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Original Article

One Year of Maintenance Metronomic Cyclophosphamide, Methotreaxate & Celecoxib in High Risk Diffuse Large B Cell Non Hodgkin Lymphoma After Complete Response.

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Background & purpose: Diffuse large B cell lymphoma (DLBCL) is the most common subtype of Non-Hodgkin lymphoma (NHL). Although cure rates are increased after the addition of Rituximab to the standard CHOP; yet there is a significant number -especially of high risk- patients get relapse and have a poor response to second-line regimens. Novel approaches are needed for DLBCL treatment. We conducted this trial to evaluate the role of metronomic maintenance chemotherapy in patients with high risk DLBCL who enter in complete response (CR) after first line standard chemotherapy.

Patients and methods: this is a prospective phase II single arm study done on 40 patients with high risk DLBCL. All patients received metronomic maintenance chemotherapy for one year (who had CR after standard R-CHOP) in the form of oral Cyclophosphamide (50 mg every day), oral Methotrexate (2.5 mg 4 times/week) and high-dose oral Celecoxib (400 mg twice daily).

Results: thirty five and 65 percent of patients presented with stage III and IV respectively, 29 patients (72.5%) had international prognostic index (IPI) score of 3 (high intermediate), while11 patients (27.5%) had IPI score of 4 or 5 (high). The most common toxicity was grade I and II hematological and gastrointestinal toxicity. Grade I fatigue was the most remarkable side effect noticed in 27.5% of patients. No thromboembolic manifestations were observed. Median follow up time was 33 months as actually less than 50% of patients reached the mid duration (95% CI: 31.95-34.05).

Conclusions: Metronomic maintenance chemotherapy for one year seems to carry beneficial outcome for high risk aggressive NHL, this regimen is well tolerated. Double randomized controlled study with a large number of patients is needed to validate these results.

Key words: diffuse large B cell NHL; high risk; metronomic chemotherapy; maintenance therapy
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Original Article

INTRODUCTION

Combination chemotherapy with Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone (CHOP) has been the mainstay of treatment for several decades with complete response rates (RR) of 45–55% and cure rates of approximately 30–35% in aggressive NHL; the addition of the immunotherapeutic agent Rituximab to chemotherapy (R-CHOP) has improved complete RR to 76–86% and OS rates significantly. Nevertheless, variability in response rates, and particularly in event free survival (EFS) and OS, have been observed in these trials, and up to one-third of all patients experience progression or relapse of their disease. Outcomes in relapsed or refractory DLBCL are poor, with a median survival approximating 6 months. Salvage chemotherapy followed by high-dose therapy and autologous stem-cell transplantation (HDT-ASCT) is widely considered the standard treatment for relapsed DLBCL, though the ORR and EFS are significantly lower in this setting as compared to those observed in upfront treatment. Many patients, moreover, are not eligible for transplant, and of those that are, about half fail to respond to salvage therapy, becoming ineligible for HDT-ASCT[1].

Recent data indicate that angiogenesis is important in the pathophysiology and prognosis of aggressive histologic subtypes of NHL[2]. Angiogenesis and angiogenic factors are increased in most lymphomas. In addition, angiogenesis has been associated with adverse outcomes or more aggressive clinical behavior in malignant lymphoma. However, the role of angiogenesis might vary in lymphoma subtypes because of the prognostic value of microvessel density and the different

expression of angiogenesis related molecules in the various lymphoma subtypes[3].

Kerbel *et al.*[4] and others[5] have shown that the use of chronic, low-dose chemotherapy administered at close regular intervals with no prolonged breaks (termed ''metronomic'' chemotherapy) may inhibit angiogenesis by targeting genetically stable endothelial cells of the neovasculature and circulating proangiogenic bone marrow derived cells, including circulating endothelial progenitor cells (CEPs)[6,7].

