Usefulness of digestive biopsy in the diagnosis of graft-versus-host disease

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ABSTRACT

Introduction. Graft-versus-host disease (GVHD) is a frequent complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT), with high morbidity and mortality rates. Intestinal clinical signs are unspecific, which means differential diagnosis with infections and drug-related etiology should be carried out. Even though intestinal biopsy is widely considered as the gold standard technique, there is no consensus as to which sampling method is best.

Objective. To assess the results of the biopsy techniques used in patients with suspected intestinal GVHD.

Materials and methods. A retrospective study of patients with suspected intestinal GVHD undergoing allo-HSCT from 2010 to 2019 was carried out. They were assessed through digestive biopsy – esophagogastroduodenal biopsy (upper GI endoscopy – UGIE) or rectal biopsy (colonoscopy or direct biopsy). Quantitative variables, expressed as median and interquartile range, and qualitative variables, expressed as absolute frequency and percentage, were collected.

Results. 23 patients were studied, 60.9% of whom were male. Median age at biopsy was 9 years (7-14 years). UGIE was used in 47.8% of patients (n= 11), colonoscopy was used in 26.1% of patients (n= 6), and direct biopsy was used in 34.8% of patients (n= 8), with GVHD positive results in 2 (18.2%), 2 (33.3%), and 4 (50%) patients, respectively.

Conclusions. Samples taken through direct biopsy stand as an effective alternative in GVHD diagnosis.

KEY WORDS: Graft-versus-host disease; GVHD; Colonoscopy; Rectal biopsy; Bone marrow transplantation.

UTILIDAD DE LA BIOPSIA DIGESTIVA PARA EL DIAGNÓSTICO DE LA ENFERMEDAD INJERTO CONTRA HUÉSPED

RESUMEN

Introducción. La enfermedad injerto contra huésped (EICH) es una complicación frecuente de los trasplantes de células precursoras hematopoyéticas alogénicos (alo-TCPH), con gran morbimortalidad. La clínica intestinal es inespecífica, planteando el diagnóstico diferencial con infecciones y etiología medicamentosa. Aunque las biopsias intestinales son el *gold standard*, no existe consenso sobre la mejor técnica para obtenerlas.

Objetivo. Evaluar los resultados de las técnicas empleadas para obtener biopsias en pacientes con sospecha de EICH intestinal.

Material y métodos. Estudio retrospectivo que incluye pacientes sometidos a alo-TCPH entre 2010 y 2019, con sospecha de EICH intestinal estudiados mediante biopsias digestivas: esofagogastroduodenales (endoscopia digestiva alta - EDA) o rectales (colonoscopia o biopsia directa). Recogimos variables cuantitativas, expresadas como mediana y rango intercuartílico; y cualitativas, expresadas en frecuencia absoluta y porcentaje.

Resultados. Estudiamos 23 pacientes (60,9% varones). La mediana de edad en el momento de la biopsia fue 9 años (7-14 años). Empleamos EDA en el 47,8% (n= 11), colonoscopia en 26,1% (n= 6) y biopsia directa en el 34,8% (n= 8); siendo positivas para EICH en 2 (18,2%), 2 (33,3%) y 4 (50%), respectivamente.

Conclusiones. Las muestras obtenidas mediante biopsia directa se plantean como una alternativa eficiente en el diagnóstico del EICH.

PALABRAS CLAVE: Enfermedad injerto contra huésped; EICH; Colonoscopia; Biopsia rectal; Trasplante de médula ósea.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one of the therapeutic options most widely used in the treatment of various hematologic diseases, both acute and chronic[1]. Graft-versus-host disease (GVHD) stands as one of the most severe complications of allo-HSCT, with an approximate incidence of 30-60% in the receptors of HLA identical siblings, and of up to 80% when using unrelated HLA identical donors[2]. GVHD is caused by an immunological reaction of the donor's immunocompetent cells against those of the receptor, with lymphoid, epithelial, hepatic, and intestinal cells being the most widely involved[3].

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Clinically speaking, cutaneous involvement is one of the most frequent manifestations. The skin is easily accessible through biopsy, which is minimally invasive, easy to perform from a technical standpoint, and little risky. However, a comprehensive assessment of which lesions are to be biopsied and when should be carried out[4]. Therefore, in cases with cutaneous involvement, biopsies should be first conducted in the skin, rather than in less accessible areas.

Intestinal GVHD is more frequent in the first 100 days following transplantation. However, this threshold is no longer used to differentiate acute GVHD from chronic GVHD[5]. The high chemotherapy doses used to prepare patients for transplantation can damage the digestive mucosa and cause diarrhea, which usually disappears in the first 20 days[6]. In this time lapse, GVHD is one of the most common causes of intestinal signs, but differential diagnosis should include infections (viruses, *C. difficile*, candida…), drug-related toxicity, and thrombotic microangiopathy, among others[3].

