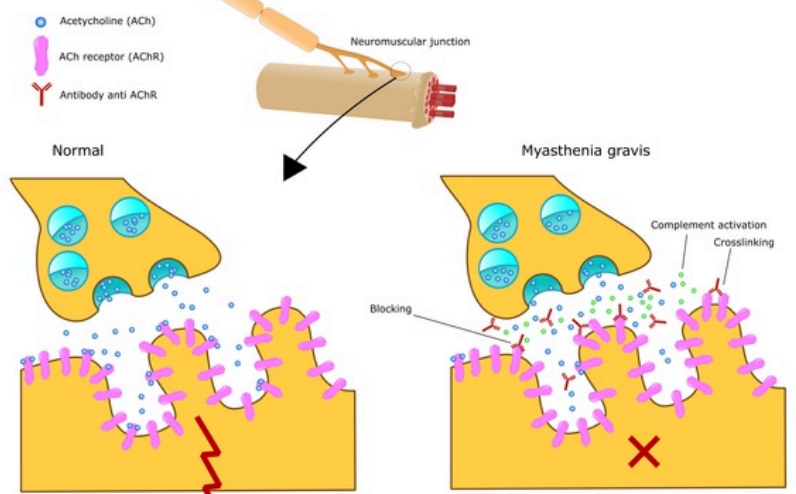


Abstract

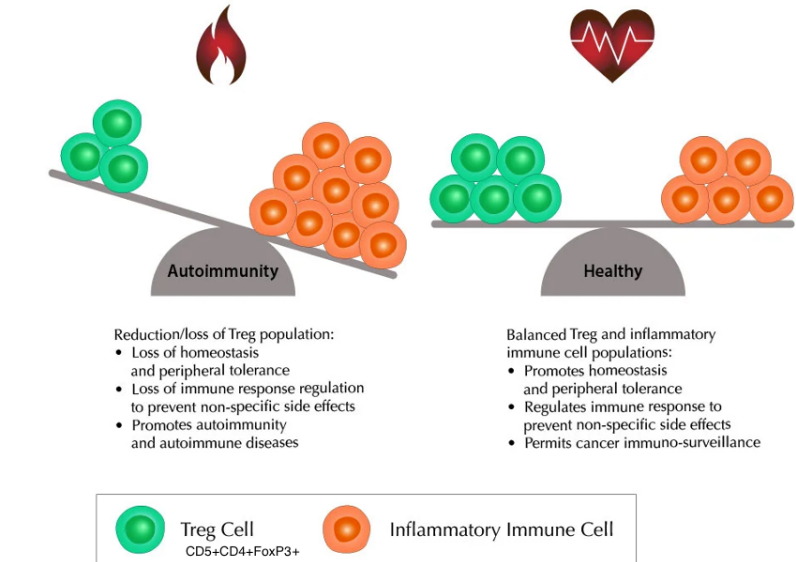
Myasthenia gravis is a T cell-dependent B cell-mediated autoimmune disease that affects multiple species in which the autoantibodies attack the acetylcholine receptors at the neuromuscular junction, resulting in muscle weakness. T lymphocytes are part of the immune system, and T regulatory cells (or Tregs) have suppressive functions that help to prevent autoimmune diseases. This study aimed to explore the relationship of T lymphocyte population identities and frequencies and Treg frequency and activation between healthy and myasthenia gravis canine patients.

Antibodies block ACh from binding to receptors on muscles



<https://ghr.nlm.nih.gov/condition/myasthenia-gravis>
7/23/20

Tregs may help to inhibit autoimmunity



<https://ghr.nlm.nih.gov/condition/myasthenia-gravis>
7/23/20

Materials, Methods, and Gating Strategy

This cell analysis study used the peripheral blood mononuclear cell samples collected, stained, and sent through a flow cytometer by Dr. Wu from dogs at the School of Veterinary Medicine of the University of Pennsylvania (Wu et. al, 2020). In this study, each dog's sample was analyzed individually using FlowJo.

