMEN

Introducción: Los trastornos gastrointestinales funcionales (TGIF) son los más comunes en la población en general. Estos trastornos pueden sobreponerse, disminuyendo la calidad de vida. Objetivo: Analizamos la prevalencia de los trastornos funcionales esofágicos (TFE) y del síndrome del colon irritable (SCI), su superposición y los factores asociados en músicos y deportistas. Métodos: Se realizó un estudio transversal por medio de cuestionarios sobre TGIF y factores asociados, administrados a cuatro grupos: instrumentistas, cantantes, deportistas y un grupo control de voluntarios sanos. Resultados: De los 161 sujetos, 62 (38,51%) tenían sólo TFE, 76 (47,2%) tenían sólo SCI y 23 (14,29%) tenían superposición de TFE e SCI. Los individuos con superposición de TFE y SCI tenían síntomas más intensos de SCI, especialmente heces duras y grumosas y estreñimiento en comparación con los que tenían sólo SCI.. El subtipo SCI fue más frecuente en el grupo de superposición, mientras que el tipo de SCI no especificado resultó menos frecuente. En cuanto al TFE, verificamos que los sujetos con superposición TFE-SCI tenían más síntomas de pirosis funcional y menos de disfagia funcional. Hubo mayor riesgo de superposición en instrumentistas y fumadores. Conclusiones: TFE y SCI son frecuentes en músicos y deportistas. Los sujetos con superposición de TFE y SCI presentaron síntomas más frecuentes y más severos. Los instrumentistas y los fumadores tienen mayor riesgo de superposición. ***Nivel de Evidencia IV; Serie de casos.***

Descriptores: *Enfermedades del esófago; Síndrome del colon irritable; Comorbilidad; Música; Rendimiento atlético.*

DOI: http://dx.doi.org/10.1590/1517-869220182401169835 Article received on 09/30/2016 accepted on 07/03/2017

INTRODUCTION

The gastroesophageal reflux disease (GERD) and the irritable bowel syndrome (IBS) are both commonly encountered conditions. They may overlap.[1,2] GERD patients may present IBS in almost 50% of cases and 40% of IBS patients have GERD.[3] Overlapping may impair the clinical state of the patients and deteriorate quality of life, because of the multiple complaints. The functional esophageal disorders (FED) may be also present in IBS patients in 16-29%.[4,5] From these, functional esophageal reflux and hypersensitive esophagus have similar symptoms with GERD.[6] Some of the professions are at risk to develop GERD, including musicians.[7,8] Stress job may be associated with FGID including IBS and/or FED. Music and sport performers are submitted to professional stress. To our knowledge there are few studies assessing IBS and FED in these vocational groups.

Thus, the aim of this study was to assess the prevalence of FED and IBS, their overlap and factors associated with the overlap in musicians and sports performers. We managed to succeed in our endeavor.

MATERIAL AND METHODS

We performed a cross-sectional survey using several questionnaires administered to four subject groups: music instrumentists, vocal singers, sportsmen and a control group. Questionnaires were administered by instructed research staff. All responders gave their informed consent and filled themselves the questionnaires. The research has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board.

The musician group comprised professionals with interpretive activity, and students in Music Academies. The vocal singers group comprised of, soloists, and students in Music Academies.

The sportsmen group comprised professional players (handball players, rugby players, judo players, bodybuilders, and athletes) and students in the Faculty of Physical Education and Sport.

The control comprised employed subjects from different companies and services, and students from different faculties (chemistry, psychology).

We excluded from the analysis those subjects that reported diseases able to bias the results i.e. other organic diseases: diabetes, cancer, endocrine diseases, chronic important alcohol abuse, drugs interfering with gastrointestinal motility or secretion.

Questionnaires

All study subjects received a questionnaire, with multiple sections. The questionnaire consisted of items regarding digestive diseases, demographic characteristics, life, and eating habits, anxiety.

For the diagnostic of the diseases of interest to this study we used the modules of the Rome III criteria: Irritable Bowel Syndrome (IBS) Module, Functional Esophageal Disorders Module.[9]

We assessed anxiety levels with the Zung Self-rating Anxiety Scale (SAS) (22).

We used a non-standardized list of questions to assess demographic data, life and eating habits (e.g. smoking, sleeping problems, eating frequency and diet, physical exercise).

Statistical analysis

Qualitative data was presented with counts and percentages; skewed continuous data was presented with median and quartiles, while normally distributed continuous data was presented with means and standard deviations. Normality of the data was assessed with quantile-quantile plots and Shapiro-Wilk test.

Associations between qualitative variables were assessed with Chi square test, or Fisher exact test. Two groups' comparisons for normally distributed continuous data were made with t-test for independent samples, while for skewed data, the comparisons were made with Wilcoxon rank-sum test.

To assess which factors are likely to be related to the overlap of the two FGIDS made a multiple logistic regression was made. The dependent variable was FED-IBS overlap versus FED or IBS alone. A full model with all the following independent variables was created: the four study groups (instrumentists, vocal singers, sportsmen vs. control), body mass index (BMI), smoking, Zung SAS score, drinking milk, eating legumes, eating bread, eating potatoes, drinking alcohol, drinking coffee. For all models we checked for multicolinearity, misspecification, and component residual plots for functional form. Odds ratios (OR) with their 95% confidence intervals (CI) were presented.

For all statistical tests a two tailed p value was used with a 0.05 level of significance.

All statistical analysis were carried out with the R environment for statistical computing and graphics (R Foundation for Statistical Computing, Vienna, Austria), version 3.2.1(23).

RESULTS

From the 1600 distributed questionnaires, 1148 (72%) were returned. 137 questionnaires were discarded since incomplete, remaining 1011. From these subjects we retained for further analysis 161 presenting criteria for FED or IBS, which represented our study group. Out of them 23 (14.29%) had FED-IBS overlap, 62 (38.51%) had FED alone, and 76 (47.2%) had IBS alone.

The distribution of subgroups was the following: 45 (27.95%) control subjects, 31 (19.25) singers, 45 (27.95%) instrumentists, and 40 (24.84%) sportsmen.

