“LYMPHOCYTE SUBPOPULATIONS HAVE PREDICTIVE VALUE IN THE DEVELOPMENT OF ANTIBODIES AFTER VACCINATION AGAINST SARS-COV-2 IN HEMODIALYSIS PATIENTS AND KIDNEY TRANSPLANT RECIPIENTS”

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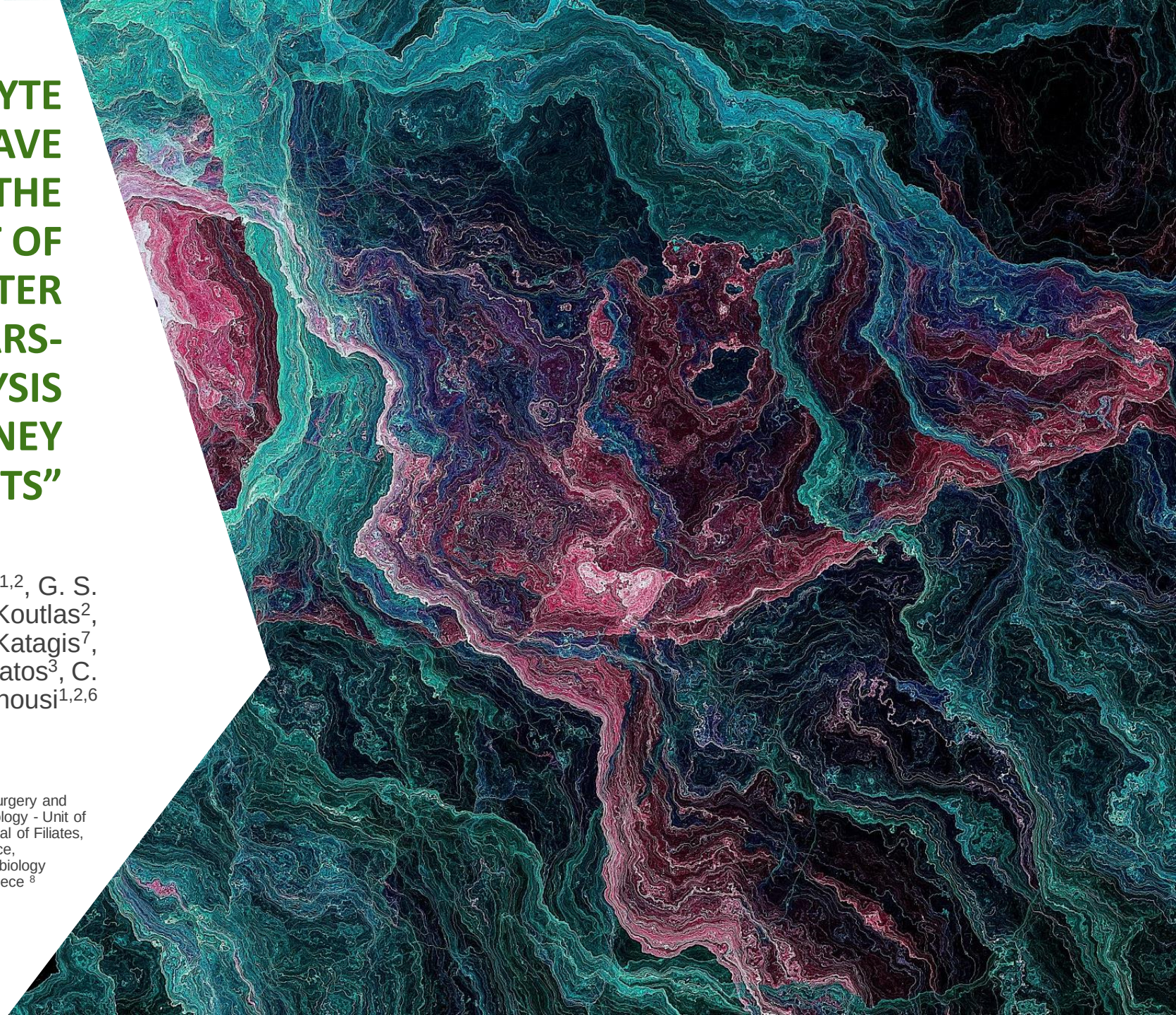

INTRODUCTION AND AIM

- During the COVID-19 pandemic, mortality due to SARS-COV-2 infection in hemodialysis (HD) patients and kidney transplant recipients (KTRs) has been reported high
- The adequacy of the respective generated immune responses is **significantly lower** than the general population
- Booster doses have been recommended by multiple health systems and the World Health Organization
- The aim of our study was to determine the predictive value of lymphocyte subpopulations in the production of antibodies against SARS-CoV-2 after the second dose of the vaccine

