



RECOVERING THE BODY IMMUNE SYSTEM AFTER QUITTING SMOKING. IS IT INFLUENCED BY USING E-CIGARETTES?

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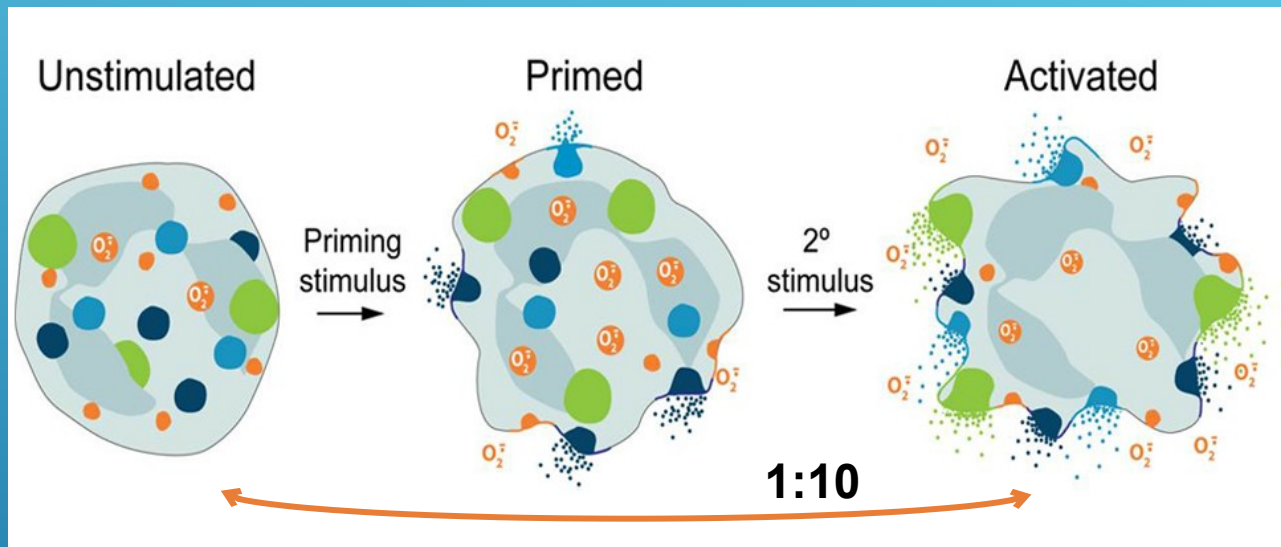
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- ❑ ASTONISHINGLY, MANY OF THE ADVERSE AND/OR BENEFICIAL (?) EFFECTS OF SMOKING MAY RESULT FROM THE ABILITY OF CIGARETTE SMOKE TO SUPPRESS THE IMMUNE SYSTEM.
- ❑ KEY INNATE IMMUNE CELLS (NEUTROPHILS) ARE SUBJECTED TO REPROGRAMMING BY SMOKING TO ADAPT TO THE STATE POTENTIALLY RESPONSIBLE FOR CHRONIC SMOKING-MEDIATED IMMUNOSUPPRESSION.
- ❑ AFTER QUITTING SMOKING, THE IMMUNITY IS SUPPOSED TO EXHIBIT A DILATORY RESTORING.
- ❑ ACCORDING TO STATISTICS, > 90% OF USERS OF ALTERNATIVE NICOTINE DELIVERY SYSTEMS (**ANDS**) ARE FORMER OR CURRENT SMOKERS.

