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ORIGINAL PAPER

Biomarkers for Graft-Versus-Host Disease in Hematopoietic Stem Cell Transplant Patients

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ABSTRACT

Introduction: One of the therapeutic strategies for hematological malignancies is hematopoietic stem cell transplantation (HSCT). However, a common and severe post-transplantation complication is graft-versus-host disease (GVHD), primarily mediated by the donor's immunocompetent cells, particularly T lymphocytes. **Objectives:** This study aims to explore potential biomarkers for the prognosis and diagnosis of GVHD following HSCT. **Methods:** This integrative review was conducted using the PubMed and Google Scholar databases. The research question was structured and refined using the PICO (Population, Intervention, Comparison, and Outcome) method, resulting in the guiding question: "Can biomarkers help in the diagnosis, prognosis, and treatment of GVHD?" The descriptors used were: "graft versus host disease," "hematopoietic stem cell transplant," and "microRNAs," combined with the Boolean operator "AND." These searches resulted in 1,942 articles, from which 33 were selected based on the English language and relevance to the main objectives of the study, after reviewing their titles and abstracts. **Results:** Among the biomarkers examined, microRNAs emerge as the most promising due to their stability and ease of extraction from body fluids. While some progress has been made in identifying such markers in recent years, further research is necessary to enhance understanding and ensure their practical application in clinical settings. **Conclusion:** The importance of finding biomarkers, considered more enlightening and less susceptible to bias compared to traditional diagnostic methods that can predict the risk of GVHD, is demonstrated. Although there are strategies to reduce the risk of developing this complication, such as T lymphocyte depletion, patients undergoing these therapies have a high risk of relapse since the effect called "graft-versus-leukemia" is compromised. Although the search for such markers has achieved some success in recent years, further studies are needed to provide additional clarification and ensure applicability in daily clinical practice. An ideal biomarker should be easily measurable, highly sensitive, and specific, enabling early detection using readily available samples.

Descriptors: Biomarkers; MicroRNAs; Graft-Versus-Host Disease.

Biomarcadores para Doença do Enxerto Contra Hospedeiro em Pacientes Transplantados com Células-Tronco Hematopoiéticas

RESUMO

Introdução: Uma das estratégias terapêuticas para neoplasias hematológicas é o transplante de células-tronco hematopoiéticas. No entanto, uma complicação comum e grave pós-transplante é a doença do enxerto contra hospedeiro, mediada principalmente pelas células imunocompetentes do doador, particularmente os linfócitos T. **Objetivos:** Este estudo tem como objetivo explorar potenciais biomarcadores para o prognóstico e diagnóstico da doença do enxerto contra hospedeiro após o transplante de células-tronco hematopoiéticas. **Métodos:** Trata-se de uma revisão integrativa realizada nas bases de dados PubMed e Google Acadêmico. A questão de pesquisa foi estruturada e refinada utilizando o método PICO (*Population, Intervention, Comparison, and Outcome*), resultando na seguinte questão norteadora: "Os biomarcadores podem auxiliar no diagnóstico, prognóstico e tratamento da DECH?" Foram utilizados os descritores: "doença do enxerto contra hospedeiro", "transplante de células-tronco hematopoéticas" e "microRNAs" combinados com o operador booleano "AND". Essas buscas resultaram em 1.942 artigos, dos quais 33 foram selecionados com base no idioma inglês e na relevância para os objetivos principais do estudo, após revisão de seus títulos e resumos. **Resultados:** Entre os biomarcadores examinados, os microRNAs surgem como os mais promissores devido à sua estabilidade e facilidade de

extração de fluidos corporais. Embora nos últimos anos tenha havido algum sucesso na identificação de tais marcadores, mais pesquisas são essenciais para melhorar a compreensão e garantir a sua aplicação prática em ambientes clínicos. **Conclusão:** Demonstra-se a importância de encontrar biomarcadores, considerados mais esclarecedores e menos suscetíveis a vieses em relação aos métodos diagnósticos tradicionais, que possam prever o risco da doença do enxerto contra hospedeiro. Embora existam estratégias que reduzam o risco de desenvolvimento dessa complicação, como a depleção de linfócitos T, os pacientes submetidos a essas terapias apresentam alto risco de recidiva, uma vez que o efeito denominado "enxerto *versus* leucemia" fica comprometido. Embora a busca por tais marcadores tenha alcançado algum sucesso nos últimos anos, mais estudos são necessários para fornecer maiores esclarecimentos e garantir a aplicabilidade na prática clínica diária. Um biomarcador ideal deve ser facilmente medido, ter alta sensibilidade e especificidade e permitir a detecção precoce a partir de amostras prontamente disponíveis.