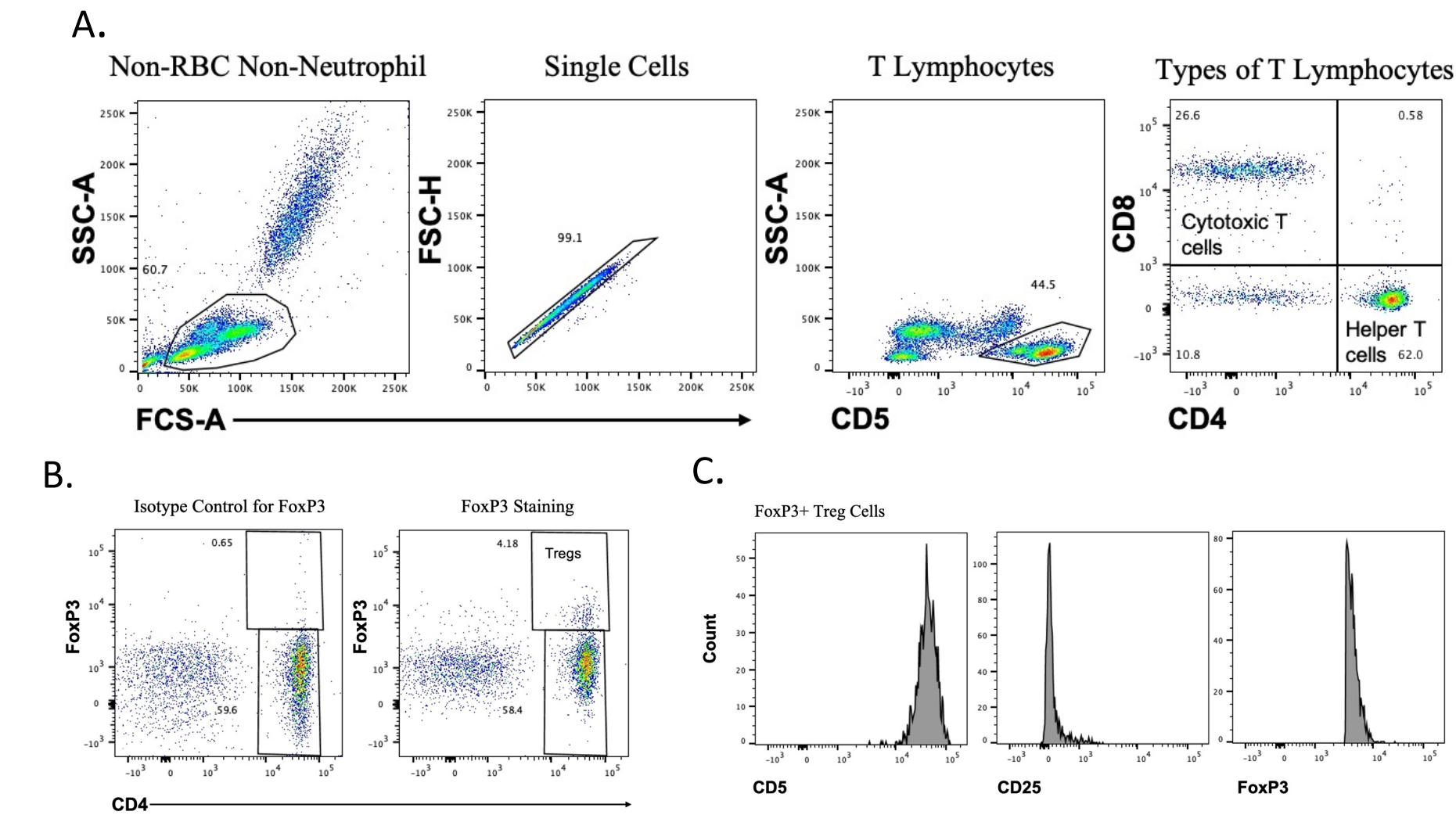


Figure 1. Gating strategy for the identification of Tregs. (A) In each dog's full sample, red blood cells and neutrophils were gated out before gating for single cells and T lymphocytes. Within the T lymphocyte population, types of T lymphocytes were identified. (B) These gates were applied to the dog's sample for isotype FoxP3. Within the T lymphocyte gate in this sample, a CD4+ FoxP3+ population and a CD4+ FoxP3- population were gated for. The gates from the isotype sample were applied to the full sample to control for FoxP3. (C) The geometric MFIs for CD5, CD25, and FoxP3 were explored in the FoxP3+ population.

