FLOW CYTOMETRY IN THE DIAGNOSIS OF VITREORETINAL LYMPHOMA

BILL SEWELL

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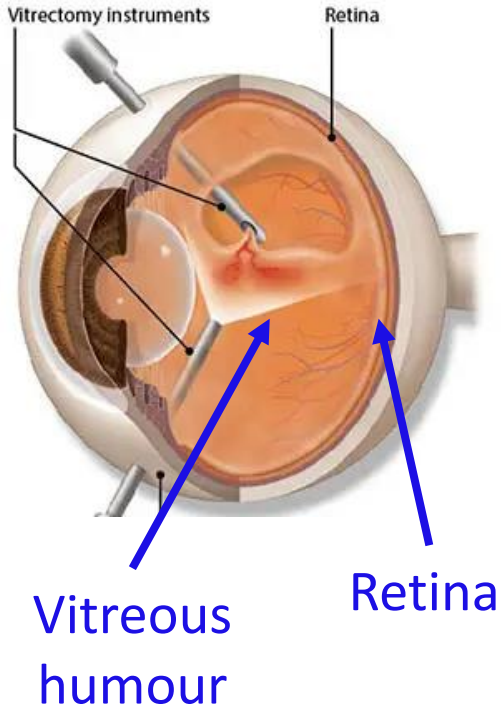
Vitreoretinal lymphoma (VRL)

- Rare
- Most cases are “Primary large B-cell lymphomas of immune-privileged sites” in the WHO classification.
- May occur concurrently with primary CNS large B cell lymphoma.
- Patients typically present with blurred vision.
- Lymphoma cells are in retina with spillover into vitreous humour.
- Best diagnostic procedure is vitrectomy.

Vitrectomy



American College of Ophthalmologists

Diagnostic vitrectomy retrospective cohort study

- 176 vitrectomies assessed at SydPath 2015-2023
- Modalities used:
  - Cytology
  - Immunocytochemistry on cell blocks
  - Flow cytometry
    - performed on 139 samples, reportable populations in 71
  - Microbiology
  - Limited role for molecular testing
- Samples for flow:
  - Washings from the vitrector cassette, delivered to flow lab 1:1 in RPMI medium.
  - 0.5 mL neat vitreous fluid

Standard flow panels for CantoII

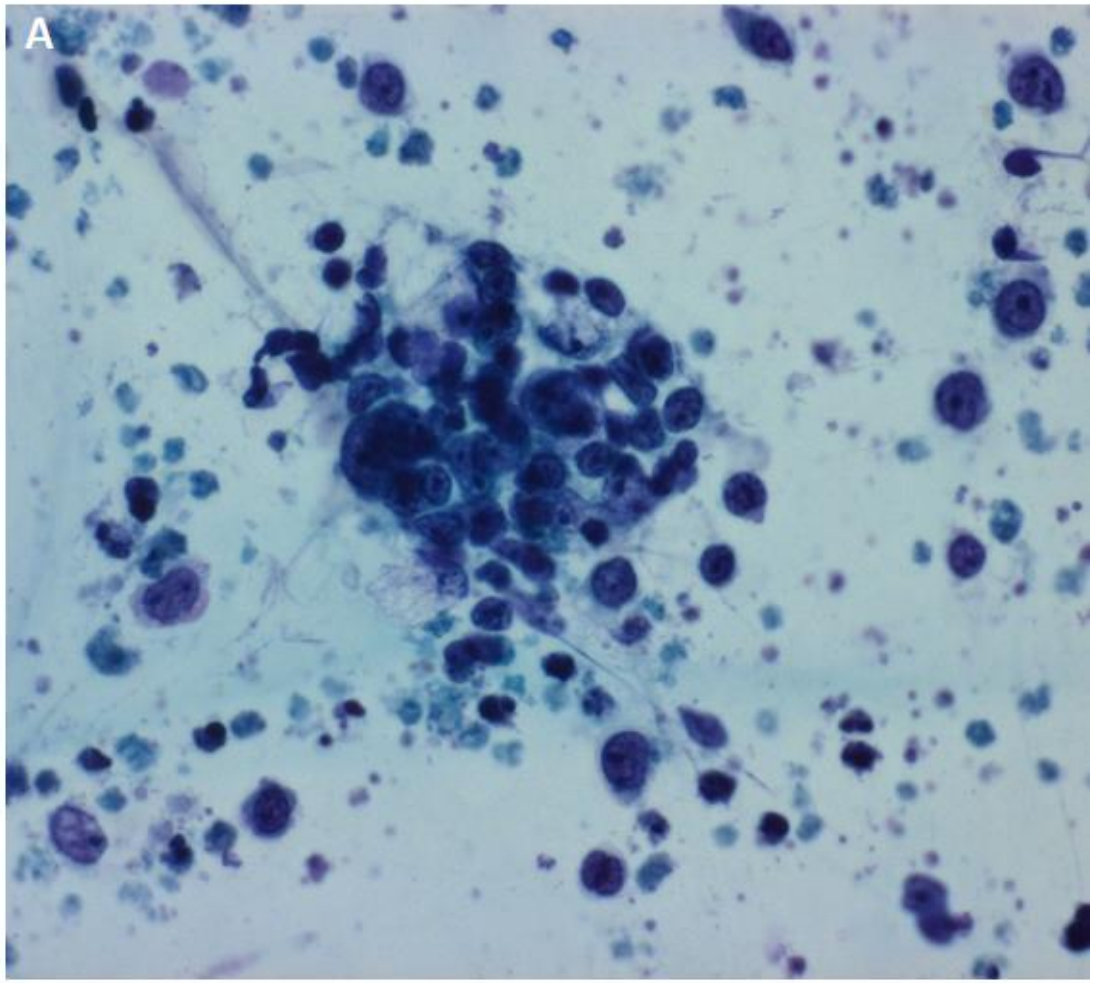
	V450	V500	FITC	PE	PERCP	PE CY7	APC	APC–H7
Tube 1	CD19 +20	CD45	CD3	CD8+ 16+56	CD4	CD5	CD7	HLA-DR
2	CD5	CD45	Kappa	Lambda	CD20	CD10	CD11c	CD19

Diagnoses

Age at presentation	mean 74 (range 10-96)
Male : female	89:87
Total no. of samples	176
Diagnostic	173
Non-diagnostic	3
Diagnostic category	
Benign	123 (71%)
Atypical	22 (13%)
Malignant	28 (16%)

