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Glycosaminoglycans as disease biomarkers: a systematic review

Glicosaminoglicanos como biomarcadores de doenças: uma revisão sistemática

Glicosaminoglicanos como biomarcadores de enfermedades: una revisión sistemática

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ABSTRACT

Objective: To identify glycosaminoglycans (GAGs) as biomarkers predictive of normal biological processes, pathological processes, and non-invasive diagnostic interventions. **Methods:** Searches were performed in the databases Acervo+ *Index base*, Scopus, MEDLINE, Web of Science, SciELO and LILACS, and experimental and review studies addressing the use of GAGs as biomarkers in diagnoses of diseases were included. For the critical analysis of the selected studies, the QUADAS-2 method was applied as a tool for evaluating methodological quality. **Results:** We selected 66 potentially relevant articles, 20 of which met the eligibility criteria. After evaluating the methodological quality, 10 studies classified with low risk of bias for narrative synthesis and 1 experimental article that studied the mechanism of GAGs in hepatic fibrosis were included. **Final considerations:** The present systematic review identified that the use of GAGs as biomarkers presents good reproducibility, can be used in non-invasive procedures (blood or urine), be performed in most laboratories, and allow for the evaluation of progress and pathophysiological processes. However, it still has limitations to differentiate intermediate stages in some diseases.

Keywords: Noninvasive diagnoses, Mucopolysaccharides, Pathological processes, Systematic review.

RESUMO

Objetivo: Identificar os glicosaminoglicanos (GAGs) como biomarcadores preditivos de processos biológicos normais, processos patológicos e intervenções diagnósticas não-invasivas. **Métodos:** Foram realizadas buscas nas bases de dados Acervo+ Index base, Scopus, MEDLINE, Web of Science, SciELO e LILACS e incluídos os estudos originais que abordam o uso de GAGs como biomarcadores em diagnósticos de doenças. Para a análise crítica dos estudos selecionados, foi aplicado o método QUADAS-2 como ferramenta de avaliação da qualidade metodológica. **Resultados:** Foram selecionados 66 artigos potencialmente relevantes, os quais 20 atendiam os critérios de elegibilidade. Após avaliação da qualidade metodológica, foram incluídos 10 estudos classificados com nível baixo no risco de viés para a síntese narrativa e 1 artigo experimental que estuda o mecanismo da GAG na fibrose hepática. **Considerações finais:** A presente revisão sistemática, identificou que o uso de GAGs como biomarcadores apresenta boa reprodutibilidade, podem ser empregados em procedimentos não invasivos (sangue ou urina), ser realizados na maior parte

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dos laboratórios e, permitem a avaliação do progresso e dos processos fisiopatológicos. Contudo, ainda possui limitações para diferenciar estágios intermediários em algumas doenças.

Palavras-chave: Diagnósticos não-invasivos, Mucopolissacarídeos, Processos patológicos.

RESUMEN

Objetivo: Identificar glicosaminoglicanos (GAGs) como biomarcadores predictivos de procesos biológicos normales, procesos patológicos e intervenciones diagnósticas no invasivas. **Métodos:** Se realizaron búsquedas en las bases de datos Acervo+ Index base, Scopus, MEDLINE, Web of Science, SciELO y LILACS y se incluyeron estudios experimentales y de revisión que abordaron el uso de GAGs como biomarcadores en diagnósticos de enfermedades. Para el análisis crítico de los estudios seleccionados se aplicó el método QUADAS-2 como herramienta para evaluar la calidad metodológica. **Resultados:** Se seleccionaron 66 artículos potencialmente relevantes y 20 cumplieron con los criterios de elegibilidad. Después de evaluar la calidad metodológica, se incluyeron 10 estudios clasificados como de bajo riesgo de sesgo para la síntesis narrativa y 1 artículo experimental que estudia el mecanismo de GAG en la fibrosis hepática. **Consideraciones finales:** La presente revisión sistemática identificó que el uso de GAGs como biomarcadores presenta buena reproducibilidad, puede ser utilizado en procedimientos no invasivos (sangre u orina), ser realizado en la mayoría de los laboratorios y permitir la evaluación del progreso y los procesos fisiopatológicos. Sin embargo, todavía tiene limitaciones para diferenciar etapas intermedias en algunas enfermedades.

Palabras clave: Diagnósticos no invasivos, Mucopolisacáridos, Procesos patológicos, Revisión sistemática.

INTRODUCTION

Biomarkers, also called biological markers, are measurable indicators of normal biological processes, which may change in pathological processes as well as a response to clinical or surgical therapeutic interventions. These biomarkers can be molecules, such as hormones, proteins, genes, among others as well as specific cells, as it occurs for some tumors (FISHEL S, et al., 2017).

