Evaluation of the role of multiparametric ultrasound study in discriminating among intestinal complications after hematopoietic stem cell transplantation. A pilot study.

ECO-STEM

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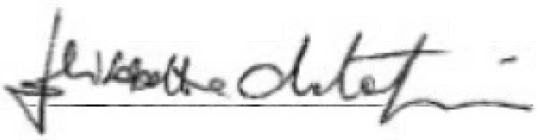

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INTRODUCTION

Allogeneic stem cell transplantation (HSCT) represents a fundamental therapeutic option for patients affected by malignant hematological diseases. One of the major life-threatening complication after HSCT is acute graft versus host disease (aGvHD), especially when gastrointestinal tract is extensively involved. All grade aGvHD occurred in 40-80% of HSCT recipients, depending on donor choice, stem cell source, conditioning regimen and GvHD prophylaxis. Among patients with aGvHD, intestinal involvement accounts for 60% of the cases (1). Approximatively one third of patients with intestinal aGvHD reached a grade III-IV stage with an increased risk of mortality and morbilities. The most common symptom of GI involvement of acute GVHD is diarrhea, secretory and usually voluminous, often reaching more than 1.5 liters per day. In severe cases, patients presented abdominal pain and gastrointestinal bleeding may occur, reflecting the mucosal ulceration (2). These symptoms are nonspecific given the fact they might be frequently observed after allo-SCT in *Clostridium difficile* colitis, bacterial colonization, cytomegalovirus or adenovirus infection, drug toxicity, or neutropenic enterocolitis (3). Weber et al (4) reported the use of contrast enhancement ultrasound imaging (CEUS) in diagnosing gastrointestinal aGvHD. The penetration of intravenous injected contrast microbubbles into the gut lumen showed a sensitivity of 92.9% and specificity of 94.4% for intestinal aGvHD diagnosis, but the specificity was only of 39.6% for advanced stage. On the other hand, sonographic criteria

such as free fluid, bowel wall thickness, color-coded Doppler sonography (CCDS), and compound elastography showed additional modifications in severe gastrointestinal GvHD. Also Schreyer et al (5), reported transmural penetration of intravenous applied microbubbles during CEUS as feasible diagnostic feature for intestinal aGvHD involvement. Contrary to nonspecific bowel wall thickening or free fluid observation, transmural penetration of contrast microbubbles allows to discriminate between aGvHD and chronic inflammatory bowel diseases, that were used as comparing group. Benedetti et al (6) were inspired by the findings of neovascularization detected in aGvHD intestinal biopsies. They observed abnormal bowel wall thickening in multiple bowel segments, due to mucosal edema, as documented also in intestinal infectious conditions. The addition of CEUS imaging revealed microcirculation enhancement of the bowel walls during arterial phase, followed by a prolonged venous phase washout. They also monitored patients' response to treatment and noted that in patients with complete response, the picture return to be normal, both with conventional ultrasound and CEUS. In patients obtaining a partial response, ultrasound showed normal bowel wall, while CEUS still detects microvascular changes at a capillary level suggesting persistent inflammatory activity.

Ultrasound evaluation on bed-side may represent a reliable tool for aGvHD diagnosis in patients with intestinal symptoms. Even if histological features is considered the gold standard for aGvHD diagnosis, a very probable diagnosis of aGvHD could be made quickly with ultrasound method allowing to start steroid treatment with more safety if compared to the only clinical suspicion. Patients with gastrointestinal aGvHD need to be promptly

diagnosed and treated given the high rate of mortality due to advanced staged disease. Ultrasound method might reduce time spent awaiting endoscopic and histological diagnosis, especially if will be confirmed a discriminating power of ultrasound for aGvHD rather than others intestinal complications.

In the concept of multiparametric ultrasound study of the intestine of these patients we would also like to introduce the use of shear wave elastography (SWE).

