Original Article

Efficacy and Safety of Cyclophosphamide Low-Dose Pre-Phase Chemotherapy in Diffuse Large B Cell Lymphoma with Gastrointestinal Involvement

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Abstract. *Background:* **Gastric Diffuse large B-cell lymphoma (DLBCL) is the most common extranodal site of lymphoma's involvement (30%-40% of all extranodal lymphomas and 55%-65% of all gastrointestinal lymphomas). However, gastric localizations are also sometimes found in systemic DLBCL. Gastric complications such as bleeding, perforation, and stenosis under chemotherapy are well documented.**
Methods: **We retrospectively analyzed 15 patients with newly diagnosed DLBCL with gastrointestinal involvement. Endoscopies were performed in these patients before and after treatment. Treatment consisted of cyclophosphamide low-dose pre-phase chemotherapy before conventional-dose chemotherapy.**
Results: **Endoscopy at staging detected ulcers in 12 patients (80%). After low-dose pre-phase chemotherapy, GI ulcers healed in 91.6% of cases (1 ulcer detected). After the whole treatment (Low-dose pre-phase + chemotherapy) 9 patients (60%) achieved complete response, 4 patients (26.6%) partial response, 2 (13,3%) patients presented disease progression. The most frequent adverse event was neutropenia (73.3%); the most frequent non-hematological adverse event was transaminases elevation (20%).**
Conclusion: **Cyclophosphamide low-dose pre-phase chemotherapy resulted in a safe and effective way to prevent adverse events in systemic DLBCL with gastrointestinal involvement.**

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Introduction. Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of aggressive non-Hodgkin Lymphoma (NHL), accounting for about 40% of all NHLs.[1] Primary gastric DLBCL (PG-DLBCL) is NHL's most common extranodal site (30%-40% of all extranodal lymphomas and 55%-65% of all gastrointestinal lymphomas).[2] Primary gastric lymphoma is a rare tumor, with an incidence of 4% to 20% of NHL and approximately 5% of primary gastric neoplasms.[3] The small intestine and ileocecal regions follow in frequency.[4] However, gastric localizations are also sometimes found in systemic DLBCL. Nowadays, R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone) has been established as the first-line treatment for DLBCL.[5] The current standard therapy for DLBCL with gastric lesions is six to eight cycles of R-CHOP.[6] It is now well documented that gastric complications such as bleeding, perforation, and

stenosis can occur under chemotherapy.[7] For this reason, several strategies have been used to minimize adverse events, like fractioned chemotherapy or pre-phase chemotherapy, with positive results.[8-9]

Given the recent evidence highlighting differences between systemic DLBCL and PG-DLBCL on histological and prognostic levels,[10-11] we decided to review newly diagnosed patients with DLBCL with gastrointestinal (GI) involvement treated with Cyclophosphamide low-dose pre-phase chemotherapy at our institution to verify if the strategies used for PG-DLBCL could have also been applied to systemic DLBCL with GI localizations.

Methods. We retrospectively analyzed newly diagnosed patients with DLBCL with secondary GI involvement in Policlinico Tor Vergata, Rome, between February 2016 and April 2020. Patients with PG-DLBCL were excluded. All patients with DLBCL and concurrent extranodal GI lesions, highlighted with CT-PET scan and histologically diagnosed, were considered. Endoscopies (Esophagogastroduodenoscopy if uptake at gastric level, Rectocolonsigmoidoscopy if uptake at colon level) were performed in these patients before and after treatment to carry out biopsies for histological exams and to evaluate the presence of ulcers or mucosal alterations. Fluorescence in situ hybridization (FISH) for MYC/BCL2 and BCL6 translocations were performed on Paraffin-embedded tissue with the dual-color break-apart FISH assay.

Blood chemistry tests were performed at diagnosis, inflammatory indices were dosed, and Helicobacter Pylori (HP) infection was investigated.

Statistical analysis was performed through IBM SPSS Statistics 27 (IBM Corp. in Armonk, NY). Mann-Whitney U test was used to compare variables. Cut-off of statistical significance was set at p <0.05.

Prior to treatment, patients signed informed consent.

Treatment. All treatments were carried out in our institution. The patients enrolled received a low-dose pre-phase therapy before conventional-dose chemotherapy (CT). Before treatment HBV, HCV, and HIV status was studied. None of the patients presented viral infections.

Low-dose pre-phase chemotherapy consisted of Cyclophosphamide 0.2 g intravenously (IV) on days 1, 3, 5, 7, and 9.

Endoscopies were then performed again to reassess the state of mucosa and the possible presence or evolution of ulcers at a minimum of 48 hours from the last Cyclophosphamide dose.

Conventional-dose CT consisted of R-CHOP, six to eight cycles.