Glycosaminoglycan molecules (GAGs), also previously referred to as mucopolysaccharides, are long linear polysaccharides consisting of repeated units of disaccharides, composed of an hexosamine (glucosamine or galactosamine) and a uronic acid residue (glucuronic acid or iduronic acid) or galactose. Depending on the type of the hexoaminse and of the other sugar residue, glycosidic linkage and sulfation pattern, GAGs can be classified as hyaluronic acid (HA), chondroitin sulfate (CS), heparan sulfate (HS), dermatan sulfate (DS), heparin (Hep) and keratan sulfate (KS) (ALEKNAVIČIŪTĖ-VALIENĖ G, BANYS V, 2022; KHAN AS, et al., 2020; MENEGHETTI MCZ, et al., 2015).

GAGs occur in the cells covalently linked to a protein constituting the proteoglycans (PG). HA is the only GAG that does not occur as a PG. The expression of GAGs is involved in important biological functions such as regulation of cell growth and proliferation, embryogenesis, interactions between cells and cell surface receptors (enzymes, cytokines, complement proteins and chemokines) and maintenance of residual hydration, anti-coagulation, among others (COULSON-THOMAS YM, et al., 2015; KHAN AS, et al., 2020).

In addition, GAGs exert influence on proteins comprised in pathological and physiological processes and can be used as biological markers (NAKAMURA-UTSUNOMIYA A, 2021; DOS SANTOS PRD, 2015), whose accumulation in multiple tissues generated by the deficiency of lysosomal enzymes can result in an organic dysfunction (KHAN AS, et al., 2020; DREYFUSS JL, et al., 2009).

Among GAGs, hyaluronic acid (HA) is one of the most used biomarkers in studies and clinical practice for the diagnosis or identification pathology occurrence risk. The HA can be found in the blood circulation (half-life of 2-5 minutes), organs, the lymphatic system, interacting to cellular receptors and the extracellular matrix (ALEKNAVIČIŪTĖ-VALIENĖ G and BANYS V, 2022).

Under normal health conditions, HA concentrations are low in serological tests, as the circulating molecule is rapidly eliminated from the blood by the liver, kidneys and spleen. However, in health conditions with pathological risks, there is an increase in the production of hyaluronic acid, whose hepatic elimination of the glycosaminoglycan molecule is decreased. Thus, the high serum concentration of HA allows for the identification of stages of liver fibrosis in patients with chronic hepatitis B virus infection, as well as liver cirrhosis. It is noteworthy that HA concentrations are proportional to the negative outcomes of diseases such as liver stiffness in clinical severity and disease activity (ALEKNAVIČIŪTĖ-VALIENĖ G, BANYS V, 2022; NEUMAN MC, et al., 2016).

The deficiency of enzymes that degrade GAGs can cause lysosomal storage disorders, which characterize Mucopolysaccharidoses (MPS) of various types (SINGH R, et al., 2020). There are different types of MPS (I to IX), which are distinguished by the deficient enzyme and, consequently, by the accumulated GAGs (NEUFELD EF, MUENZER J, 2001).

Among the most common MPS are MPS I (Hurler/Scheie syndrome) and MPS II (Hunter syndrome), and MPS type III (MPS III), also called Sanfilipo syndrome with is divided into 4 different syndromes according to the deficient enzyme involved in the degradation of heparan sulfate (HS). In addition, the pathological process of the disease also acts on secondary storage products, resulting in biochemical and cellular changes, such as neuroinflammation and progressive deterioration of nervous system functions (JAKOBKIEWICZ-BANECKA J, et al., 2016).

For the diagnosis of Sanfilipo syndrome, HS can be used as a biomarker in diagnostic and predictive procedures that use urine and plasma (JAKOBKIEWICZ-BANECKA J, et al., 2016; SINGH R, et al., 2020). In summary, GAG biomarkers have also been employed in the diagnosis of cancerous and musculoskeletal diseases (Dupuytren's contracture, rheumatoid arthritis, osteoarthritis, intervertebral disc degeneration, Peyronie's disease) (BRATULIC S, et al., 2022; MARTEL-PELLETIER J, et al., 2017; NASCIMENTO PCH, et al., 2016; RODRIGUES LMR, et al., 2019; SZEREMETA A, et al., 2018; UETA RHS, et al., 2018; WATANABE MA, et al. 2017).

In the diagnosis of diseases using GAGs as biomarkers, a biochemical evaluation of patient samples can be performed, including the qualitative and quantitative evaluation of serum GAGs, followed by a determination of enzymatic activities and molecular diagnosis. Non-invasive detection techniques using chromatography, spectrometry, spectrophotometry or electrophoresis have been employed to detect GAGs, among which, liquid chromatography coupled with tandem mass spectrometry is the fastest, most specific, sensitive and economical method (KHAN AS, et al., 2020).

