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REVIEW ARTICLE

Biomarkers in high-grade gliomas: A systematic review

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Abstract

Background: *Biomarkers are a subcategory of clinical signs that can be measured and reproduced with precision and influence to predict outcome. Tissue, cells, and fluid conform the biological process. Biomarker usefulness is to determine and specify illness predisposition counting with variability and validity. Process systematization can reduce operative costs. To date, four major biomarkers have been described for high-grade gliomas: 1p/19q deletion, O6-methylguanine-DNA methyltransferase (MGMT) promoter mutation, IDH1/IDH2 mutation, and microRNA. In this manuscript we present a systematic review according to the MOOSE protocol to establish the bases to describe the utility of biomarkers in high-grade tumors.* **Materials and methods:** *We conducted a systematic review of the literature according to the PRISMA and MOOSE guides of all the published data from January 2004 to November 2014 with the key words: "biological markers" and "glioblastoma" that included OR and 95% CI. One researcher performed data extraction and analysis.* **Results:** *A total of 169 articles were found in three major medical search engines: PubMed (42), Embase (30) and Ovid (96).* ***Conclusion:*** *Biomarkers are tools designed for early detection of specific illnesses such as high-grade glioma. Lack of methodological standardization slows down the speed of progress.* (Gac Med Mex. 2016;152:76-81)
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Introduction

A biomarker is defined as a subcategory of medical signs that can be accurately measured and reproduced. Overall, biomarkers are characteristics that are objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. The World Health Organization defines them as any substance, structure, or process that can be measured in the body (or its products) and influence or predict the incidence of outcome or disease[1].

The use of biomarkers in basic and clinical research has turned into an essential axis of clinical trials and some of them have been accepted as valid. Specific biomarkers have been characterized and have been repeatedly shown to provide predictive information on the outcome of a variety of treatments.

By definition, biomarkers are objective instruments and quantify the characteristics of biological processes in tissues, cells or fluids. However, they do not necessarily correlate with a patient's experience or sense of wellbeing. The advantage offered by the use of biomarkers as surrogate endpoints is evident in survival studies, since they can provide researches evidence about the

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safety and efficacy of a treatment. In addition, it allows researchers to reduce the number of study subjects.

To identify surrogate endpoint biomarkers, their relevance and validity are required to be established. Relevance of a biomarker is defined as the ability to provide clinically relevant information on the question of interest. Validity refers to the need to characterize a biomarker's effectiveness as an endpoint[2].

In practice, biomarkers include auxiliary tools and techniques to identify the cause, diagnosis, progression, regression and outcome of a disease[1].

In the central nervous system, there is a wide array of techniques to obtain information on the state of the brain, either healthy or ill. Biological media measurement can be direct (blood, cerebrospinal fluid [CSF]) or indirect through imaging studies that do not require for a biological sample to be obtained but that can change its composition[1].

Their use in research has increased with the need to obtain direct systems to measure exposure in a disease with the least possible bias and with the potential to provide information on exposure. Molecular biomarkers offer the clinical and research physician sufficient information to understand the spectrum of neurological diseases[1].

Biomarker classification[3]

- Type 0: measures the natural history of a disease and must be correlated with clinical indices' time of expression.
- Type 1: has effect on the intervention.
- Type 2: these are surrogate endpoint markers.

Exposures, modifiers and risk factors

When there is the suspicion of a disease as a consequence of exposure, the investigators analyze exposure factors. External exposure is measured as the concentration of toxin in the subject's immediate environment. Questionnaires identify historical exposure, but direct measurement (air, water, soil, food) of the dose, which offers the most accurate information, is required. When these toxins are identified in tissues or fluids, they become biomarkers. A biomarker that measures the biological effect of the dose is usually related to the amount of toxin or chemical substance lodging in the target organ[1].

Genetic susceptibility

Environmental influence on disease can be analyzed through epidemiological studies. Variants in alleles and polymorphisms may be related, but they are not determining. This biomarker exists prior to disease or outcome and is independent from other exposures[1].

Intermediate biomarkers

These biomarkers are strongly related with the disease through pathways leading to the cause. They can be dependent on other unknown cause and can be related to an already identified exposure or represent an alteration caused by disease-entailed exposure[1].

Biomarkers of disease

The potential use of these biomarkers includes the capacity to identify an individual destined to suffer from certain disease or being at its preclinical phase, as well as to reduce heterogeneity of disease in clinical trials or epidemiological studies; they reflect the natural history of the disease, including the induction, latency and detection phases, and the target in a clinical trial[1].

