

1    **Type of article- Original research paper**

2    **A pilot, randomized sham control trial of autologous bone marrow derived**  
3    **mononuclear cells in acute ischemic central retinal vein occlusion**

4    **Summary**

5    In this pilot, sham controlled randomized control trial (RCT) in patients with ischemic central  
6    retinal vein occlusion (CRVO), we studied the safety and efficacy of intravitreal injection of  
7    autologous bone marrow derived mononuclear cells and found that both patients who  
8    received stem cell injections did not develop anterior segment neovascularization at 1 year  
9    follow up. Except for some sterile inflammatory reaction in the initial follow up, no long term  
10   injection related serious adverse events (SAEs) were observed. Based on our observations we  
11   recommend a larger, multicentric study to further establish the safety and efficacy of this  
12   treatment in patients with ischemic CRVO.

13   **Abstract**

14   **Purpose:** To study the safety and efficacy of autologous bone marrow derived mononuclear  
15   cells injected intravitreally in patients with ischemic CRVO

16   **Study design:** Randomized sham controlled trial

17   **Method:** 4 cases with ischemic CRVO were recruited into the study. 2 cases were  
18   randomized into intervention group and 2 into control group. Baseline investigations included  
19   best corrected visual acuity (BCVA), intra ocular pressure (IOP), fundus fluorescein  
20   angiography (FFA), gonioscopy and optical coherence tomography (OCT). Patients in the  
21   intervention group received intravitreal injection of autologous bone marrow derived

22 mononuclear cells (MNCs) and those in control group received sham injection. Patients were  
23 followed up over a 12-month period.

24 **Main outcome measures:** Development of anterior segment neovascularization.

25 **Results:** Both patients in the intervention group did not develop anterior segment  
26 neovascularization over a follow up period of 12 months. 1 patient in control group  
27 developed neovascularization of iris and elevated intra ocular pressure over a follow up  
28 period of 6 weeks and required trabeculectomy for control of IOP. The other patient in  
29 control group was lost follow up after 2 weeks.

30 **Conclusions:** Our initial observations suggest that intravitreal injection of mononuclear cells  
31 may reduce the risk of developing anterior segment neovascularization in patients with  
32 ischemic central retinal vein occlusion. A larger, multicentric study would be valuable to gain  
33 further evidence to our preliminary observations.

#### 34 **Key words**

35 Mononuclear cells, vascular occlusion, retina, intravitreal injection, neovascular glaucoma

#### 36 **INTRODUCTION**

37 Retinal vein occlusion is the most common vascular cause of visual loss after diabetic  
38 retinopathy.<sup>1</sup> Central retinal vein occlusion (CRVO) is the occlusion of central retinal vein at  
39 or just behind the lamina cribrosa.<sup>2-4</sup> Based on the studies of May and colleagues,<sup>5</sup> CRVO is  
40 classified as ischemic CRVO and non-ischemic CRVO. Ischemic CRVO is defined as central  
41 retinal vein occlusion characterized by more than 10 disc areas of retinal non perfusion or  
42 more than 50% of capillary non perfusion areas in a 30 degree fundus photograph by fundus  
43 fluorescein angiography.<sup>5</sup> Major complications of ischemic CRVO are macular edema and  
44 anterior segment neovascularization with subsequent development of neovascular glaucoma.

45 Treatment options available for the management of ischemic CRVO include pan retinal  
46 photocoagulation, intravitreal anti-VEGF (vascular endothelial growth factor) agents and  
47 corticosteroids. CRVO study has shown that pan retinal photocoagulation causes regression  
48 of neovascularisation in 56% of cases if instituted after early evidence of neovascularization  
49 of iris (NVI). But neither pan retinal photocoagulation nor macular grid can help in  
50 improvement of vision.<sup>6</sup> RAVE trial (Rubeosis Anti-VEGF trial) has shown that after  
51 intravitreal injection of ranibizumab monthly for 9 months, at 6 months of follow up 90% of  
52 cases had resolution of macular edema, 60% of cases had improvement in visual acuity by  
53 four lines and none of the patients developed neovascularization of iris. At 3 years of follow  
54 up however, patients had deterioration of visual acuity and 30% of cases developed  
55 neovascular glaucoma.<sup>7</sup> So far there is no established treatment algorithm for ischemic  
56 CRVO.