Even though diarrhea is the most frequent clinical sign of intestinal GVHD, the latter can be associated with a wide array of unspecific symptoms (anorexia, nausea and vomiting, abdominal pain…), which complicates accurate diagnosis. Therefore, a pathological study is required to confirm clinical suspicion. In spite of this, empirical treatment can be immediately initiated in severe cases highly suggestive of GVHD, without having to wait for histological confirmation. Today, there is no consensus as to which sampling method is best[6].

Direct rectal biopsy can be achieved without the need for full colonoscopy, thus reducing the risks associated with intestinal preparation, colonoscopy itself, and general anesthesia, which is required when performing this technique in children[7-9].

The objective of this work was to describe the results of suspected intestinal GVHD cases at our healthcare facility, as well as to assess the efficacy of various techniques used to perform digestive biopsy in this pathology.

MATERIALS AND METHODS

Inclusion and exclusion criteria

A retrospective descriptive study of all patients aged 0-18 years old undergoing one or various allo-HSCTs from January 2010 to December 2019 was carried out.

Patients not undergoing digestive biopsy due to the following reasons were excluded:

- Lack of intestinal involvement.
- Digestive clinical signs highly suggestive of GVHD that were empirically-treated without previous histological sampling.
- Concomitant cutaneous clinical signs allowing diagnosis to be achieved by means of skin biopsy.
- Allo-HSCT failure or patient death.

This means a total of 23 patients with clinical signs suggestive of intestinal GVHD undergoing digestive biopsy in 1 or more different locations were included. Patients were divided into 3 groups based on whether biopsy had been performed at the upper digestive tract through upper GI endoscopy (UGIE), at the rectum through colonoscopy, or through direct rectal biopsy.

Biopsy sampling procedure

Upper digestive tract biopsies were carried out through upper GI endoscopy using endoscopic biopsy forceps.

Rectal biopsies were conducted either at the operating room through colonoscopy using endoscopic biopsy forceps, or directly. Direct rectal biopsies can be performed at the operating room if a second procedure requiring general anesthesia needs to be carried out, or under bedside sedation, using endoscopic forceps through rectal examination up to the posterior rectal semicircumference.

In all 3 cases, specimens were submitted as formalin samples for pathological study, and as fresh samples for microbiological study. Viral infection study was performed through immunohistochemistry and/or PCR.

Statistics

For data analysis purposes, quantitative variables, expressed as median and interquartile range, and qualitative variables, expressed as absolute frequency and percentage, were collected. Version 25 of the *SPSS Statistics* software was used.

RESULTS

Of the 108 patients undergoing allo-HSCT in the study period, digestive biopsy was performed in only 23 of them given the suspicion of intestinal GVHD. Epidemiological data are featured in Table 1.

To analyze biopsy pathological results, patients were divided into 3 groups (Table 2):

- UGIE was performed in 11 patients, with the following samples: esophagus (63.6%), stomach (90.9%), and duodenum (81.8%). Diagnosis was achieved in 3 of them – 2 patients were diagnosed with GVHD and 1 with adenovirus infection.
- Rectal biopsy through colonoscopy was carried out in 6 patients, with diagnosis being achieved in 5 cases. 2 patients were diagnosed with GVHD. Alternative diagnoses included cytomegalovirus infection in 2 cases and adenovirus infection in 1 case.
- Direct rectal biopsy was conducted in 8 patients, 4 of whom were diagnosed with GVHD.

In two patients, simultaneous UGIE and colonoscopy biopsies were carried out, with the same diagnosis being achieved in each case – adenovirus infection in 1 patient, and inconclusive result in the other.

Table 1. Sample epidemiological data.

Number of patients	23
Median age, years (interquartile range)	9 (7-14)
Male patients, n (%)	14 (60.9%)
Diagnosis, n (%)	
ALL	17 (74%)
AML	4 (17.4%)
MDS	1 (4.3%)
Thalassemia	1 (4.3%)
Type of allo-HSCT, n (%)	
UI	4 (17.4%)
UNI	8 (34.8%)
HI	4 (17.4 %)
RI	6 (26.1%)
RNI	1 (4.3%)

ALL: acute lymphoid leukemia; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; UI: unrelated identical; UNI: unrelated non-identical; HI: haploidentical; RI: related identical; RNI: related non-identical.

DISCUSSION

GVHD is one of the most frequent complications arising from bone marrow transplantation and requires early diagnosis and treatment. The most widely involved cells are those of the digestive tract, the skin, and the liver. Typically, hepatic and intestinal involvement are not isolated or occur as the first manifestation[10].

However, given that intestinal clinical signs are often associated with or mistaken for infections as a result of the immunosuppression state of the patient, identifying intestinal GVHD based on symptoms only is a difficult task. Therefore, histological examination is needed for diagnostic confirmation purposes, except in highly suggestive cases requiring immediate empirical treatment initiation[3].

