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Pulse Monthly Cyclophosphamide Therapy in Refractory Autoimmune Hemolytic Anemia: A New Frame Of Mind

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Abstract

High-dose cyclophosphamide, without stem cell rescue, has been used successfully to treat aplastic anemia and other autoimmune disorders. Treatment of steroid refractory Autoimmune Hemolytic Anemia (AIHA) is challenging especially with no evidence based consensus guidelines and limited resources. To determine the safety and efficacy of high dose pulse cyclophosphamide-1 g/month for four consecutive months-in our patient with severe refractory AIHA secondary to adult onset stills disease. Our patient failed to respond to high dose of steroid therapy ± azathioprine. Mean hemoglobin level, reticulocyte count and LDHwere assessed before and after cyclophosphamide treatment every month. After the 4th cycle of cyclophosphamide, our achieved complete response. The mean hemoglobin level was significantly increased after the 1st, 2nd, 3rd and 4th months of pulse cyclophosphamide therapy when compared to before treatment and the mean reticulocyte (%) were significantly decreased after the 2nd, 3rd and 4th months. We conclude that pulse cyclophosphamide therapy is well tolerated and induces durable response in patients with severe refractory AIHA.

Introduction

High-dose cyclophosphamide was initially chosen as conditioning for allogeneic bone marrow transplantation because of its potent immunosuppressive properties [1,2]. Lymphocytes are highly sensitive to cyclophosphamide, but primitive hematopoietic progenitors are resistant to its cytotoxic effects because they contain high levels of aldehyde dehydrogenase, an enzyme that confers resistance to cyclophosphamide [3]. Others showed that high-dose cyclophosphamide without stem cell transplantation induces durable treatment-free remissions in patients with variety of other refractory autoimmune conditions [4-6]. And can eliminate alloantibodies [7].

Autoimmune Hemolytic Anemia (AIHA) is a rare disease. In a recent population-based study the incidence was 0.8/1,00,000/year, but the prevalence is 17/1,00,000 [8].The disease is classified as primary (idiopathic) or secondary if there is an underlying disorder. Primary (idiopathic) AIHA is less frequent than secondary AIHA. Secondary cases are often challenging because not only AIHA but also the underlying disease(s) must be diagnosed and treated [9]. The associated antibody that causes hemolysis is either a warm antibody or a cold agglutinin [10]. The diagnosis of AIHA is usually straightforward and made on the basis of the following laboratory findings: normocytic or macrocytic anemia, reticulocytosis, low serum haptoglobin levels, elevated Lactate Dehydrogenase (LDH) level, increased

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indirect bilirubin level, and a positive Direct Antiglobulin Test (DAT) .Treatment with glucocorticoids results in improvement in the majority of cases, but relapse iscommon [11]. For patients whose disease becomes refractory or who do not respond to glucocorticoids, splenectomy is often employed as a second-line treatment [12]. Subsequent salvage treatments include intravenous immunoglobulin, danazol, [12] and a variety of immunomodulating agents including low-dose cyclophosphamide, azathioprine, cyclosporine, and vincristine [11]. Unfortunately, many patients become refractory to multiple therapeutic approaches and develop complications of chronic high-dose steroid therapy. Cold agglutinin autoimmune hemolytic anemia is particularly refractory to treatment. Because of its success in other severe autoimmune disorders, high-dose cyclophosphamide was studied in-patient with severe AIHA that was refractory to standard therapies.

