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**ESTABLISHMENT OF METHODOLOGY FOR COVID-19
CONVALESCENT PLASMA COLLECTION AND TESTING AT
CROATIAN INSTITUTE OF TRANSFUSION MEDICINE**

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No conflict of interest to declare.

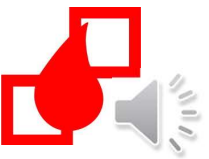


BACKGROUND

I.

Passive immunotherapy is the century-old practice of administering antibodies from an exposed convalescent or vaccinated person to a patient susceptible to the disease in question.

Experience from prior outbreaks with other coronaviruses (SARS-CoV-1) shows that such convalescent sera contain neutralizing antibodies (NAb) against relevant virus and that their use was beneficial in the treated patients.



II.

Collection of COVID-19 convalescent plasma (CCP) at Croatian Institute of Transfusion Medicine (CITM) started in July 2020 and first unit for clinical use was issued in December.

Clinicians in Croatia started using CCP in second wave of pandemics, mostly for patients with hematological malignancies.

Such patients are not able to fight the SARS-CoV-2 infection by producing their own antibodies.



III.

- CCP with individually checked levels of NAb, as an external source of antibodies, showed promising results in improving condition of these patients during SARS-CoV-2 infection.



AIM

- To present the establishment of relevant methodology to properly evaluate SARS-CoV-2 neutralization capacity testing and to assess the correlation of donor disease severity and demographic characteristics with antibody level.



METHODS

I.

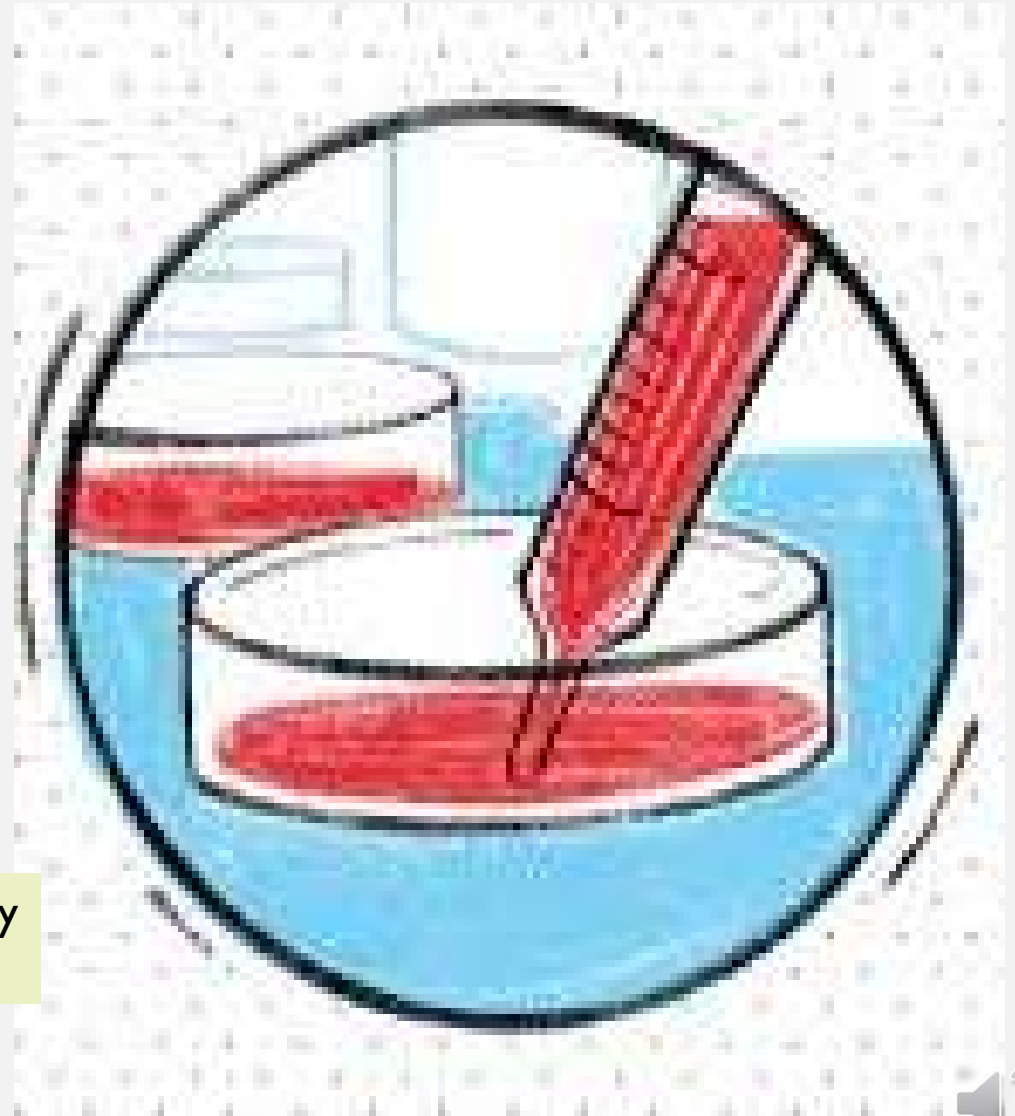
- All plasma units were donated from recovered and healthy COVID-19 patients with documented history of SARS-CoV-2 infection.
- Each donor fulfilled donor eligibility.
- All plasma units were collected by apheresis procedure.