Biopsies can be performed in various locations when it comes to histological sampling. In the presence of cutaneous manifestations, skin biopsies are usually preferred because they are easier and associated with lower morbidity rates[4].

Patient age is one of the main issues found in the literature. Most studies published were carried out in adults, which means pediatric results can only be extrapolated. Regarding the most appropriate intestinal biopsy technique, there is no consensus in the literature as to which sampling method is best, or which intestinal segment should be chosen. Therefore, attitudes vary according to the healthcare facility – some institutions prefer UGIE[11], others favor colonoscopy[12], and others use both irrespective of the symptoms. Nomura[3] observed no differences between the ascending colon, the descending colon, and the ileum in terms of GVHD diagnosis, with a 92.3% PPV for GVHD grades 3 and 4 in the presence of intestinal mucosa exfoliation. Thompson[13] showed that, in patients with nausea and vomiting, colonoscopy with ileum exploration and UGIE with sigmoidoscopy were equally effective and more sensitive combined than separately, concluding that sigmoidoscopy could be useful as an initial test. Other groups advocate the use of UGIE, such as Cox[14], who demonstrated that UGIE gastric biopsy is the most sensitive, even when diarrhea predominates over nausea, vomit, and anorexia.

Rectal biopsy for GVHD has been used for more than 40 years. Sale et al.[1] demonstrated that, in the adequate clinical context, pathological rectal biopsy is highly supportive of GVHD diagnosis, whereas inconclusive biopsy should be a reason for other etiologies to be searched for. Epstein et al.[15] concluded that rectal biopsy is an accurate method when it comes to detecting intestinal involvement in acute GVHD, as long as performed once chemotherapy or radiation effects have worn off. Numerous publications report higher sensitivity rates in sigmoidoscopy biopsy than in full colonoscopy, ileoscopy, gastroscopy, etc. biopsy[6,18-21]. Some authors even consider rectal biopsy to be the most sensitive area[6,18,19], such as Nydegger, who pointed out sigmoidoscopy added little information to rectal biopsy[17].

However, direct rectal biopsy does not allow the intestinal mucosa to be visualized. According to some publications, there is a high correlation between macroscopic findings at endoscopy and positive results at biopsy, both in acute and chronic forms of GVHD, but samples of involved and normal intestinal mucosa should be independently taken[3,11].

One of the greatest advantages of biopsy lies in the fact it allows microbiological studies to be performed in order to rule out some of the most frequent alter-

Table 2. Digestive biopsy results.

Type of test	*GVHD diagnosis*	*Alternative diagnosis*	*Total biopsies*
UGIE	2 (18.2%)	1 (9.09%)	11
Colonoscopy	2 (33.3%)	3 (50%)	6
Direct rectal biopsy	4 (50%)	1 (12.5%)	8

native pathologies. Up to 13% of diarrheas in patients undergoing allo-HSCT are of infectious etiology, with viruses (adenovirus, cytomegalovirus, etc.) being the most common microorganisms[14]. In our sample, viral infections were diagnosed in up to 20% of GVHD negative biopsies.

In our series, UGIE allowed diagnosis to be achieved in 27.27% of cases only, which makes it the least effective technique. However, in rectal biopsies, colonoscopy and direct biopsy had a higher proportion of positive results (83.3% and 62.5%, respectively). Even though colonoscopy allowed diagnosis to be achieved in a higher percentage of cases, these are different techniques carried out in different patient groups, which means they cannot be compared to one another. Therefore, according to our results, it cannot be concluded that colonoscopy is superior to direct biopsy in terms of intestinal GVHD diagnosis.

Regarding study limitations, our study was a retrospective one with a low number of patients. In addition, the lack of a standardized biopsy protocol hinders data comparison and prevents statistically significant results from being achieved.

On the other hand, most publications discussing the best location for colonoscopy biopsy study the whole colon down to the ileum. In the literature, there is no consensus as to how far the colon should be examined at colonoscopy, which is key in patients with higher incidence of thrombocytopenia and higher risk of hematoma[18]. However, we found great difficulty in completing colonoscopy, since we could explore beyond the ileocecal valve in one patient only (GVHD positive result in all colon segments biopsied). This technical difficulty can most likely be explained by the fact patients were in a poor general condition and they were shorter than those from other series, which were carried out in adults.

In the future, we advocate the use of both direct and colonoscopy rectal biopsy in all patients with suspected intestinal GVHD, as well as the creation of a standardized protocol for patient preparation and selection of the most adequate biopsy site. This would allow comparable groups to be established, thus elucidating whether any given technique is superior to the others.

CONCLUSIONS

Given the potential risk that UGIE and/or colonoscopy entail in patients undergoing allo-HSCT, direct rectal biopsy should be the first test when it comes to assessing suspected intestinal GVHD, since it is easier to perform and potentially less dangerous. If adequately identified, GVHD can be treated early, whereas if samples are inconclusive, the endoscopic study can be completed at a later stage.

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