Primary Granulocyte Colony Stimulating Factor (G-CSF) prophylaxis was used in patients with age > 65

years and/or renal/liver dysfunctions and/or open wounds and/or bone marrow involvement and/or with high infection risk.

HP eradication treatment was given to all patients positive for HP infection as determined by the ^{14}C-Urea breath test or histological features.

Treatment response was assessed according to Lugano criteria.[12]

Table 1. Characteristics of Population.

Characteristics of population			
	Mean	No	%
Age (years)	57,8±14,2		
Gender	M	8	53.3
	F	7	46.6
Comorbidities	0	7	46.7
	1	5	33.3
	≥2	3	20
Bone marrow involvement	Yes	3	20
	No	8	53.3
	Not investigated	4	26.7
CNS involvement	Yes	0	0
	No	10	66.7
	Not investigated	5	33.3
B symptoms			
	Positive	5	33.3
	Negative	10	66.7
Ki-67 (%)	77,5±12,5		
IPI ≥ 2		9	60
IPI	High	3	20
	High-Intermediate	6	40
	Low-Intermediate	5	33.3
	Low	1	6.7
Hp infection			
	Positive	4	26.7
	Negative	11	73.3
Albumine	3,4±0,5		
Fibrinogen	415±167		
LDH	396,3±263,6		
COO according to Hans algorythm			
	GC	8	53.3
	Non-GC	7	46.7
IHC double expressor	Positive	5	33
	Negative	7	46
	No data	3	21
Present state			
	Alive	15	100
	Dead	0	0

CNS: Central Nervous System; COO: Cell of Origin; IHC: Immunohistochemistry

Results.

Demographics/Staging. Major patient characteristics are shown in **table 1**. The population consisted of 15 patients. The mean age was 57.8 years (range 27-75 years), 53.3% male, 46.6% female. Seven patients did not have any comorbidity, five patients had one comorbidity, three patients had at least two comorbidities. B symptoms were experienced by 33.3% of patients. Mean Ki67 expression was 77.5% (range 60%-95%). All patients

presented with stage IV of Ann-Arbor classification (Gastrointestinal involvement al staging PET-TC scan). No patients had a history of prior anti-lymphoma therapies or hematological diagnoses (including low-grade lymphomas and subsequent transformation). International Prognostic Index (IPI) score was high in 20% of patients, high-intermediate in 40% of patients, low-intermediate in 33.3%, low in 6.6%.[13] Central Nervous System-IPI (CNS-IPI) was also calculated in every patient: 10 of them had high risk CNS-IPI. Diagnostic Lumbar Puncture was performed in every high-risk patient and resulted in negative in all cases.

Eleven patients received bone marrow biopsy; in 3 (20%), bone marrow involvement was documented.

Mean LDH was 396.3 UI/L (range 98-910 UI/L). Serum albumin was 3.4 g/dL (range 2.2-4.38 g/dL). 4 patients were positive for HP infection.

Imaging studies. Staging CT-PET was performed in all patients. Results are summarized in **table 2**. All patients presented at least another site of pathologic uptake and GI involvement; uptaking tissues, in the absence of other possible causes, were considered to be referred to DLBCL involvement even in the absence of histological exam. The most common sites were lymphadenopathies, detected in all patients.

Table 2. Diagnostic test results.

Patient	Pet uptake	Ulcers at EGDS	Ulcers at RSCS
1	Lymphadenopathies, gastric localizations.	No	Not performed
2	Lymphadenopathies, gastric, pancreatic, peritoneal, uterine, thyroid localizations.	Yes	Not performed
3	Lymphadenopathies, pleuric, gastric localizations.	Yes	Not performed
4	Lymphoadenopathies, soft tissue, gastric, pancreatic localizations.	Yes	Not performed
5	Lymphoadenopathies, ileal, colon localizations.	Not performed	Yes
6	Lymphoadenopathies, ileal localizations. colon localizations.	Not performed	No
7	Lymphoadenopaties, gastric localizations.	Yes	Not performed
8	Lymphoadenopaties, gastric, lungs localizations.	Yes	Not performed
9	Lymphoadenopaties, gastric, bone localizations.	Yes	Not performed
10	Lymphoadenopaties, gastric, spleen localizations.	Yes	Not performed
11	Lymphoadenopaties, gastric, spleen, soft tissue localizations.	Yes	Not performed
12	Lymphoadenopaties, gastric localizations.	Yes	Not performed
13	Lymphoadenopaties, gastric, ileal localizations.	No	Not performed
14	Lymphoadenopaties, gastric, ileal localizations.	Yes	Not performed
15	Lymphoadenopaties, gastric, spleen, bone localizations.	Yes	Not performed

Pathology findings. 8 patients presented Germinal Centre DLBCL (GC-DLBCL) (53.3%), and 7 presented non-Germinal Centre DLBCL (46.6%) (NGC-DLBCL), by Hans' algorithm.[14]

Six patients (40%) were affected by "double expressor" lymphoma (Positive Immunohistochemistry for MYC and BCL2). BCL2 was expressed in 9 cases (60%), whereas MYC was expressed in 7 patients (46.6%).