The findings of Wun *et al.*[8] reveal that human Burkitt-type B-cell lymphoma cell lines express elevated levels of cyclooxygenase 2 (COX-2), and this fits with earlier studies indicating that B-lineage cells are capable of expressing COX-2. COX-2 is a prostaglandin synthase enzyme that has been implicated in tumorigenesis. One of the proposed mechanisms by which this may occur is by stimulating angiogenesis through the production of proangiogenic factors including VEGF, basic fibroblast growth factor, platelet-derived growth factor, transforming growth factor-h1, and endothelin-1. COX-2 inhibitors, such as celecoxib, have been shown to reduce the incidence of neoplastic lesions in familial adenomatous polyposis and therefore may have a role in the treatment of established malignancies. Furthermore, COX-2 is overexpressed in some lymphomas and is of potential prognostic importance. These preclinical data provide the rationale for using low-dose chemotherapy together with a selective COX-2 inhibitor in the treatment of aggressive NHL.

The combination of cyclophosphamide with celecoxib is based on: the postulated importance of angiogenesis in NHL, the in vitro and in vivo antiangiogenic and antineoplastic properties of celecoxib, the ''antivascular'' properties of chemotherapy delivered continuously in mouse xenografts, especially when given in combination with anti-VEGF therapy and not as monotherapy, and early clinical experience in other cancers when standard maximally tolerated dose chemotherapy has failed[9].

Maintenance therapy is prolonged treatment given after the initial chemotherapy course has taken effect and reduced the cancer. The goal of maintenance therapy is prolonging the length of time before relapse. Maintenance therapy has been researched and shown to improve outcomes of many cancers subtypes including NHL[10].

There is only two published studies regarding the use of metronomic chemotherapy in treatment of relapsed and/or refractory NHL, one by Buckstein *et al.*[9], while the other one by Abd El Bary *et al.*[11] in which patients were enrolled to receive oral combination of high-dose celecoxib and low-dose Cyclophosphamide, The preclinical data provide the rationale for using low dose chemotherapy together with selective COX-2 inhibitors in the treatment of DLBCL. The combination of low dose cyclophosphamide and methotrexate was chosen in this combination based on preclinical and clinical data. Celecoxib was selected for inclusion in this regimen because of its commercial availability, ease of administration, accepted side-effect profile and putative antiangiogenic effects.

This study aims to evaluate the role of metronomic maintenance chemotherapy in patients with high risk DLBCL NHL.

PATIENTS AND METHODS

This is a prospective phase II single arm study that included 40 patients with high risk DLBC NHL presented to the clinical oncology department, Menofia University from January 2009 till March 2011 after approval from the faculty of medicine ethical committee at Menofia University. All included patients were those achieving complete response to front line standard chemotherapy.

The Primary objective was to determine the RFS & OS after receiving continuous daily oral high-dose celecoxib and oral low-dose Cyclophosphamide & Methotrexate for one year following first line chemotherapy (R-CHOP) in these patients population. The Secondary objective was to detect the toxicity & tolerability to the previously mentioned treatment.

Inclusion criteria:

Patients were considered eligible if they have the following criteria: Age above 18 years old, EGOC performance status ≤ 2, High risk DLBCL (IPI 3, 4, 5), Advanced stage disease (stage III or IV according to Ann Arbor staging system), Achieved complete response after finishing the first line standard chemotherapy (6-8 cycles of R-CHOP) according to the international working group (IWG 1999)[12], Adequate hematological count (hemoglobin count > 8.5g/dl, absolute neutrophil count > 1.000/ mm^3, platelet count > 75.000/mm^3), Normal serum creatinine, normal hepatic functions. A written consent was taken from every patient.

Exclusion criteria:

Patients were considered ineligible for the study if they have the following criteria: Receiving chemotherapy within the preceding 2 weeks, EGOC performance status ≤ 2, Evidence of histological transformation from indolent lymphoma (s), Uncontrolled hypertension, Unstable cardiovascular or significant renal disease, Previous history of active peptic ulcer and current continuous

treatment by steroids, non-steroidal anti-inflammatory drugs (NSAIDs), or by anti-coagulants.

Pre-treatment assessment:

Full history taking, complete physical examination, computed tomography (CT) neck, chest, abdomen and pelvis, bone marrow aspiration and biopsy, Doppler echo cardiography, complete blood picture (CBC), liver function tests, kidney function tests, lactate dehydrogenase (LDH) and erythrocyte sedimentation rate (ESR).

Treatment plan:

All eligible patients received the following schedule of metronomic maintenance chemotherapy:

- Cyclophosphamide tab 50mg p.o. daily on an empty stomach.
- Methotrexate tab 2.5mg p.o. twice per day, 2 days weekly.
- Celecoxib tab 400mg p.o. bid daily (with food).