In this context, the present systematic review seeks to explore the use of GAGs as disease biomarkers and non-invasive diagnoses, highlighting their potential for a prediction of clinical severity and prognosis, and enabling a broad view of therapeutic monitoring and disease screening.

METHODS

The systematic review, registered in the PROSPERO database (record no. CRD42023414144), was conducted in accordance with PRISMA recommendations (*Preferred Reporting Items for Systematic Reviews and Meta-analysis Statement*) (MOHER D, et al., 2009).

Five bibliographic databases were consulted: Acervo+ Index base, Scopus, *Medical Literature Library of Medicine On-Line* (MEDLINE) by the PubMed platform, *Web of Science*, *Scientific Electronic Library Online* (SciELO) and Latin American and Caribbean Literature (LILACS).

Search strategy

The search strategy included searching for the unique identifier of the Health Sciences descriptor registry (DeCS). The descriptors used were combined with the boolean operator AND: (g*lycosaminoglycans* AND *biomarkers* AND *diseases*), (g*lycosaminoglycans* AND *biomarkers*), (g*lycosaminoglycan*s AND non-invasive diagnostic), (*mucopolysaccharides* AND *biomarkers* AND *diseases*), (*mucopolysaccharides* AND *biomarkers*)

and (*mucopolysaccharides* AND *non-invasive diagnostic*), present in the titles, summaries and keywords, to which temporary and linguistic filters were added. The non-invasive diagnostic descriptor does not have a unique DeCS/MeSH record identifier, so it was used as an uncontrolled descriptor.

Eligibility criteria

The inclusion criteria were: original articles, in Portuguese, English or Spanish, available as open access and with a publication period between 2013 and 2023, whose search was updated up until March 2023. Articles reporting glycosaminoglycans as biomarkers of different diseases were included.

Duplicate studies and those that did not present at least two of the descriptors in the abstract or in the title were excluded. After the critical reading, articles that were not relevant to the proposed objective were also excluded.

Literature search process

After importing the articles, intra- and inter-base duplicates were checked, followed by the evaluation of the titles, abstracts and keywords of the relevant articles. The selected articles were submitted to full reading and methodological quality. The narrative synthesis was structured from the data of the use of glycosaminoglycans as biomarkers of pathological processes and non-invasive diagnostic strategies.

Methodological quality assessment a

The articles were evaluated and considered according with the impact of bias and analysis of the findings of experimental, cohort and exploratory studies. Thus, the methodological quality of the studies was evaluated using the QUADAS-2 tool (*Quality Assessment of Studies of Diagnostic Accuracy Included in Systematic Reviews*).

In this system, methodological quality is classified in relation to the risk of bias at high, low or uncertain levels, which comprise the patient selection domains; index test; standard/reference test and patient flow, and time of index and reference tests (WHITING P, et al., 2003).