Case presentation

A 22 years old pleasant Muslim, housewife hailing from rural Bangladesh presented to us with the complaints of pain in the multiple joints of both upper and lower limbs for last 6 months involving mainly small joints of hands and feet, wrists, ankles. Joint involvement was symmetrical and non-migratory. There was associated inactivity stiffness that lasted for more than an hour and relieved partially with activity and taking analgesics. Joint pain was associated with high-grade intermittent fever (max recorded temperature was 1030 F), not associated with chills and rigor and subsided after taking anti pyretic. Joint pain was not associated with any redness or increased warmth of the joints. She also denied any low back pain, buttock or groin pain, sole pain or pain over heel. There was also no H/O sexual exposure, urethral discharge or bloody diarrhoea preceding the illness. No red eye, visual impairment, proximal muscle pain or stiffness, photosensitive rash, oral ulcer, genital ulcer, papulopustular or acne form or scaly skin lesions were present either. There was also no history of dry mouth or dry eye, loss of scalp hair, venous thrombosis, tightening of skins of body, dysphagia, heartburn, altered bowel habit or any history suggestive of Raynaud's phenomenon. Nevertheless, she had weight loss of 5 kg in the last 3 months and she also admit development of transient rash over her trunk during spike of temperature. On further query, there was no family history of such illness. She had no significant drug history prior the illness except she took ceftriaxone for 7 days before admission. But she had transfusion of eighteen units of whole blood in last three months. Her menstrual history was also noncontributory. She had neither any sexual promiscuity nor had any history of tuberculosis or any contact with the patient with active tuberculosis. On examination, she was ill looking, hyperpigmented, febrile (temperature 1010 F) with stable vitals. There were macular rash all over her trunk. She was moderately anemic but non-icteric and non-cyanosed. Skin was not thinned with no bruising or telangiectasia. Eyes including both fundi were normal. There were multiple enlarged cervical lymph nodes, Thyroid gland was not enlarged. There was no edema, bony tenderness or any nail or peri-ungualvasculitic changes. On musculoskeletal system examination, GALS screening was done. Gait and spine examination revealed no abnormalities. On upper limb examination, MCP, PIP and wrists of both sides were swollen, tender and there was painful restriction of both active and passive movements of these joints. DIP's were spared. On lower limb examination, ankles and small joints of feet of both sides were swollen, tender and there was painful restriction of both active and passive movements of these joints. There were neither any deformities nor any evidences of sacroilitis or enthesitis. Oral cavity examination revealed no ulcers. But there were hepto-splenomegaly5 cm from right costal margin in right mid clavicular line and 4 cm along its long axis respectively. There were no clinical evidences for serositis (ascites, pleural or pericardial effusion). Other systemic examination was noncontributory.

On investigation there was normocytic normochromic anemia of Hb- 8.70 gm% (MCV 85, MCH-29.6), ESR92 mm in 1st hour, TC-11940/cmm (N-38%, L-54%), TPC-520000/cmm, CRP. 108.5 mg/dl, PBF- normocytic normochromic anemia with large number of spherocytes and marked polychormasia with thrombocytosis. Urine R/E revealed; proteinuria (+), no RBC or no casts. S albumin 31 gm/L, UTP- 1.01 gm/day, ALT- 74 U/L, S. ferritin 15435 ng/L (normal 10-291 ng/L). Renal function tests, RBS, CPK, IgG, and Blood C/S Urine C/S all were noncontributory. ALT was 93U/L (normal less than 31 U/L). On immunological test, ANA, Anti ds DNA, RA factor, Anti CCP, ENA (extractable nuclear antigen)profile all came negative.C4 was low 0.45 g/l. CXR P/A, Echocardiography, X- ray of both hands revealed no abnormalities. USG of whole abdomen revealed mild ascites, hepatosplenomegaly with bilateral mild pleural effusion. Serological investigations for HBV and HCV were negative, VDRL non-reactive. Direct coomb's test was strongly positive. Further work up for hemolysis revealed s. indirect bilirubin 1.8 mg/dl (normal 0.2-0.8 mg/dl), LDH 632 U/L (normal 142-280 U/L), reticulocyte count 4.6% (normal 0.5%-1.5%). Not do serum haptoglobincould. So diagnosis of Adult Onset Stills Disease with Auto Immune Hemolytic Anemia was made.