Dose reduction of celecoxib to 200 mg p.o. bid then to 100 mg p.o. bid is done with more than grade III nausea and vomiting or a 50% increase in serum creatinine or liver enzymes. Any gastrointestinal bleeding > grade II resulted in study discontinuation. Similarly, Cyclophosphamide could be reduced twice (to 25 mg p.o. daily then 25 mg p.o. on alternate days) for greater than grade III neutropenia or thrombocytopenia. Dose reduction of Cyclophosphamide could be also carried if patient develops severe renal impairment (glomerular filtration rate (GFR) less than 10 ml/min) or severe hepatic dysfunction (transaminases >2-3 times the upper limit of normal). Methotrexate discontinuation was done in the following conditions: diarrhea more than grade II, and ulcerative stomatitis (that may result in dehydration) till recovery, significant reduction in blood counts, significant increase in serum transaminases levels, pulmonary syndrome (especially dry nonproductive cough or nonspecific pneumonitis), or active infection.

The toxicity was assessed according to NCI Common Terminology Criteria for Adverse Event (CTCAE) v 3.0 Publish Date: August 9, 2006.

Patients follow up: Clinical examination was done every month or according to patient's condition. CBC, liver function tests, kidney function tests were repeated every month to assess toxicity. Blood levels of LDH & ESR were repeated every month while CT scans were done every 3 months, to assess relapse.

Statistical analysis:

The data were analyzed using SPSS program 17, statistical package. RFS was measured as the interval between the start of treatment (metronomic chemotherapy) and the date of the first clinical or radiological evidence of relapse. The RFS was estimated using Kaplan-Meier estimate.

RESULTS

Patient & disease characteristics:

Forty patients were enrolled with a median age 54 years (34 years to 75 years). Male to female ratio was 1:1.8.

All of them had pathologically diagnosed DLBCL. Sixty five percent presented in stage IV disease. Only 9 patients had extra-nodal involvement at presentation. B symptoms were presented in 16 (40%) patients of the study group. Among patients within the study, 29 patients (72.5%) had high intermediate IPI score; while11 patients (27.5%) had high IPI score.

LDH level above the normal range was encountered in 28 (70.0%) patients, while patients with ESR level above 100 represented 15% (6 patients) Table 1.

Treatment related toxicity:

The treatment was generally well tolerated in the out patient setting. No treatment-related deaths were reported.

Fatigue was the most remarkable side effect noticed in the study group. Eleven patients had grade I fatigue, while five patients had grade II fatigue & one patient had grade III fatigue. No dose reduction or treatment interruption was done except for the patient with grade III fatigue in which treatment was interrupted for one week then resumed on the usual dose.

Table 2 summarizes most of grade I and II toxic effects observed. Gastrointestinal toxicities were mild and didn't affect the compliance and ranged from grade I to grade II with no grade III or IV toxicities. Seven patients (17.5%) had grade I nausea and three patients had grade II nausea (7.5%), while 4 patients had grade I vomiting representing (10%) of cases.

Neutropenia was observed in 10 patients with 20% had grade I and 5% had grade II neutropenia. Anemia was noticed in 7 patients, five patients (12.5%) had grade I, while two patients had grade II anemia (5%). Grade I thrombocytopenia was observed in only 3 patients representing 7.5% of cases.

Two patients (5%) had grade I elevated serum creatinine level with no dose reduction. One patient had

(2.5%) grade II elevated serum creatinine level with dose reduction of celecoxib.

Peripheral neuropathy was observed in three patients (7.5%). All of them had grade I which responded to medical treatment.

Grade I elevation in liver enzymes was encountered in two patients. Three patients (7.5) had grade I edema which responded to medical treatment (short course of diuretics) with no treatment interruption.

Survival analysis:

Median follow up time was 33 months as actually less than 50% of patients reached the mid duration (95% CI: 31.95-34.05) Fig. 1& 2.