Variability

It occurs individually and independently if the biomarker represents exposure. Intersubject variability is the consequence of the amount of external exposure or the form whereby toxins are metabolized. Intrasubject variability is related to laboratory errors or other conditions. There is also group variability, which usually is sought as a prognostic indicator[1].

Validity

It has to be considered and measured as any other variable. Laboratory errors can cause mistakes in the classification and identification of causative agents. Pilot studies have to be run to establish reasonable reliability. The degree of agreement has to be used by means of the κ analysis for binary or dichotomous variables and the intraclass correlation coefficient to assess test-retest agreement and consistency[1].

In the validity assessment of a biomarker, which is complex, three fundamental aspects should be contemplated:

- Validity contents: it demonstrates the degree of accuracy of a biomarker to reflect the biological phenomenon under study.
- Construct validity: it represents other characteristics of the disease; for example, another biomarker or disease manifestation.

– Validity criteria: they demonstrate a biomarker's correlation degree with a particular disease; usually, this validity is measured through sensitivity, specificity and predictive values (positive and negative).

Practical considerations: measurement errors

This type of errors decrease validity towards the disease, and can occur inside or outside the laboratory. They can occur during the material's collection or the transportation of specimens to the laboratory, with an impact on the biomarker's measurement; inadequate storage can affect the result as well. Training of personnel is essential. A manual of standard operating procedures with precise details on storage, monitoring and maintenance record can help to decrease this type of errors[1,4].

Confounders

Confounders are all those factors that alter a biomarker's measurement. They can be internal (e.g., patient's weight) or external (e.g., materials used in the laboratory). Biomarkers' individual properties must influence on interpretation in order for them to be included in the research. Biological stability is particularly important if preserving a biomarker for a long time is intended. The storage of tissues or extracted genetic material is expensive, and the required storage time interval should be evaluated[1].

Costs

Costs should be part of the decisions in biomarker research. In clinical trials with small samples perhaps costs are not significant, but in an epidemiological study with large recruitment of patients elevated costs can be reached, unless the laboratory already has an automated system in place, which can even reduce costs by volume[1].

Tissues and fluids candidate to be biomarkers

Nearly any body tissue or fluid sample is a good candidate to become a biomarker. In some specific tests, such as DNA methylation, they can be studied using paraffin blocks. For RNA extraction, the preservation process requires more care. In most primary tumors, information is obtained from a biopsy, but for early detection of cancer and other non-transmittable diseases, it can be obtained by means of body fluids such as peripheral venous blood, oral cavity epithelium or saliva, urine, feces, bronchial aspirate and, in some cases, muscle and adipose tissue[5,6].

Systematic reviews and meta-analyses are useful tools to summarize current evidence on a research question in order to improve its usefulness in the research area. They have to be presented in a clear and truthful form[7]. For this reason, this work is intended to gather the necessary information to set the basis for the search of biomarkers for primary tumors of the brain, which represent the most common primary tumor of the central nervous system. Survival is estimated to reach no more than 3 years, with an average of 18 months[8]. These malignancies affect nearly 20,000 patients of the USA every year[9].

Biomarkers in high-grade gliomas

IDH1/IDH2 mutation

High-grade gliomas sequencing has identified mutations in the genes that codify for IDH1 and IDH2. IDH mutation is specific to high-grade gliomas; the IDH2 mutation has been found in acute myeloid leukemia. The mutation has been correlated with gliomagenesis early phase[10]. This IDH1 mutation is found in 80% of grade II and III gliomas according to the WHO classification and in 10% of primary glioblastomas. The IDH2 mutation has been described in gliomas, but less frequently[10,11].

1p/19q

The loss of heterozygosity by translocation of the centromere on chromosome 1p/19q has been identified as an atypical marker for primary tumors of the central nervous system, with a frequency of 80% for low-grade gliomas, 60% for anaplastic oligodendrogliomas, 30-50% for oligoastrocytomas, 30% for anaplastic oligoastrocytomas and 10% for high-grade gliomas[10].

Methylation of the MGMT-promoting site

The *MGMT* gene codifies for a DNA-repairing protein by removing the alkyl groups from the O6 position of guanine as a result from alkylating chemotherapy agents, such as temozolomide[12-14]. The process to identify the methylation status is carried out by methylation-specific PCR by means of bisulfite conversion (non-methylated conversion to uracil)[8,15,16]. MGMT is a repair enzyme that removes the alkylating agent from the guanine O6 position, which causes mismatch during cell replication, inducing apoptosis, which entails an increased survival[17].