Characteristics of the respondents

There was no statistically significant difference between the demographic characteristics of the FED-IBS overlap versus FED alone or IBS alone (Table 1). Females were more numerous than males, in all three groups, in the overlap group being the most frequent. The median age, and BMI was similar in all three groups. Instrumentists were more frequent in the FED-IBS overlap group than in FED alone and IBS alone groups (about half as frequent in both groups). Sportsmen, singers and controls were less frequently in the FED-IBS overlap group, than in FED alone and IBS alone groups (with similar frequencies). High education levels were more frequent in the FED-IBS overlap group, than in FED alone and IBS alone groups (with similar frequencies)

Symptoms and subtypes comparisons between FED-IBS overlap and FED or IBS alone

IBS symptoms and IBS subtypes distribution in FED-IBS overlap group and IBS alone group is presented in Table 2. Regarding symptoms the only statistically significant difference was for hard or lumpy stools symptoms which were more intense in the overlap group. Almost all the symptoms scores were higher in the overlap group compared to IBS alone, except for more frequent bowel movements when discomfort or pain started, and loose, mushy or watery stools. Regarding IBS subtypes, we observed statistically significant differences. The IBS with constipation was more frequent in the overlap group, while IBS with diarrhea and unspecified IBS were less frequent in the overlap group.

The comparison of FED subtypes frequencies in FED-IBS overlap with FED alone is presented in Table 3. Functional heartburn was more frequent, and functional dysphagia was less frequent in the overlap group, compared to the FED alone group, the results being statistically significant. Functional chest pain of presumed esophageal origin, and

Table 1. Demographic characteristics of the subjects in FED-IBS overlap, FED alone, and IBS alone.

	FED-IBS overlap (n=23)	FED alone (n=62)	Overlap vs. FED alone P-value	IBS alone (n=70)	Overlap vs. IBS alone P-value
Gender (f/m), n. (%)	17 (73.91) / 6 (26.09)	37 (59.68) / 25 (40.32)	0.226	53 (69.74) / 23 (30.26)	0.7
Age (years), median (IQR)	22 (21 - 34)	21 (20 - 32)	0.414	22 (20 - 25.25)	0.259
BMI (kg/m2), median (IQR)	22.04 (20.58 - 23.98)	23 (20.29 - 24.79)	0.533	21.34 (19.31 - 23.62)	0.267
Group, n. (%)					
control	5 (21.74)	19 (30.65)	0.07	21 (27.63)	0.094
instrumentist	12 (52.17)	14 (22.58)		19 (25)	
singers	3 (13.04)	13 (20.97)		15 (19.74)	
sportsmen	3 (13.04)	16 (25.81)		21 (27.63)	
Educational levels, n. (%)					
low	0 (0)	2 (3.23)	0.791	3 (3.95)	0.663
middle	14 (60.87)	41 (66.13)		50 (65.79)	
high	9 (39.13)	19 (30.65)		23 (30.26)	

BMI – body mass index (weight in kg/ height in m²), IQR – interquartile range.

Table 2. IBS symptoms and subtypes overlap in symptoms of FED-IBS overlap and IBS alone.

	FED-IBS overlap (n=23)	IBS alone (n=76)	P-value
Symptoms:			
discomfort or pain anywhere in abdomen, mean (SD)	4.17 (0.94)	3.82 (0.89)	0.098
discomfort or pain get better or stop after bowel movement, mean (SD)	2.22 (0.95)	1.91 (1.13)	0.238
when discomfort or pain started, there were more frequent bowel movements, mean (SD)	0.96 (0.98)	1.12 (0.97)	0.484
when discomfort or pain started, there were less frequent bowel movements, mean (SD)	1.26 (1.18)	1.04 (0.99)	0.37
when discomfort or pain started, stools were looser, mean (SD)	1.3 (1.02)	1.09 (1.06)	0.398
when discomfort or pain started, stools were harder, mean (SD)	1.39 (1.03)	1.29 (0.96)	0.663
hard or lumpy stools, mean (SD)	1.7 (1.11)	1.2 (0.98)	0.041
loose, mushy or watery stools, mean (SD)	0.83 (0.78)	1.08 (0.93)	0.241
IBS type, n. (%)			0.03
IBS-C: n (%)	8 (34.78)	11 (14.47)	
IBS-D: n (%)	0 (0)	11 (14.47)	
IBS-M: n (%)	14 (60.87)	43 (56.58)	
IBS-U: n (%)	1 (4.35)	11 (14.47)	

SD – standard deviation, IBS - Irritable Bowel Syndrome (IBS subtypes: C – with constipation, D – with diarrhea, M - mixed, U - unspecified).

Table 3. FED subtypes in symptoms of FED-IBS overlap and FED alone.

Overlap irritable bowel syndrome – functional esophageal disorders:	FED-IBS overlap (n=23)	FED alone (n=62)	P-value
Functional heartburn, n. (%)	18 (78.26)	34 (54.84)	0.049
Functional chest pain of presumed esophageal origin, n. (%)	4 (17.39)	10 (16.13)	1
Functional dysphagia, n. (%)	1 (4.35)	16 (25.81)	0.033
Globus, n. (%)	2 (8.7)	5 (8.06)	1

Factors associated with overlap.

globus were similar in frequency in both groups, with no statistically significant differences observed.

The logistic regression results that assessed factors associated with FED-IBS overlap, compared to FED or IBS alone is presented in Table 4. The model adjusted for all the variables identified two statistically significant factors associated with higher odds of overlap: being instrumentist versus control, and smoking.

Table 4. Factors associated with overlap of symptoms of FED-IBS versus FED or IBS alone.

	OR adjusted	(95% CI)	p
BMI (kg/m2)	0.96	(0.81 - 1.12)	0.606
Group (instrumentist vs. control)	5.77	(1.22 - 34.83)	0.037
Group (singers vs. control)	1.06	(0.12 - 7.68)	0.953
Group (sportsmen vs. control)	0.5	(0.07 - 3.2)	0.474
Zung SAS score	1.01	(0.97 - 1.07)	0.545
Smoking	9.57	(2.27 - 55.95)	0.005
Alcoholic drinks	0.3	(0.06 - 1.18)	0.102
Coffee	0.24	(0.04 - 1.06)	0.071
Milk	0.44	(0.09 - 2.06)	0.277
Legumes of any kind	5.0e+7	(0 - inf)	0.995
Potatoes	2.2e+6	(0 - 9.9e+191)	0.991
Bread	1.03	(0.15 - 9.16)	0.98

BMI – body mass index (weight in kg/ height in m²), OR – odds ratio, CI – confidence interval, Zung SAS score - Zung Self-rating Anxiety Scale.

DISCUSSION

To our knowledge there are very few studies assessing the overlap of IBS with functional dysphagia, or globus. The majority of the studies that assess the overlap of IBS with FED, focus on functional heartburn. Our study looked into all these relations, and also assessed factors associated with this overlap.