Canine Participants

Name	Classification	Age, Sex	Breed	anti-AChR titer
Shiro	Healthy control	10yo, male	Mix	
Harley	Healthy control	5yo, female	Pointer Mix	
Juno	Healthy control	2yo, female	Mastiff	
Kate	Healthy control	13yo, female	Weimaraner	
Louise Scavello	Healthy control	12yo, male	Mix	
Elisee	Healthy control	7yo, female	Mix	
Girny	Symptomatic	2yo, female	Boxer	1.25 nmol/L
Kobi sample 1	Symptomatic	2yo, male	Boxer	2.92 nmol/L
Kobi sample 2	Symptomatic			N/A
Kobi sample 3	Symptomatic			2.11 nmol/L
Flapjack	Symptomatic	6yo, male	Boxer	4.56 nmol/L
Kiya sample 1	Symptomatic	9yo, female	GSD	4.02 nmol/L
Kiya sample 2	Symptomatic			3.3 nmol/L
Kiya sample 3	Symptomatic			0.81 nmol/L
Kiya sample 4	Symptomatic			1.44 nmol/L
Ricky sample 1	Symptomatic	10yo, male	Mix	2.86 nmol/L
Ricky sample 2	Symptomatic			0.84 nmol/L
Ricky sample 3	Symptomatic			2.26 nmol/L
Leo Fattelli	Symptomatic	1yo, male	HAV	8.59 nmol/L
Lily	Immunological remission	8yo, female	Pitbull	0.26 nmol/L
Zuma	Immunological remission	2yo, male	Mix	0.35 nmol/L
Kasper	Immunological remission	6yo, male	GSD	0.56 nmol/L

- Healthy control: no MG symptoms, anti-AChR titer < .6 nmol/L
- Symptomatic: MG symptoms, anti-AChR titer > .6 nmol/L
- Immunological remission (IR): MG symptoms, anti-AChR titer < .6 nmol/L

Do myasthenia gravis patients have higher T lymphocyte frequencies than healthy controls?

T lymphocyte frequency does not differ among clinical groups, but changes over disease course

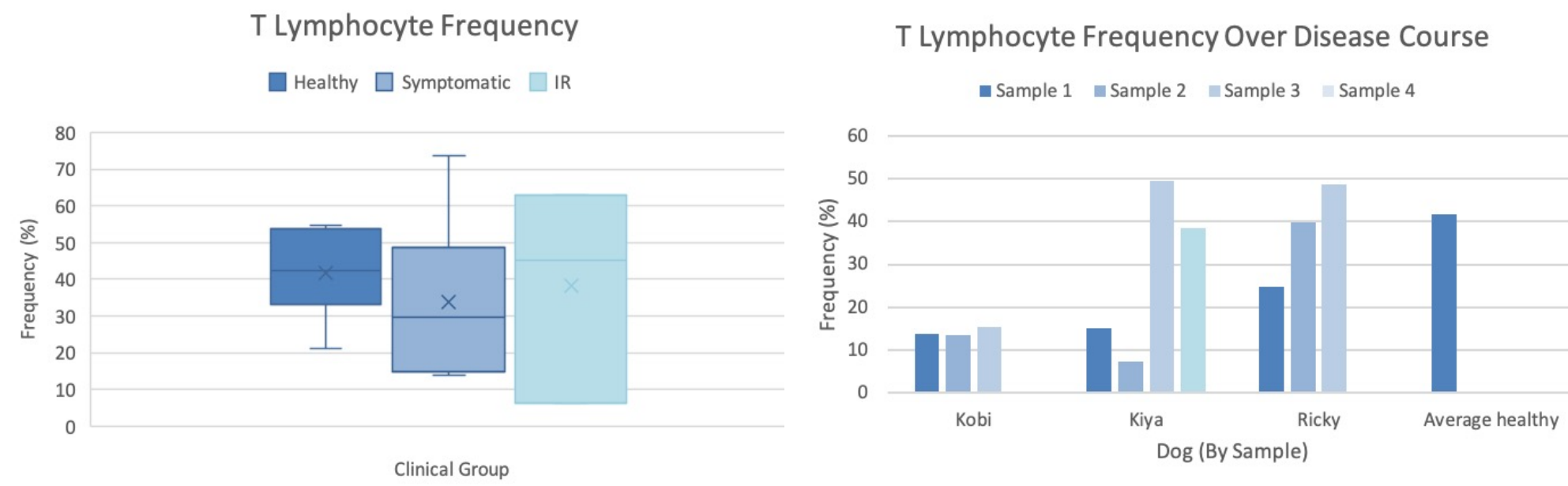


Figure 2. T lymphocyte frequency among clinical groups using highest AChR titer sample.