During hospital, stay she was started systemic steroid (oral prednisolone 1 mg/kg/day) with DMARD (azathioprine). Regular monitoring of both hematological and biochemical parameters were done. On 5th day, she was discharged, as there was financial constraints. During discharge, her Hb%-8.1gm/L, Total count-4200/cmm(PMN- 68%, L- 30%), ESR- 44 mm in 1sthr, TPC -392000/cmm, CRP- 44.92 mg/dl, Urine R/E- no protein, no casts, no RBC, no WBC, S. Ferritin-2243 ng/ml. After three weeks, on OPD follow up, she presented with severe prostration. But her joint symptoms subsided completely. On examination. There was severe pallor. Icterus was present Complete blood count revealed Hb%-2.9gm/L, Total count-5700/cmm (PMN- 78%,L- 20%),ESR- 32 mm in 1sthr, TPC -262000/cmm, Peripheral blood film revealed plenty of spherocytes.. S. indirect bilirubin 4.6 mg/dl (normal 0.2-0.8 mg/dl), LDH 2672 U/L (normal 142-280 U/L) , reticulocyte count 9.6% (normal 0.5%-1.5%). 3 units of fresh whole blood was transfused. As the patient was poor, further treatment options for AIHA with rituximab and splenectomy could not be undertaken. So, after explaining all the possible adverse effect of Cyclophosphamide (CYC), pulse monthly CYC (1 gram/dose) therapy was planned. Mesna (sodium 2-mercaptoethanesulfonate) 10 mg/kg was given at 3, 6, and 8 hours following each cyclophosphamide dose. Granulocyte colony-stimulating factor at 5 g/kg was initiated 6 days after the completion of treatment with high-dose cyclophosphamide and continued until an Absolute Neutrophilcount (ANC) of 1000/microL or greater was attained. Table 1 showing hemoglobin (gm%), reticulocyte count and LDH before and end of each cycle of pulse CYC therapy.

Figure 1: Hemoglobin (gm%), reticulocyte count and LDH before and end of each cycle of pulse CYC therapy.

Parameter	Before starting pulse CYC therapy	On 2nd month before 2nd cycle of CYC therapy	On 3rd month before 3rd cycle of CYC therapy	On 4th month before 4th cycle of CYC therapy	On 5th month, 1 month after 4th cycle of CYC therapy	On 6th month, 2 months after 4th cycle of CYC therapy
Hemoglobin (gm %)	2.9	8.6	8.9	9.1	9.9	10.6
S. Reticulocyte (%)	9.6	4.5	3.9	3.2	2.3	1.9
LDH(U/L)	2672	1132	1090	867	546	214

The patient is still on regular monthly follow up and she is doing well regarding her joint symptoms and hematological parameters.

Discussion

Autoimmune Hemolytic Anemia (AIHA) occurs when antibodies directed against the person's own Red Blood Cells (RBCs) cause them to burst (lyse), leading to an insufficient number of oxygen-carrying red blood cells in the circulation. The lifetime of the RBCs is reduced from the normal 100-120 days to just a few days in serious cases [13,14]. The intracellular components of the RBCs are released into the circulating blood and into tissues, leading to some of the characteristic symptoms of this condition. The antibodies are usually directed against high-incidence antigens, therefore they also commonly act on allogenic RBCs (RBCs originating from outside the person themselves, e.g. in the case of a blood transfusion) [15]. AIHA is a relatively rare condition, with an incidence of 5-10 cases per 1 million persons per year in the warm-antibody type and 0.45 to 1.9 cases per 1 million persons per year in the cold antibody type [16].

The terminology used in this disease is somewhat ambiguous. Although MeSH (Medical Subject Headings) uses the term "autoimmune hemolytic anemia" [17], some sources prefer the term "immunohemolytic anemia" so drug reactions can be included in this category [18]. The National Cancer Institute considers "immunohemolytic anemia", "autoimmune hemolytic anemia", and "immune complex hemolytic anemia" to all be synonyms [19].