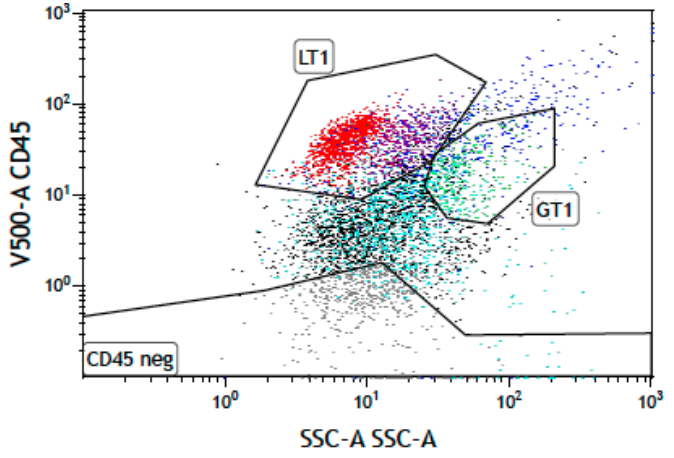
Diagnostic Category	Number of Cases	% of Total
Benign	123	71
Infectious	16	9
Inflammatory	88	50
Haemorrhage	14	8
Degenerative	5	3
Atypical	22	13
Lymphocytosis	12	7
Other	10	6
Malignant	28	16
Lymphoma	21	12
Carcinoma/Melanoma	7	4