II.

- Titre of NAb was measured with the SARS-CoV-2 neutralization assay for quantification of SARS-CoV-2 NAb in BSL3 conditions.
- Test has been performed on **Vero E6 cell** suspensions with home working stocks of SARS-CoV-2 virus - clinical isolate of the Laboratory working stock label **SARS-CoV-2 297/20 Zagreb virus**.

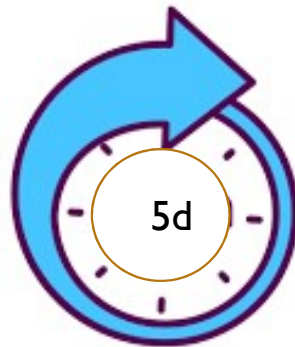
Examination of SARS-CoV-2 NAb by neutralization assay is considered the gold standard !!!



The neutralization test is a very complex and time-consuming procedure !!!



Waiting for results

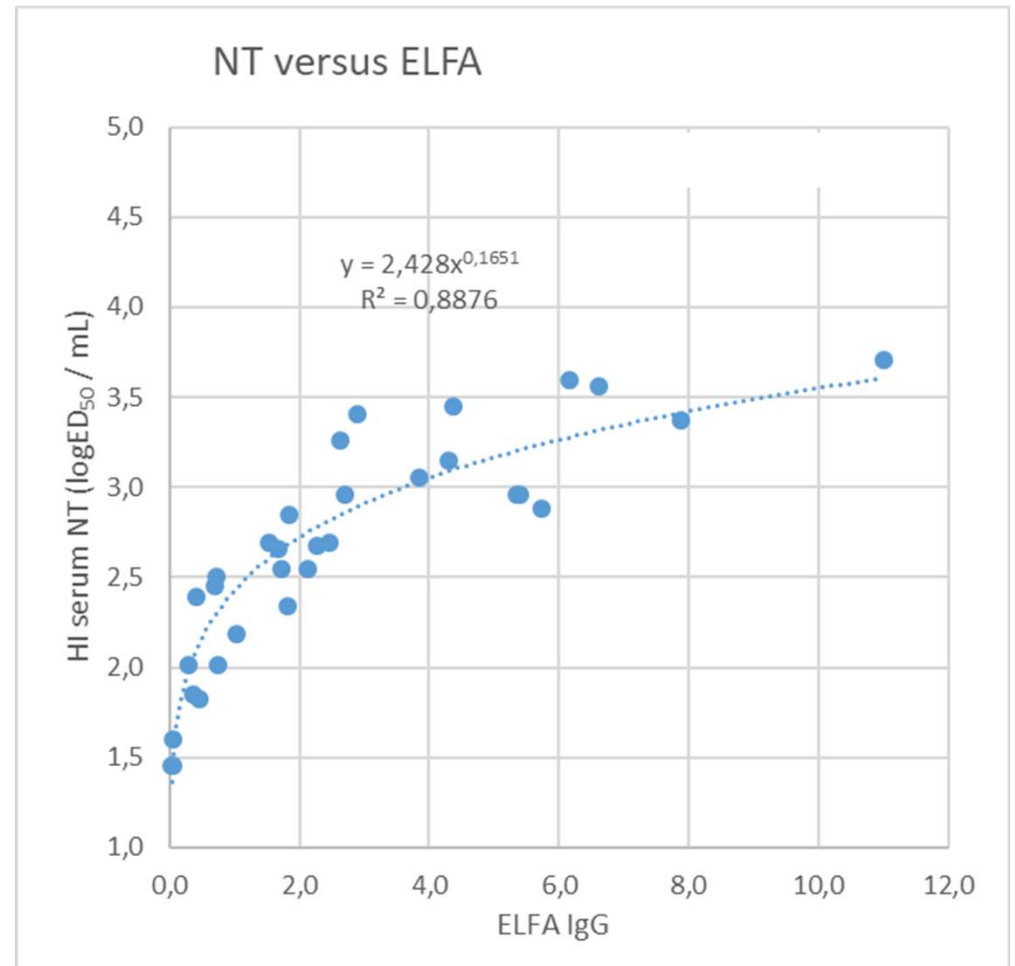


To avoid and shorten the procedure...



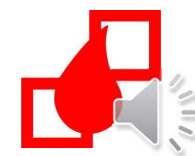
III.

- The regression analysis for Vidas SARS-CoV-2 IgG (**RBD of Spike SARS-CoV-2 protein**) test was done. This resulted in high correlation coefficient with neutralization assay.
- The regression equation has been used for the estimation of threshold for high NAb level.
- High level was determined as ED50/ml > 1200.



RESULTS

Follow-up period	8 months	28 th July 2020 to 28 th March 2021	
N° of units	256		
Age	M 36 (18-62)		
Male	Female	60.6%	30.4%