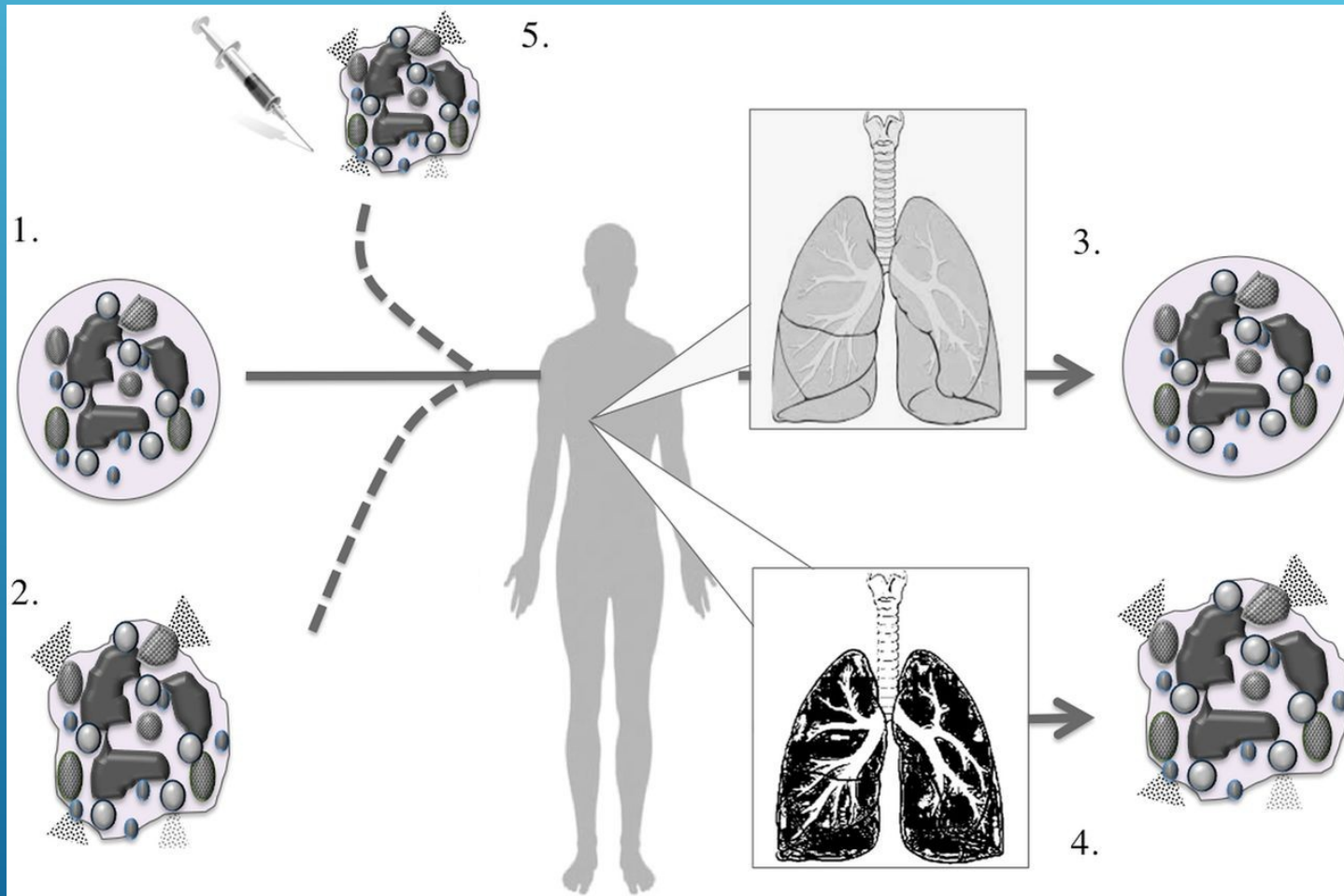
PURPOSE OF OUR WORK: STUDYING THE ACTIVITY OF PERIPHERAL-BLOOD NEUTROPHILS IN ANDS USERS AND SMOKERS



- ✓ There are clear associations between neutrophils and many chronic inflammatory diseases as diverse as COPD, myocardial infarction, type 2 diabetes and inflammatory bowel disease.
- ✓ Neutrophils exist in one of three states: quiescent, primed or active. Priming and activation appear distinct. In the primed state, there is no increase in oxidase activity; however, subsequent stimulation is able to provoke a response that is **10-fold larger** than in non-primed activated cells.
- ✓ Total particulate matter (TPM) from cigarette smoke reduce the expression of NADPH oxidase component gp91 and iNOS, molecules important for bacterial killing.

CIGARETTE SMOKE IS ABLE TO PRIME NEUTROPHILS FOR AN INCREASED BURST RESPONSE.

Priming and cytoskeletal rearrangement render the neutrophil more stiff, hindering its passage through the pulmonary circulation and favouring retention and diapedesis into the lung tissue.