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ORIGINAL RESEARCH article

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The Humoral Immune Response to BNT162b2 Vaccine Is Associated With Circulating CD19+ B Lymphocytes and the Naïve CD45RA to Memory CD45RO CD4+ T Helper Cells Ratio in Hemodialysis Patients and Kidney Transplant Recipients

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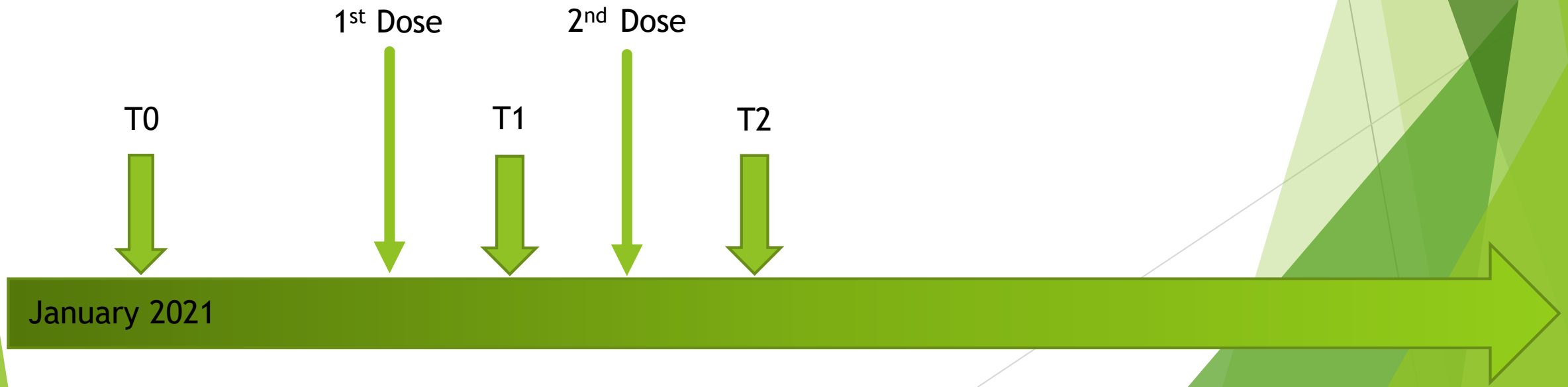
Results: 31 HD patients (91.8%) and 16 KTRs (29.6%) became seropositive at T2. HD patients who became seropositive following the first dose displayed higher CD19+ B lymphocytes compared to their seronegative HD counterparts. A positive correlation was established between CD19+ B cells counts and antibody titers at all time-points in both groups ($p < 0.001$). KTRs showed higher naïve CD4+CD45RA+ T helper cells compared to HD patients at baseline and T2 whereas HD patients displayed higher memory CD45RO+ T cells compared to KTRs at T2. The naïve CD4+CD45RA to memory CD4+CD45RO+ T helper cells fraction was negatively associated with antibody production in both groups.

METHODS

- 34 HD patients
- 54 KRTs

- Multicenter, prospective study from January 2021 and still ongoing…
(ClinicalTrials.gov, NCT04932876)
- Study population: **34 HD patients and 54 KTRs** who received two doses of the BNT162b2 (Pfizer–BioNTech)
- Exclusion criteria included previous infection by SARS-CoV2 as well as infection during study follow-up
- Lymphocyte subpopulations (B cells, CD4+ and CD8+ T cells as well as naïve and memory T lymphocytes subpopulations) were analyzed



METHODS

- Titers >50 arbitrary units (AU)/ml were considered positive for seroconversion at T1 and at T2.

- A multiple linear regression model was applied, separately to the two subgroups of patients

Results -KTRs

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B		Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	4869.236	2122.817		2.294	.024	648.507	9089.965					
	CD19+	514.817	126.760	.382	4.061	.000	262.784	766.850	.384	.403	.382	1.000	1.000
	CD3+CD16+CD56+	-225.894	84.458	-.252	-2.675	.009	-393.819	-57.970	-.245	-.279	-.252	.997	1.003
	CD4+CD45RO+	-138.886	64.581	-.203	-2.151	.034	-267.290	-10.481	-.186	-.227	-.203	.997	1.003

a. Dependent Variable: SARS COV-2 ANTIBODIES (T2)

- The mean age of the kidney transplanted recipients was 58,5 years of age
- The populations of CD19+, CD3+CD16+56+ and CD4+CD45RO have predictive role on antibody formation (p-ANOVA<0.001) based on the multiple regression model:

❖ **Ab=4869+519*CD19-226*CD3+CD16+56-139* CD4+CD45RO**.