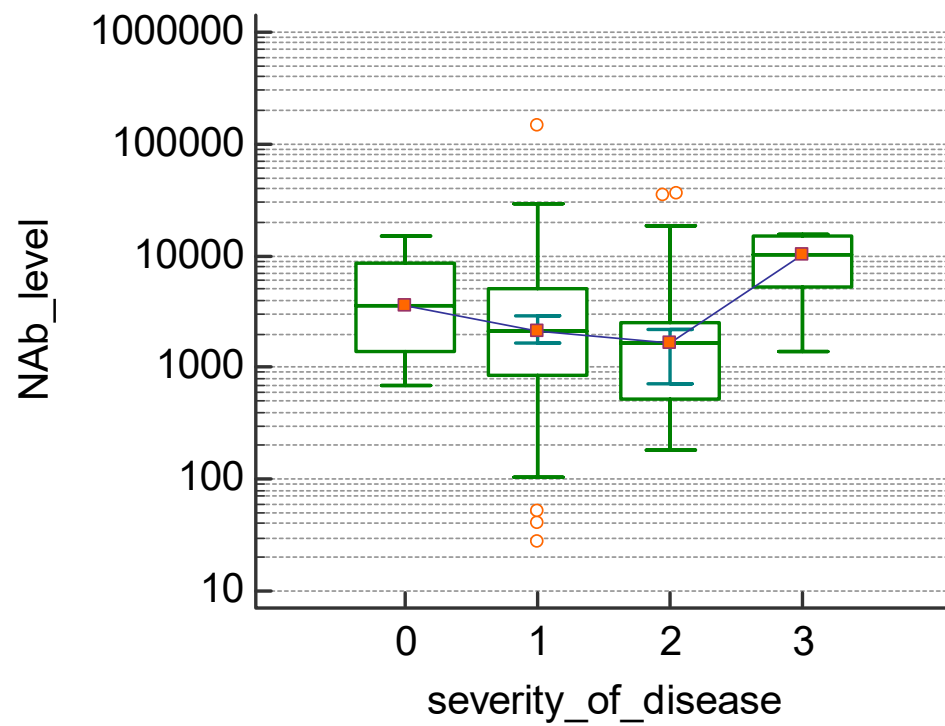


Blood group	%	COVID-19 symptoms	%	NAb level	%
A	43	asymptomatic	2.5	high	75.4
O	33	mild	75	low	24.6
B	12	medium	19.4	Median - ED50/ml 2,560 (274 -145,218)	
AB	12	severe	3.1		



- To assess a relation of donor disease severity and demographic characteristics with antibody level, Chi-squared, Kruskal-Wallis and Mann-Whitney tests were performed for statistical analysis.
- Statistically significant results were considered when $P < 0.05$.

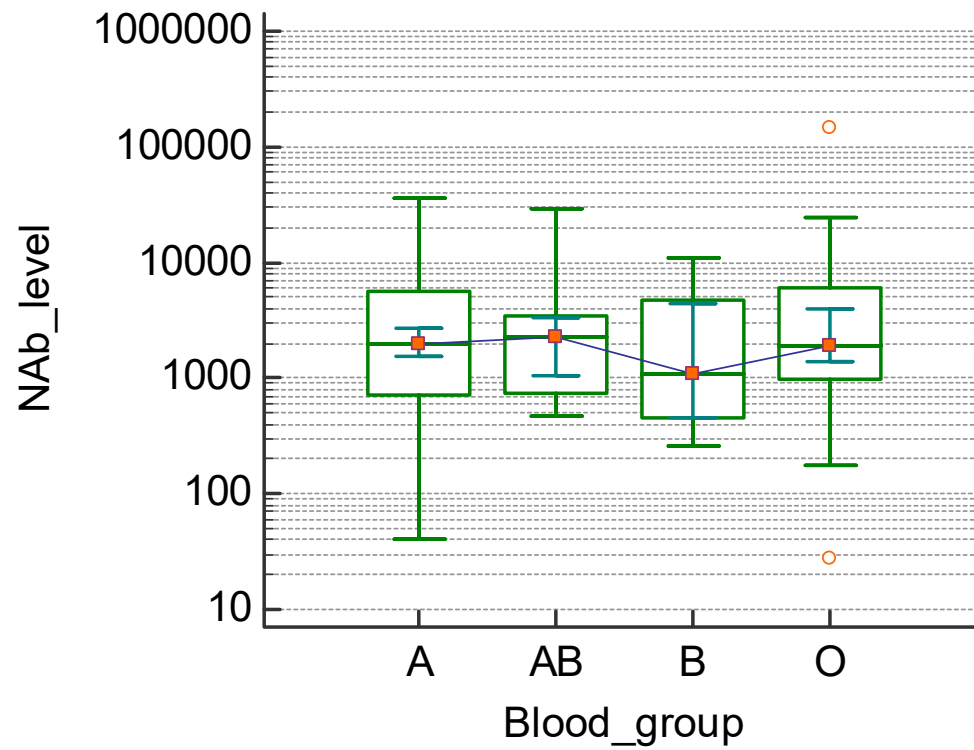




- NAb level did not statistically differ between severity of symptoms.