Overlap differences

The comparison between subjects with FED-IBS overlap and IBS alone showed that higher symptoms scores of IBS, especially hard and lumpy stools, corresponding to constipation IBS subtype were more frequent in the overlap group, while unsubtyped IBS was less frequent. Thus, subjects with overlap between IBS and FED to have intense symptoms, similar to findings of other FGIDs overlapping studies,[10,11] as well as poor quality of life.[12]

When looking into FED subtypes frequencies in FED-IBS overlap group compared with FED alone, those with overlap had more functional heartburn and less functional dysphagia symptoms. Our results are close to those found in a Chinese outpatients study[4] of IBS – functional heartburn overlap. Another Chinese study in hospitalized patients[5] found higher percentages of overlap. These differences are normal since both our study and the first mentioned Chinese study, were symptom based studies, while the last Chinese study - excluding the organic diseases could get closer to the truth.

Associated factors

In our study we found a statistically significant association between instrument players and smoking with higher odds of overlap. It is known from the literature that instrument players[8] have higher odds to have

gastroesophageal reflux disease, and we looked also into heartburn overlapping with IBS.

As any other study, this one has its own limitations. The most important limitation of this study is that we used self-completed questionnaires, not followed by clinical check-up. To prevent this impact on accuracy, we used many exclusion criteria for known organic diseases. As an advantage, we have a large sample of two professions which commonly are rarely investigated.

CONCLUSIONS

FED and IBS are encountered frequebtly in music and sport performers, both professions submitted to professional stress. Overlap between FED and iBS may exist also in these categories. Subjects with FED-IBS overlap compared to those without overlap show higher symptoms scores of IBS, more frequent IBS constipation subtype, more frequent functional heartburn and less functional dysphagia symptoms. There were higher odds of overlap in instrument players and in smokers

ACKNOWLEDGEMENTS

None. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

All authors declare no potential conflict of interest related to this article.

AUTHORS' CONTRIBUTIONS: Each author made significant individual contributions to this manuscript. SN (0000-0001-7396-4394)*: contributed to the study design, collected and performed acquisition of data, interpreted the results, and contributed to the writing of the paper; DCL (0000-0003-4218-8622)*: contributed to the study design, gave the original idea, performed the statistical analysis, and contributed to the writing of the paper; DLD (0000-0001-5404-7662) *: contributed to the study design, interpreted the results, and contributed to the writing of the paper. All authors read and approved the final version of the manuscript. *ORCID (Open Researcher and Contributor ID).

REFERENCES

1. Drossman DA, Hasler WL, Rome IV-Functional GI disorders: disorders of gut-brain interaction. Gastroenterolog. 2016;150(6):1257-6.
2. Rasmussen S, Jensen TH, Henriksen SL, Haastrup PF, Larsen PV, Søndergaard J, et al, Overlap of symptoms of gastroesophageal reflux disease, dyspepsia and irritable bowel syndrome in the general population. Scand J Gastroenterol. 2015; 50(2):162-9.
3. Nastaskin I, Mehdikhani E, Conklin J, Park S, Pimentel M, Studying the overlap between IBS and GERD: a systematic review of the literature. Dig Dis Sci. 2006;5(12):2113–20.
4. Yao X, Yang YS, Cui LH, Sun G, Peng LH, Wang WF, et al. The overlap of upper functional gastrointestinal disorders with irritable bowel syndrome in chinese outpatients: a multi center study. J Gastroenterol Hepatol. 2016;31(9):1584-93.
5. Sichuan Da Xue Xue Bao Yi Xue Ban. [Symptom overlaps between functional heartburn, functional dyspepsia, and irritable bowel syndrome]. 2014;45(3):489-92.
6. Aziz Q, Fass R, Gyawali CP, Miwa H, Pandolfino JE, Zerbib F. Functional esophageal disorders. Gastroenterology. 2016; pii: S0016-5085(16)00178-5.
7. Cammarota G, Masala G, Cianci R, Palli D, Bendinelli B, Galli J, et al. Reflux symptoms in wind instrument players. Aliment Pharmacol Ther. 2010;31(5):593-600.
8. Cammarota G, Masala G, Cianci R, Palli D, Capaccio P, Schindler A, et al. Reflux symptoms in professional opera choristers. Gastroenterology. 2007;132(3):890-8.
9. Drossman DA, Corazziari E, Delvaux M, Spiller RC, Talley NJ, Thompson WG, et al. Rome III: The functional gastrointestinal disorders. 3rd ed., McLean, Virginia: Degnon Associates; 2006.
10. Talley NJ, Dennis EH, Schettler-Duncan VA, Lacy BE, Olden KW, Crowell MD. Overlapping upper and lower gastrointestinal symptoms in irritable bowel syndrome patients with constipation or diarrhea. Am J Gastroenterol. 2003;98(11);2454–9.
11. Stanghellini V, Tosetti C, Partenicò A, De Giorgio R, Barbara G, Salvioli B, et al. Predominant symptoms identify different subgroups in functional dyspepsia. Am J Gastroenterol.1999:94(8);2080–5.
12. Si JM, Wang LJ, Chen SJ, Sun LM, Dai N. Irritable bowel syndrome consulters in Zhejiang province: the symptoms pattern, predominant bowel habit subgroups and quality of life. World J Gastroenterol. 2004;10(7);1059–64.

Int J Clin Pharm
DOI 10.1007/s11096-016-0378-2

RESEARCH ARTICLE

Combined use of renin-angiotensin-aldosterone system-acting agents: a cross-sectional study

Andreea Farcas[1] · Daniel Leucuta[2] · Camelia Bucsa[1] · Cristina Mogosan[1] · Dan Dumitrascu[3]