Results -KTRs

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	CD19+	CD3+CD16 +CD56+	CD4+CD45R O+
1	1	3.307	1.000	.00	.02	.03	.00
	2	.439	2.744	.00	.15	.82	.00
	3	.226	3.829	.03	.79	.11	.07
	4	.028	10.907	.96	.04	.04	.93

a. Dependent Variable: SARS COV-2 ANTIBODIES (T2)

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	CD4+CD45R O+, CD19+, CD3+CD16 +CD56+[b]	.	Enter

a. Dependent Variable: SARS COV-2 ANTIBODIES (T2)

b. All requested variables entered.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.496[a]	.246	.220	4401.14534	1.645

a. Predictors: (Constant), CD4+CD45RO+, CD19+, CD3+CD16+CD56+

b. Dependent Variable: SARS COV-2 ANTIBODIES (T2)

- The multiple regression model meets the following criteria:
  1. No multicollinearity of the variables was observed (all VIF<10)
  2. No autocorrelation of the residuals was found (D>du>1.505)
  3. Normality and homoscedasticity of the independent variables
- The regression model explains the variation of the dependent variable (Ab), according to the adjusted R^2 index, at a rate of **24%**

Results-HD patients

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B		Correlations			Collinearity Statistics	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	20313.002	6350.829		3.198	.003	7324.098	33301.906					
	CD19+	846.344	245.654	.425	3.445	.002	343.924	1348.764	.691	.539	.364	.732	1.366
	CD45RA + CD45RO +	-287.609	100.816	-.345	-2.853	.008	-493.801	-81.416	-.264	-.468	-.301	.762	1.313
	CD4+CD45RO+	-372.592	117.148	-.381	-3.181	.003	-612.187	-132.998	-.408	-.509	-.336	.776	1.289
	CD4/CD8 +	833.133	367.995	.252	2.264	.031	80.499	1585.766	.356	.388	.239	.897	1.115
	CD3-CD16+56+	-194.868	83.894	-.290	-2.323	.027	-366.450	-23.287	-.160	-.396	-.245	.713	1.402

a. Dependent Variable: SARS COV-2 ANTIBODIES (T2)

- The mean age of HD patients was 68,5 years of age
- The analysis of HD patients revealed that the populations of CD19+, CD45RA+CD45RO, CD4/CD8, CD3-CD16+56+ and CD4+CD45RO can predict antibody formation (p-ANOVA<0.001) based on the multiple regression model:

Ab=20267+835.3*CD19-286*CD45RA+CD45RO-

375.2*CD4+CD45RO+851*CD4/CD8-187.3*CD3-CD16+56+

Results-HD patients

Collinearity Diagnostics[a]

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions (Constant)	CD19+	CD45RA + CD45RO +	CD4+CD45RO+	CD4/CD8 +	CD3-CD16+56+
1	1	5.146	1.000	.00	.01	.00	.00	.01	.00
	2	.348	3.845	.00	.06	.00	.00	.46	.15
	3	.218	4.863	.00	.54	.00	.00	.43	.03
	4	.212	4.930	.00	.06	.11	.02	.08	.31
	5	.068	8.723	.00	.00	.43	.30	.02	.07
	6	.009	23.940	.99	.33	.46	.67	.01	.43

a. Dependent Variable: SARS COV-2 ANTIBODIES (T2)

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.027E+9	5	205452066	12.137	.000[b]
	Residual	490912642	29	16928022.2		
	Total	1.518E+9	34			

a. Dependent Variable: SARS COV-2 ANTIBODIES (T2)

b. Predictors: (Constant), CD3-CD16+56+ , CD4/CD8 +, CD4+CD45RO+, CD45RA + CD45RO +, CD19+

The multiple regression model meets the following criteria:

1. No multicollinearity of the variables was observed (all VIF<10)
2. No autocorrelation of the residuals was found (D>du>1.671)
3. Normality and homoscedasticity of the independent variables

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.823[a]	.677	.621	4114.36777	2.493

a. Predictors: (Constant), CD3-CD16+56+ , CD4/CD8 +, CD4+CD45RO+, CD45RA + CD45RO +, CD19+

b. Dependent Variable: SARS COV-2 ANTIBODIES (T2)

The regression model explains the variation of the dependent variable (Ab), according to the adjusted R^2 index, at a rate of **67%**

Limitations of the study

- Relatively small sample size may cause the model to “overfit” the data, reducing the model’s generalisability
- As always a multiple regression model can only establish correlations and not causations
- Many other confounding factors may affect the regression model results (KTRs –immunosuppressants)

Conclusions

- Quantification of lymphocyte subpopulations by flow cytometry appears to have a significant prognostic value regarding development of antibodies after vaccination against SARS-CoV-2 in KTRs and HD patients

- Of particular interest is the significant difference in lymphocyte populations affecting antibody production between HD patients and KTRs, as more populations appear to influence antibody generation in HD patients than in KTRs

- More studies are needed to validate these predictive models

Thank you for your attention!