RESULTS AND DISCUSSION

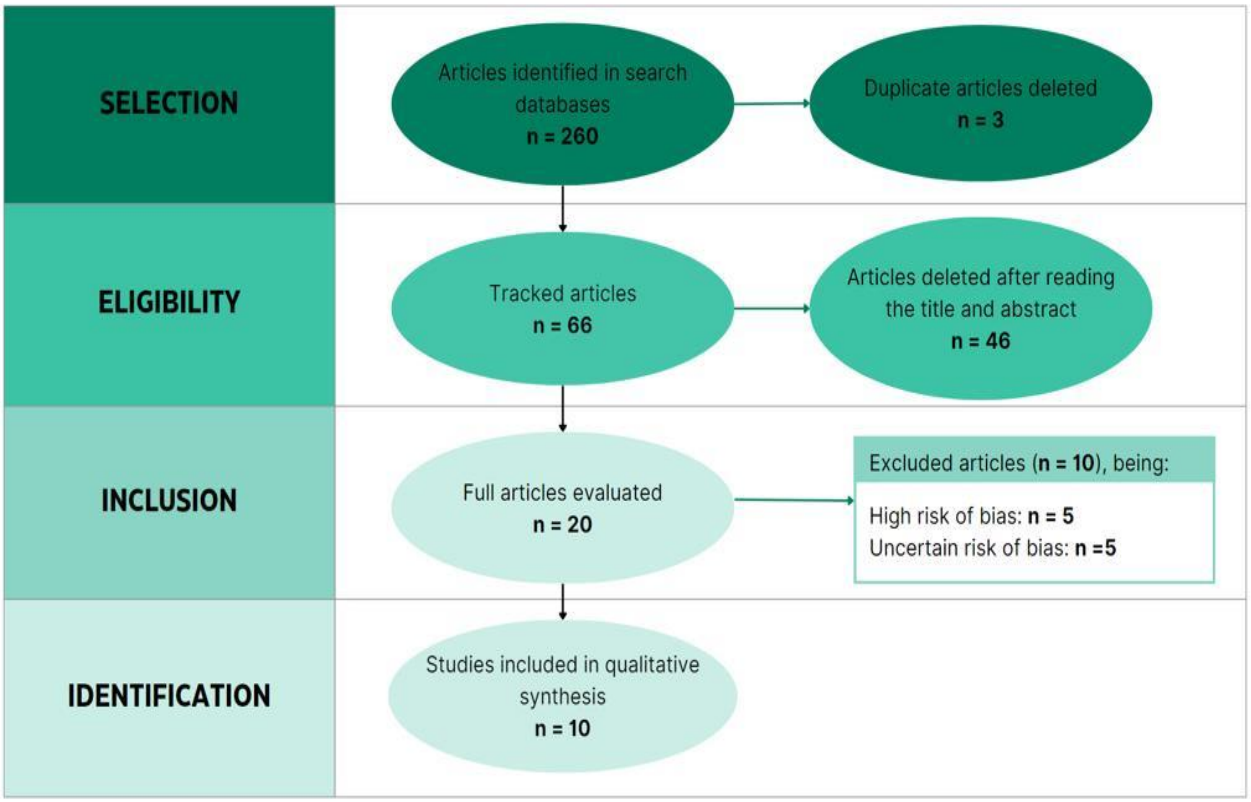
This systematic review sought to explore the use of GAGs as biomarkers of cancer, musculoskeletal, neurometabolic and hepatic diseases through invasive and non-invasive diagnoses. The originality of this study in relation to previous systematic reviews refers to the use of the PRISMA and QUADAS-2 methodology to sinter high impact and methodological quality works, indicating the potential results and their limitations.

Applying the inclusion and exclusion criteria, 260 potentially relevant articles were identified (**Figure 1**). After the exclusion of duplicate articles, 66 studies were selected for full reading, of which 20 met the eligibility criteria and 10 were included in the narrative synthesis after the analysis of methodological quality.

The methodological evaluation of the analyzed studies resulted in 10 articles classified with low risk of bias (50%) (**Table 1 and Figure 2**), 5 with risk of uncertain bias (25%) and 5 with high risk of bias (25%). An experimental study of risk of uncertain bias was used in narrative synthesis to investigate the mechanism of GAGs in liver fibrosis.

The articles with uncertain risk of bias did not present enough information to allow for judgment, while those with high risk of bias did not present a reference standard for comparison of results and the interpretation of the index test could have introduced a bias to the study. We emphasize that we chose to keep the name of the authors and their respective works classified as uncertain and high-risk bias, thus presenting only those that were classified with low risk of bias.

Figure 1 - Flowchart of the study selection process.



Source: Lenzi LGS, et al., 2023.

The general characteristics of the selected studies are described in **Table 2**. With the exception of the experimental studies of Nascimento PCH, et al. (2016) and Yang YM et al. (2019). The number of patients for the diagnostic tests ranged from 50 to 1260 people, of both genders (predominantly men) with an average age of 65 years, with the exception of 2 studies that extended their research to an age group from 0 to 18 years.