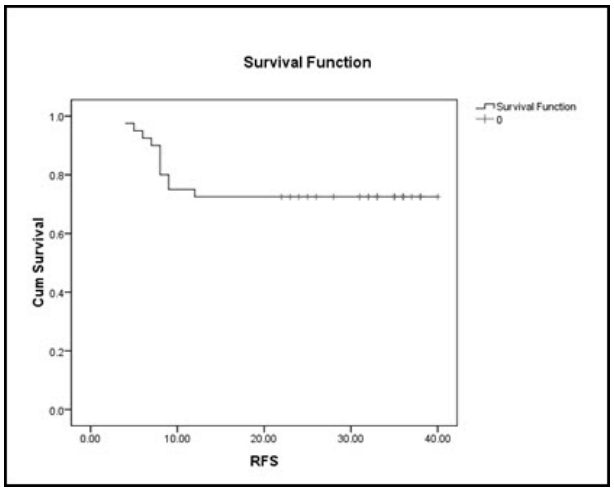

Figure 1: The Kaplan–Meier of Relapse-free survival (RFS) in the 40 eligible patients with high risk diffuse large B cell NHL.

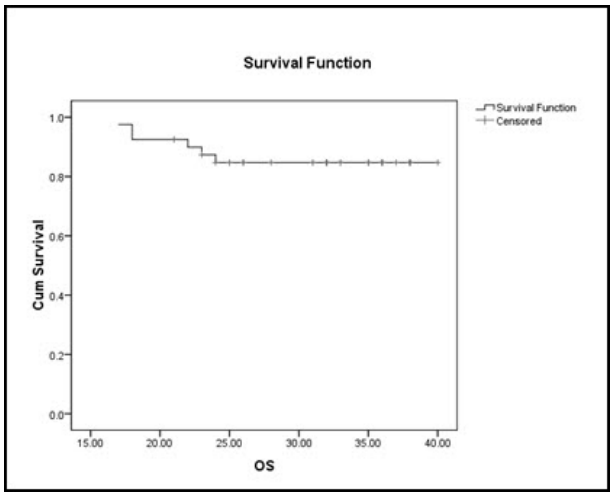

Figure 2: The Kaplan–Meier of overall survival (OS) in the 40 eligible patients with high risk diffuse large B cell NHL.

Table 1: Patients & disease characteristics of the 40 eligible patients.

Items	No. of patients & (%)
Sex	
Male	14 (35)
Female	26 (65)
Age in years	
Median	54
Range	34-75
B symptoms	
Absent	24 (60)
Present	16 (40)
Clinical stage	
III	14 (35)
IV	26 (65)
Extra-nodal involvement	
Yes	9 (22.5)
No	31 (77.5)
LDH level	
Low	12 (30)
High	28 (70)
IPI	
High intermediate (3)	29 (72.5)
High (4,5)	11 (27.5)

Table 2: Treatment-related toxicity of metronomic maintenance chemotherapy.

Toxicity	Grade	No. & %
Nausea	Grade I	7 (17.5)
	Grade II	3 (7.5)
Vomiting	Grade I	4 (10)
Dyspepsia	Grade I	6 (15)
	Grade II	4 (10)
Oral mucositis	Grade I	4 (10)
	Grade II	1(2.5)
Hematologic toxicity		
Anaemia	Grade I	5 (12.5)
	Grade II	2 (5)
Thrombocytopnia	Grade I	3 (7.5)
Neutropnia	Grade I	8 (20)
	Grade II	2 (5)
Renal toxicity		
Serum creatinine elevation	Grade I	2 (5)
	Grade II	1 (2.5)
Peripheral neuropathy	Grade I	3 (7.5)
Edema	Grade I	3 (12.5)
Liver enzymes elevation	Grade I	2 (5)

DISCUSSION

Ideally, maintenance therapy would be limited to patients in CR or with minimal disease after initial therapy. The impact of these strategies on overall & EFS is important because the use of ineffective maintenance therapy adds the burden of additional cost and morbidity. Another hallmark of maintenance therapy is the impact on patient. Ideally, a maintenance regimen would be nontoxic, easy to administer (e.g. oral) & demonstrate a survival benefit over administration of the same agent in the relapsed disease setting. Historically, the use of maintenance therapy for lymphoma was limited to interferon (IFN) after standard therapy in the setting of radiological CR, or plateau phase for patients with indolent/low grade lymphoma. More recent approaches have focused on the use of the monoclonal antibody (MoAB) Rituximab with varying schedules[13].

Up to our knowledge there is no study used metronomic chemotherapy as maintenance in DLBC NHL.