Shear wave velocity US or shear wave elastography is another ultrasonographic method for measuring tissue stiffness. Instead of direct pressure, it applies energy to the tissues through a pulse wave generated by the US probe. These vibrations displace tissue across multiple geometrical planes instead of only deforming it perpendicularly. They are propagated through tissues as a shear wave, which travels faster in denser and stiffer materials.

The literature reports various experiences on the use of SWE in the study of the intestine, especially in patients with chronic inflammatory bowel diseases (7-9). But no data are reported on the use of the method in patients with graft versus host disease. Considering the simplicity of the method, we think it can be applied to an ultrasound study of the intestine to try to understand the potential of SWE to further define the characteristics of the intestinal segments involved.

OBJECTIVES

Primary objective

Primary objective of the study is to evaluate the use of bed-side multiparametric ultrasound study in the diagnosis of gastrointestinal aGvHD in patients underwent to allogenic transplant.

Secondary objectives

Secondary objective of the study is to evaluate the use of bed-side multiparametric ultrasound study in distinguishing complications different from aGvHD (i.e., infectious, drug-related o neutropenic colitis) in patients underwent to allogenic transplant as compared to auto-transplant.

METHODS

Study design

This is an ambispective interventional study with device.

Setting

The study will be realized at Fondazione Policlinico Universitario Agostino Gemelli IRCCS in Rome.

Study duration

The enrollment will be start after ethic committee acceptance and will enroll during a period of 12 months. Patients will be enrolled consecutively. Data will be collected prospectively during the study course and until all patients will reached 100 days after HSCT. Data will be analyzed subsequently throughout a period of six months.

Population

Study population will include all patients submitted to allogenic stem cell transplantation who developed diarrhea > 500 ml per day suggestive for aGvHD until 100 days from transplant date. Patients submitted to autologous stem cell transplant who developed diarrhea between transplant date and engraftment achievement will constitute control population as for other infectious complications assessment.

Inclusion criteria

- Age ≥18 years;
- Submitted to first allogeneic stem cell transplant during the 12 months period of enrollment of the study;
- Diarrhea onset until day +100 from transplant ;
- Signed informed consent.

Exclusion criteria

- Previous allogeneic stem cell transplantation;
- Patients refusing to sign informed consent for the study;
- Patients affected by severe cardiomyopathy and respiratory distress/insufficiency;
- Patients unable to fully understand and accept study protocol for various problems.

Variables and Procedures

Within 24-48 hours from symptoms, onset full panel of stool cultures will be performed together with Clostridium toxin assessment. Multiparametric ultrasound study will be performed at the onset of the symptoms and then weekly to monitoring intestinal changes accordingly to symptoms evolution during treatment. In the case with high suspicion for aGvHD (stool colture negative, persistent or progressive diarrhea, ultrasound imaging closely suggestive for aGvHD), as per common clinical practice, colonoscopy will be required to evaluate internal macroscopy features of the bowel and to obtain histological samples for conclusive diagnosis. In the meantime steroid therapy will be started, as per common clinical practice. The only modification of this protocol to common clinical practice is represented by the addition of a non-invasive bed-side method of evaluation of the patients with diarrhea. None of the routinely performed diagnostic and therapeutic process will be modified.

All patients submitted to autologous stem cell transplant who developed diarrhea between transplant date and engraftment achievement will constitute control population. Also in this case full panel of stool culture and clostridium toxin assessment will be performed at the onset of the symptoms. Multiparametric ultrasound study will be made al symptoms onset and then weekly until symptom resolution.

Multiparametric ultrasound study will be performed by expert qualified staff as following:

The examinations will be performed at patient's bed, in supine position, with a MyLab™9 Platform (Esaote, Italy) system. Fasting is required.

US for bowel requires both low-frequency convex probes (3.3-5 MHz) and high-frequency (5–17 MHz) linear assay probes to increase the spatial resolution of the intestinal wall and to assess the wall diameter and wall layer.