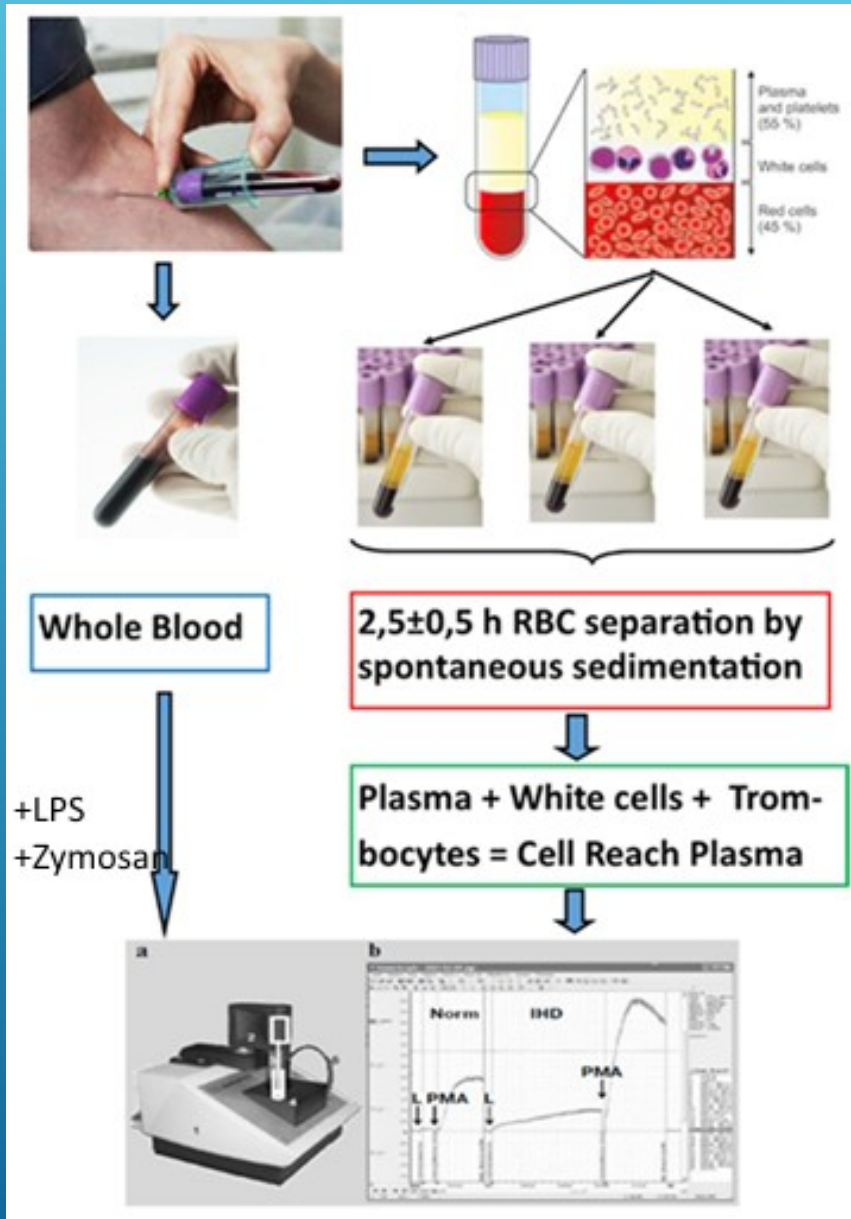
Factors that may influence neutrophil transit time through the pulmonary circulation. In health, there may be no delay and no significant retention of neutrophils in the pulmonary circulation (1, 3). Primed neutrophils (caused by systemic or pulmonary inflammation or infection) will be retained in the pulmonary circulation; (2) however, some priming is reversible and once de-primed by the pulmonary endothelium, quiescent cells will be released back into the circulating blood. (3) In inflammatory diseases of the lungs, this de-priming mechanism fails, leading to the release of primed cells (4) that may also have potential systemic inflammatory consequences. In experimental models, methods of neutrophil isolation or labelling may cause priming, leading to neutrophil retention within the pulmonary capillaries (5).