57 Studies have shown that injection of autologous bone marrow derived mononuclear stem  
58 cells in ischemic stroke results in axonal plasticity and functional recovery in both  
59 experimental models and patients.<sup>8-10</sup> The neuro-protective effect of stem cells is presumably  
60 due to expression of neurotrophic factors like insulin like growth factor, basic fibroblast  
61 growth factor and epidermal growth factor which rescue the injured neuron. Similarly,  
62 intravitreal injection of mononuclear bone marrow derived stem cells in animal model of  
63 retinal ischemia have shown reduction in development of pre-retinal neovascular tufts.<sup>11</sup>

## 64 **Methods**

65 Approval was obtained from institutional committee for stem cell research and therapy (letter  
66 enclosed) and institute ethics committee (**IESC/T-448/30.11.2012**), AIIMS. Patients were  
67 recruited from the Retina clinic services at Dr. Rajendra Prasad Centre for Ophthalmic  
68 Sciences, A.I.I.M.S (All India Institute of Medical Sciences). They were evaluated by both

69 retina (PV) and glaucoma specialists (RS). Bone marrow aspiration was carried out by  
70 specialists in the department of hematology (TS). Mononuclear cell layer separation was  
71 performed in the Department of Stem cell facility. Four cases with ischemic CRVO  
72 confirmed by fundus fluorescein angiography without evidence of anterior segment  
73 neovascularization or glaucoma or other concurrent ocular pathology such as cataract or  
74 diabetic retinopathy were recruited into the study. All four patients were adults; two of them  
75 had hypertension as a risk factor for the development of CRVO. Two patients were of the  
76 male gender. By simple randomization using enclosed chits, two of the four cases were  
77 enrolled into intervention group and two others into study group. All patients underwent  
78 thorough ocular examination including measurement of best corrected Snellen visual acuity  
79 and intra ocular pressure (by Goldmann applanation tonometry), slit lamp biomicroscopy for  
80 anterior segment evaluation. Goldmann single mirror gonioscopy was performed to rule out  
81 anterior chamber neovascularization. All patients also underwent fundus fluorescein  
82 angiography (Carl Zeiss Meditec AG, FF450 plus Fundus Camera), to note the retinal  
83 perfusion status and SD-OCT (Carl Zeiss Meditec AG, Cirrus 500 HD OCT) to note central  
84 macular thickness. Retinal ischemia was more than 70% in all four cases.

85 Patients in intervention group underwent bone marrow aspiration by a standard technique. In  
86 lateral decubitus position, skin over the iliac bone was cleaned with antiseptic solution and  
87 draped. Skin and soft tissue down to periosteum was infiltrated with local anaesthetic 1%  
88 lignocaine with 1:1000 adrenaline. Approximately 40 ml of bone marrow was aspirated with  
89 15G bone marrow aspiration needle from posterior superior iliac spine. Patients in control  
90 group underwent sham bone marrow aspiration in which patients were similarly positioned,  
91 parts cleaned and draped, and the skin over the posterior superior iliac spine was pressed with  
92 hub of syringe (and no needle) to produce sensation of pain.

93 Bone marrow aspirate was immediately transferred to the Department of stem cell facility.  
94 Bone marrow mononuclear cell layer was separated by Ficoll density separation method.  
95 Bone marrow sample was layered over lymphocyte separation medium (Bio Whittaker) and  
96 centrifuged at a speed of 1500 rpm for 25 min. Mononuclear cells were aspirated and washed  
97 thrice in heparinized normal saline to remove the traces of Ficoll. All the procedures were  
98 done under strict aseptic condition. The harvested mononuclear cells were evaluated for  
99 viability, total count, morphology and Giemsa staining. Total cell count was calculated by  
100 counting the cells in the Neubaur counter under the microscope. Cell viability was identified  
101 by using trypan blue dye exclusion test. More than 90% viability was considered as  
102 acceptable. Giemsa stain was used to assess cell morphology.