Descritores: Biomarcadores; MicroRNAs; Doença Enxerto Hospedeiro.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a potentially curative therapeutic strategy for hematological malignancies.[1] This modality involves the administration of healthy hematopoietic stem cells to patients with dysfunctional bone marrow. It can be categorized as syngeneic transplantation, which occurs between identical twins, autologous transplantation, where bone marrow products are removed from the patient and reinfused after purification, and allogeneic transplantation (allo-HSCT), which encompasses grafts performed between individuals of the same species. Syngeneic transplantation is recognized as the safest due to its high genetic compatibility, thus minimizing the possibility of rejection. However, its application is restricted due to the limited occurrence of identical twins. Allo-HSCT can be subdivided into related allo-HSCT, which involves donation between relatives, and unrelated allo-HSCT, which includes unrelated or non-family donors. There is also haploidentical transplantation, an alternative for patients who need a transplant but do not have donors with a fully compatible human leukocyte antigen (HLA).[2]

A frequent and serious complication after HSCT, mediated by the donor's immunocompetent cells – primarily T lymphocytes – is graft-versus-host disease (GVHD). This complication can occur due to the presence of incompatible minor antigens that have not been previously detected, even if there is complete HLA compatibility. Although GVHD represents an important cause of mortality among transplant patients, these T cells mediate a potent beneficial anti-tumor effect, known as graft-versus-leukemia (GVL), which reduces the chances of disease recurrence. One way to reduce the incidence of GVHD would be the depletion of T lymphocytes, however, patients undergoing this technique have lower GVL effect results and a high risk of recurrence.[3] Therefore, predicting the risk of GVHD after HSCT using biomarkers would be an extremely useful alternative resource.[4]

Since GVL diagnosis is still primarily based on clinical symptoms and treatment may be ineffective in some patients, the present study aims to review and update knowledge about this complication in transplant patients, emphasizing research into the existence of biomarkers that can contribute to the development of more efficient and safe protocols and the reduction of failures in HSCT.

METHODS

The present study consists of an integrative literature review aimed at investigating potential biomarkers useful for the prognosis and diagnosis of GVHD following HSCT. The research question was structured and refined using the PICO method (Population, Intervention, Comparison, and Outcome), defined as follows:

- Population: patients diagnosed with GVHD;
- Intervention: use of biomarkers to predict risk, diagnose, or monitor the response to treatment;
- Comparison: comparison among biomarkers;
- Outcome: relevance and efficiency of biomarkers in managing this disease.

Based on this framework, the guiding question was: "Can biomarkers assist in the diagnosis, prognosis, and treatment of GVHD?"

Searches were conducted in databases indexed in digital libraries such as PubMed and Google Scholar from June 2023 to November 2024, using the following descriptors: "graft-versus-host disease," "hematopoietic stem cell transplantation," and "microRNAs," combined with the Boolean operator "AND." These searches resulted in two articles from PubMed and 1,940 articles from Google Scholar. From these, 33 articles were selected based on their English language and relevance to the study's

main objectives, after a review of their titles and abstracts. Priority was given to studies published within the last 15 years. Eight of the selected works were used to prepare Table 1, which summarizes the main studies on potential GVHD biomarkers.

Table 1. Main biomarkers investigated for diagnosis, prognosis and risk for GVHD and the main results found.