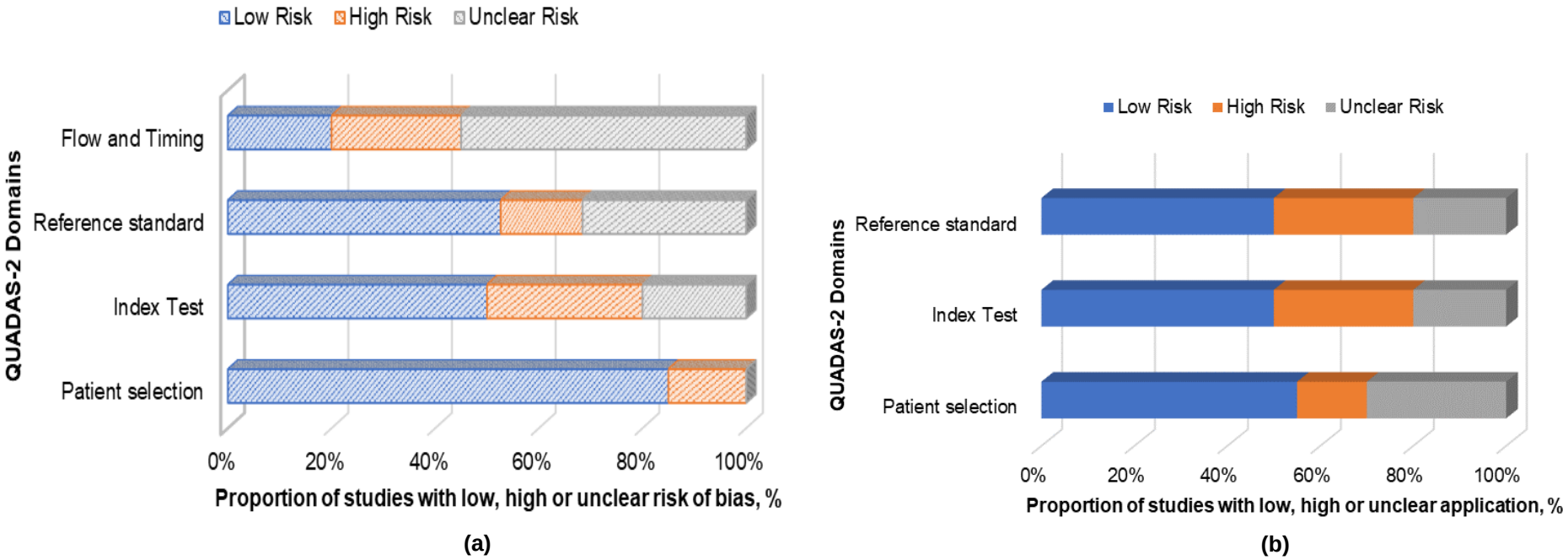
The study designs were based on cohort, exploratory and experimental studies of carcinogenic, neurometabolic, musculoskeletal and hepatic diseases. Most studies investigated the concentration of serum GAGs for the diagnosis of mucopolysaccharidoses type I (Hurler Syndrome), type II (Hunter Syndrome), type III (Sanfilippo Syndrome), type IV (Morquio Syndrome) and type VI (Maroteaux-Lamy Syndrome).

Table 1 - Evaluation of the methodological quality of the articles evaluated in the review according to the QUADAS-2 tool (Quality Assessment of Diagnostic Accuracy Studies).

Study by	Risk of Bias				Applicability Concerns		
	PS	IT	RS	F&T	PS	IT	RS
Amendum PC, et al. (2021)	Low risk	Low risk	Low risk	Unclear risk	Unclear risk	Low risk	Low risk
Bratulic S, et al. (2022)	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Gatto F, et al. (2022)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Gatto F, et al. (2018)	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Herbest ZM, et al. (2022)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Kantarcioglu B, et al. (2022)	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Khan AS, et al. (2018)	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Lin H, et al. (2018)	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Low risk
Martel-Pelletier J, et al. (2017)	Low risk	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk
Sabir E, et al. (2020)	Low risk	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk
ID 001*	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
ID 002*	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
ID 003*	Low risk	High risk	Unclear risk	Unclear risk	Low risk	High risk	Unclear risk
ID 004*	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Unclear risk	Unclear risk
ID 005*	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
ID 006*	Low risk	High risk	High risk	High risk	Low risk	High risk	High risk
ID 007*	Low risk	High risk	High risk	High risk	Low risk	High risk	High risk
ID 008*	High risk	High risk	Unclear risk	High risk	High risk	High risk	Unclear risk
ID 009*	High risk	High risk	High risk	High risk	High risk	High risk	High risk
ID 010*	High risk	High risk	High risk	High risk	High risk	High risk	High risk

Note: PS: Pacient Selection; **IT**: Index Test; **RS**: Reference Standard; **F&T**: Flow and Timing; *Author identification preserved**. Source:** Lenzi LGS, et al., 2023.

Figure 2 - Proportion of studies with (a) risk of bias and (b) applicability of the articles evaluated in the review according to the QUADAS-2 tool.



Source: Lenzi LGS, et al., 2023.

Table 2 - General characteristics of the studies covered in the review.