103 Using flow cytometry, the mononuclear cells were characterized using the following  
104 antibodies- CD-34, CD-45 (all antibodies from BD PharMingen). During flow cytometry,  
105 approximately 0.5 million mononuclear cells were stained with the above antibodies at 4  
106 degree Celsius for 30 minutes. Isotope controls were also stained in parallel. Analysis was  
107 done using FACS LSR-II and FACS DIVA (BD Biosciences) software.<sup>12</sup>

108 Intravitreal injection was given within 2 hours of mononuclear cell isolation. Standard  
109 procedure was followed for intravitreal injection. In brief, pupil was dilated with Tropicamide  
110 1% eye drops and ocular surface was anaesthetized using Proparacaine 0.5% drops. Eye was  
111 cleaned with povidone iodine and draped. Bone marrow derived cell preparation of 0.09 ml  
112 suspended in normal saline (containing 6-8 million mononuclear cells) and 0.01 ml of  
113 triamcinolone acetonide (containing 0.04 mg of the drug) was drawn into a tuberculin  
114 syringe. The mixture was injected at a distance of 3.5-4.0 mm from limbus with a 26G needle  
115 in the inferotemporal quadrant. Anterior chamber paracentesis was done to normalize  
116 intraocular pressure intraoperatively. For patients in control group, a sham injection was  
117 performed; eye was cleaned, draped and the globe was pressed with the syringe hub to

118 produce sensation of discomfort. Postoperatively all patients were prescribed Moxifloxacin  
119 0.5% eye drops to be used four times daily for one week. Patients in the intervention group  
120 received only one injection of autologous bone marrow stem cells. None of the patients  
121 received any additional intravitreal injections like bevacizumab, ranibizumab or  
122 triamcinolone. Also, no periocular injection of corticosteroids was used in the follow up  
123 period.

124 Patients were followed up over a period of 12 months at 1 week, 2 weeks, 4 weeks, 8 weeks,  
125 12 weeks, 6 months and 12 months. At each visit we recorded best corrected visual acuity,  
126 and intra ocular pressure. Slit lamp biomicroscopy for evidence of intra ocular inflammation,  
127 and iris neovascularization along with gonioscopy for angle neovascularization was also  
128 performed. SD-OCT was done in every follow up while fundus fluorescein angiography was  
129 undertaken only at week 4 and week 24. For detailed summary of patient baseline and follow  
130 up results please refer to supplementary data sheet.

## 131 **Results**

132 Case 1 (intervention group): 41 year old female with no systemic risk factors and symptoms  
133 of 2 weeks duration, baseline BCVA of 1/60 Snellen equivalent and central macular thickness  
134 of 764 $\mu$ . On first post-operative day there were 2+ cells in AC, which resolved by 2 weeks  
135 with topical prednisolone acetate eye drops. At 6 months follow up there was no evidence of  
136 anterior segment neovascularization and patient had a BCVA of 6/12 Snellen equivalent and  
137 central macular thickness of 262 $\mu$  (Figure 1) Intraocular pressure was within the normal  
138 range at all follow-ups.

139 Case 2 (Intervention group): 64 year old hypertensive female with symptoms of 12 week  
140 duration, baseline BCVA of 3/60 Snellen equivalent and presence of fine epimacular  
141 membrane. On first postoperative day patient had central vitreous haze, which persisted till 4

weeks. It soon cleared spontaneously without the need for any additional therapy. At the end of 12 months patient had no evidence of anterior segment neovascularization with BCVA of 6/60 and pseudohole with pre-existing epimacular membrane (Figure 2). Intraocular pressure remained normal throughout the follow up.

Case 3 (Sham group): 74 year old male with symptoms of 12 week duration with hypertension with baseline visual acuity of 1/60 Snellen equivalent and central macular thickness of 1151 $\mu$ . There was no evidence of anterior segment neovascularization on 4 week follow up but patient presented at 6 week with complaints of ocular pain. Intra ocular pressure was recorded to be 42 mm of Hg on Goldmann applanation tonometer. There was no evidence of NVI but gonioscopy revealed presence of NVA. Patient underwent pan retinal photocoagulation. Trabeculectomy with Mitomycin C 0.02% was done to control intraocular pressure, as adequate control was not achieved with medical measures.

Case 4 (sham group): 70 year old male with baseline BCVA of 1/60 Snellen equivalent with central macular thickness of 760 $\mu$  and history of 10 weeks. Patient was lost to follow up after 2 weeks. At 2 weeks there was no evidence of anterior segment neovascularization and intraocular pressure was normal.

## **Discussion**

Ischemic CRVO is a major cause of neovascular glaucoma. Pan retinal photocoagulation after the appearance of anterior chamber neovascularization involving 2 clock hours of iris is the current standard of care. Intravitreal injection of bone marrow derived mononuclear cells has shown some benefit in mouse models of inherited retinal degenerations and retinitis pigmentosa.<sup>13, 14</sup> These are chronic disease processes and an end point is difficult to establish. In contrast, CRVO is an acute event that has a well established natural history which evolves over the initial one year. Human studies involving intravenous injection of autologous bone

166 marrow derived stem cells have been done in patients with ischemic stroke, which is also an  
167 acute event, and of vascular origin.