- ($P = 0.072, \chi^2 = 6.99$)





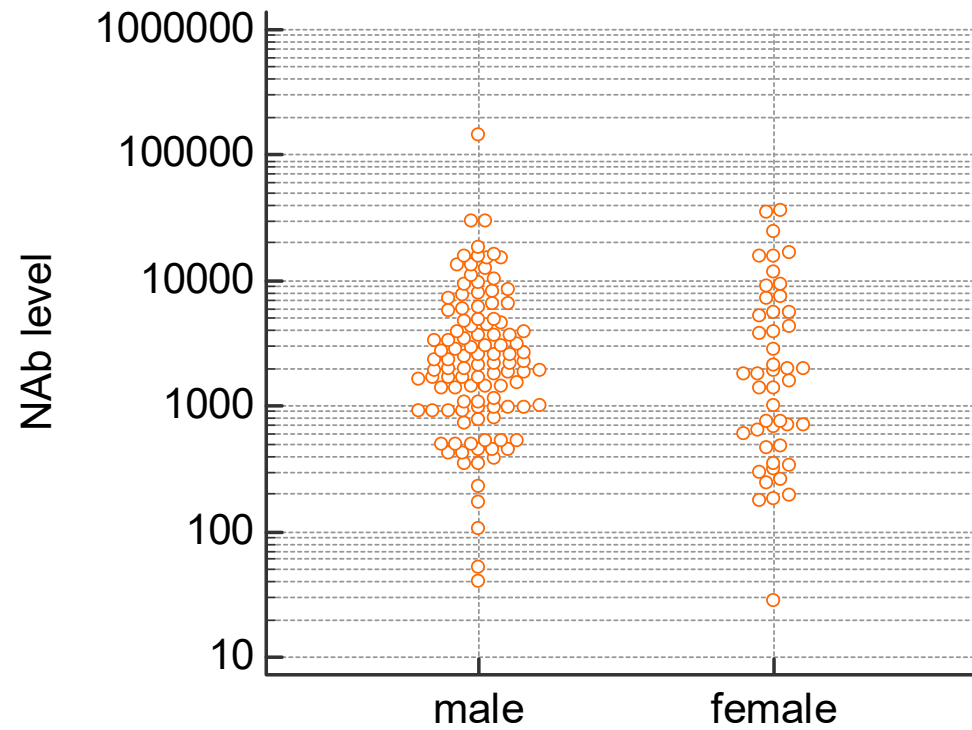
- NAb level did not statistically differ between blood groups.

- ($P = 0.842$, $\chi^2 = 0.83$)



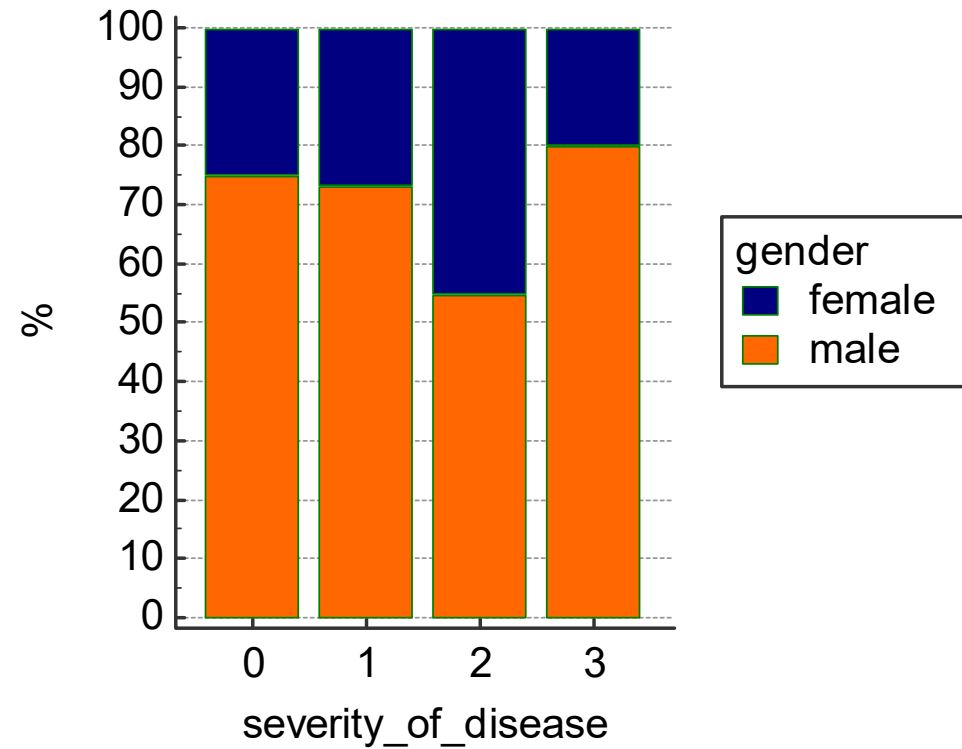
- NAb level did not statistically differ between genders.