Figure 3. T lymphocyte frequency per dog with multiple samples over course of disease compared to average healthy frequency.

Do myasthenia gravis patients have increased levels of activated Tregs compared to healthy controls?

Treg frequency does not differ among clinical groups

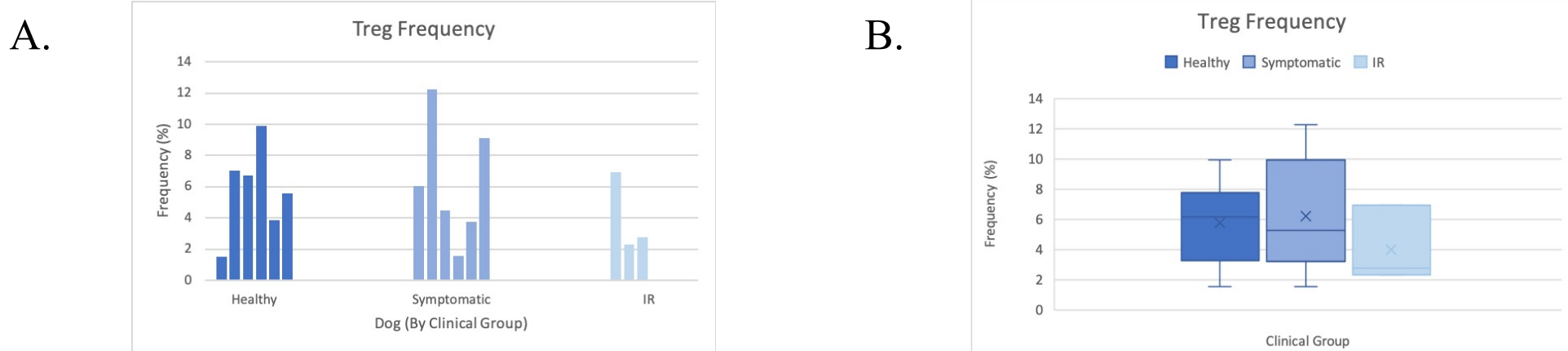


Figure 4. Frequency of Tregs among CD4+ cells among clinical groups using highest AChR titer sample. (A) Each bar represents an individual dog. (B) Clinical groups.

Treg frequency changes over disease course Activation status does not differ among clinical groups

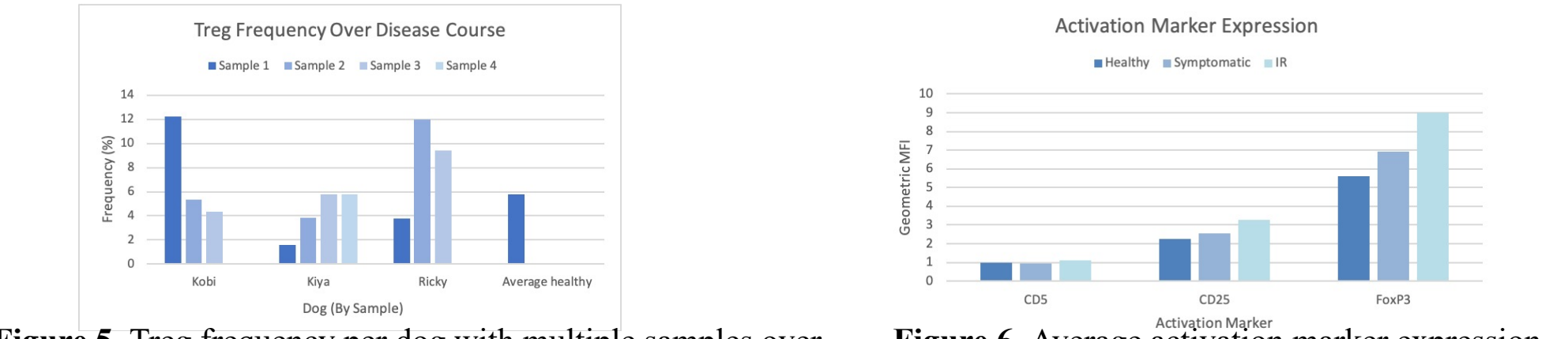


Figure 5. Treg frequency per dog with multiple samples over course of disease compared to average healthy frequency.