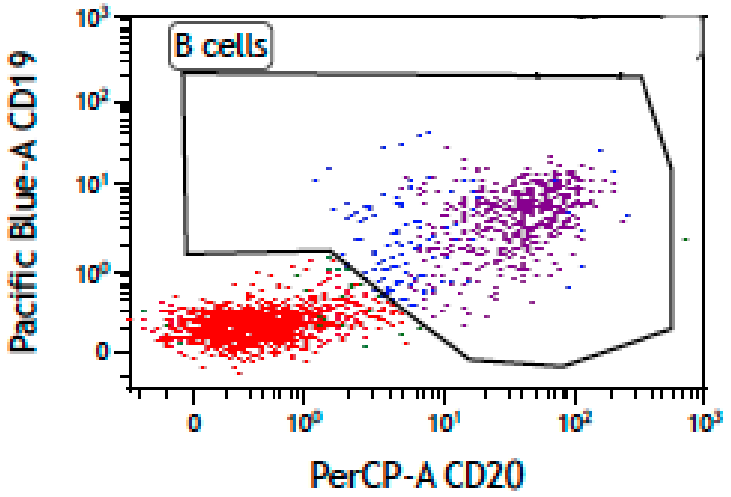
Cytology of DLBCL

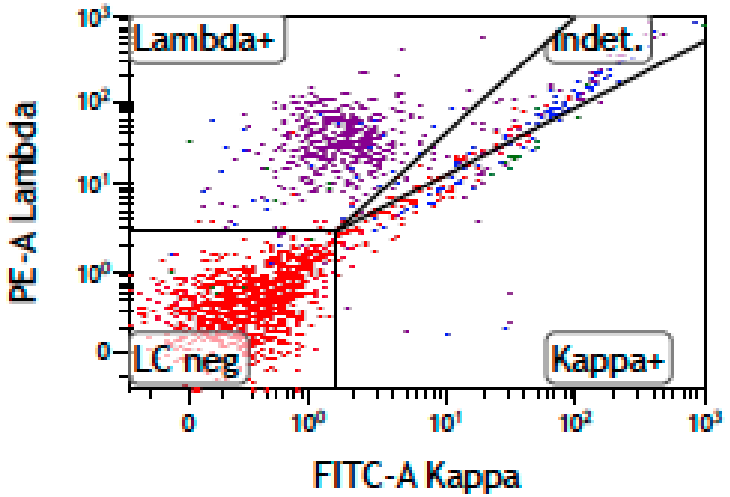


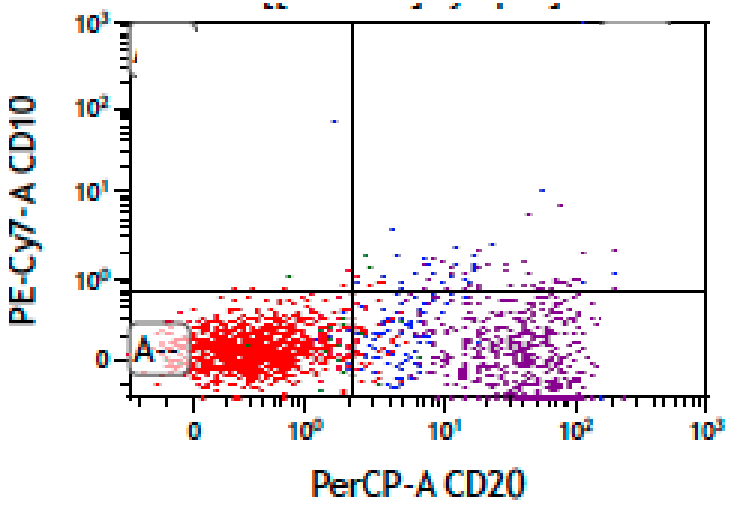
F 53 Large B cell lymphoma

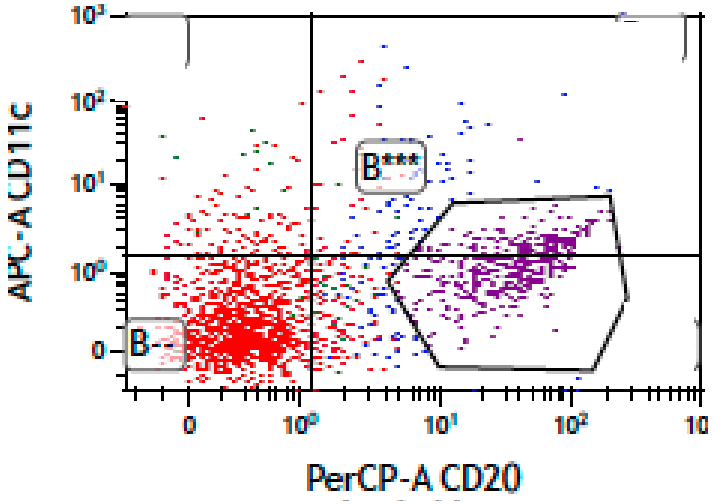
Vitreous Washings

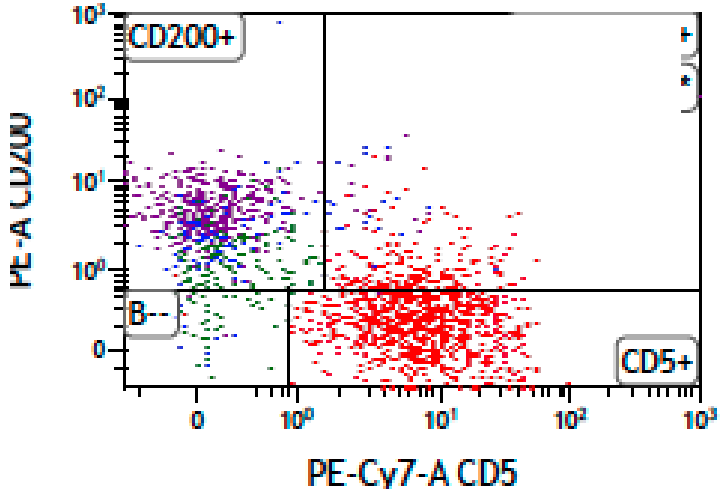






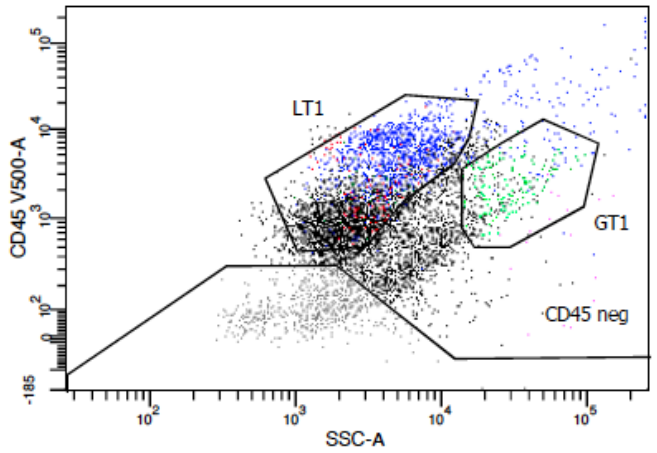


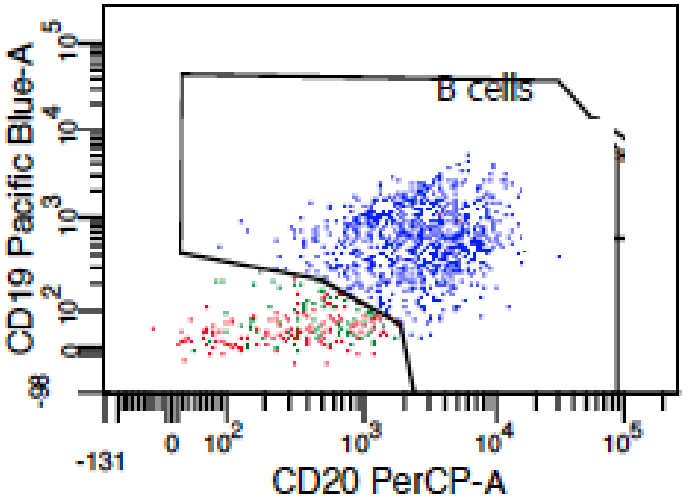


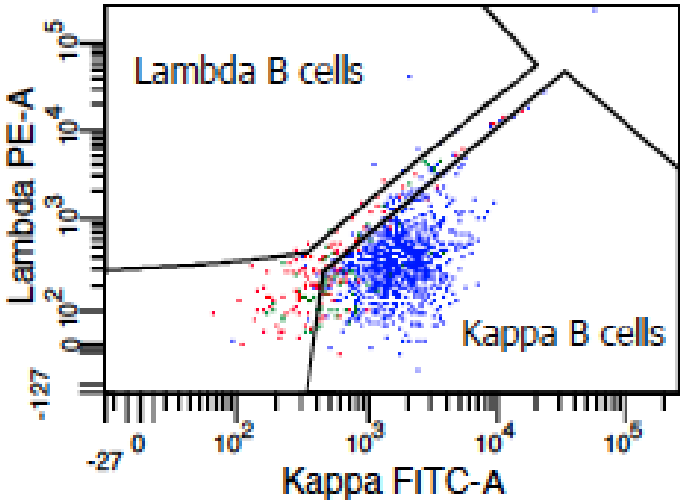


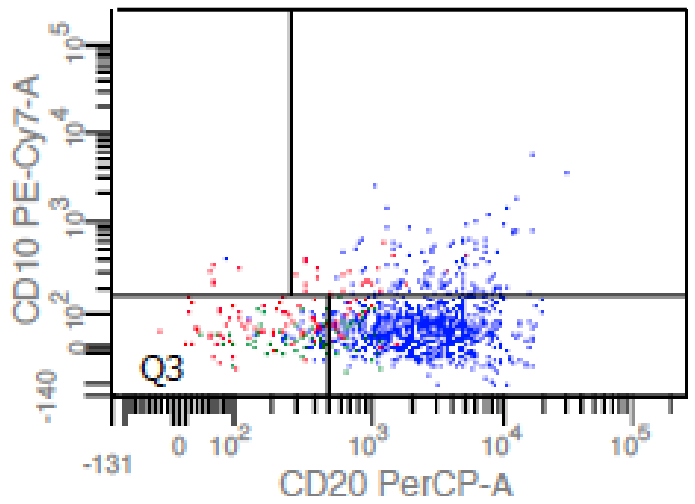
F 63 Large B cell lymphoma

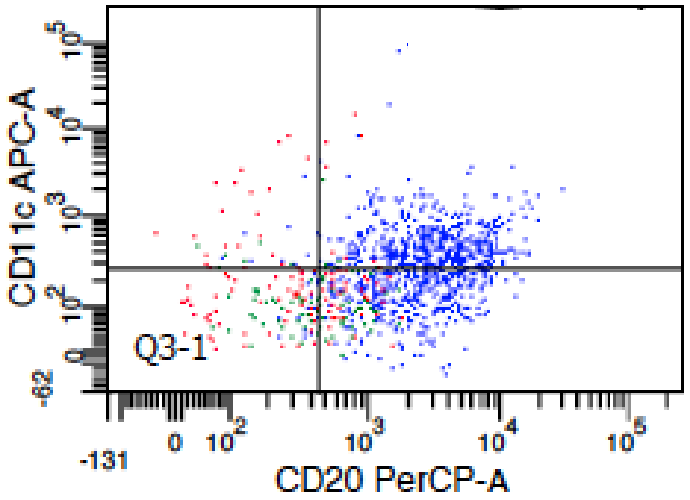
Vitrectomy

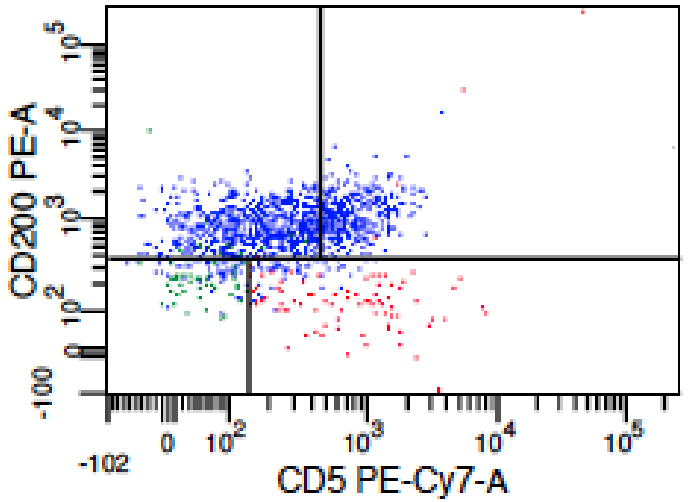






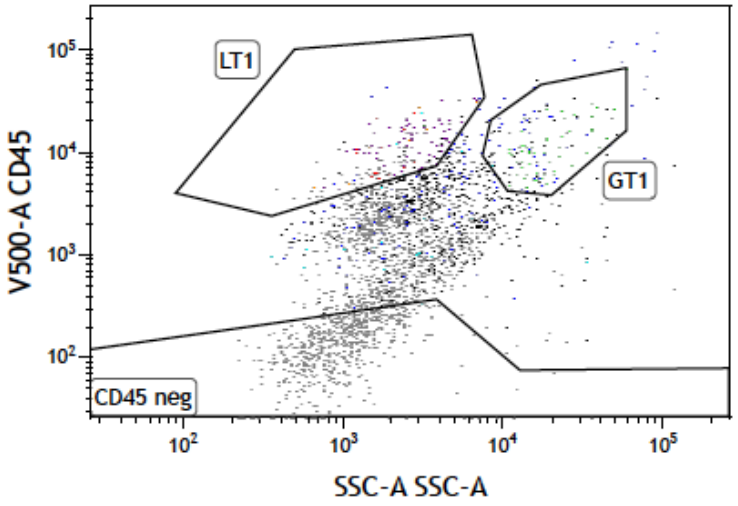