Table 1. Description of articles employed for the review

Author (year)	Cases	Country	Histology
Laxton et al. (2013)[40]	288	United Kingdom	Glioblastoma multiforme
Collet et al. (2011)[41]	5	France	Glioblastoma multiforme
Medina Villaamil et al. (2011)[42]	28	Spain	High-grade glioma Low-grade glioma
Yakut et al. (2007)[43]	37	Turkey	High-grade glioma
Demirci et al. (2012)[44]	44	Turkey	High-grade glioma
Jha et al. (2010)[45]	101	India	High-grade glioma
Ma et al. (2008)[46]	72	Germany/China	High-grade glioma Low-grade glioma

Micro-RNA

Micro-RNAs are short, non-coding RNA portions (19-24 nucleotides) that post-transcriptionally regulate a gene's expression. They act as key regulators of multiple biological processes, such as cell proliferation, cell differentiation and apoptosis. They can act as tumor suppressors or oncogenes[6,8,14,16,18-21].

Material and methods

This is a literature systematic review according to the PRISMA guidelines proposed by Liberati et al., as well as the MOOSE guidelines for analysis of systematic reviews and meta-analyses[22,23].

Primary search

All relevant review articles exclusively published in English in PubMed, Medline and Embase, from January 2004 through November 2014, containing 95% OR and 95% CI, were included. The search used the following search terms (MeSh): *biological markers and glioblastoma.* The articles were restricted to studies in humans and adult population.

Inclusion and exclusion criteria

All those observational articles identifying biomarkers with high-grade gliomas, regardless of the size of the population, with the following characteristics, were included: 95% OR and 95% CI, the studies should be unrelated, and in the case of articles overlapping population resources, the most recent article or the one with the largest population was chosen.

Data extraction

A single investigator extracted the information using a standardized sheet for quality assessment. In case of discrepancy, consensus was reached though a second independent investigator. The following was extracted out of each article: main author, publication year, country of origin, number of patients, OR and 95% CI (Table 1).

Quality control

To assess methodological quality of the research, the ordinal scale system proposed by Steels et al. was used[24].

Statistical method

The results regarded as being significant had a p-value < 0.05 comparing the distribution by groups. Since the study was about a literature review, no parametric or non-parametric tests were used.

Results

Characteristics of included articles

The purpose of this work was to identify the scientific basis to consider the use of biomarkers in high-grade gliomas. A flow-chart was created (Fig. 1) to identify eligible works according to the inclusion criteria using MeSH terms. Forty-two articles were found in PubMed, 30 in Embase and 96 in Ovid[25-24]. Of 168 identified articles, only 6 met the search criteria for the review (main author, publication year, country of origin, number of patients, OR and 95% CI).