E. Sapey and R.A. Stockley, *Thorax* 2014, 69, 606-608

▮ EXPERIMENTAL PART

▮ METHODS

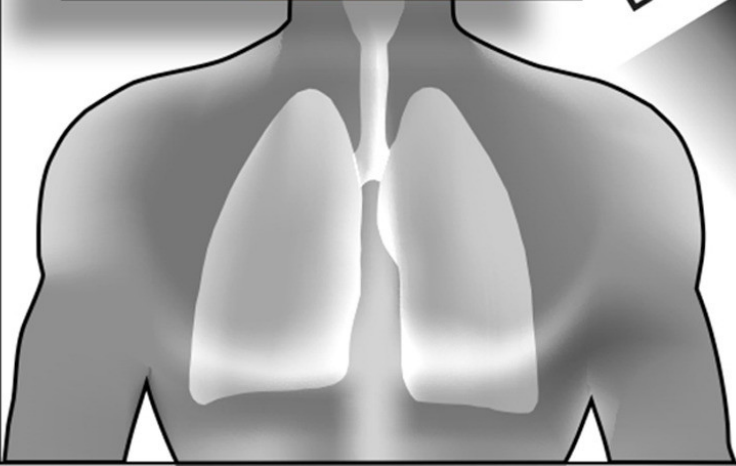




Characteristics	Smoking Status			P Value
	Never	Former	Current	
Number, N	11	11	11	
Age (years)	37.3 ± 7/2	39.6 ± 5.2	41.9 ± 8.2	ns
BMI (kg/m ²)	24.5 ± 3.9	26.4 ± 4.5	26.1 ± 5.0	ns
Sex, m/f	6/5	5/6	6/5	
Smoking stage or cessation, years	-	3.9 ± 1.2	23.4 ± 5.8	
FEV1, %	99.4 ± 9.0	98.3 ± 7.4	92.1 ± 9.6*	<0.05
FEV1/FVC, %	90.7 ± 8.5	89.8 ± 7.9	87.9 ± 10.1	Ns
WBC count, 10 ⁹ /L	6.0 ± 1.2	5.9 ± 1.4	7.7 ± 2.2*	<0.05
Exhaled CO, ppm	2.1 ± 0.5	1.9 ± 0.6	9.2 ± 1.4*	<0.001
Cotinine (ng/ml)	<30	<30	>200	
Heart rate, bpm	63 ± 4	61 ± 3	60 ± 5	ns
Systolic blood pressure, mmHg	122.9 ± 13.3	120.3 ± 11.7	116.5 ± 12.6	ns
Diastolic blood pressure, mmHg	78.8 ± 6.9	79.9 ± 7.1	73.4 ± 7.6*	<0.05
Peripheral oxygenation, %	95 ± 2	95 ± 3	94 ± 3	ns

A MAJOR PROBLEM OF STUDYING ISOLATED NEUTROPHILS IS THE INADVERTENT PRIMING OF THE CELLS CAUSED BY THE ISOLATION PROCESS ITSELF!