168 Both patients who received intravitreal injection of mononuclear cells had minimal  
169 intraocular inflammation in the first week which resolved without any complication. So, it  
170 gives a little evidence that the risk of severe intraocular inflammation after intravitreal  
171 injection of bone marrow derived mononuclear cells is less likely. The immediate post-  
172 injection sterile reaction was minimal and well controlled due to concurrent injection of  
173 microdose of triamcinolone. Thus the combined dose of 0.09mL of autologous bone marrow  
174 derived mononuclear cells and 0.01mL of triamcinolone was found to be safe and well  
175 tolerated.

176 In both patients in intervention group, anterior segment neovascularization did not develop  
177 over a follow up period of 12 months. The best result was observed in case 1 in whom the  
178 injection was given within 2 weeks of developing symptoms. Visual acuity in this patient  
179 improved from 1/60 to 6/12. This suggests that early intervention with stem cells may be able  
180 to aid better functional recovery. Better visual recovery following early intervention has also  
181 been observed following intravitreal anti-VEGF injection.<sup>15</sup> In animal models of stroke and  
182 some initial stem cell trial in patients with neuronal stroke too, it is believed that early  
183 intervention with stem cells is likely to improve functional recovery.<sup>16</sup>

184 An important limitation of our study is the small sample size owing to which it would be  
185 difficult to ascertain if the absence/ presence of anterior segment neovascularization was a  
186 result of the known natural history of CRVO.<sup>17</sup>

187 **Conclusion** In this pilot study we found that a single intravitreal injection of autologous bone  
188 marrow derived mononuclear cells produces an early sterile reaction. However, in the long  
189 term it was well tolerated and may help to reduce the risk of anterior segment



neovascularization in patients with acute onset CRVO. Multicentric and large clinical trials are suggested to add further evidence to our initial observations.