- ($P = 0.262, Z = 1.12$)

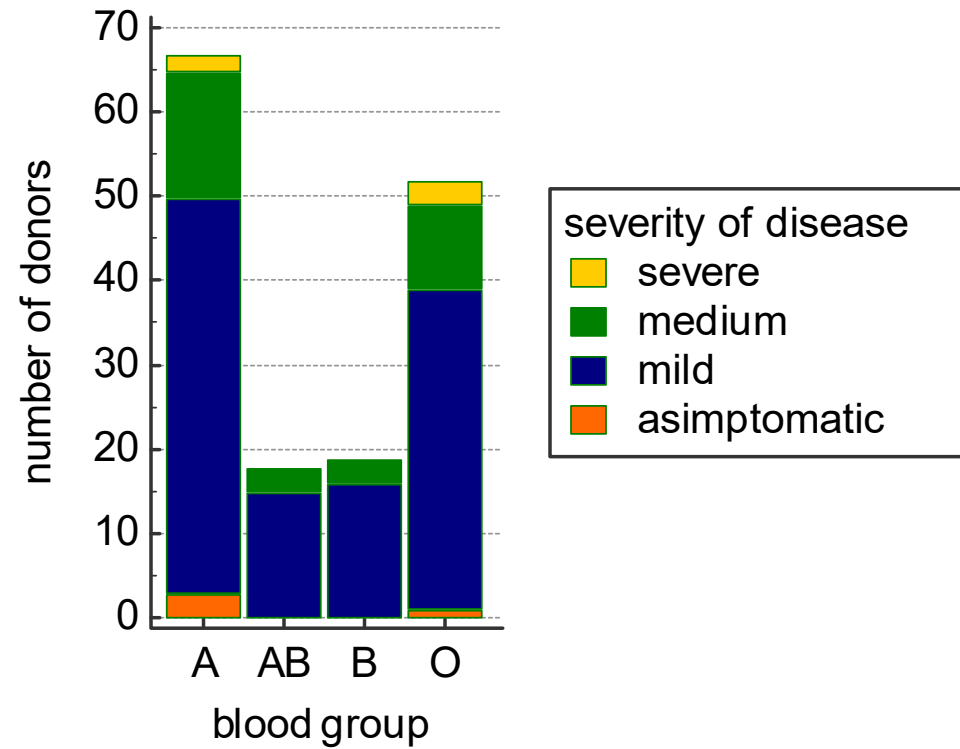


- Severity of disease did not statistically differ between genders.

- ($P = 0.23, \chi^2 = 4.26$)



- Severity of disease did not statistically differ between blood groups.
- ($P = 0.803, \chi^2 = 5.34$)



SUMMARY

- Application of serological test (Vidas SARS-CoV-2 IgG) highly correlated with the neutralization assay and allowed quicker issuing of CCP for clinical use which is very important for due time therapy of COVID-19 patients.
- 3/4 of collected CCP had high NAb level.
- NAb level was not dependent on gender, symptom severity nor blood groups.
- Severity of disease was not dependent on blood groups nor gender.



THANK YOU FOR
YOUR ATTENTION!

- Zagreb - the Capital of Croatia



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