Received: 21 April 2016 / Accepted: 6 September 2016

Abstract *Background* Due to recent EU warnings and restrictions on the combined use of renin-angiotensin-aldosterone system (RAAS)-acting agents, and the seriousness of the associated harm, we analyzed the prescription of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) as dual therapy or associated with spironolactone. *Setting* An administrative claims database of a regional hospital in Romania. *Methods* We retrospectively included all adult patients hospitalized during 18 months in 2013–2014, discharged with a prescription of a RAAS-acting agent. *Main outcome measures* Counts of ACEIs and ARBs co-prescription, of ACEIs or ARBs combined with spironolactone, co-morbidities, co-medication, creatinine, and electrolytes assessment and values. *Results* Out of 1697 patients with a prescription of a RAAS-acting agent, 24 (1.4 %) were co-prescribed ACEIs and ARBs, and 416 (24.5 %) ACEIs or ARBs with spironolactone. Patients prescribed dual ACEI/ARB therapy *and* the ones with ACEI or ARB-spironolactone combination had significantly higher prevalence of increased creatinine level before discharge, *compared to* the ACEI *and* ARB monotherapy groups (48 *and* 31 % *compared to* 17 *and* 27 %). Subjects with diabetes, heart failure, ischaemic heart disease, or urea $\geq$40 mg/dL had higher odds of having ACEI or ARB-spironolactone combination *compared to* monotherapy, while hypertension and renal disease subjects had lower odds. Similar findings were comparing dual ACEI/ARB therapy *to* monotherapy except heart failure (not statistically significant). *Conclusion* Overall, the prevalence of use of dual therapy was low. The combined use of RAAS-acting agents was higher in patients with known risk factors for further renal function deterioration, compared to the ones without.

Keywords Potential harm · Renin-angiotensin-aldosterone system-acting agents · Romania · Serum creatinine and potassium monitoring · Utilization

Impacts of findings on clinical practice

- Despite earlier evidence on related harm (severe hyperkalaemia and renal impairment), the co-prescription of ACEIs with ARBs and of spironolactone with an ACEI or ARB is more likely to occur in special cohorts of population that might be at risk, such as patients with diabetes mellitus.
- Prescribing practice in the secondary care Romanian population does not appear to be influenced by the results from different clinical trials published before EU safety warnings.
- In order to prevent potential harm, there is a need for greater awareness on the potential renal toxicity and for close monitoring of serum electrolytes in patients co-prescribed a potassium-sparing diuretic and an ACE inhibitor or an angiotensin receptor blocker.

✉ Daniel Leucuta
dleucuta@umfcluj.ro

[1] Drug Information Research Center, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

[2] Medical Informatics and Biostatistics Department, "Iuliu Hatieganu" University of Medicine and Pharmacy, 6 Pasteur Street, 400349 Cluj-Napoca, Romania

[3] 2nd Medical Department, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Published online: 27 September 2016

Introduction

Randomized clinical trials (RCTs) have demonstrated the efficacy of angiotensin-converting enzyme inhibitors (ACEIs) in patients with chronic heart failure (CHF) associated with left ventricular systolic dysfunction, by reducing mortality [1, 2]. There are also data that angiotensin receptor blockers (ARBs) can provide a similar reduction in mortality, compared to ACEIs [3, 4].

Concomitant therapy with both ACEIs and ARBs for more complete renin-angiotensin inhibition was also proposed several years ago in specific patients groups (heart failure and diabetic patients) [5, 6]. The clinical benefits of such dual therapy compared with ACEIs or ARBs monotherapy were hypothesized, and proved up to some extent, to be reduced morbidity in patients with heart failure (HF), additional blood pressure reduction in patients with hypertension, and reduced proteinuria in patients with chronic kidney disease (CKD). However the dual therapy came along with concerns on hyperkalemia and worsening of renal function at a higher rate than the rate seen with the use of ACEIs/ARBs monotherapy. These concerns were clarified only recently with the ONTARGET (Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial) [7] and VA-NEPHRON-D (Combination Angiotensin Receptor Blocker and Angiotensin Converting Enzyme Inhibitor for Treatment of Diabetic Nephropathy) [8] trials. Based on these trials and on the Makani et al. meta-analysis [9] the dual inhibition of the renin-angiotensin system is not recommended since September 2014, unless if considered absolutely essential, and carried out under specialist supervision with close monitoring of renal function, electrolytes and blood pressure [10].

Concomitant use of spironolactone in patients already receiving background therapy with ACEIs can be of benefit in CHF patients as demonstrated by the randomized aldactone evaluation study (RALES) trial, where a 30 % decrease in mortality was observed [11]. However, due to the potassium-sparing effect of spironolactone, this therapy poses a risk for hyperkalemia, especially in patients taking other medicines acting on the renin-angiotensin-aldosterone system (RAAS). This kind of co-prescription also requires regular blood test monitoring for hyperkalemia and renal dysfunction, especially in elderly patients, those suffering from diabetes, or those with high serum creatinine and potassium levels before treatment [12, 13].

There are not many observational studies assessing in clinical practice the prescription of ACEIs/ARBs, as dual therapy or associated with spironolactone, and the status of serum creatinine (SCr) and potassium monitoring [14–16]. This information is lacking in Romania.

Aim of the study

Our study aimed to evaluate the prescription of ACEIs/ARBs as dual therapy or combined with spironolactone, to see to what extent SCr and potassium are assessed before prescription, and to see what factors are associated with the combined use of RAAS-acting agents.

Ethics approval

For this type of study formal consent is not required.

Methods

This study is a retrospective analysis of an administrative claims database of a regional hospital in Nord-West Romania, covering all medical specialties. We included all adult patients hospitalized during 18 months in 2013–2014, discharged with a prescription of a RAAS-acting agent. Four patient groups were defined: (1) patients with an ACEI alone, (2) patients with an ARB alone (3) patients with a co-prescription of an ACEI and an ARB without spironolactone and (4) patients with an ACEI or an ARB combined with spironolactone. We extracted patient information and variables like demographics, co-morbidities, co-medications and proportion of patients with serum creatinine, urea, sodium and potassium assessed, and their values before discharge. We considered the prescription of chronic polypharmacy defined as the exposure to five or more different drugs. Based on the RALES trial increased potassium (K) was defined as any $K \geq 5.5$ mEq/L [11]. Increased serum creatinine was defined as any SCr $\geq$ 1.2 mg/dL, and severely increased if SCr $\geq$1.7 mg/dL. Patients with diabetes mellitus (DM) or with renal disease were identified by searching the medical records using the 10th revision of the international classification of disease (ICD) codes as recorded for each patient hospitalization in the study period. ICD codes E10–E14 were used to identify patients with DM; ICD codes N17–N19 were used to identify patients with renal dysfunction (chronic or acute).

Statistical analysis

Comparisons between groups for categorical variables were made with Chi square test, and Fisher exact test; for continuous variables were made with ANOVA one way test followed by Tukey–Kramer post hoc tests, or with Kruskal–Wallis test followed by a nonparametric post hoc test. When multiple comparisons were made for categorical variables we used the Bonferroni correction.