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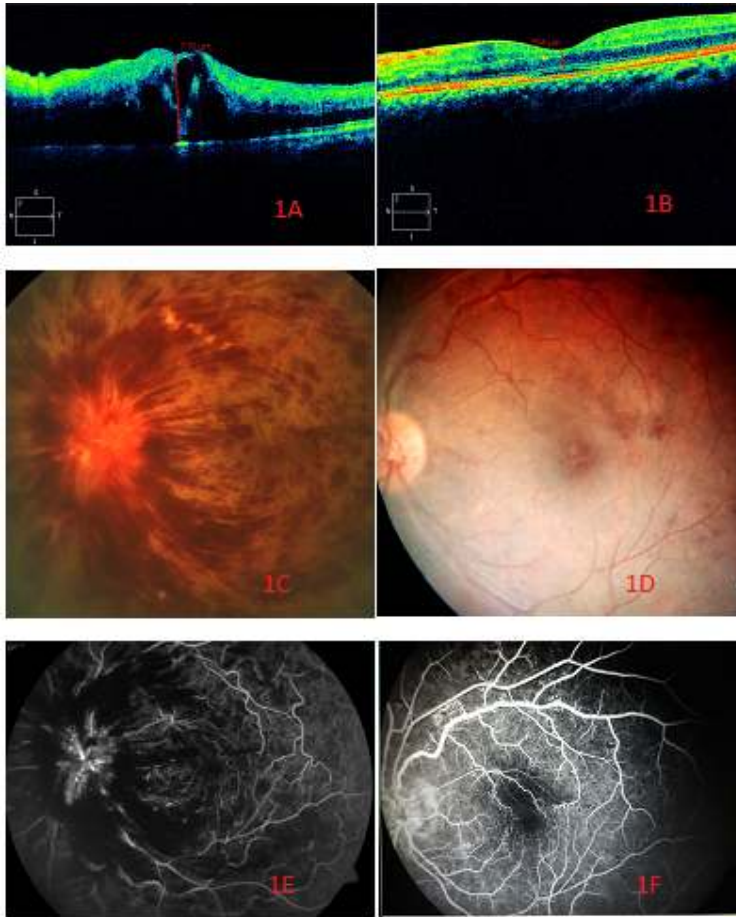
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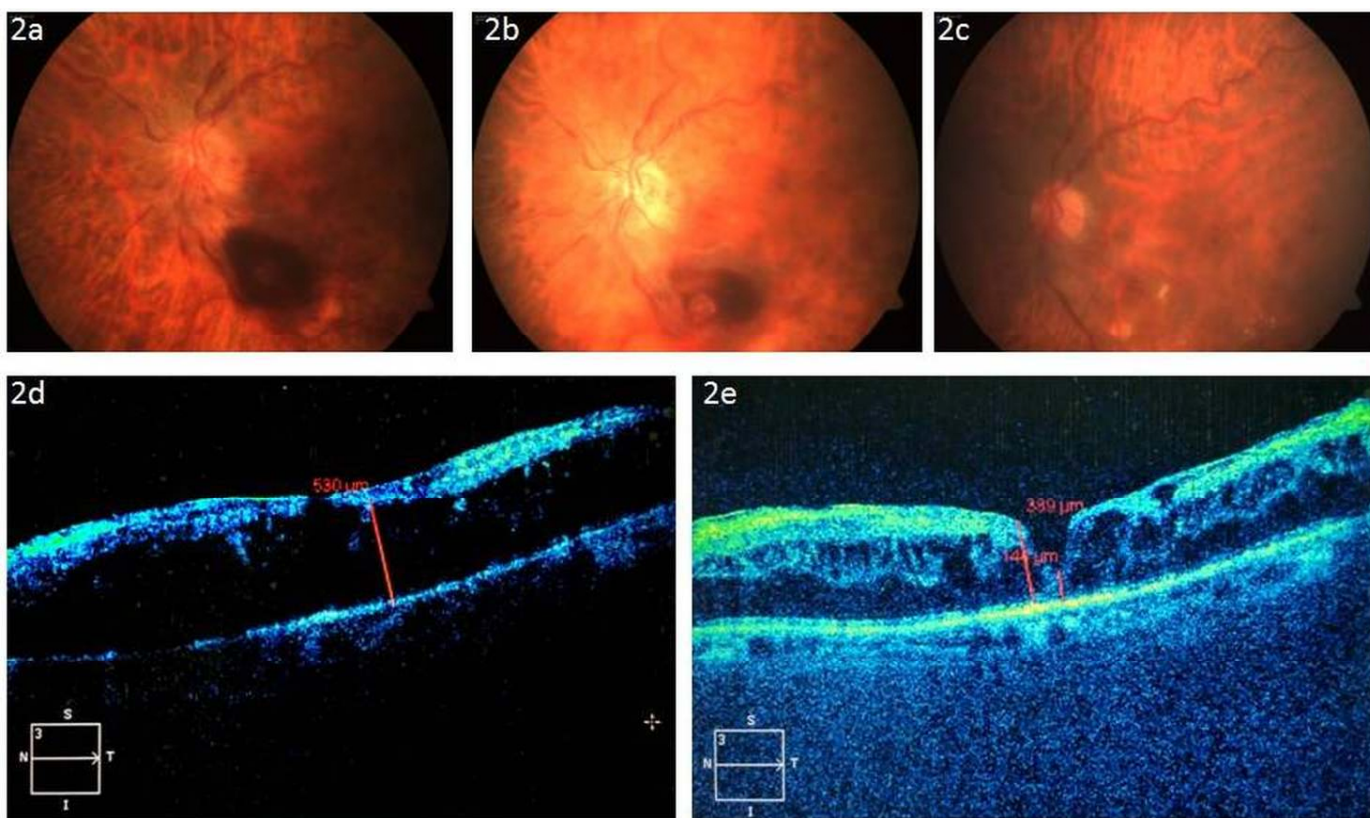
#### **Figure legend**

Figure 1: 1A- Pre intervention SD-OCT showing macular edema with cystic spaces. 1B- 24 week post intervention SD-OCT showing resolution of macular edema. 1C- Pre-intervention fundus photograph. 1D- 24 week post-intervention fundus photograph. 1E- Pre intervention fundus fluorescein angiography. 1F- 24 week post-intervention fundus fluorescein angiography.

Figure 2: 2a- Pre intervention fundus photograph. 2b- 4 week post-intervention fundus photograph. 2c- 24 week post-intervention fundus photograph. 2d- Pre intervention SD-OCT showing macular edema with cystic spaces. 2e- 24 week post intervention SD-OCT showing pseudohole with pre-existing epi macular membrane.



**figure-1**



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**Figure 2**