Biomarker	Condition	Clinical importance	Reference
CK-18	Acute hepatointestinal GVHD	Increase in serum levels in patients with hepatointestinal aGVHD, 7 to 14 days before symptoms appear.	Sauer et al.[20]
sC5b9	Thrombotic microangiopathy (TA-TMA) in patients with high-grade aGVHD	sC5b9 showed considerably higher mean levels in TMA cases at the onset of aGVHD.	Li et al.[21]
CD146, CD31, and CD140-y	GVHD	CD146 was associated with a 60% increased risk, whereas CD31 and CD140-y were associated with a reduced risk of approximately 40 and 60%, respectively, for developing GVHD.	Lia et al.[23]
hsa-miR-29c-3p, hsa-miR-374b-5p, and hsa-miR-630	cGVHD	Levels of hsa-miR-630 and hsa-miR-374b-5p were 4.1- and 2.7-fold lower, respectively, while hsa-miR-29c-3p levels were 5.8-fold higher in patients with cGVHD.	Lacina et al.[25]
miR-365-3p, miR-148-3p, and miR-378-3p	cGVHD	A highly specific 3-miRNA signature for cGVHD was developed.	Reikvam et al.[28]
miR-34-5p and miR-148a-3p	Predicting response to therapy for GVHD	The miRNAs miR-34-5p and miR-148a-3p present high levels in patients with GVHD before extracorporeal photopheresis and significantly decreased after this therapy.	Montoya et al.[29]
CXCL9	Severe cGVHD	The increase in CXCL9 at the onset of cGVHD preceded the development of severe cGVHD.	Giesen et al.[30]

Source: Elaborated by the authors.

HSCT

HSCT has become an established method for treating various hematological malignancies, offering the potential for cure and being the most widely used cellular immunotherapy.[5] Thus, HSCT remains a standard of care for different subtypes of leukemia. In 2020, in the United States, around 5,200 allo-HSCTs were performed in patients with leukemia, out of a total of 22,000 HSCTs.[6] The HSCT modalities and their respective cell sources are described in Table 2.

Table 2. Main HSCT modalities.

HSCT modality	Stem cell source	Donor
Autologous HSCT	Bone marrow Peripheral blood	Patient himself
Syngeneic HSCT	Bone marrow Peripheral blood	Identical twin
Allogeneic HSCT (allo-HSCT)	Bone marrow Peripheral blood Umbilical cord blood	Related: familiar Unrelated: anyone with no family ties to the patient
Haploidentical HSCT	Bone marrow Peripheral blood	Usually first degree family member with HLA at least 50% incompatible

Source: Elaborated by the authors.

According to Koreth et al.,[7] the results of allo-HSCT for acute myeloid leukemia in first complete remission were better compared to high doses of chemotherapy and autologous transplantation for intermediate- and high-risk patients. However, this method is not recommended for low-risk patients.[7]

The availability of compatible donors is one of the main challenges for performing allo-HSCT.[8] Therefore, using an HLA-haploidentical donor, that is, a related donor who shares an HLA haplotype, is an alternative for patients undergoing treatment for hematological malignancies who require a transplant.[9] Haploidentical HSCT (haplo-HSCT) presents its main advantage as the almost universal availability of donors; however, it is accompanied by bidirectional alloreactivity that results in high incidences of graft failure, GVHD, and mortality unrelated to relapse.[8]

The intrinsic antileukemic effect of GVL from HSCT depends on the genetic disparity between donor and recipient; however, as a result, it is closely associated with GVHD,[10] a significant and relevant complication that can occur after the transplantation

procedure. It is essential to carefully monitor and manage this complication and the patient's immunological reconstitution to ensure the best post-transplant outcomes.[3]

GVHD

GVHD is a systemic syndrome resulting from the activation of donor T lymphocytes by recipient histocompatibility antigens. This complication of HSCT is classified into acute GVHD (aGVHD) and chronic GVHD (cGVHD) forms, according to the time to manifestation, clinical findings, and the immunological mechanisms involved. In this context, aGVHD is the main fatal complication during the first months after HSCT, while cGVHD is related in the long term to increased morbidity and reduced quality of life for patients.[11]