The following ultrasound parameters will be collected:

- Intestinal wall thickening (normal values < 3 mm; a mean of two measurements for section will be taken, in longitudinal and transverse sections).
- Intestinal wall stratification;
- Intestinal wall flow at colorDoppler;
- Strictures;
- Free fluid (FF) within the peritoneal cavity;
- Enlarged mesenteric lymph nodes;
- Mesenteric hypertrophy.

The contrast agent is administered intravenously and excreted through the lungs without risk of nephrotoxicity or ionizing radiation as in the case of computed tomography contrast agent; it does not remain in the body longer than 15 min. It consists of microscopic bubbles of gas (size 3–5 lm), which are encapsulated in a shell of flexible and stiff phospholipids: they enhance the backscatter signal from blood cells, oscillating when exposed to a low-intensity US field, thereby demonstrating tissue perfusion in real-time ''blood-pool''

imaging. SonoVue (SV, Bracco, Italy) is actually the second-generation US contrast agent used for CEUS, consisting of phospholipid-stabilized microbubbles filled with sulphur hexafluoride. The recommended dose of SonoVue is 2.4 mL. Every injection should be followed by a flush with 5-10 mL of 9 mg/mL (0.9%) sodium chloride solution. Approximately, 10–15 s after intravenous injection, the SonoVue arrives to the intestinal wall, thereby achieving the maximum concentration (peak intensity) after approximately 30 s. This stage is followed by a venous phase, and it is finally excreted by the lungs. No adverse event was reported with SonoVue use: the only contraindication is severe cardiomyopathy (7-9).

The following CEUS parameters will be collected:

- Time-intensity curve (TIC) included basic intensity (B), wash-out slope and/or decent slope (K), wash-in slope or rise slope (C), time to peak (TTP), area under the gamma curve (Area), arrive time (ATM), peak intensity (PI), change of peak intensity (I).
- Any exam will be recorded;
- Acquisition time: 5'

Moreover, each single evaluation will be completed with the use of SWE in order to further evaluate the characteristics of the inflammatory bowel.

SWE of the bowel wall will be performed according to manufacturer guidelines

ENDPOINTS

Primary endpoint

Primary endpoint of the study is to evaluate the accuracy of bed-side multiparametric ultrasound study in the diagnosis of gastrointestinal aGvHD in patients underwent to allogenic transplant in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Secondary endpoints

- To assess potential associations between bed-side multiparametric ultrasound measurements weekly performed and aGvHD diagnosis in patients underwent to allogenic transplant.
- To assess whether bed-side multiparametric ultrasound measurements at baseline differ in distinguishing complications different from aGvHD (i.e., infectious, drug-related o neutropenic colitis) in patients underwent to allogenic transplant as compared to auto-transplant.

Sample Size calculation

The literature reports various experiences on the use of SWE in the study of the intestine, especially in patients with chronic inflammatory bowel diseases. But no data are reported on the use of the method in patients with graft versus host disease. Hence, we consider this as a pilot study. As such, no formal sample size calculation is needed. However, based on common rules on pilot studies [10], the minimum sample size required would be of 20 subjects. In our department, given the median annual number of allogenic stem cell transplant, we plan to enroll 75 patients. As for the secondary endpoints, an equal number of age-matched patients undergone to autologous transplant will be enrolled.

Statistical Analysis

All variables included in the study will be first analyzed by descriptive statistic techniques. In depth, qualitative variables will be described as absolute and relative percentage frequencies. The Shapiro Wilk's test will be used to assess the distribution of quantitative variables. Data will be then expressed either as mean and standard deviation (SD), whether normally distributed, or as median and interquartile range (IQR), otherwise.

Between groups differences (allogenic vs. autologous transplant) will be assessed by the Fisher exact test and the Chi-square test, with Yates correction, as appropriate, in the case of qualitative variables. Quantitative data, indeed, will be assessed either by the Student's t test or the non-parametric Mann Whitney U test, as appropriate.

