Immunocytochemical analysis in cell block method using vitreous infusion fluids in patients with vitreoretinal lymphoma

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Background:

Vitreoretinal lymphoma (VRL) patients are often diagnosed as diffuse large B-cell lymphoma (DLBCL). However, only a small amount of undiluted vitreous fluid may be obtained safely. The aim of this study is to analyze immunocytological findings in vitreous cell block (CB) preparations using waste cassette vitreous fluids in patients with VRL .

Patients & Methods:

This is a retrospective observational study involving 13 eyes of 13 cases with VRL who presented vitreous opacity and underwent diagnostic vitrectomy. CB specimens were prepared in all 13 eyes from waste diluted fluids containing shredded vitreous. These specimens were then submitted for hematoxylin-eosin (HE) staining as well as immunocytological analysis with antibodies against the B cells marker CD20, and the T cell marker CD3. This study further examined immunoreactivity to MUM-1, Bcl-2, Bcl-6 and CD10 to determine systemic DLBCL cell type based on Hans algorism (Blood 2004). Diagnosis of VRL was confirmed based on the results of cytology, concentration of interleukin (IL)-10 and IL-6 in undiluted vitreous, and immunoglobulin heavy chain gene rearrangements.

Results:

All CB specimens in VRL contained large lymphoma cells and small reactive lymphocytes with necrotic background. Immunoreactivity to CD20 was observed in the cell membrane of lymphoma cells in 12 VRL cases, whereas small lymphocytes were positive for CD3 but not CD20. Nuclear immunoreactivity to Bcl-2 and MUM-1 was detected in 4 out of 4, and 2 out of 4 cases examined, respectively. In contrast, no cases positive for Bcl-6 or CD10 were noted .

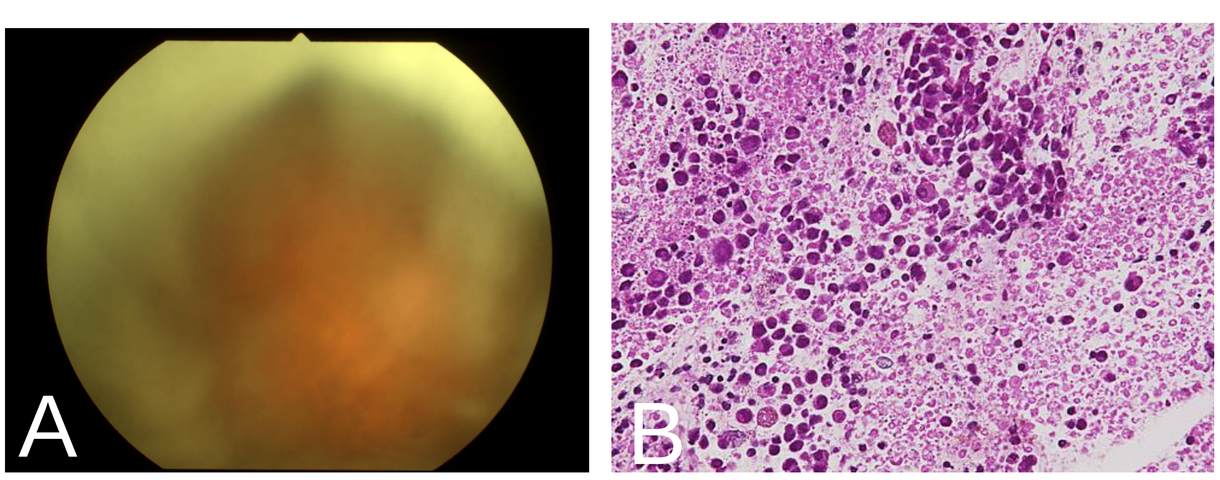


Figure 1. A representative case of VRL and the pathological findings of cell block preparation
Severe opacity of the vitreous is noted where appaerance of the optic disc is vauge (A). The cell block specimen reveals a variety of cell populations with large nuclear/cytoplasmic ratio and atypical nuclei. There is a characteristic necrotic background. Some macrophages and small reactive lymphocytes are intermingled.