Figure 6. Average activation marker expression per clinical group.

Do myasthenia gravis patients have higher ratios of types of T lymphocytes compared to healthy dogs?

CD4:CD8 ratio is significantly higher in MG

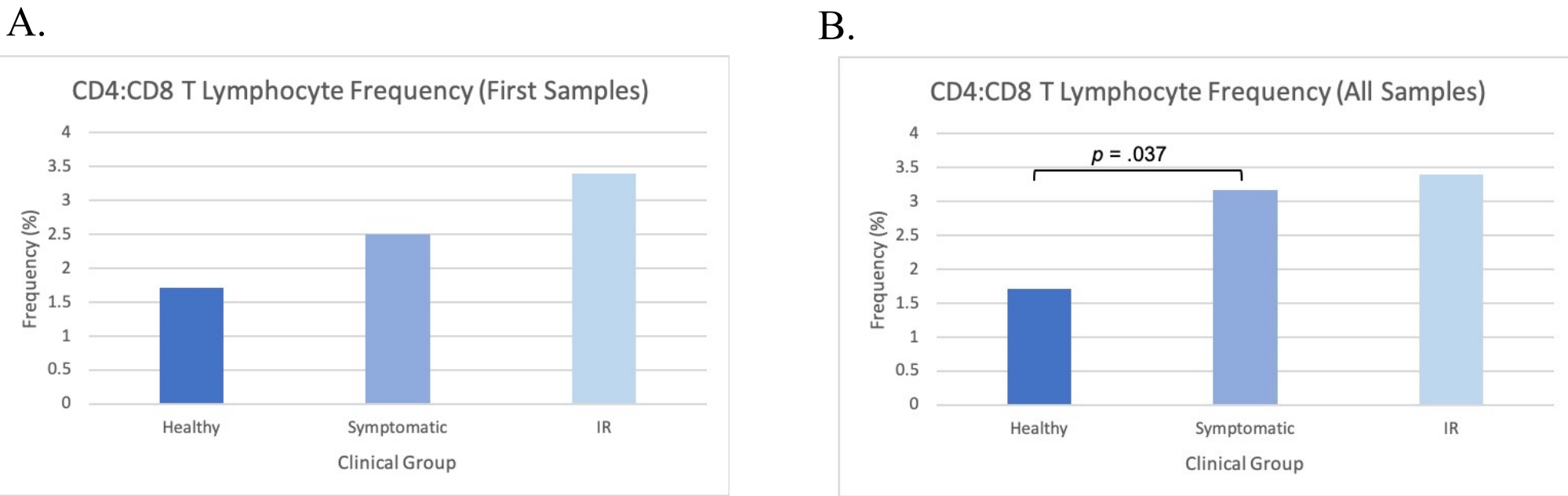


Figure 7. (A) Average CD4:CD8 ratio among clinical groups is not significant without all samples. (B) Average CD4:CD8 ratio among clinical groups is significant when using all samples. $p < .05$ by a one-tailed t test with variance.

Conclusions, Limitations, and Future Directions

Conclusions

- T lymphocyte frequencies do not differ among clinical groups, but increase in those that recover over the course of their disease
- We confirmed Dr. Wu's findings that Treg frequencies do not differ among clinical groups, but we also see a trend towards average healthy frequency over disease course
- CD4:CD8 T cells is significantly higher in myasthenia gravis

Limitations

- Small cohort size
- Relatively few markers

Future Directions

- Confirm T lymphocyte and Treg findings with a larger cohort size
- Follow myasthenia gravis patients for longer over course of disease and measure T lymphocyte and Treg frequencies
- Explore the meaning of an increased CD4:CD8 T cell ratio in relation to MG

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Contact

Carly Seligman
University of Pennsylvania Schools of Arts and Sciences
Email: carlyds@sas.upenn.edu
Phone: 610-952-7958