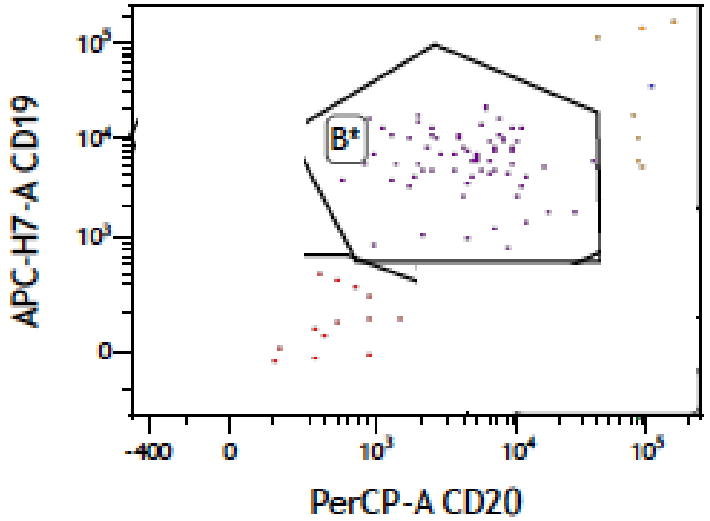


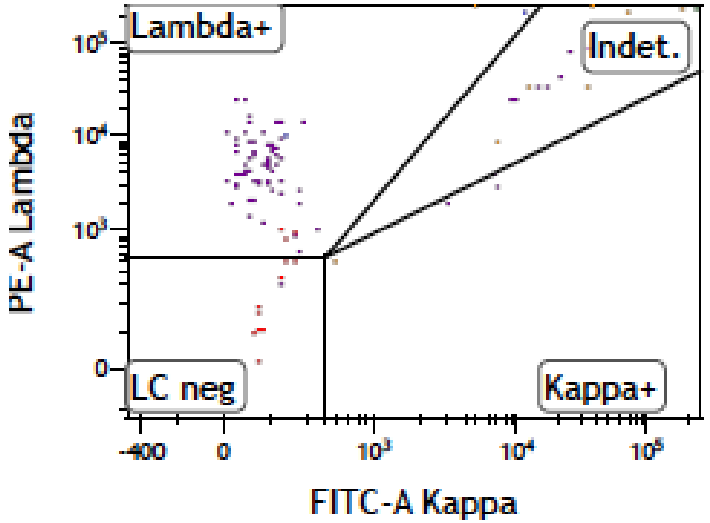


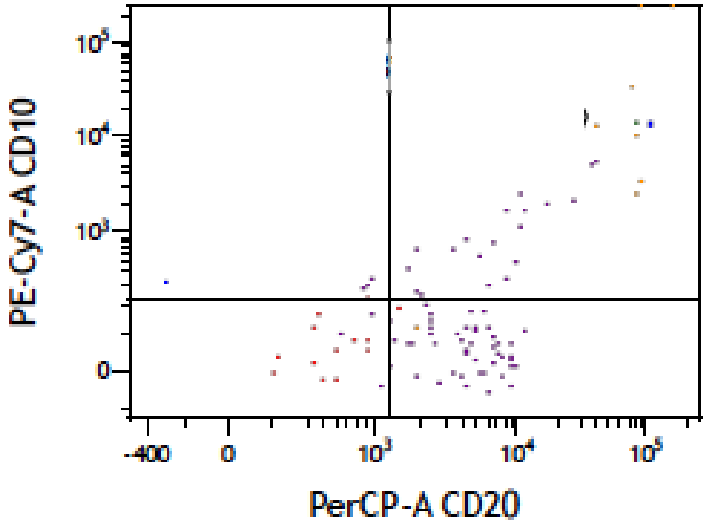
M 69 Large B cell lymphoma

Vitreous washings









Summary of lymphoma cases

Total lymphoma cases	21
T cell lymphoma	1
B cell lymphoma	20
Not further classified	6
DLBCL	14
DLBCL, not further classified	8
DLBCL, ABC type	4
DLBCL, GC type	2
B cell lymphoma Interpretable by flow	16
CD19+	14
CD20+	13
CD5+	3
CD10+	5

Flow cytometry of B cell lymphoma cases

Total cases	20
Interpretable by flow	16
Light chain restriction	14
kappa	7
lambda	7
light chain negative	2
CD5+	2
CD10+	5
CD11c+	6