N.	Author (year)	Number of individuals	% women	% men	Average age (years)	Follow-up time	Design
1	Amendum PC, et al. (2021)	276[1,*]	42,4	56,6	0-2.9 years: 51% 3-4.9 years: 13% 5-9.9 years: 19% 10-14.9 years: 11% 15-19.9 years: 2% 20+ years: 4,0%	-	Retrospective cohort
2	Bratulic S, et al. (2022)	724 sick 536 healthy	47 sick 55 healthy	53 sick 45 healthy	62 sick 60 healthy	-	Retrospective and prospective cohort
3	Gatto F, et al. (2022)	50	34	66	68,5	3 years	Prospective cohort
4	Gatto F, et al. (2018)	175 sick 19 healthy	31	59	60	4 years	Retrospective cohort
5	Herbest ZM, et al. (2022)	60	NA	NA	1,5	3 years	Experimental
6	Kantarcioglu B, et al. (2022)	101	48,5	51,5	63	-	Exploratory cohort
7	Khan AS, et al. (2018)	109	NA	NA	6.24 (0 to 20 years)	-	Exploratory
8	Lin H, et al. (2018)	79	NA	NA	9.1 (1 month to 49 years)	5 years	Retrospective cohort
9	Martel-Pelletier J, et al. (2017)	97	-	100	61	2 years	Exploratory
10	Sabir E, et al. (2020)	368[2] healthy 47 sick	50.3 healthy 46.8 sick	49.7 healthy 53.2 sick	*Healthy:* 1-12 months: 10,3% 1- 2 years: 9,3% 2- 4 years: 14,1% 4- 6 years: 13,6% 6- 10 years: 14,1% 10-16 years 14.1% 16-18 years: 9,5% 18+ years: 15,0% *Sick:* 1 to 15 years	7 weeks	Exploratory

Subtitles: [1]Japanese patients;*1% of people were not discriminated against by gender; [2]Moroccan patients;* **NA:** Not evaluated**.**

Source: Lenzi LGS, et al., 2023.

In the studies, compounds HA, CS and DS were analyzed as the main GAG biomarkers for diseases, mostly from serum samples (plasma and urine), using chromatographic detection techniques associated with mass spectroscopy, spectrophotometric assays or by laser-induced fluorescence capillary electrophoresis analysis (**Table 3**). All authors identified an increased concentration of GAGs in the pathological process of different types of diseases.

Table 3 - Main findings of the studies selected for the review.

Author	Disease	GAG Biomarkers	Sample Type	Detection method	Conclusion
Amendum PC, et al.	MPS (I, II, IIIA, IIIB, IVA, IVB and VII) and Encephalopathy	DS and QS	Plasma	LC-TMS	High levels of serum GAGs have been detected in patients with MPS of various types, viral and non-viral encephalopathy, being a potential biomarker for diseases other than MPS. However, the mechanisms of encephalopathy associated with increased GAGs should still be studied. As well as a validation study of disease groups with a larger sample size.
Bratulic S, et al.	Cancer (breast, bladder, cervix, colorectal, head and neck, small cell lung, prostate); Chronic lymphocytic leukemia; Diffuse glioma; Diffuse large cell lymphoma; Carcinomas (endometrial, ovarian and renal cell); and, neuroendocrine tumor of the small intestine	HA, CS and HS	Plasma and Urine.	Liquid biopsy with UHPLC-MS	The authors observed GAG alterations in all cancers and that five GAG characteristics could extract significant information about the spatial and temporal status of the cancer from the early stage.
Gatto F, et al.	mRCC	CS, HA and HS	Plasma and Urine.	UHPLC-MS	They concluded that changes in plasma- and urine-free GAGs (40%) can be correlated to a progressive disease. Thus, serum-free GAGs are a potential biological marker for MRCC response. However, the understanding of therapeutic problems has yet to be determined.
Gatto F, et al.	nmRCC	CS, HA and HS	Plasma	Capillary electrophoresis with laser-induced fluorescence	The authors concluded that plasma GAGs are highly sensitive for the diagnosis and prognosis of nmRCC.

Author	Disease	GAG Biomarkers	Sample Type	Detection method	Conclusion
Herbest ZM, et al.	MPS-II	HS and DS	Dry blood	Methods of internal and endogenous disaccharides by LC-TMS	The endogenous biomarker method was able to differentiate MPS-II patients from healthy ones.
Kantarcioglu B, et al.	Pulmonary ebullism	Endogenous GAGs	Plasma	Spectrophotometric assay using heparin red and immunoenzymatic serological test	GAGs are related to the pathological process of PE, presenting an increase in the organism. In addition, GAGs correlate with inflammatory biomarkers in patients.
Khan AS, et al.	MPS (II, III, IVA and IVB)	DS, HS and QS	Plasma and Urine.	LC-TMS with enzymatic digestion	Serum GAGs have the potential to diagnose different types of MPS. In addition, the detection method allowed for the separation of GAGs with the same molecular weight and subclasses with specificity and accuracy.
LIN Y, et al	MPS (I, IIA, IIB, III, IV and VI)	DS, HS and QS	Urine	LC-TMS	Urinary GAGs have the potential to diagnose different types of MPs. In addition, the detection method allowed it to be a reliable and sensitive tool in the diagnosis, identification of subclasses and therapeutic monitoring when compared to the spectrophometric assay.
Martel-Pelletier J, et al.	Osteoarthritis	HA	Plasma	Immunoenzymatic serological test	The biomarker HA was effective as a predictive biomarker to assess the inflammation levels of OA patients.
Sabir E, et al.	MPS (I, II, IIIA, IIIB, IVA and VI)	CS	Urinário	Spectrophotometric assay using 1,9-dimethylmethylene blue	Genetic and environmental factors, habitual diet, and socioeconomic status all influence ethnic differences in GAG biomarker concentrations, demonstrating that each population should have specific and updated patterns so as not to lead to an inaccurate interpretation of clinical trial results. In addition, GAGs also decrease with age, presenting a higher reference value in individuals up to 1 year.