A multinomial logistic regression analysis was performed trying to investigate possible associations between the prescription of dual ACEI/ARB therapy and ACEI or ARB combined with spironolactone therapy versus monotherapy with the variables listed in Table 3 (the full model). Using a stepwise backward/forward variable selection procedure using Akaike information criterion, the final model was obtained.

For all tests a two tailed p value below 0.05 was considered statistically significant. All statistical analyses were carried out with the R environment for statistical computing and graphics, version 3.2.0 [17].

Results

Of 10,315 patients hospitalized during the study period, 1697 (16.5 %) were prescribed RAAS agents on hospital discharge: 1003 (59 %) were prescribed an ACEI, 254 (15 %) an ARB, 24 (1.4 %) were co-prescribed an ACEI and an ARB without spironolactone, and 416 (24.5 %) an ACEI or an ARB combined with spironolactone.

The demographic, co-morbidity and co-medication characteristics of the study patients' groups are presented in Table 1. Most of these patients were hospitalized in internal medicine departments (91 %) and the rest in the rheumatic diseases department. More than half of the patients were female (62 %). Hypertension affected 1417 of the patients (84 %), ischemic heart disease 924 (54 %), heart failure 620 (37 %), diabetes 400 (24 %), cerebrovascular disease 311 (18) and renal disease 127 (7 %).

Ninety-seven (6 %) of these patients were discharged with polypharmacy. Polypharmacy was more frequent in the ACEI/ARB dual therapy group (25 %) and in the group with combined spironolactone (18 %).

Prescription of ACEIs/ARBs with or without spironolactone

Patients with ACEI/ARB dual therapy or the ones with combined spironolactone therapy were slightly older compared to the monotherapy groups ($p < 0.001$).

In the dual ACEI/ARB therapy group more patients had renal and diabetic disease compared to ACEI and ARB monotherapy groups (21 vs. 6 %, $p = 0.04$, and vs. 11 %, $p = 0.34$), and (58 vs. 19 %, $p \leq 0.001$, and vs. 28 %, $p = 0.004$). More patients having spironolactone combined with an ACEI or an ARB were with heart failure compared

Table 1 Demographic, clinical characteristics and co-medication at discharge of the study patients groups

Characteristics	ACEI (n = 1003)	ARB (n = 254)	ACEI *and* ARB without spironolactone (n = 24)	ACEI *or* ARB with spironolactone (n = 416)
Demography				
Age [years (mean, SD)]	67.71 (±11.64)	68.19 (±11.79)	69.38 (±10.77)	71.57 (±10.72)
Male [n, (%)]	380 (37.89)	83 (32.68)	10 (41.67)	170 (40.87)
Co-morbidities, ICD code [n (%)]				
Hypertension, I10	828 (82.55)	224 (88.19)	20 (83.33)	345 (82.93)
Ischemic heart disease, I20–I25	463 (46.16)	148 (58.27)	19 (79.17)	294 (70.67)
Heart failure, I50	223 (22.23)	88 (34.65)	8 (33.33)	301 (72.36)
Diabetes, E10–E14	192 (19.14)	71 (27.95)	14 (58.33)	123 (29.57)
Cerebrovascular disease, I60–I69	191 (19.04)	36 (14.17)	6 (25)	78 (18.75)
Renal disease N17–N19	62 (6.18)	27 (10.63)	5 (20.83)	33 (7.93)
Myocardial infarction I21, I22	38 (3.79)	16 (6.3)	2 (8.33)	29 (6.97)
Co-medication at discharge [n, (%)]				
Beta-blockers	544 (54.24)	169 (66.54)	15 (62.5)	258 (62.02)
Calcium channel blockers	276 (27.52)	112 (44.09)	13 (54.17)	136 (32.69)
Diuretics	410 (40.88)	118 (46.46)	11 (45.83)	393 (94.47)
Statins	358 (35.69)	111 (43.7)	11 (45.83)	127 (30.53)
Antithrombotics	28 (2.79)	8 (3.15)	1 (4.17)	10 (2.4)
Oral anticoagulants	132 (13.16)	40 (15.75)	1 (4.17)	123 (29.57)
Number of medicines				
Medicine number, median (IQR)	3 (2–3)	3 (2–4)	4 (3.75–5)	4 (3–5)
≥5 medicines, n, (%)	7 (0.7)	8 (3.15)	6 (25)	75 (18.03)

SD standard deviation, *IQR* interquartile range

Table 2 Laboratory monitoring before discharge in the study patient groups

Characteristic	ACEI (n = 1003)	ARB (n = 254)	ACEI *and* ARB without spironolactone (n = 24)	ACEI *or* ARB with spironolactone (n = 416)
Patients with parameter monitored [n, (%)]				
Creatinine	897 (89.43)	232 (91.34)	22 (91.67)	386 (92.79)
Potassium	887 (88.43)	233 (91.73)	21 (87.5)	397 (95.43)
Sodium	883 (88.04)	232 (91.34)	20 (83.33)	394 (94.71)
Urea	902 (89.93)	230 (90.55)	21 (87.5)	384 (92.31)
Median values[a] (IQR)				
Creatinine (mg/dL)	0.92 (0.76–1.11)	0.96 (0.79–1.21)	1.16 (1.05–1.44)	1.01 (0.83–1.24)
Potassium (mEq/L)	4.11 (3.8–4.5)	4.17 (3.81−4.6)	4.1 (3.62–4.68)	4.2 (3.8–4.5)
Sodium (mEq/L)	140.33 (138–142)	141 (138.54−143)	142.06 (139–144)	140.36 (138.42–142.1)
Urea (mg/dL)	37.5 (28–47.38)	40.3 (31–53.2)	47 (41–71.38)	46.75 (36.55–60)
Patients with increased values[a] (n, [%])				
Creatinine ≥1.2 mg/dL	172 (17.46)	66 (26.72)	11 (47.83)	122 (30.5)
Creatinine ≥1.7 mg/dL	26 (2.64)	17 (6.88)	3 (13.04)	16 (4)
Creatinine ≥2.5 mg/dL	7 (0.71)	3 (1.21)	1 (4.35)	1 (0.25)
Potassium ≥5.5 mEq/L	10 (1.03)	5 (2.02)	0 (0)	5 (1.22)
Sodium ≥145 mEq/L	26 (2.94)	5 (2.16)	1 (5)	18 (4.57)
Urea ≥40 mg/dL	425 (42.93)	126 (51.43)	17 (77.27)	262 (65.83)

SD standard deviation, *IQR* interquartile range

[a] As per the last measurement before discharge

to the monotherapy ACEI and ARB groups (72 vs. 22 %, $p \leq 0.001$, and vs. 35 %, $p \leq 0.001$).