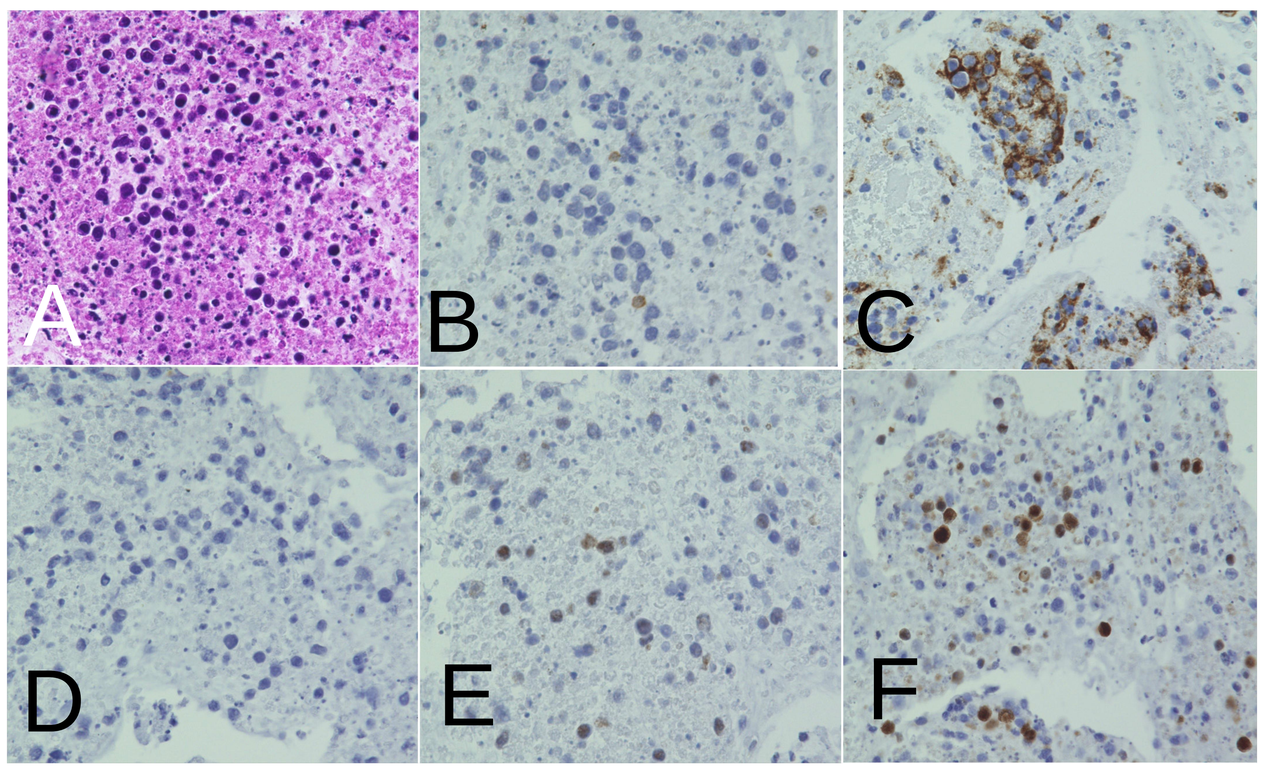
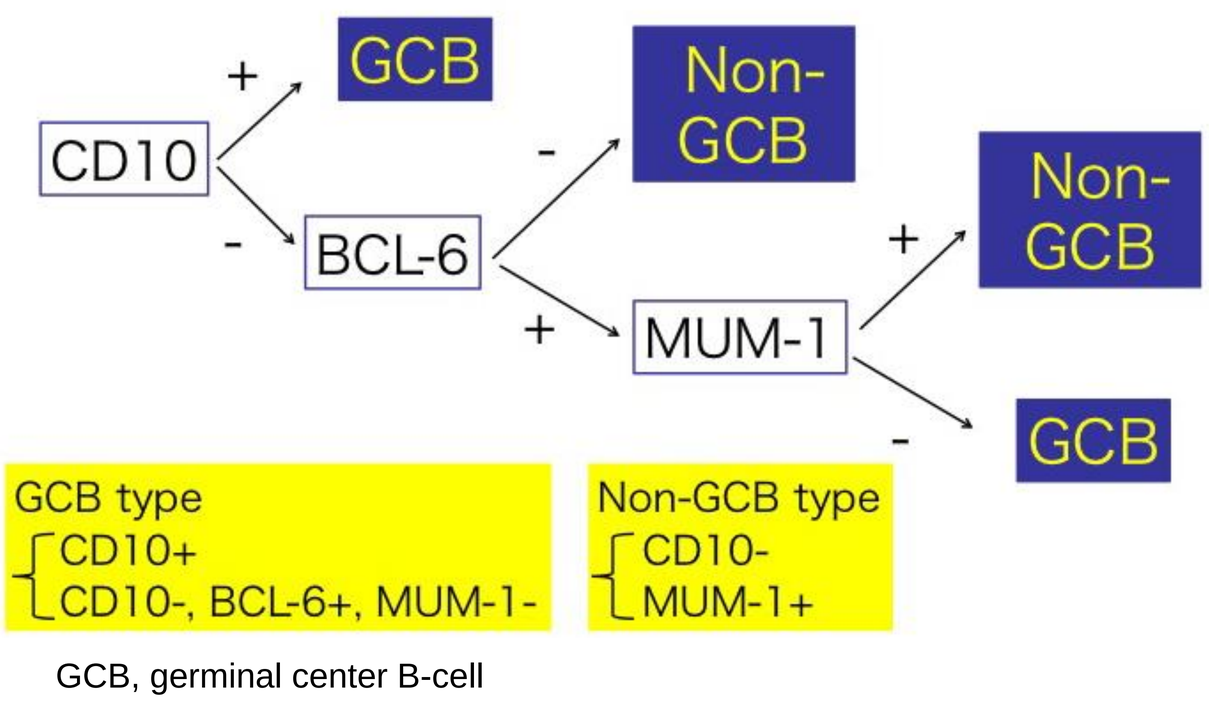