aGVHD is the main factor in mortality after HSCT and occurs in almost 50% of patients who receive a transplant.[12] The development of the acute form is characterized by clinical manifestations in the skin, liver, and gastrointestinal tract, including rashes, elevated bilirubin levels, and diarrhea, respectively. However, there is evidence that aGVHD can affect other organs where damage is less apparent or more difficult to differentiate from drug toxicity, such as the central nervous system, ovaries and testes, bone marrow, lungs, thymus, and kidneys.[13]

cGVHD is characterized by heterogeneous and pleomorphic symptoms, with patients typically presenting more than three affected organs. The treatment of cGVHD is based on the prolonged use of immunosuppressive agents, impacting the quality of life of transplant recipients. Furthermore, although prophylaxis strategies to reduce this complication have been studied, their effect on patients' immunological reconstitution remains a challenge.[14]

The main risk factor that predisposes transplant patients to developing GVHD is HLA disparity,[15] and its diagnosis is based mainly on clinical manifestations, which can be supported by tissue biopsies to help differentiate from other conditions that mimic GVHD, including drug reactions and viral infections such as hepatitis and colitis.[16]

Biomarkers for GVHD

Biomarkers can be conceptualized as genetic, immunological, and biochemical elements that correlate with the manifestation of pathological processes. In neoplastic diseases, these variables are related to the activity or remission of the pathology, helping to direct treatment.[17]

Compared to traditional methods for diagnosing GVHD, such as tissue biopsy, biomarkers are considered more enlightening and less susceptible to bias, making their use possible to establish an early diagnosis.[18] Since invasive techniques like tissue biopsies may not always reveal sufficient histopathological characteristics to distinguish the etiologies of dysfunctions after HSCT, the concern regarding serum biomarkers in GVHD has increased significantly in the last decade.[19]

Among the substances that demonstrated positive correlations with clinical results of GVHD are cytokines, cytokine receptors, and T cell surface markers. The first systemic biomarkers validated for aGVHD were interleukin (IL)-2Ra, tumor necrosis factor (TNF)R-1, IL-8, and hepatocyte growth factor (HGF). In addition to markers of systemic inflammation, proteins associated with tissue damage have been identified, such as elafin, an elastase inhibitor characteristic of cutaneous GVHD.[19]

In 2018, Sauer et al.[20] correlated cytokeratin 18 (CK18), a product of apoptotic manipulation, with hepatointestinal aGVHD. The authors observed a serum increase in this protein 7 to 14 days before the manifestation of clinical symptoms of hepatointestinal aGVHD, guiding the use of this specific biomarker in the prediction and diagnosis of this condition. Furthermore, studies are also looking for biomarkers for possible complications in patients predisposed to having aGVHD. Thus, Li et al.,[21] through a case-control study with 208 participants, investigated biomarkers for transplant-associated thrombotic microangiopathy (TA-TMA), a condition that presents a predisposing factor to high-grade GVHD. In this scenario, among the markers analyzed, sC5b9 was considerably higher in cases of TMA compared to cases without TMA at the beginning of aGVHD. This elevation occurred approximately 2 weeks after transplantation, in cases of TMA, and remained over time.[21]

Extracellular vesicles (EVs) are also important biomarkers, as their removal from biological fluids requires relatively non-invasive procedures.[22] In this context, Lia et al.,[23] in a study with 41 patients with multiple myeloma undergoing allogeneic HSCT, observed a correlation with three biomarkers expressed on the surface of EVs. The membrane protein CD146, also known as MCAM, was associated with a 60% increased risk of developing GVHD, while the proteins CD31 and CD140-γ were associated with a reduced risk of about 40 and 60%, respectively.

Although several studies propose validated biomarkers for aGVHD, biomarkers for cGVHD are still scarce due to a greater variety of manifestations that differentially influence the prognosis, the longer course of the manifestation, and the lack of sufficient patients for multicenter trials.[24] In this sense, Lacina et al.[25] investigated the role of microRNAs (miRNAs) as biomarkers for cGVHD and found that they were differentially expressed in these patients. MiRNAs are small endogenous non-coding single-stranded RNA molecules, composed of 19 to 25 nucleotides, which have important regulatory effects on gene expression at the post-translational level.[25] These microparticles act as regulators by binding to the 3' untranslated region of messenger RNAs, allowing the reduction of protein levels of their target genes.[18]