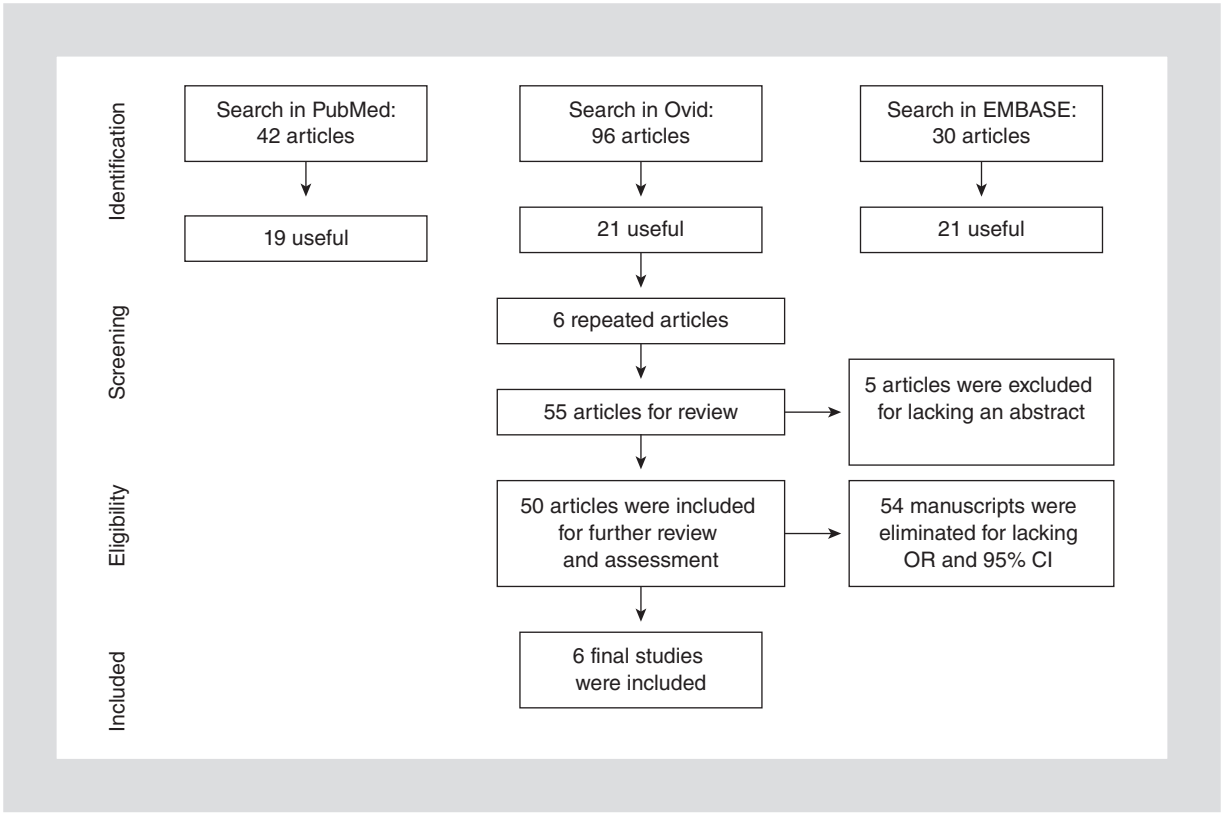

Figure 1. *Flow-chart for the retrieval and assessment of articles for analysis.*

In the first phase, 6 papers were eliminated because they were duplicated, 5 lacked an abstract and 54 articles did not contain a results section; thus, only 6 articles were finally further analyzed. No paper of the American continent was identified. The publication with the largest population had 288 patients, all corresponding to glioblastoma histology.

Discussion

Biomarkers represent a challenge to improve the development of diagnostic and therapeutic methods for different conditions, with particular interest in high-grade gliomas. Understanding measurable biological processes and their clinical meaning is indispensible to broaden the choice of treatment for diseases. Over the past 30 years, large clinical trials have been conducted to identify biomarkers in major diseases, such as heart conditions and cancer. Research on this field has been encouraged in basic and clinical science.

In high-grade gliomas, the deletion of 1p/19q has been investigated as a diagnostic and prognostic marker by means of in situ hybridization techniques. There are groups that use other methods to detect the deletion, such as PCR with microsatellites, which shows larger proportions of 1p deletions. However, the impact of 1p/19q loss is not consistent in the literature, and the best diagnostic method for this test is fluorescent in situ hybridization (FISH) with OR 0.39 (0.25-0.60). The loss of 1p and 19q resulting from centromeric translocation was initially associated with sensitivity to chemotherapy with alkylating agents and subsequently was regarded as a response to radiotherapy. The loss of 1p can predict survival in low-grade gliomas.

Methylation of the MGMT-promoting site has been observed to have predictive capacity for treatment response and survival in patients with high-grade gliomas. The survival difference between patients with MGMT high and low expression is 8 versus 29 months ($p = 0.0002$)[35]. IDH1 mutation is a reliable marker to distinguish between primary glioblastomas, secondary glioblastomas and anaplastic glioblastomas.

Survival has been favorable, as high as 31 months for patients with IDH mutation, with an OR of 0.33 and 95% CI of 0.25-0.42. In a group of 301 patients, the best prognostic factors were IDH mutation, MGMT-promoting site methylation and age[36]. Most studies on MGMT methylation have been performed with extraction from paraffin blocks; however, more reliability

on results has been shown when the sample is freshly processed[36,37]. Biomarkers can be obtained in an outpatient setting through a peripheral blood or CSF sample[21,38,39].

Conclusions

Biomarkers are promising tools for early detection of conditions such as high-grade glioma. However, lack of standardization in methodological procedures has delayed its advance and replication of clinical trials to obtain clinical validity that allows establishing an accurate prediction of the outcome for patients with cancer, particularly with high-grade gliomas.

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