Smoker

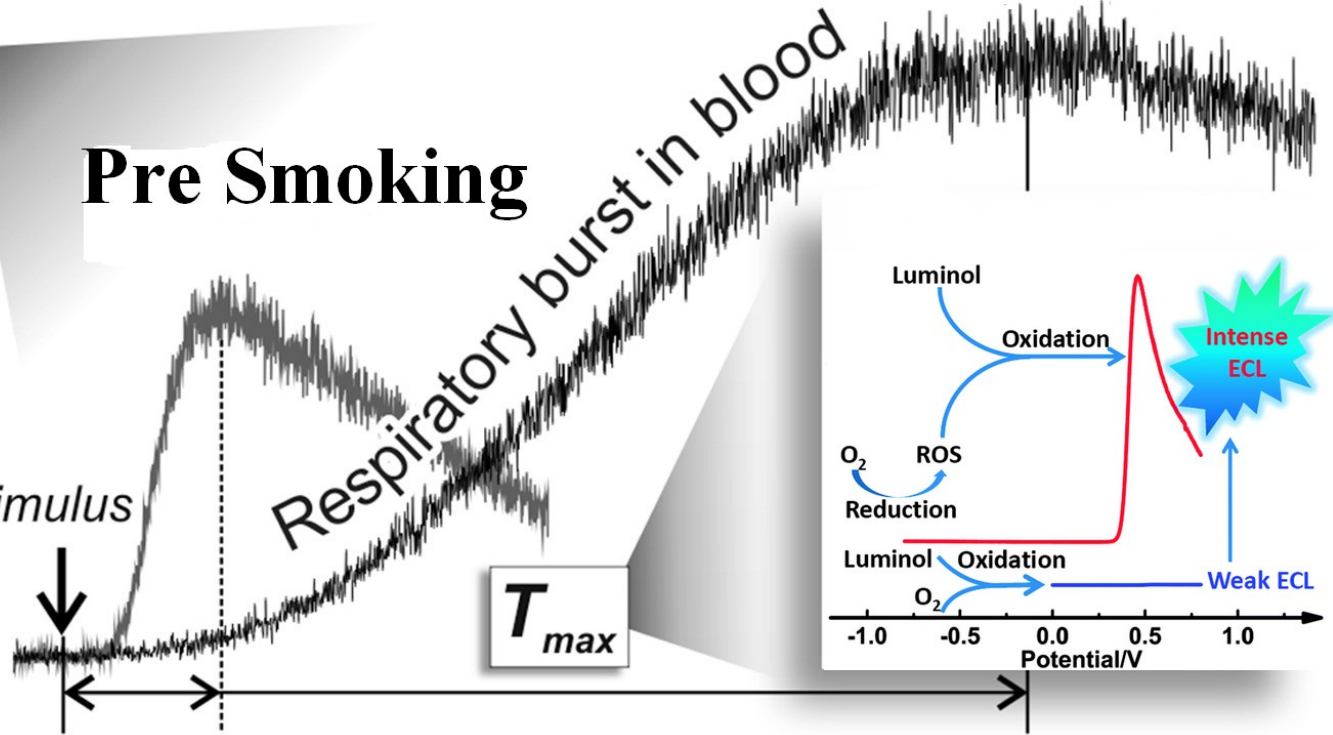


Blood

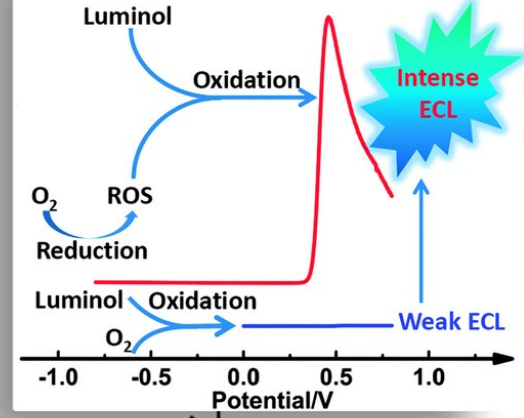


Pre Smoking

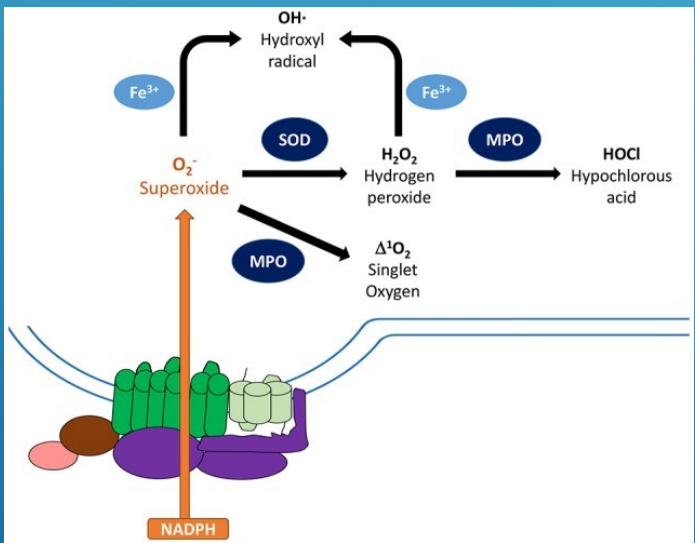
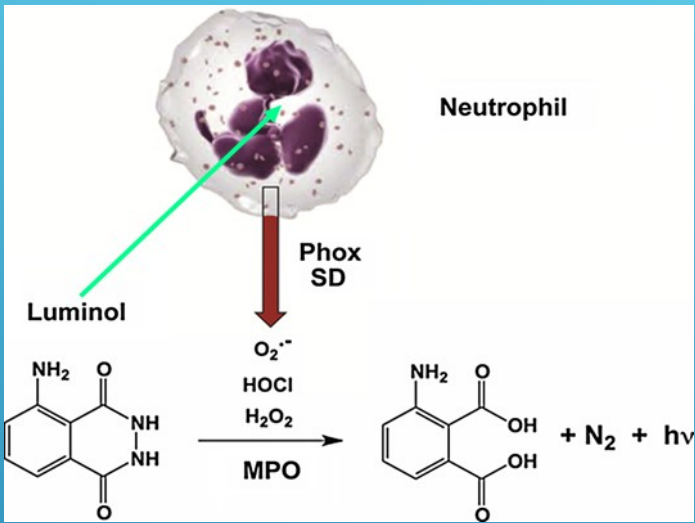
Stimulus