Subtitles: **HA:** hyaluronic acid; **nmRCC:** non-metastatic renal cell carcinoma; **LC-TMS:** Liquid Chromatography coupled with tandem mass spectroscopy; **mRCC:** metastatic renal cell carcinoma; **GAG:** glycosaminoglycans; IIA: type 2-moderate; IIB: type II-severe; IVA: type IV-moderate; IVB: type IV-severe; **MPS:** mucopolysaccharidoses; **OA:** osteoarthritis; **CS:** chondroitin sulfates; **DS:** dermatan sulfate; **HS:** heparan sulfate; QS: Keratan sulfate, **UHPLC-MS:** Ultra-high-efficiency liquid chromatography coupled with mass spectrometry. **Source:** Lenzi LGS, et al., 2023.

Multi-cancer Early Detection (MCED) by plasma and urinary free GAG biomarkers (>0.1 µg/mL) was investigated by Bratulic S, et al. (2022) for 14 types of cancers. The authors analyzed 2064 samples using UHPLC-MS. The study showed a sensitivity to any type of low-grade cancer between 41.6-62.3% and specificity of 95%, whose sensitivity could be increased by combining with genomic biomarkers.

The prospective single-center cohort study conducted by Gatto F, et al. (2022) evaluated the correlation of free GAGs of urine and plasma in patients with metastatic renal cell carcinoma (mRCC), whose study design was based on a longitudinal follow-up with the patients every three months. In the study, free GAG was measured in 279 plasma or urine samples (>0.1 µg/mL) using a standardized UHPLC-MS/MS kit and compared to radiological assessments, as free GAG provides information at the molecular level reflecting pathology metabolism.

As a result, the progressive disease was correlated with the increase in the concentration of CSs, whose response to treatment was compatible with the detectable characteristic changes of free GAGs (40%). In addition, through the development of biomarker progression scores in plasma and urinary tests, it is possible to determine progressive disease with a sensitivity and specificity of 94% and 84% for plasma and 92% and 91% for urine, as well as 85% and 97% when combined, being a valid non-invasive diagnostic procedure at the beginning of treatment or after 6-8 weeks.

Prior to this study, Gatto F, et al. (2018) conducted a retrospective design to evaluate the sensitivity and specificity of plasma GAGs for detection of early-stage non-metastatic renal cell carcinomas and prediction of recurrence after surgery. The study consisted of an analysis of data with longitudinal follow-up between 1 and 30 months after surgery. The study then presented for GAGs a sensitivity and specificity of 93.5% and 94.7%, respectively, to discriminate non-metastatic carcinomas from healthy samples. Although the properties of GAGs do not correlate with tumor stage, grade and histology, the results indicated that GAGs have promising potential as a biomarker of the disease. However, the limitations of the study should be taken into account, such as the possibility of changes in GAG concentrations and composition in retrospective sample collections, especially when the “age” of the sample is not known, being an important factor for GAG stability.

GAGs have a high expression in the pathological process of neurometabolic diseases such as mucopolysaccharidosis, encouraging research on biomarkers for different types of the disease. Herbest ZM, et al. (2022) used the internal disaccharide method and endogenous method for analysis of MPS-II biomarkers from the dried blood of newborns and detected through LC-TMS. In the study, the methods were efficient as a screening protocol for the disease, reducing the level of false positives from enzyme assays. However, a study with a larger population of patients must be reproduced to validate the protocol.

Amendum PC, et al. (2021) carry out a retrospective cohort study to investigate GAG biomarkers in the diagnosis of 5 types of MPS; encephalopathy (viral and non-viral), epilepsy; respiratory, renal, developmental disorders; fatty acid, and hepatic metabolism; hypoglycemia; myopathy, and acidosis, through serum samples analyzed by LC-TMS. The largest population analyzed was children between 0 and 9 years old (83%), who had high levels of GAGs in their blood.