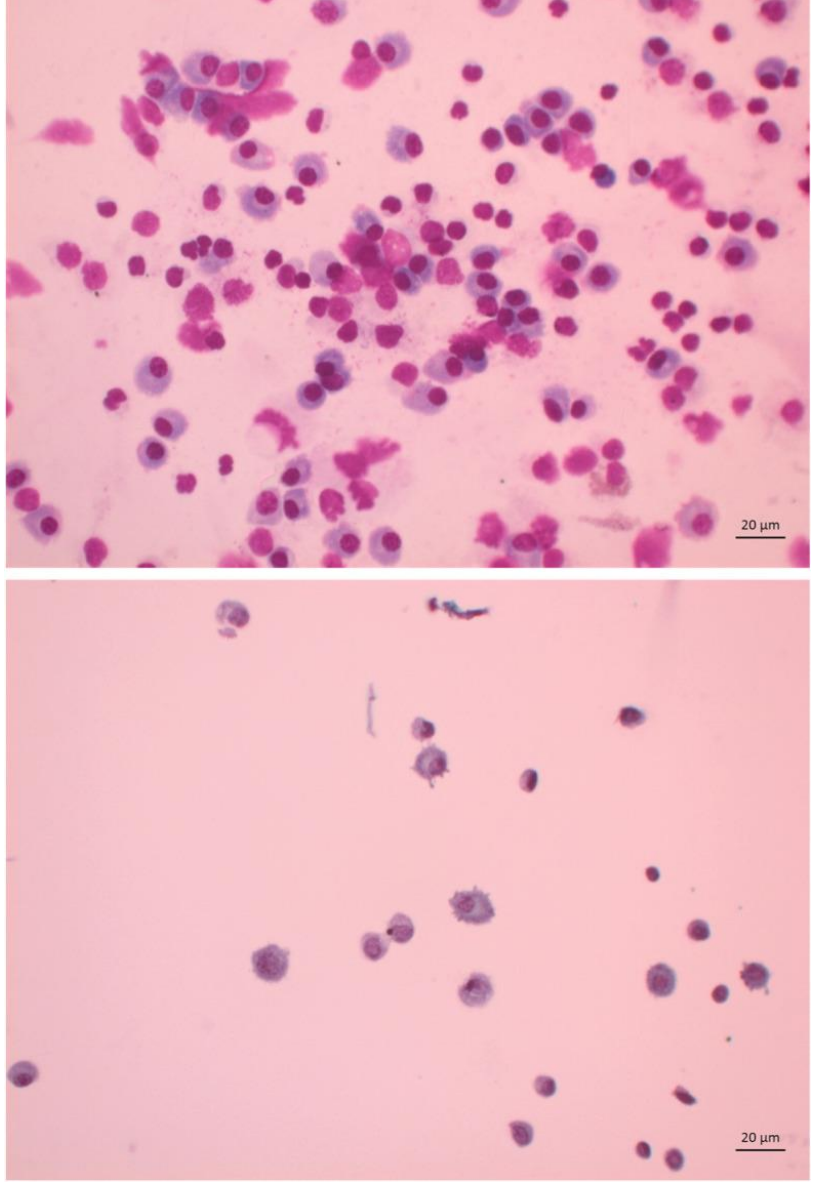
Features of atypical cases

Total atypical cases	22
Cytology performed	22
Atypical lymphocytes detected	13
Flow cytometry performed	13
Interpretable sample	9
B cell light chain restriction	1
Predominantly T cells	8
CD4:CD8 T cell ratio (mean)	4.38 : 1

UNUSUAL LYMPHOMAS AND BENIGN CASES

M 79

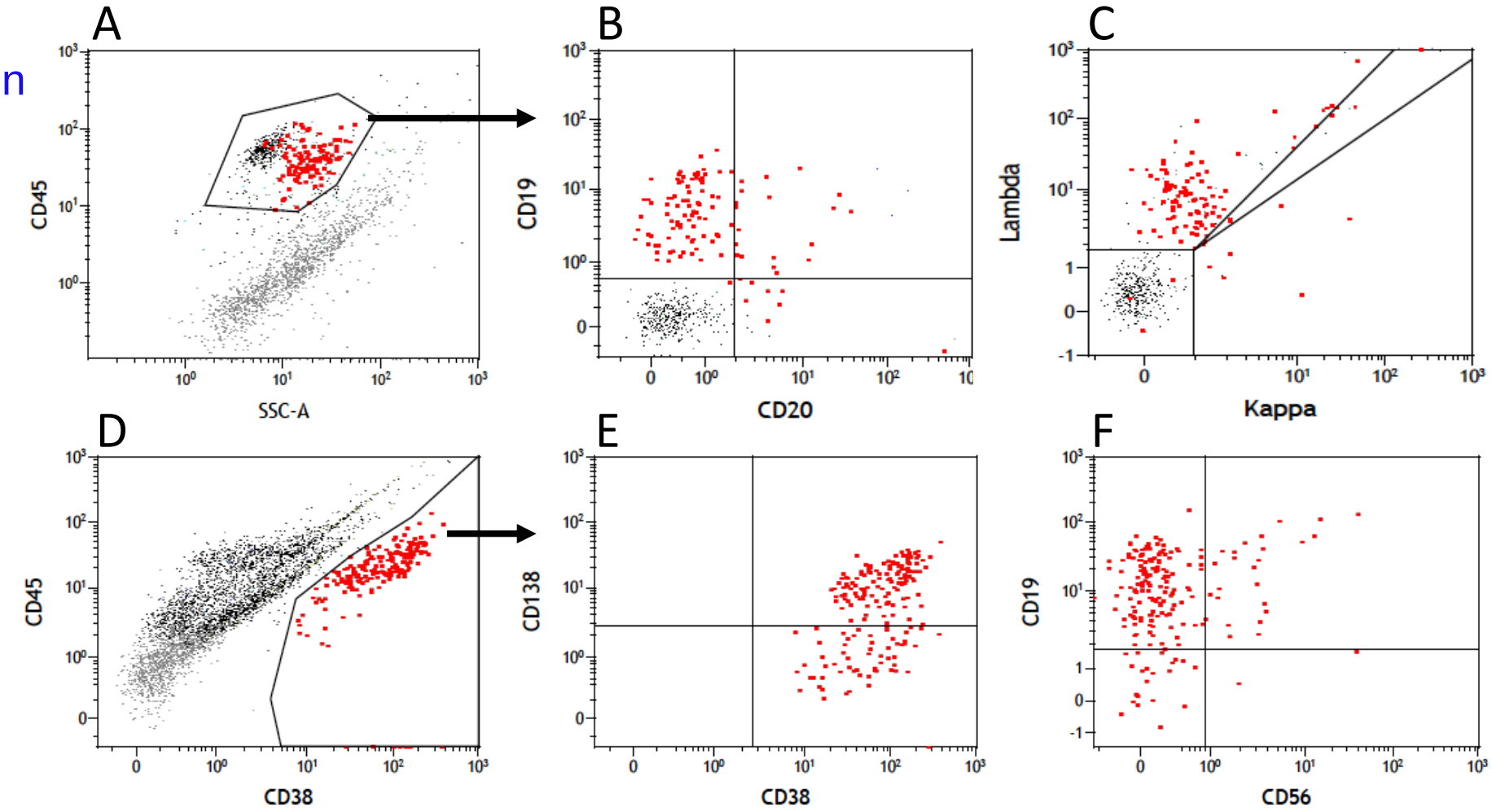
- 2 year history of worsening vision right eye, refractory to standard therapies
- Cells/inflammation noted in anterior chamber right eye
- Diagnostic Vitrectomy
  - The cytospins were highly cellular with numerous plasma cells, some with atypical findings including binucleation, nuclear pleomorphism and conspicuous nucleoli, in addition to small to intermediate sized lymphocytes and eosinophils.



Ayton T, Cherepanoff S, Gottlieb D, Sewell WA, Smith S, Hooper C. Intraocular solitary extramedullary plasmacytoma. BMC Ophthalmol. 2021 Jan 30;21(1):66.

M 70 Vitrectomy Flow plots

- clonal lambda-restricted population with phenotype largely consistent with plasma cells, except that the lambda positivity was detectable by surface staining.
- normal T cells
- no normal or lymphomatous B cells.