The diagnosis of AIHA is usually straightforward and made on the basis of the following laboratory findings: normocytic or macrocytic anemia, reticulocytosis, low serum haptoglobin levels, elevated Lactate Dehydrogenase (LDH) level, increased indirect bilirubin level, and a positive Direct Antiglobulin Test (DAT). However, there are pitfalls, particularly in secondary cases, because not always are all of the typical laboratory findings of AIHA present [20]. For the diagnosis of secondary AIHA a careful history, including information on the onset (acute or insidious), history of infections, information on recent transfusions, exposure to drugs or vaccination, signs of immune disease (arthritis), and general clinical condition are helpful. The exclusion of a drug-induced hemolytic anemia is particularly important, because stopping the drug is the most effective therapeutic measure in this situation. A clinical examination (to rule out lymphadenopathy, splenomegaly) is obligatory. History, clinical findings, and the type of antibody must determine the need for additional investigations. Extended work-up relevant for treatment decisions may include abdominal examination by computed tomographic scan (to search for splenomegaly, abdominal lymphomas, ovarian dermoid cysts, renal cell carcinoma), quantitative determination of immunoglobulins, a search for a lupus anticoagulant in case of warm antibodies, or a bone marrow examination [21]. Treatment with glucocorticoids results in improvement in the majority of cases, but relapse is common. For patients whose disease becomes refractory or who do not respond to glucocorticoids, splenectomy is often employed as a second-line treatment [11]. Subsequent salvage treatments include intravenous immunoglobulin, danazol [12] and a variety of immunomodulating agents including low-dose cyclophosphamide, azathioprine, cyclosporine, and vincristine [11]. The other option for second-line treatment is the anti-CD20 antibody rituximab. The standard regimen is 375 mg/m^2 on days 1, 8, 15, 22 for four doses. There is no doubt that the short-term benefit/risk ratio for rituximab is high and that rituximab is certainly the best option for patients who are not qualified for or who refuse splenectomy. The problem is the small number of selected patients, the heterogeneity of patient population, and the lack of systematic long-term data on efficacy and safety in the published reports. In ITP (immune thrombocytopenic purpura) it has been shown that patients with a CR after rituximab can have long remission durations and that splenectomy can be avoided or postponed [22]. Such data are not available for rituximab in AIHA [21]. Azathioprine and cyclophosphamide are both immune suppressors leading to a decrease of autoantibody production. The addition of these drugs can be considered if steroid therapy does not lead to a sufficient result, when a steroid maintenance dose of more than 20 mg/day is needed or steroid doses must be tapered due to side effects. Cyclophosphamide (100 mg/day) or azathioprine (100-150 mg/day) can be administered as monotherapy or in combination with steroids. Due to their myelosupressive effects, peripheral blood cell counts must be monitored regularly and if needed dosage must be modified. From the pretreatment data in rituximab trials, it appears that, in the era before rituximab, azathioprine and cyclophosphamide were popular as second-line therapy, but we have used immunosuppressant's rarely because of doubts about efficacy and the fear of side effects [21] .Unfortunately, many patients become refractory to multiple therapeutic approaches and develop complications of chronic high-dose steroid therapy. Because of its success in other severe autoimmune disorders, high-dose cyclophosphamide was studied in patients with severe AIHA that was refractory to standard therapies [23]. Intermittent intravenous cyclophosphamide (pulse therapy) has been reported as an effective treatment for various autoimmune diseases including nephritis associated with systemic lupus erythematosus [24].

The mechanism of action of pulse cyclophosphamide in autoimmune disorders appears to be suppression of both T and B lymphocyte numbers and function, which leads to diminished autoantibody production [25]. High-dose cyclophosphamide was initially chosen as conditioning for allogeneic bone marrow transplantation because of its potent immunosuppressive properties [2]. Lymphocytes are highly sensitive to cyclophosphamide, but primitive hematopoietic progenitors are resistant to its cytotoxic effects because they contain high levels of aldehyde dehydrogenase, an enzyme that confers resistance to cyclophosphamide [3]. High-dose cyclophosphamide without stem cell transplantation induces durable treatment-free re-

missions in patients with severe aplastic anemia [26]. This approach also has activity in a variety of other refractory autoimmune conditions [6], and can eliminate alloantibodies [7].