Serum creatinine and potassium monitoring

High percentages of patients in all four groups had their creatinine, potassium, sodium and urea assessed during hospitalization, before prescribing the discharge therapy (Table 2). The highest percentages were seen in the ACEI/ARB—spironolactone combination group. However, we observed slightly lower percentages of patients assessed for potassium and sodium in the dual ACEI/ARB therapy group, compared to the ACEI and ARB monotherapy groups.

Serum creatinine was significantly raised in the dual ACEI/ARB therapy group compared to ACEI ($p < 0.001$) and ARB ($p = 0.032$) monotherapy groups. Also a significant difference was seen for urea values between dual ACEI/ARB therapy group compared to ACEI monotherapy group ($p = 0.018$). There were no significant differences in the values of potassium and sodium between the four groups.

Compared to the ACEI and ARB monotherapy groups, more patients of the dual ACEI/ARB therapy group had increased creatinine and urea values before discharge. This was seen regardless of the cut-off value defined for increased creatinine: 48 % patients in the dual ACEI/ARB therapy group versus 17 and 27 % patients in the ACEI and ARB monotherapy groups ($p \leq 0.001$ and $p = 0.06$) had SCr ≥1.2 mg/dL and 13 % patients in the dual ACEI/ARB therapy group versus 3 and 7 % patients in the ACEI and ARB monotherapy groups had SCr ≥1.7 mg/dL.

The prevalence of raised urea levels was significantly higher in the dual therapy group (77 %) compared with the ACEI (43 %) and the ARB (51 %) monotherapy groups (both $p < 0.05$). The prevalence of increased creatinine (≥1.2 mg/dL) and urea was also higher in the group that was prescribed spironolactone in addition to an ACEI or an ARB, compared to ACEI and ARB monotherapy groups: 31 % patients versus 17 and 27 % patients ($p \leq 0.001$ and $p = 0.60$) with raised creatinine and 66 % patients versus 43 and 51 % patients (both $p \leq 0.001$) with raised urea. There were few patients with potassium levels ≥5.5 mEq/L in all four groups, with no significant differences between them.

Factors associated with ACEI/ARB prescription-regression models

Diabetes and ischemic heart disease were associated with the co-prescription of ACEIs and ARBs as shown in Table 3. Compared to the monotherapy groups, patients with diabetes had higher odds of dual therapy prescription (OR 4.51, 95 % CI 2.20–9.26, $p < 0.001$), and so did patients with ischemic heart disease (OR 2.82, 95 % CI 1.18–6.75, $p = 0.020$). Also raised urea, but not creatinine, were associated with a higher odds of co-prescription of

Table 3 Factors associated with the prescription of dual ACEI/ARB therapy and ACEI or ARB combined with spironolactone therapy (univariate and multivariate multinomial logistic regression results)

Explanatory variable	ACEI *and* ARB without spironolactone versus monotherapy		ACEI *or* ARB with spironolactone versus monotherapy	
	Unadjusted OR (95 % CI), *p*	Adjusted OR (95 % CI), *p*	Unadjusted OR (95 % CI), *p*	Adjusted OR (95 % CI), *p*
Age (years)	1.03 (0.99–1.07), $p = 0.21$	–	**1.03** (1.02–1.04), $p < 0.001$	–
Gender, male	1.06 (0.43–2.57), $p = 0.9$	–	1.21 (0.96–.53), $p = 0.1$	–
Hypertension	0.87 (0.40–1.89), $p = 0.7$	**0.35** (0.14–0.86), $p = 0.022$	0.94 (0.76–1.17), $p = 0.58$	**0.69** (0.53–0.91), $p = 0.009$
Ischemic heart disease	**2.91** (1.34–6.32), $p = 0.007$	**2.82** (1.18–6.75), $p = 0.020$	**1.94** (1.63–2.31), $p < 0.001$	**1.26** (1.01–1.57), $p = 0.033$
Heart failure	1.16 (0.59–2.28), $p = 0.662$	0.661 (0.31–1.39), $p = 0.276$	**4.46** (3.71–5.36), $p = 0.000$	**4.23** (3.43–5.21), $p < 0.001$
Diabetes	**4.21** (2.20–8.06), $p < 0.001$	**4.51** (2.20–9.26), $p < 0.001$	**1.42** (1.18–1.70), $p < 0.001$	**1.257** (1.01–1.56), $p = 0.038$
Cerebrovascular disease	1.53 (0.78–3.01), $p = 0.218$	–	1.04 (0.85–1.28), $p = 0.694$	–
Renal disease	2.64 (1.28–5.46), $p = 0.009$	1.83 (0.83–4.03), $p = 0.133$	1.07 (0.79–1.45, $p = 0.665$	**0.57** (0.41–0.80), $p = 0.001$
Myocardial infarction	1.88 (0.66–5.37), $p = 0.239$	–	1.48 (1.05–2.08), $p = 0.025$	–
Creatinine ≥1.2 mg/dL	2.53 (1.35–4.75), $p = 0.004$	–	1.42 (1.18–1.71), $p < 0.001$	–
Creatinine ≥1.7 mg/dL	2.79 (1.14–6.82), $p = 0.025$	–	1.06 (0.70–1.60), $p = 0.790$	–
Potassium ≥5.5 mEq/L	0.02 (0.0–3.05), $p = 0.870$	–	1.03 (0.5–2.12), $p = 0.945$	–
Sodium ≥145 mEq/L	0.54 (0.07–4.18), $p = 0.557$	–	0.60 (0.30–1.10), $p = 0.096$	–
Urea ≥40 mg/dL	2.76 (1.27–6.03), $p = 0.011$	**2.32** (1.03–5.21), $p = 0.042$	1.80 (1.51–2.51), $p < 0.001$	**1.58** (1.29–1.93), $p < 0.001$

SD standard deviation, *IQR* interquartile range

Values in *bold* depict statistical significance

ACEIs and ARBs (OR 2.32, 95 % CI 1.03–5.21, $p = 0.042$).The same three factors, and in addition heart failure were associated with the prescription of spironolactone combined with an ACEI or an ARB. Compared to the monotherapy groups, HF was associated with higher odds of associating spironolactone to ACEI or ARB therapy (OR 4.23, 95 % CI 3.43–5.21, $p < 0.001$).