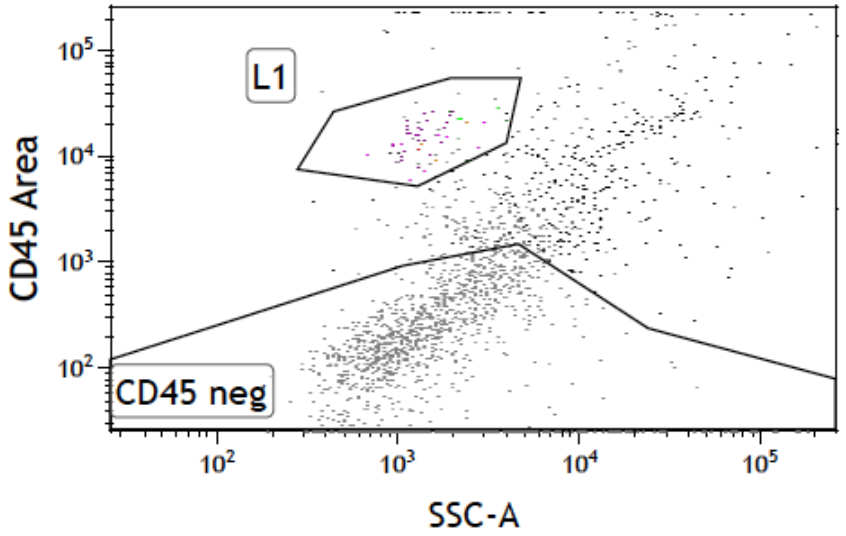


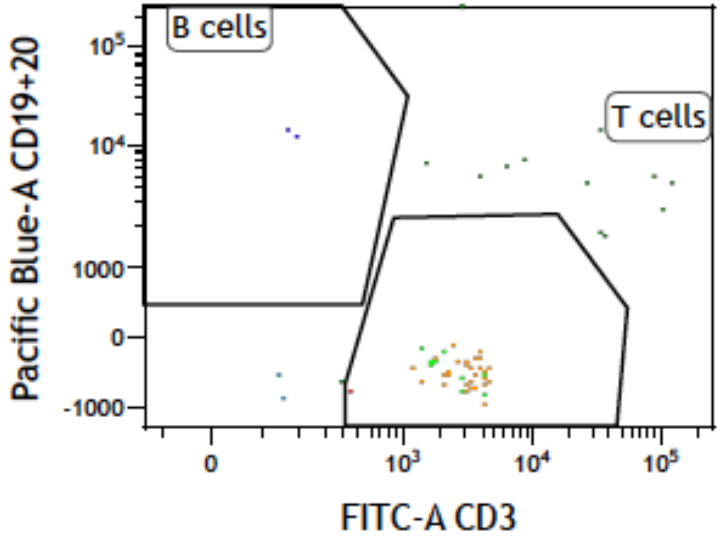
Clinical Details

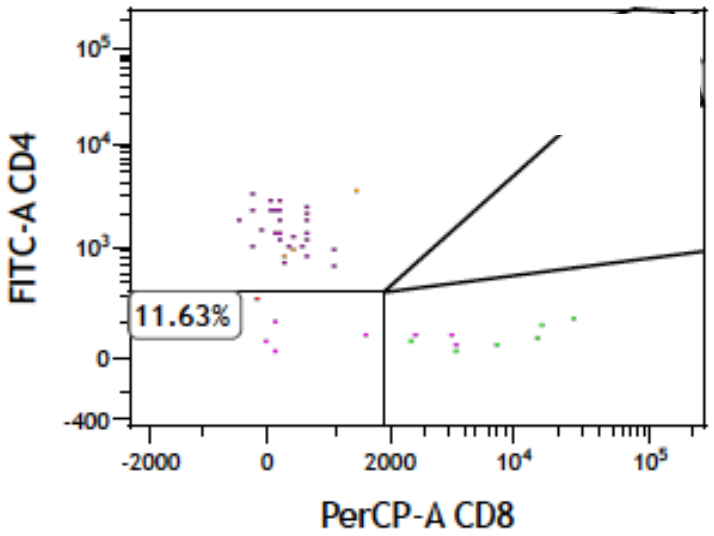
- Haematology review was negative, including:
  - PB flow cytometry (an irrelevant low count CLL-type MBL population was detected)
  - BMA including flow cytometry
  - PET-CT
  - MRI of brain and orbits
  - CSF including flow cytometry
- Diagnosis of Solitary Extramedullary Plasmacytoma (SEP) was made.
  - Treated with local radiotherapy.
  - 2 yrs later patient had 6/60 vision in treated eye and no evidence of haematological recurrence.

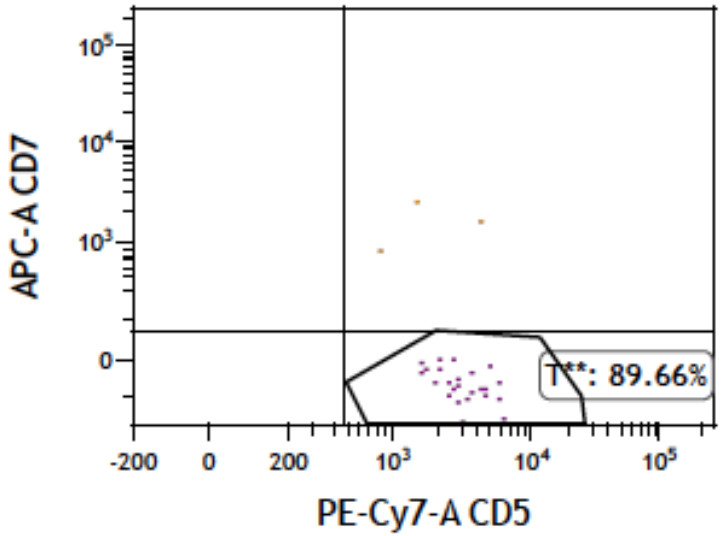
F 86 T cell lymphoma

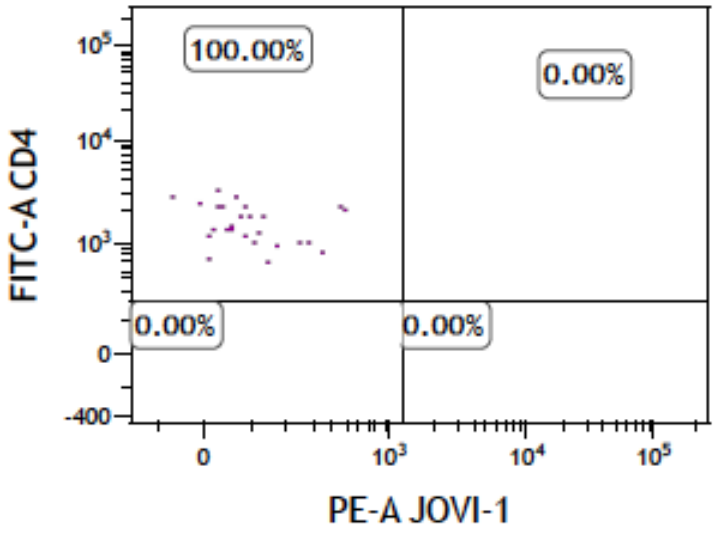
Vitrectomy. History of cutaneous T cell lymphoma