Complete immunohistochemistry data were not available in 4 of 15 patients. In 7 out of 15, FISH was performed searching BCL2, BCL6, and MYC translocation. None of them resulted translocated. In cases where FISH was not performed, three patients were classified as GC-DLBCL by Hans' algorithm and MYC by immunohistochemistry was performed in 4 of them (3 positives, 75%).

Endoscopic findings. Esophagogastroduodenoscopy (EGDS) was performed in all patients and detected ulcers in 11 of them (73.3%) (**Figure 1**). In patients who did not have ulcers, gastric involvement was supposed by PET positivity and confirmed by biopsy (**Table 2**). In these patients (2), EGDS showed gastric erosions, and in 1 of them, mucosal thickening. Rectocolonsigmoidoscopy (RSCS) was performed in 2 patients due to PET positivity and detected colon ulcers in 1 one of them (6.6%); performed biopsies confirmed the lesions as lymphoma localizations (**Table 2**).

After low-dose pre-phase chemotherapy, GI ulcers healed in 91.6% of cases (1 ulcer detected) (**Table 3**).

During low-dose pre-phase chemotherapy, one patient, the only one with colon ulcers, presented rectorrhagia.

Table 3. Efficacy of Low-dose pre-phase chemotherapy.

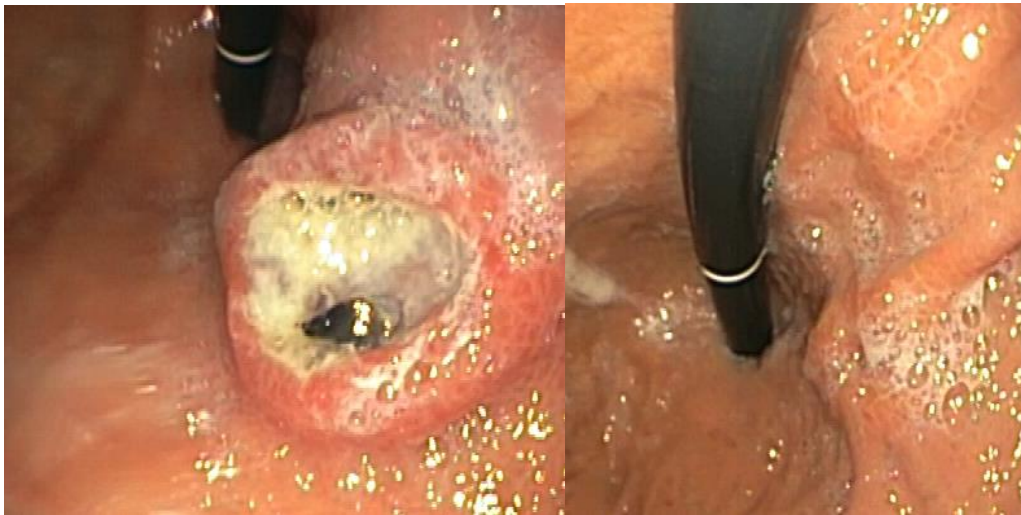|	Onset of Disease		After Low-dose pre-phase	
	No	%	No	%
Ulcer at Gastroscopy	11	73	1	6,6
Ulcer at Colonscopy	1	6,6	0	0
No ulcers detected	3	20		

Figure 1. EGDS from one of the patients with gastric ulcer. Before (Left) and After (Right) Cyclophosphamide chemotherapy pre-phase.

Table 4. Response Rates.

Response Rates		
	No	%
Complete Response	9	60
Partial Response	4	26,6
Progression of Disease	2	13,3

Therapy. Response rates to the whole treatment (Low-dose pre-phase + R-CHOP) are shown in **Table 4**. 9 patients (60%) achieved complete response, 4 patient (26.6%) partial response, 2 (13,3%) patients presented progression of the disease.

Endoscopies were repeated at the end of R-CHOP treatment. All resulted negative for DLBCL involvement. Non/partial responders' GI mucosal involvement was not detected.

There were no significant differences between complete responders and non/partial responders for age (p=0.5), cell of origin according to Hans algorithm (p=0.5), fibrinogen at onset (p=0.7), LDH at onset (p=1), albumin at onset (p=0.6), ki67 (p=0.8), IPI score (p=0.8) and number of involved sites (p=0.8).