1,5h After Smoking



NON-OPSONIZED ZYMOSAN-INDUCED AND LUMINOL-ENHANCED CHEMILUMINESCENCE IN A CONTINGENTLY WHOLE BLOOD

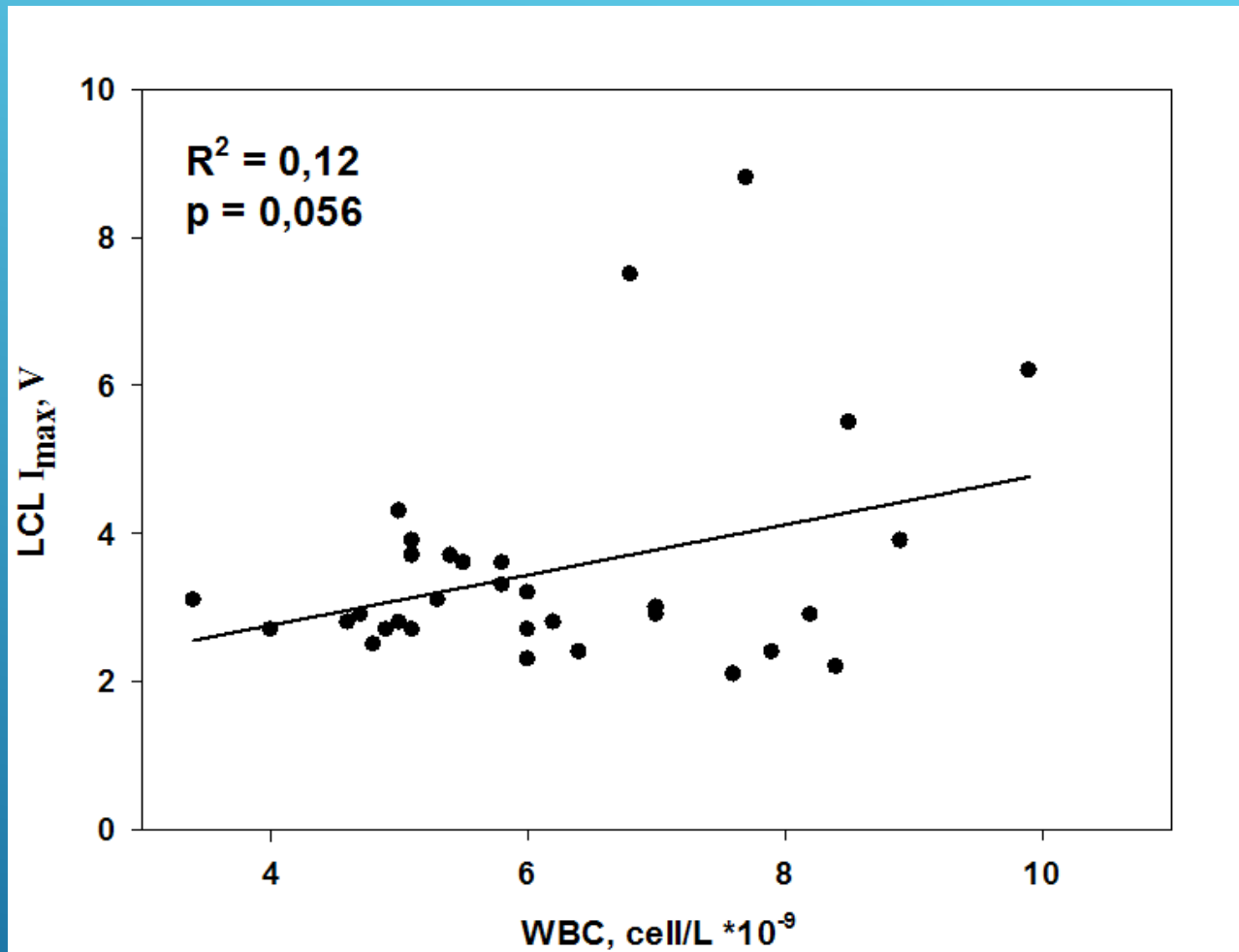


- Luminol chemiluminescence (CL) induced by phagocytosis of particle matters (zymosan-endotoxin complex) was studied in a system consisting of the whole (contingently whole) blood, luminol and apyrogenic physiological saline only.
- The major producers of ROS in a circulating bloodstream are neutrophils in the activated state. Activated NADPH oxidase catalyzes the transfer of electrons from NADPH to molecular oxygen generating superoxide anions as the primary product.
- The whole-blood CL assay is a simple, rapid and facile tool for monitoring the phagocytic function and priming of neutrophils.