Receiver Operating Curve (ROC) analysis will be used to assess the accuracy of multiparametric bedside ultrasound in aGvHD diagnosis as compared to the istologic diagnosis (gold-standard, used as reference). Positive (PPV) and Negative predictive values (NPV), sensitivity and specificity will be computed, as well as the area under the ROC curve (AUROC).

To assess potential associations between bed-side multiparametric ultrasound measurements and aGvHD diagnosis in patients underwent to allogenic transplant uni- and multivariable logistic regression models will be used.

Logistic regression models will be also applied to assess whether bed-side multiparametric ultrasound (US) measurements differ in distinguishing complications different from aGvHD (i.e., infectious, drug-related o neutropenic colitis) in patients underwent to allogenic transplant as compared to auto-transplant. More in depth, to evaluate the combined effects between US measurements and transplant group, multivariable interaction models will be fitted, and the interaction odd ratios (IOR) evaluated. In particular, for each US measurement, one interaction logistic regression model will be fitted. In summary, the coefficients of the main effects (in exponential terms) will be interpreted as ORs of the outcome by considering the allo-transplant group from control ($OR_{autologous}$), by

fixing US measurements values to 0 (null), and ORs of the outcome by considering a unit increase of the US measurements in the auto-transplant group ($OR_{US\_measurements}$). The interaction parameters (IOR) will be interpreted as difference (in OR terms) of the US measurements variations between transplant groups (autologous transplant as reference category).

Statistical significance will be set at P value < 0.05. P values between 0.05 and 0.10 will be further reported as suggestive. All analyses will be performed by using R software (v. 4.1.2, R Core Team, 2021) [11].

Data Collection

Each patient transplanted at Hematology and stem transplant Unit of the Fondazione Policlinico Universitario Agostino Gemelli IRCCS had to provide informed consent for enrollment. Data on patients and transplant characteristics will be recorded prospectively: age, underlying disease, conditioning regimen, donor type, stem cell source, GvHD prophylaxis, diarrhea features, treatment for diarrhea, microbiological and radiological findings, ultrasound findings, endoscopic finding and histological report.

Adverse events definition and management

Adverse events, or any harmful clinical event that occurs in subjects involved in a clinical study, regardless of the event is or is not related to the product under study, will be recorded and communicated to the Competent Authority and the Ethics Committee in accordance to what required by current legislation for medical devices (EU Regulation 2017/745 and

related texts), and for medicinal products, (DM 30 April 2015 and GVP-EMA module VI) according to the flow of pharmacovigilance postmarketing.

In the case of a medical device, we mean • defect, any deficiency in the identity, quality, durability, reliability, safety or performance of a device under investigation, including malfunction, errors in its use or inadequacy of the information provided by the manufacturer; • accident, any malfunction or alteration of the characteristics or performance of a device made available on the market, including the error in using caused by the ergonomic characteristics, as well as any inadequacy in the information provided by the manufacturer and any side effect. A serious accident is defined as any accident which, directly or indirectly, has caused, may have caused or may cause one of the following consequences: a) the death of a patient, user or another person; b) the serious deterioration, temporary or permanently, of the health conditions of the patient, the user or another person; c) a serious threat to public health. In the case of a medicinal product, a suspected adverse reaction means a harmful and adverse effect resulting from the use of a drug, compliant or not compliant with the indications contained in the marketing authorization, including overdose, misuse, abuse, therapeutic error and events resulting from exposure for professional reasons. A suspected adverse reaction is defined as serious when it has resulted in death or life threatening, hospitalization or prolonged hospitalization, severe or prolonged disability or incapacity, a congenital anomaly or a birth defect, the onset of another clinically relevant condition "

The reference documents for the study are among others: Summary of the product characteristics of the SonoVue and technical data sheets and user manual for MyLabTM9 Esoate. The diagnostic investigation with contrast medium sulfur hexafluoride microbubbles, Sonovue is of common / consolidated use in clinical practice for gastrointestinal diagnostics diagnostics.

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