No adverse effects or particular toxicity were observed during the cyclophosphamide low-dose pre-phase. **Table 5** summarizes the whole pre-phase and chemotherapy-related toxicities. No treatment was delayed because of toxicities. The most frequent adverse event was neutropenia (73.3%); the most frequent non-hematological event adverse was transaminases elevation (20%).

All patients are still alive. The mean time of follow-up was 35.6 months (median 31 months).

Discussion. GI tract is the most common extranodal site involved in NHL, especially in DLBCL, the most frequent histotype among aggressive hematological malignancies of the gastrointestinal tract.[15]

GI involvement in systemic DLBCL is a negative prognostic factor for the risk of bleeding, perforation, or

Table 5. Treatment-related toxicity.

Treatment-related toxicity		
	No	%
Neutropenia grade 3/4	11	73,3
Anemia grade 3/4	5	33,3
Trhombocytopenia grade 3/4	2	13,3
Perforation/Bleeding	1	6,6
Sepsis	1	6,6
AST/ALT elevation grade 3/4	3	20
Neuropaty grade 3/4	1	6,6

stenosis (risk reported between 6.2% and 43% by different authors) and for the patient's impossibility to feed effectively, resulting in defedation and worsening of performance status.[16,17]

Therefore, it is evident the importance of treating these forms safely, rapidly, and effectively.

Cui et al. described a treatment strategy using a low-dose pre-phase chemotherapy (Cyclophosphamide 0.2 g and Vincristine 1 mg intravenously twice a week for 2-4 weeks) in patients with PG-DLBCL and a stomach ulcer. After the pre-phase, the patients underwent conventional-dose chemotherapy. Compared to cases treated in the same center with only conventional-dose chemotherapy, this strategy proved safer, effective on the ulcers (85.7% of ulcer healing), and better response and overall survival outcomes.[8]

Even if the experience described by Cui et al. is the only pre-phase chemotherapy strategy we found in the literature, it is not easy to compare it with ours. Indeed, we utilized only Cyclophophamide with a different schedule, the patients treated in our institution were all stage IV of Ann Arbor classification with a likely higher IPI score (Median IPI 2.6 ± 1 vs. IPI ≤ 2 in 71.4% of patients), and not all the patients presented ulcer at the diagnosis (Pre-phase chemotherapy was also performed in a patient with gastrointestinal involvement at PET-TC scan without mucosa lesions).

Ann Arbor stage is an independent prognostic factor

and is included in IPI as a prognostic index of outcome and, as Cui et al. highlighted, of bleeding and/or perforation in gastric lymphomas treated with chemotherapy.[8,9]

Despite the high mean IPI (2.6), only one of our patients experienced mild, non-lethal rectorrhagia, and the ulcer healing rate was 91.6%.

Furthermore, the complete response rate was 60%, with an overall response of 86.6% (**Table 4**). These data are in line with response rates reported by the International Non-Hodgkin's Lymphoma Prognostic Factors Project (67% complete response rate in IPI 2, 55% in IPI 3. Mean IPI of our populations (2.6) and, more recently, by Nowakowski and Czuczman, who report 40% of patients with refractory disease or disease relapsing after an initial response, with important differences between DLBCL molecular subtypes.[9,18] In this regard, about half of our patients presented GC-DLBCL (53.3%) while the other half NGC-DLBCL (46.6%), in line with the findings of Nagakita et al. who examined 49 primary gastrointestinal DLBCL, half of which (49%) was non-GCB-like phenotype by Hans' algorithm.[19]

The most frequent treatment-related toxicity in the present study was neutropenia, which occurred more frequently than in the study of Cui et al. (73.3% vs. 60.7%). The most frequent non-hematological adverse event was transaminases elevation, which occurred more frequently than in the study of Cui et al. (20% vs. 7.1%) (**Table 5**).[8]

Myelosuppression, and in particular neutropenia, is a common adverse effect in patients treated with R-CHOP. [20] The higher incidence of neutropenia in our study probably relies on the different number of cycles of conventional chemotherapy after the low-dose pre-phase (six to eight in our protocol vs. four to six). The high incidence in both the studies of transaminases elevation (20% and 7.1%) is probably due to the hepatotoxic effect of Cyclophosphamide, used in more massive dosages

than conventional chemotherapy.[21]

Conclusions. Our experience gives evidence that low-dose pre-phase chemotherapy could be a safe and effective way to prevent adverse events not only in G-DLBCL, as Cui et al. highlighted, but also in systemic DLBCL with gastrointestinal involvement.[8]

High IPI confirms to be a useful tool to predict adverse events in DLBCL gastrointestinal involvement.

Issues still pending are the best drug or combination of drugs to use in pre-phase chemotherapy, the most appropriate schedule, and if PET-TC uptake without mucosa lesions constitutes a real risk of adverse events.

Thus, more studies on a larger scale are necessary to clarify these aspects.

Compliance with Ethical Standards.

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