MiRNAs were initially associated with cancer due to their location close to chromosome 12 breakpoints and the deregulation of their expression levels in several malignancies.[26] Thus, miRNAs from malignant tissue are up or downregulated compared to healthy tissue, being considered oncogenes or tumor suppressors, respectively.[27]

In recent years, miRNAs have proven to be important in the pathogenesis of neoplasms and hematological malignancies, and their potential usefulness as biomarkers in HSCT complications, such as the development of GVHD, is being investigated.[4]

Reikvam et al.[28] also investigated the role of miRNAs in the pathophysiological process of cGVHD. The study included 79 allotransplant patients and analyzed the serum miRNA profile. Among the study participants, 50 showed symptoms of cGVHD in the follow-up carried out for 1 year after HSCT, with five different miRNAs being elevated in the serum of these patients: miR-365-3p, miR-148-3p, miR-122-5p, miR-378-3p, and miR-192-5p. Furthermore, the miRNAs miR-365-3p, miR-148-3p, and miR-378-3p were demonstrated to be potential diagnostic biomarkers for cGVHD in a subsequent analysis, which developed a highly specific signature of these miRNAs.[28]

Furthermore, Montoya et al.[29] studied the change in miRNAs expression during extracorporeal photopheresis, an immunomodulatory therapy performed in patients with aGVHD or cGVHD, aiming to analyze whether these biomarkers could predict the response to this treatment. Thus, in three different cohorts, peripheral blood samples were collected before therapy administration and after 6 months of treatment. The study demonstrated that the miRNAs miR-34-5p and miR-148a-3p presented high levels in patients with GVHD before extracorporeal photopheresis and significantly decreased after this therapy. Therefore, these miRNAs are potential biomarkers for monitoring responses to this therapy treatment.[29]

Also studying cGVHD but investigating proteins as biomarkers, Giesen et al.[30] measured the CXCL9 protein in the serum of 480 patients who survived at least 6 months after allogeneic HSCT. The results demonstrated that serum CXCL9 levels measured 100 days after HSCT were not associated with the severity of subsequent cGVHD episodes, but its levels at the onset of non-severe cGVHD symptoms predicted the development of severe cGVHD later. Thus, the authors suggested that CXCL9 levels at the onset of cGVHD could help predict severe disease courses and optimize the personalized administration of immunosuppressive therapy.[30]

Table 1 presents a summary of the main biomarkers investigated for the diagnosis, prognosis, and risk of GVHD, along with the main results found.

CONCLUSION

GVHD represents a significant cause of mortality after HSCT. However, it is important to emphasize the relevance of HSCT in the GVL effect, resulting from the interaction between donor T lymphocytes and residual tumor cells from the host, which reduces the chances of disease recurrence. Although there are strategies that reduce the incidence of GVHD, such as T lymphocyte depletion, patients undergoing these strategies have a high risk of recurrence, as the GVL effect is compromised. Thus, the ideal condition would be to find biomarkers that could predict the risk of GVHD, to avoid HSCT complications and provide more efficient and safer treatments for patients with leukemia.

Therefore, studies on the pathogenesis of GVHD allow us to better understand biomarkers, which are considered more enlightening and less susceptible to bias than traditional diagnostic methods. Of all the biomarkers studied, the most promising seem to be miRNAs, since they have advantages related to their high stability and the ease of obtaining them from body fluid samples.

Although the search for such markers has achieved some success in recent years, more studies are needed to provide further clarification and ensure applicability in daily clinical practice. An ideal biomarker should be easily measurable, have high sensitivity and specificity, and allow early detection from readily available samples.

CONFLICT OF INTEREST

Nothing to declare.

AUTHOR'S CONTRIBUTION

Substantive scientific and intellectual contributions to the study: Costa IBR, Melo ACV, Leão MVP, Callera F; **Conception and design:** Costa IBR, Melo ACV, Leão MVP; **Data analysis and interpretation:** Costa IBR, Melo ACV; **Article writing:** Costa IBR, Melo ACV; **Critical revision:** Leão MVP, Callera F; **Final approval:** Costa IBR.

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