BLOOD CHEMILUMINESCENCE MECHANISM WITH THE INVOLVEMENT OF NEUTROPHILS

.....MAIN RESULTS



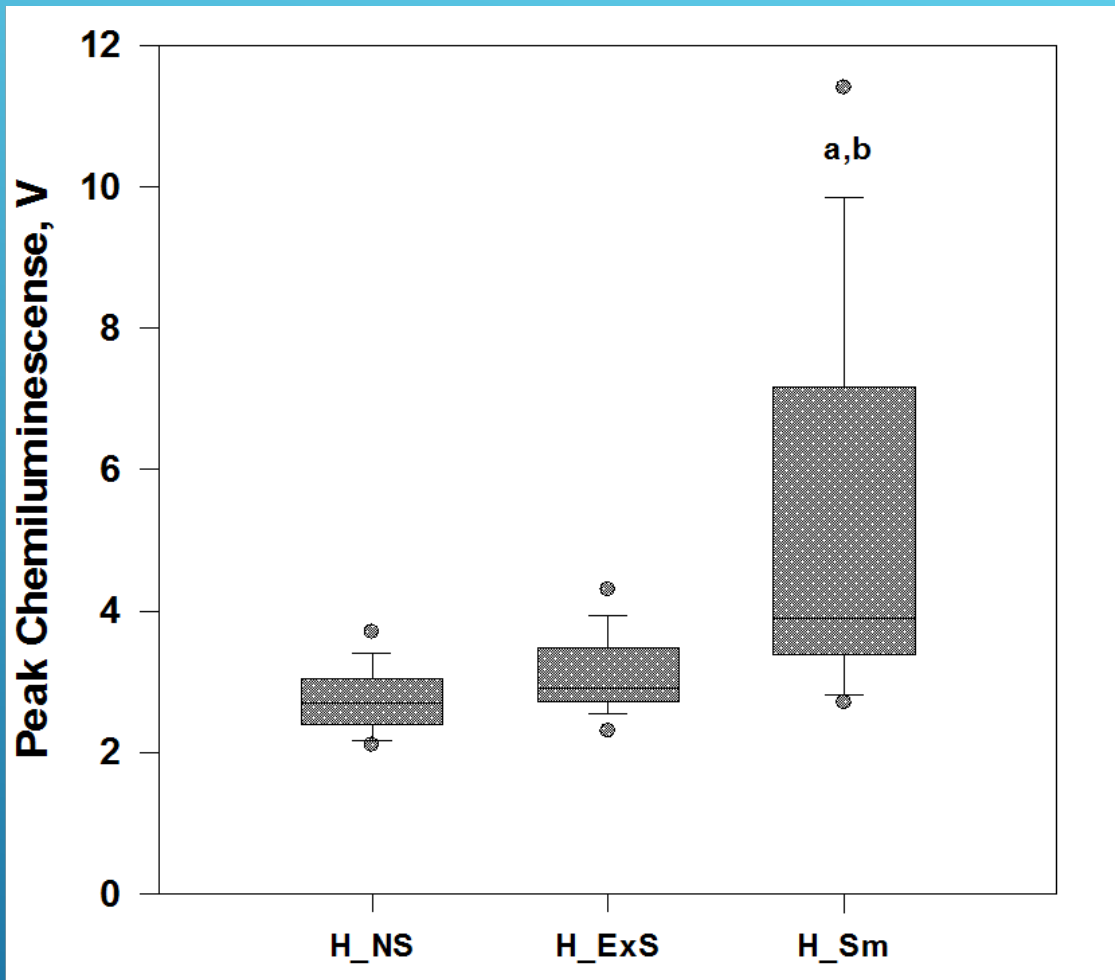


The correlation between the maximum rate of the ROS generation (manifested by the maximum luminol chemiluminescence intensity, LCL I_{max}) by the cells of the contingently whole blood and the concentration of leukocytes in the peripheral blood. The variability of the peak LCL I_{max} value is **determined only up to 12 % by the variability of the concentration of leukocytes.**

THE CORRELATION BETWEEN BLOOD CHEMILUMINESCENCE AND WHITE CELL COUNT.

▮ **VERY GOOD NEWS FOR EX-SMOKERS**





Zymosan-induced peak ROS generation in the contingently whole blood in three groups of volunteers (NS - nonsmokers, ExS - ex-smokers, Sm - smokers^{*}). Statistically significant differences between the groups: a - relative to the non-smoking group, $p < 0.01$; b - relative to the group of ex-smokers $p < 0.05$. There is no difference between the groups of non-smokers and ex-smokers at the level of $p = 0.129$.

^{*}) Blood sampling from smokers was done not earlier than **10 hours** after the contact with cigarette smoke!

^^ During the **first year** after quitting no significant changes in the activity of neutrophils were observed.

^^ After a longer period of abstinence (**from 2.5 years**) changes have occurred, and for the better.

^^ In smokers, neutrophils expectedly were predominantly in a pre-activated state

What about:

- E-cigs users?
- Tobacco heating systems users?
- Dual users (cigarette and ASDN)?