The present case was to evaluate the efficacy of pulse cyclophosphamide therapy using (1 g/month) intravenously for four consecutive months in patient with severe refractory AIHA who had failed to respond to high dose of steroid therapy.

Treatment of steroid refractory AIHA is challenging especially in patients who failed to respond to maximum dose of steroids ± azathioprine ± intravenous immunoglobulin ± oral cyclophosphamide, also their preference to avoid surgery (splenectomy), restrictions imposed by health funding authorities to provide rituximab and the unavailability of compatible blood transfusion even washed RBCs superadded more difficulties and put the patients in a critical situation, so our trial using pulse cyclophosphamide therapy monthly showed good response with no detectable hazardous effects in our patient.

AIHA frequently has an acute onset, but in most cases, it must be considered as a chronic disease with few exceptions. In primary WAIHA, there is only a low chance of spontaneous or drug induced long-term remission or cure. Thus, the primary goal of treatment is to keep the patient clinically comfortable and to prevent "hemolytic crises" with the use of medical interventions with the lowest possible short- and long-term side effects [21]. It is surprising and regrettable that treatment of AIHA is still not evidence-based, but essentially experience-based. There are no randomized studies and only a few prospective phase 2 trials, otherwise, only retrospective studies. There is no formal consensus on the definition of Complete (CR) or Partial (PR) hematologic remission and refractoriness [21]. There is little consensus on how to manage AIHA when corticosteroid therapy fails and when splenectomy is ineffective or is not an option [11]. Treatments for these patients include low-dose cytotoxic therapy [27] danazoland intravenous immunoglobulin [28]. Most of these treatments are only partially successful, with many patients becoming dependent on glucocorticoid maintenance therapy, and eventually suffering the consequences of chronic steroid administration [10]. However, progress in treatment has been much slower [29]. Several investigators have reviewed therapy, but no treatment guidelines have yet been published [30].

For patients with AIHA in whom glucocorticoid treatment fails, splenectomy is frequently offered as second-line treatment [27]. However, this approach is limited because splenectomy is less effective and have a higher complication rate in secondary AIHA [31]. There is lack of systematic long-term data on efficacy and safety in the published reports for rituximab in AIHA, also rituximab therapy has to be repeated every 1-3 years, and this may increase the risk of infections, including progressive multifocal leukoencephalopathy PML [21]. In practice, the choice of the sequence of second line treatments in patients with WAIHA mainly depends on the personal experience of the physician, patient factors such as age and co morbidity, the availability and cost of drugs, and the preference of the patient. The main factor for the selection of any drug should be safety, because the curative potential of all these drugs is low, and treatment may be more dangerous for the patient than the disease to be treated. Decisions are always made on an individual basis after discussion of experienced hematologists and then with the patient [21].

The opinion that cyclophosphamide is highly effective appears to be based on data from two earlier articles [32,33]. Those studies provided overall results but no specific patient details

Our results were in concord with the study by Moyo et al., [23] which stated that, the use of high-dose cyclophosphamide (50 mg/kg ideal body weight per day) intravenously in combination with mesna and G-CSF for 4 consecutive days is well tolerated and effective in patients with refractory AIHA and they added that further study of this approach as treatment for refractory AIHA is warranted. In our study, a nearly similar results to that [23] were obtained with relatively small dose (1 g/month for four consecutive months) without use of mesna and G-CSF. The hazards of the high-dose cyclophosphamide they used was nausea, vomiting, transient alopecia, and neutropenic fever were not observed in our patients. More studies with large number of patients are recommended to compare between the two regimens.

Conclusion

Pulse cyclophosphamide therapy with relatively high dose (1 g/month for four consecutive months) induces remission in patients with severe refractory AIHA, provides reasonable option for AIHA away from the risks of splenectomy and toxicity and the higher costs of rituximab, and needed to be studied in large scale of patients.

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