Similarly, Khan AS, et al. (2018), explored the levels of DS, KS and HS simultaneously in 4 types of MPS in patients of different ages whose serum samples were analyzed by LC-TMS with enzymatic digestion. The authors observed that biomarkers are able to distinguish the different types of MPS by age according to the concentration of serum GAGs, of which KS was the one with the highest positive correlation in MPS II, IVA and VI. The detection technique used by the authors allowed them to simultaneously investigate the levels of serum GAGs with the same molecular weight and by subclasses, allowing them to differentiate the MPS, diagnose, perform prognosis and evaluate therapeutic efficacy. However, in addition to considering the age of the patient during diagnosis and prognosis, the mechanism related to the increase in KS for these diseases should be investigated.

The efficiency of the LC-TMS technique for the identification and quantification of GAGs was also observed in the study by LIN H, et al. (2018). The study investigated the levels of urinary GAGs in patients who had clinical manifestations of different types of MPS, which observed an increase in DS levels in patients with

hernia, joint stiffness, clawed hands, heart valve disease and hepatosplenomegaly. HS was elevated in patients with intellectual disability and in patients with severe MPS-II, while KS was elevated in patients with hypermobile joints. These data were obtained by the LC-TMS detection technique, which were more accurate and reliable compared to the spectrophotometry of the dimethylethylene blue assay.

In addition to a study with a larger population, the need to determine diagnostic reference values for different ethnicities for inaccurate interpretations in diagnostic test results is emphasized. The study by Sabir E, et al. (2020), for example, demonstrated that the reference values for GAG concentration are different for the Moroccan population when compared to the Spanish and Indian population. This is because ethnic differences in GAG biomarker concentrations can be influenced by environmental and genetic factors, habitual diet, and socioeconomic conditions. In addition, the study showed that the reference concentration of biomarkers decreases with age and should be taken into account for the effectiveness of screening and treatment of patients.

Increased endogenous GAG in the blood can also be observed in the pathology of acute pulmonary embolism. In the exploratory cohort study by Kantarcioglu B, et al. (2022), it was observed that in addition to GAGs increasing during disease, they also correlate with inflammatory biomarkers, whose Spearman correlation ranged from 0.007 to 0.381, assuming a direct or indirect interrelationship between endogenous GAGs, inflammatory biomarkers and blood cell index. However, these interrelationships need to be investigated to understand the functions of blood cells in the process of endogenous release into circulation.

HA has been used as a biomarker of musculoskeletal diseases such as osteoarthritis. In the exploratory study of a randomized controlled clinical trial by Martel-Pelletier J, et al. (2017), HA was used as a plasma biomarker to evaluate the response to OA treatment with GAG-CS in reducing cartilage volume loss, which was able to discriminate the extent of systemic cartilage inflammation. In the experimental study by Nascimento PCH, et al. (2016), samples were collected from 23 patients diagnosed with Dupuytren's disease. Tissue collection was performed through fasciotomy with Bruner-type incisions for electrophoresis analysis, whose GAGs were identified by comparing the electrophoretic migration of the sample with known and purified patterns, as well as quantitative determination of the compounds by optical densitometry. As a result, individuals with the disease had higher expression of DS and CS.

Yang YM, et al (2019), studied the mechanism of HA deposition in the pathological process of liver fibrosis, since this GAG is also a biomarker of cirrhosis. The mouse study observed that HA is degraded into lower molecular weight (pro-inflammatory) species through HA synthase 2 (HAS2). HAS2 expression is influenced by hepatic stellate cells (HSCs), which are regulated by the fibrotic mediator TGF-β (transforming growth factor beta), resulting in invasive phenotypes of HSCs that migrate to inflammatory and fibrotic sites and destroy the basement membrane. Thus, the synthesis of HA promoted by HAS2 increases the concentration of serum HA and activates hepatic stellate cells, consequently inducing inflammation and lesions in liver tissue that characterize liver fibrosis.

In summary, the results of all studies have demonstrated the direct relationship of serum GAG levels with the diseases, it being possible to detect and quantify them mainly by chromatographic techniques. However, it is noteworthy that the present systematic review presented limitations in relation to the methodological quality of potential articles, and it is necessary to discard many studies for narrative synthesis. Thus, it is impossible to infer that GAGs should be incorporated into predictive protocols in diagnoses, since it was not possible to evaluate experimental studies with larger sample sizes and that did not use a retrospective cohort design, since the retrospective cohort has limitations in relation to the availability of clinical data of the subjects considered for the study.

FINAL REMARKS

This systematic review made it possible to identify advances in the use of plasma and urinary GAG biomarkers for various types of diseases, demonstrating their promising potential as a non-invasive diagnostic tool. In addition, it was possible to verify the need to understand the association of GAGs with the pathological

processes of the diseases, so that the biomarkers have, in addition to their predictive value, a prognostic and discriminatory value.

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