Figure 2. Cell block specimen of Hematoxylin and eosin staining (A), and immunoreactivity for CD5 (B), CD20 (C), CD10 (D), Bcl-6 (E) and MUM-1 (F) in another VRL case.
As shown in Figure 1, this case also reveals collection of relatively large atypical lymphoid cells with large nuclear/cytoplasmic ratio and necrotic background. Immunoreactivity for CD5 is not observed (B). In contrast, cytoplasmic immunoreactivity for CD20, a marker for B cells, is strongly detected in the atypical lymphoid cells whereas reactive small lymphocytes do not show the immunoreactivity (C). CD10 immunoreactivity is not observed in the atypical lymphoid cells in this case (D). Nuclear immunoreactivity for Bcl-6 (E) and MUM-1 (F) is strongly detected in a variety of atypical cells in the cell block specimen.



GCB, germinal center B-cell

Figure 3. Molecular lassification of When the lymphoma cells represent with CD10-positive or CD10-negative, BCL-6-positive and MUM-1-positive, cases are classified as germinal center B-cell (GCB) type. In contrast, when the tumor cells were negative for CD10, but were positive for MUM-1, cases are classified as non-GCB type. Basically, GCB type tends to show favorable patients' prognosis compared to non-GCB type (see ref. 1).

Conclusions:

CB specimens showed us not only cellular membrane but also nuclear antigens clearly. Present study suggested that VRL is derived from a non-germinal center B-cells, indicating aggressive tumor burden with poor prognosis complicated with systemic DLBCLs.

References:

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