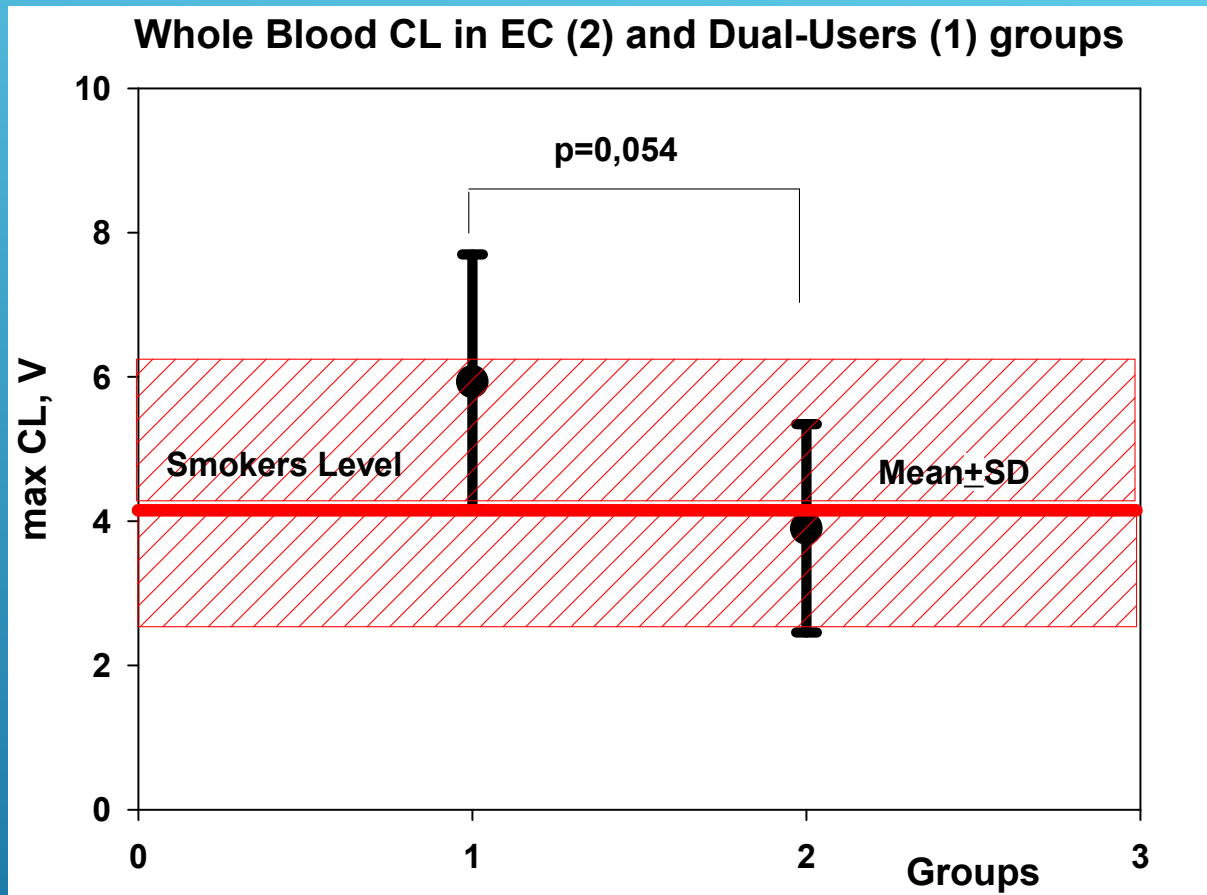
HOW ABOUT ASDN USERS?

THE MAIN INTRIGUE IS COMING...

By this moment, we have investigated:

- **the blood of merely 6 ASDN users (ex-smokers) including 3 E-cigs users and 3 users of tobacco heating devices with more than one-year experience.**
- **the blood of 6 dual users (combining smoking regular cigarettes and ANDS use) with more than two years of experience.**

PRELIMINARY DATA!



IN CIRCULATING BLOODSTREAM OF DUAL USERS, PROOXIDANT POTENTIAL OF NEUTROPHILS IS HIGHER THAN IN CASES OF ORDINARY SMOKERS AND ANDS USERS, WHILE ANDS USERS COMPARED TO SMOKERS EXIBITED ONLY SLIGHTLY BETTER RESULTS AFTER ONE YEAR OF SWITCHING TO ANDS.

STUDY IN CURRENTLY IN PROGRESS...

UNEXPECTED FINDING

- ▮ The **optimistic result** of our study consists in a finding that the increased reactivity and concentration of circulating cells of the immune system, characteristic for smokers, normalizes to the state typical for non-smokers in 2 to 3 years after quitting smoking.
- ▮ Switching to ANDS without using conventional cigarettes for **one year** after quitting smoking leads to merely slight modulation of the priming of circulating neutrophils.
- ▮ **Unexpected result** was obtained for **dual users**, preferring to combine regular cigarettes and ANDS. At a minimum, one can say that the combination of conventional cigarettes and ANDS does not give essential advantages in terms of immunity modulation.

SUMMARY

THANK YOU FOR YOUR ATTENTION!

...And good health to everyone!

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