Discussions

Our results show that while the proportion of patients with a prescription of a RAAS acting agent was quite high, up to 16 % of the overall population (with ACEIs being the most prescribed), only a small proportion (0.23 %) of this population was co-prescribed an ACEI and an ARB, reflecting the current therapeutic guidelines in use in Europe, where co-prescription is not recommended anymore [18]. These results are similar with the results obtained in a European Medicine Agency study conducted in UK, Germany and France, where, in 2012, 12.3, 18.3, and 10.3 % patients were treated with a renin-angiotensin system acting drug and 0.1, 0.3 and 0.1 %, respectively were co-prescribed different drug classes acting on this system [19]. However, our proportion of patients co-prescribed an ACEI and an ARB is much lower than the one in a population based longitudinal analysis among elderly patients where 5.4 % received the combination therapy [20].

In the renal disease and diabetic patients' subgroups significantly more patients were prescribed the dual ACEI/ARB therapy compared to ACEI or ARB monotherapy. This could be also due to the fact that numerous studies have suggested that combined renin-angiotensin system blockade offers additional renoprotective, antiproteinuric effects, independent of blood pressure reduction [21].

Significantly more patients with HF were prescribed spironolactone in addition to an ACEI or an ARB, probably due to the fact that the RALES trial proved that addition of an aldosterone antagonist to the standard heart failure therapy (with an ACEI or an ARB) conferred powerful relative risk reduction for both morbidity and mortality [11].

Notably, the most interesting results from a clinical perspective are those concerning the blood tests. We found that electrolytes (sodium, potassium), SCr, and urea were checked in the majority of patient groups before discharge prescription of the study observed medication. Despite the fact there are limitations in the use of SCr in estimating the glomerular filtration rate, especially in the elderly, most of the trials, including the RALES trial, assessed creatinine, potassium and sodium [11]. So appropriate monitoring of the renal function in patients with ACEI/ARB and spironolactone implies evaluation of the above three mentioned blood tests. Although the majority of patients had these tests evaluated, the values of SCr and urea, as per the last assessment before discharge, were already higher before prescription in the dual ACEI/ARB therapy group and in the spironolactone co-prescription group, compared to the monotherapy groups. This, of course, poses the risk of further increase of these values and deterioration of the renal function with combination therapy, after discharge. Moreover, our study population is of older age, the oldest patients belonging to the two combination groups. Older age is *per se* a risk factor for lower glomerular filtration rates and predisposition for potassium retention. Among older outpatients with heart failure co-prescribed ACEIs/ARBs and spironolactone, 38 % suffered a worsening of the renal function and 6 % developed hyperkalemia after prescription [22]. Furthermore, serum creatinine levels increase more commonly in patients with decreased rather than with preserved baseline renal function, when co-prescribed ACEIs and ARBs, as it was recently demonstrated [23].

Of particular concern is our finding that prescription of dual ACEI/ARB therapy and the co-prescription of spironolactone with ACEIs or ARBs was frequent even though the serum creatinine and urea levels were raised (regardless of the cut-off value for creatinine in the dual therapy patients' group). Kurnik et al. demonstrated that dual renin-angiotensin blockade is associated with higher absolute risks for hyperkalemia and renal dysfunction, especially in patients with reduced baseline renal function defined as serum creatinine higher than 1.5 mg/dL [24]. Shah et al. [15] also found that a baseline serum creatinine level ranging as low as 1.5–1.9 mg/dL can still predict a high risk (35 %) of hyperkalemia, and subsequently renal dysfunction in patients with heart failure that were co-prescribed spironolactone. Even the ACEI monotherapy is strongly associated with acute increases in serum creatinine of up to 30 % in patients with creatinine values greater than 1.4 mg/dL, although it stabilizes within the first 2 months of ACEI therapy [24]. RALES trial also excluded patients with a creatinine level higher than 2.5 mg/dL as a safety measure [11]. Nowadays dual ACEI/ARB therapy and co-prescription of spironolactone to an ACEI or an ARB is not recommended in patients with raised serum creatinine and urea as they are at higher risk of developing hyperkalemia and worsening of renal function. We should mention though that this study assessed prescriptions before the first clear recommendations for restrictions on combined use of medicines affecting the renin-angiotensin system were available in September 2014. Earlier evidence backing up recommendations was available from different clinical trials and meta-analysis [8, 9] and might have influenced the treatment practice, but having no data on prescription trends we cannot know if there was a change in prescription prevalence.

Multivariate analysis showed that patients with diabetes were more likely to be co-prescribed an ACEI with an

ARB. This might be explained by the rationale for dual inhibition of the renin-angiotensin system in type 2 diabetic patients aiming at slowing the progression of proteinuric diabetic nephropathy—dual inhibition that was tested for efficacy and safety in the VA-NEPHRON-D trial. However, this trial was stopped early due to an increased risk of adverse events (all-cause mortality, hyperkalemia, and acute kidney injury) among these patients [8]. Multivariate analysis also found that patients with HF were those with greater odds for having spironolactone associated to an ACEI or an ARB. These findings outline the need for greater awareness on the potential renal toxicity of the association of spironolactone with ACEIs or ARBs. These results should be seen in the context of recurrent warnings on the associated harm related to this association, the latest being issued early in 2016 by the UK Medicines and Healthcare products Regulatory Agency. The agency outlines again the fact that concomitant use of spironolactone with ACEI or ARB is not routinely recommended because of the risks of severe hyperkalaemia, particularly in patients with marked renal impairment and that monitoring of blood electrolytes is essential in patients co-prescribed a potassium-sparing diuretic and an ACEI or an ARB for heart failure [25].

The potential limitations of our study should be noted. First, this being a retrospective study, carried out in one regional hospital, the generalizability of our findings should be limited. Second, we have no data on patients' outcomes and outpatients' physician monitoring after discharge with the study drugs' prescription. Third, as we could not assess medication at admission, we do not know if there was a change in prevalence of use of combined therapy. Fourth, the fact that the majority of patients had their creatinine, urea and electrolytes monitored before prescription might be due to the fact that our study patients were in-patients, where laboratory monitoring is done routinely. This might not be the case in an outpatient setting. And fifth, misclassification of diabetes and renal failure patients could be possible, but given the fact that the data comes from a hospital covering all medical specialties, where patients have inter-department clinical evaluation, we consider the risk of misclassification being low. Nonetheless these limitations, the population-based data in our study may contribute to the assessment of the public health impact of the safety concerns in relation to the combined use of RAAS-acting agents. Few comprehensive drug utilization studies have been published to assess the extent and pattern of the co-medication of medicinal products acting on the RAAS system. Further evidence on these potentially harmful associations at the EU level would yield more results in broader population and data on the effectiveness and implementation of the regulatory warnings and recommendations.