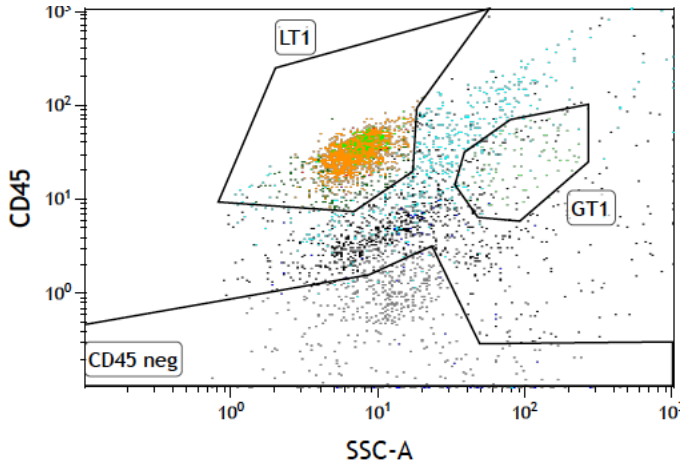


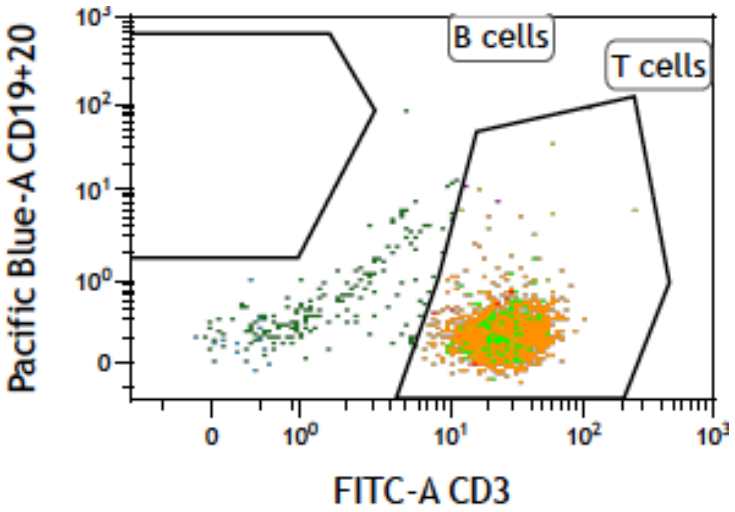


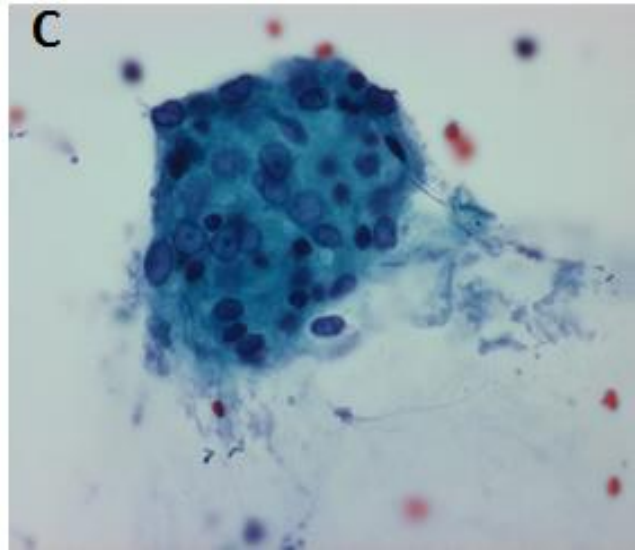


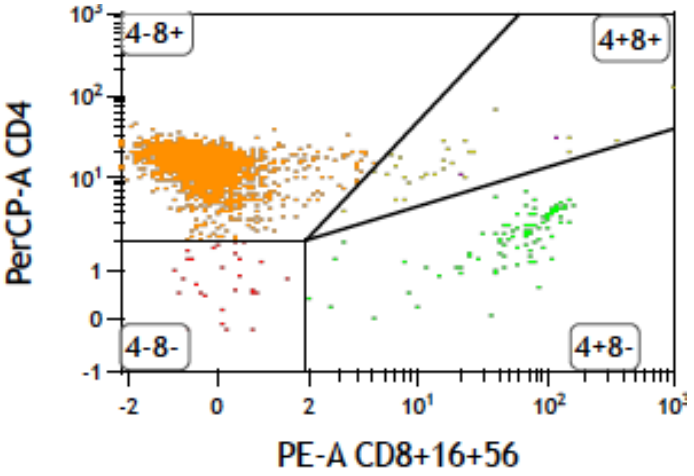
M 74 Granulomatous Inflammation

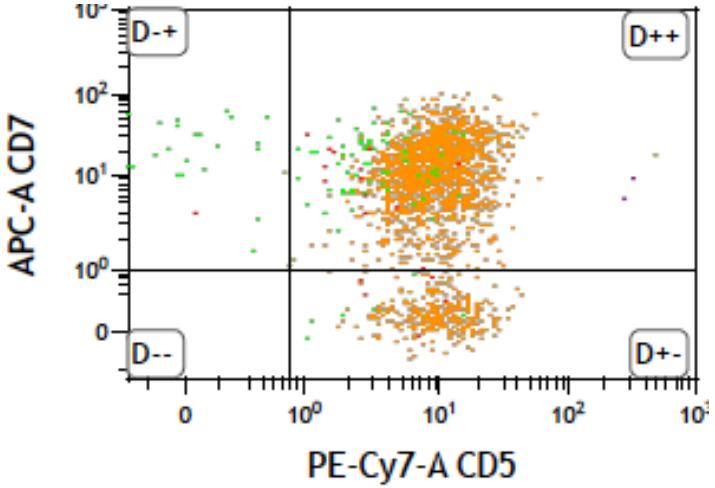
Vitrectomy





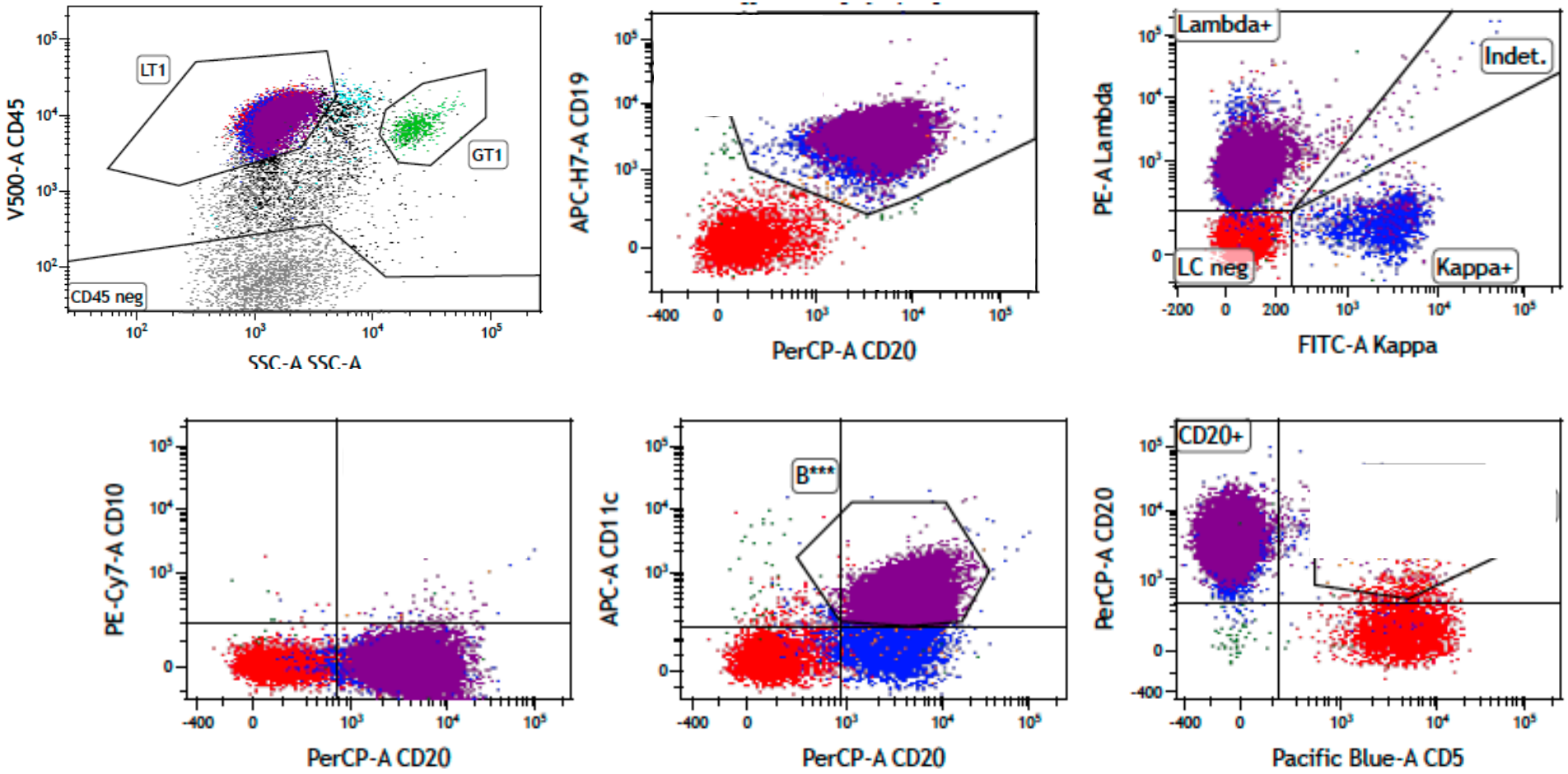




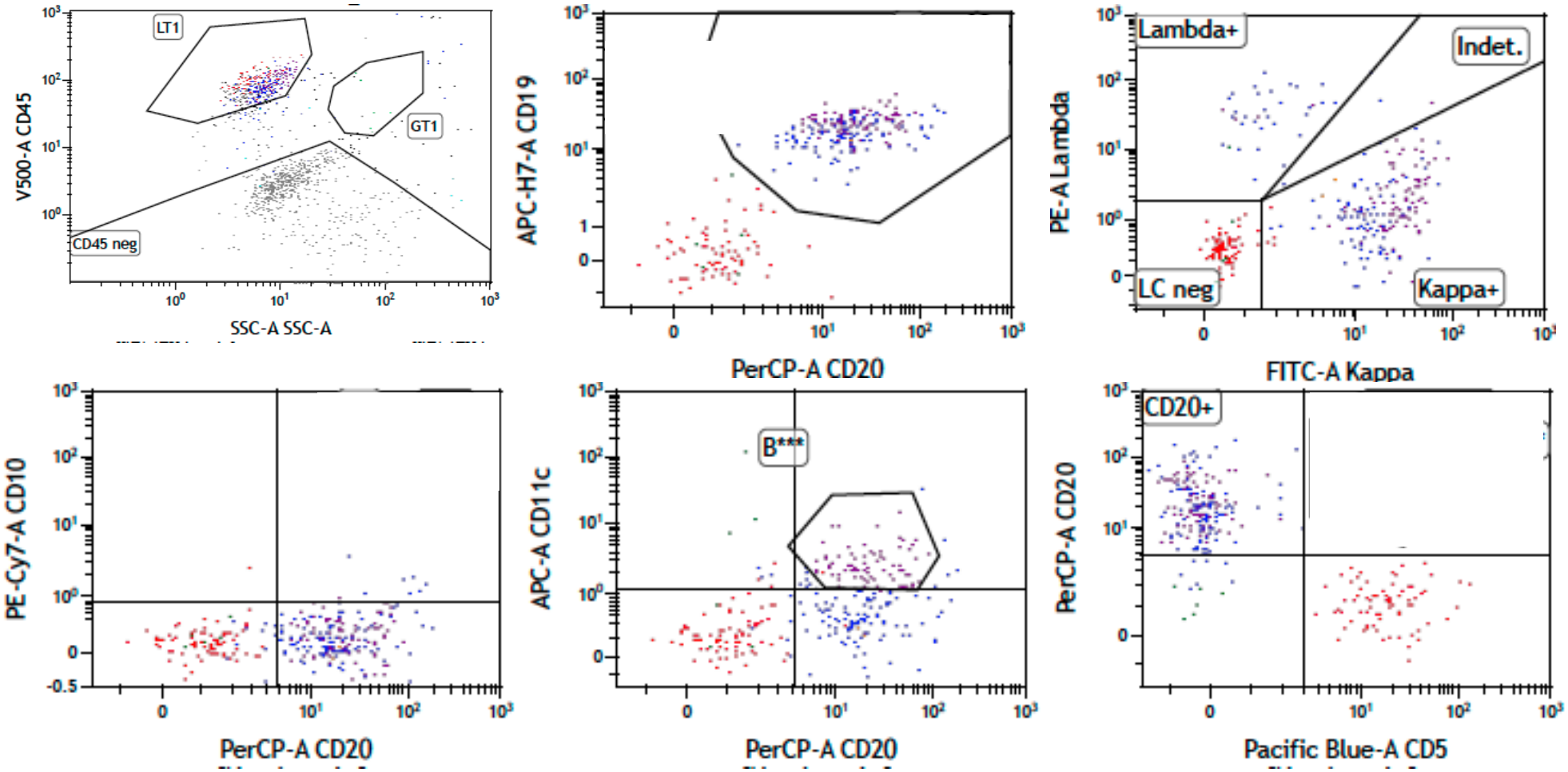


- 2019 – before assessment of clonality by TRBC1.
- Diagnosis – “granulomatous vitritis”
- Sarcoidosis considered but criteria not satisfied.

M 70 Inferior orbital tumour



M 51 conjunctival incisional biopsy



CONCLUSIONS

- Flow cytometry is feasible on vitrectomy and other ophthalmic samples
- Often cell numbers are low
- Samples should be aliquoted in the cytology lab not in theatre.
- Great care is required in sample processing and analysis.
- The study includes a few cases that were:
  - atypical by microscopy, but confirmed as lymphoma because of monoclonal B cell population detected by flow.
  - atypical by microscopy with no monoclonal B cell population detected by flow.
- Although case numbers are low, the study suggests that flow cytometry is a useful addition to microscopy on vitrectomy samples.