Interferon in Diffuse large B cell lymphoma:

The evidence supporting the benefit of maintenance IFN in DLBCL is marginal which is probably to the faster rate of tumor cell growth in aggressive NHL[13]. Avileas A. *et al.* reported results from a trial treating 96 patients randomized to receive IFN or observation after intensive therapy for high risk NHL. The estimated 5-year OS and EFS for patients who received IFN were 71% (95% CI: 61 ± 83%) and 57% (95% CI: 39 ± 69%), respectively, values which were not statistically different from the control group: 69% (95% CI: 63 ± 79%) and 54% (95% CI: 37 ± 63%), respectively (*P*= 0.2)[14]. Another trial by Avileas A. *et al.* randomized 223 patients with intermediate or high intermediate risk DLBCL to receive IFN maintenance or observation. Again there was no difference in EFS or OS between the 2 treatment arms[15]. Finally Giles *et al.* reported on a large randomized trial comparing CHOP with CHOP and IFN. The group receiving CHOP and IFN also received and additional year of IFN maintenance. Among the 214 patients randomized to receive CHOP alone, The Overall RR was 81% compared to 80% in the group receiving CHOP and IFN; after a median follow up of 36 months, there was no apparent difference in survival between the 2 treatment arms[16].

Rituximab in Diffuse large B cell lymphoma:

The largest series of patients treated with a maintenance schedule of Rituximab in the setting of DLBCL comes from the recently reported ECOG 4494 trial. This trial involved two stage randomization evaluating the addition of Rituximab to CHOP induction, followed by randomization to maintenance Rituximab (MR) or observation only in those patients who responded to induction treatment (CHOP and R-CHOP). A significant difference in the effect of maintenance therapy was observed according to the type of induction therapy (HR= 2.10; 95% CI, 1.01 to 4.36; *P*= 0.05). MR significantly prolonged Failure-free survival (FFS) after CHOP (HR= 0.45; 95% CI, 0.29 to 0.71; *P*= 0.0004) but not after R-CHOP (HR= 0.93; 95% CI, 0.53 to 1.66; *P*= 0.81). The estimated 2-year FFS rates after second random assignment were 77%, 79%, 74%, and 45% after R-CHOP, R-CHOP+MR, CHOP+MR, and CHOP, respectively. These data indicate that Rituximab as part of induction therapy or as maintenance in responding patients results in a significant prolongation of FFS (*P*= 0.001). There were no statistically significant survival differences with MR after CHOP (*P*= 0.27) or R-CHOP (*P*= 0.48)[17].

The median age in our study was 54 years, with advanced stage disease at presentation (stage 3 or 4) in 35% and 65% respectively. While in ECOG 4494 trial the median age was 69 years. Female patients represented 65% of our study, with male to female ratio1:1.8. Opposite to data from IFN and Rituximab maintenance trials were the majority of patients were males (52% in CHOP-R arm in the first trial and 55% in the second trial)[14,17].

Regarding safety the metronomic maintenance chemotherapy regimen is well tolerated with a low incidence of hematological, gastrointestinal and renal toxicities, with fatigue the most remarkable side effect noticed in our study, grade I fatigue was observed in eleven patients (27.5%), Five patients (12.50%) had grade II fatigue, while one patient (2.5%) had grade III fatigue. These results were comparable to Abd El Bary study in which grade I, II, III fatigue was observed in 34.1%, 24.4% and 2.4% of patients respectively. Gastrointestinal and Hematologic toxicities were mild, ranging from grade I to grade II with no grade III or IV toxicities observed, which is similar to Abd El Bary study. No cases experienced skin rash in our study; however, in Buckstein study skin rashes seen with grade I, II, and III in (four, seven and three patients respectively) while in Abd El Bary study only 3 patients experienced grade I skin rash. In Buckstein study, three patients developed venous thrombosis this was explained as a recognized complication in the treatment of NHL, especially at advanced stage. There was no such side effect observed in our study nor Abd El Bary study[9,11]. The main limitation of the study is the lack of control arm, small sample size and short follow up time.

Recommendation:

Enrollment of a larger number of patients is recommended with longer follow up duration, evaluation of VEGF levels in the blood as a predictor marker of

response to treatment & measuring COX-2 in NHL samples by immunohistochemistery may help to predict the response to celecoxib.

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