Conclusion

Overall, the prevalence of use of dual therapy was low. The co-prescription of an ACEI and an ARB was higher in patients with diabetes and renal disease, being prescribed in more patients with altered creatinine values compared to patients receiving monotherapy. Spironolactone combined with an ACEI or an ARB was prescribed more frequently in patients with altered creatinine. The clinical implication of these associations should be foreseen by each prescribing physician, as these patients with decreased renal function, even if only minor, are at risk, and require close monitoring and follow-up as they are prone to further renal function deterioration.

Funding This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007–2013, Project No. POSDRU/159/1.5/136893. The funding source had no involvement in the conduct of the study.

Conflicts of interest All authors declare that they have no conflict of interest.

References

1. The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. N Engl J Med. 1991;325(5):293–302.
2. Garg R, Yusuf S. Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure. Collaborative Group on ACE Inhibitor Trials. JAMA. 1995;273(18):1450–6.
3. Pitt B, Poole-Wilson PA, Segal R, Martinez FA, Dickstein K, Camm AJ, et al. Effect of losartan compared with captopril on mortality in patients with symptomatic heart failure: randomised trial—the Losartan Heart Failure Survival Study ELITE II. Lancet. 2000;355:1582–7.
4. Pfeffer MA, Swedberg K, Granger CB, Held P, McMurray JJ, Michelson EL, et al. Effects of candesartan on mortality and morbidity in patients with chronic heart failure: the CHARM-Overall programme. Lancet. 2003;362:759–66.
5. Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-WilsonPA, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). Eur Heart J. 2008;29(19):2388–442.
6. American Diabetes Association. Standards of medical care in diabetes–2012. Diabetes Care. 2012;35(Suppl 1):S11–63.
7. Yusuf S, Teo KK, Pogue J, Dyal L, Copland I, Schumacher H, et al. (ONTARGET Investigators). Telmisartan, ramipril, or both in patients at high risk for vascular events. N Engl J Med. 2008;358(15):1547–59.
8. Fried LF, Emanuele N, Zhang JH, Brophy M, Conner TA, Duckworth W, et al. (VA NEPHRON-D Investigators). Combined angiotensin inhibition for the treatment of diabetic nephropathy. N Engl J Med. 2013;369(20):1892–903.

9. Makani H, Bangalore S, Desouza KA, Shah A, Messerli FH. Efficacy and safety of dual blockade of the renin-angiotensin system: meta-analysis of randomised trials. BMJ. 2013;346:f360. doi:10.1136/bmj.f360.
10. Restriction of combined use of medicines affecting the renin-angiotensin system (RAS). EMA/554928/2014. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/referrals/Renin-angiotensin_system_(RAS)-acting_agents/human_referral_prac_000026.jsp&mid=WC0b01ac05805c516f.
11. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized aldactone evaluation study investigators. N Engl J Med. 1999;341:709–17.
12. Wei L, Struthers AD, Fahey T, Watson AD, Macdonald TM. Spironolactone use and renal toxicity: population based longitudinal analysis. BMJ. 2010;340:c1768.
13. Svensson M, Gustafsson F, Galatius S, Hildebrandt PR, Atar D. How prevalent is hyperkalemia and renal dysfunction during treatment with spironolactone in patients with congestive heart failure? J Card Fail. 2004;10:297–303.
14. Bilotta C, Franchi C, Nobili A, Nicolini P, Djade CD, Tettamanti M, et al. New prescriptions of spironolactone associated with angiotensin-converting-enzyme inhibitors and/or angiotensin receptor blockers and their laboratory monitoring from 2001 to 2008: a population study on older people living in the community in Italy. Eur J Clin Pharmacol. 2013;69(4):909–17.
15. Shah KB, Rao K, Sawyer R, Gottlieb SS. The adequacy of laboratory monitoring in patients treated with spironolactone for congestive heart failure. J Am Coll Cardiol. 2005;46(5):845–9.
16. Wang PT, Huang YB, Lin MY, Chuang PF, Hwang SJ. Prescriptions for angiotensin-converting enzyme inhibitors/angiotensin receptor blockers and monitoring of serum creatinine and potassium in patients with chronic kidney disease. Kaohsiung J Med Sci. 2012;28(9):477–83.
17. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria; 2015. http://www.r-project.org.
18. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2013;34(28):2159–219.
19. Trends in co-prescribing of renin-angiotensin system (RAS)—acting agents in France, Germany and the UK during 2001–2012. EMA drug utilisation study using IMS Health electronic health records. http://www.encepp.eu/encepp/viewResource.htm?id=4617 Accessed Apr 15 2015.
20. McAlister FA, Zhang J, Tonelli M, Klarenbach S, Manns BJ, Hemmelgarn BR, et al. The safety of combining angiotensin-converting-enzyme inhibitors with angiotensin-receptor blockers in elderly patients: a population-based longitudinal analysis. CMAJ. 2011;183(6):655–62.
21. Werner C, Pöss J, Böhm M. Optimal antagonism of the Renin-Angiotensin-aldosterone system: do we need dual or triple therapy? Drugs. 2010;70(10):1215–30.
22. Goland S, Naugolny V, Korbut Z, Rozen I, Caspi A, Malnick S. Appropriateness and complications of the use of spironolactone in patients treated in a heart failure clinic. Eur J Intern Med. 2011;22(4):424–7.
23. Kurnik D, Vesterman-Landes J, Bialik M, Katzir I, Lomnicky Y, Halkin H, et al. Hyperkalemia and renal function during monotherapy and dual renin-angiotensin blockade in the community setting. Clin Ther. 2011;33(4):456–64.
24. Bakris GL, Weir MR. Angiotensin-converting enzyme inhibitor-associated elevations in serum creatinine: is this a cause for concern? Arch Intern Med. 2000;160(5):685–93.
25. Spironolactone and renin-angiotensin system drugs in heart failure: risk of potentially fatal hyperkalaemia. Drug Safety Update volume 9 issue 6 February 2016: 2. https://www.gov.uk/drug-safety-update/spironolactone-and-renin-angiotensin-system-drugs-in-heart-failure-risk-of-potentially-fatal-hyperkalaemia Accessed Feb 18 2016.