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A new approach to extraordinary efficient protection against COVID 19 based on nanotechnology

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SUMMARY

A new approach to the protection against infections caused by bacteria and various viruses, including SARS-CoV-2 is described. In concrete example, protective filters and ALBO nanosilver masks showed extraordinary efficiency in protection against *Staphylococcus aureus.* These results show that it highly overcomes the efficiency of ordinary surgical masks. Besides, systematic meta-analysis given for ordinary surgical masks and filters N95 for masks and respirators, showed no statistical difference between them in the case of SARS-CoV-2. On the base our experimental data and systemic meta-analysis given in this paper, it can be concluded that ALBO nanosilver masks have significant advantages, and show a very perspective concept of developing new protective gear.

Keywords: COVID 19; bacteria; viruses; ALBO nanosilver masks; N95 masks; surgical masks

Background about COVID-19 (disease caused by SARS-CoV-2)

At the end of 2019, three bronchi alveolar lavage samples were collected from a patient with pneumonia of unknown etiology and examined by Real-time PCR (RT-PCR) assays that were positive for pan-Beta coronavirus. Furthermore, using Illumina and nanopore sequencing, the whole genome sequences of the virus were determined, approving similarity between SARS and SARS-CoV-2 virus, which crown-like shape and its cytopathic effects (CPE) were confirmed 96 hours after inoculation. Investigations on transgenic human ACE2 mice and Rhesus monkey induced multifocal pneumonia with interstitial hyperplasia [1].

Histological examination of post-mortem samples of a 50-year old male patient from Wuhan shows bilateral diffuse alveolar damage with cellular fibromyxoid exudates. Besides, his lung showed also acute respiratory distress syndrome (ARDS), desquamation of pneumocytes, and pulmonary edema, while interstitial mononuclear inflammatory infiltrates, dominate in both lungs. Besides, multinucleated syncytial cells with atypical enlarged pneumocytes characterized by large nuclei, amphophilic granular cytoplasm, and prominent nucleoli were identified in the interalveolar spaces, showing viral cytopathic changes [1–6].

As it is now well known, COVID-19 is transmitted via droplets during close unprotected contact between an infector and infected, while the airborne spread has not been reported. Its human-to-human transmission is largely occurring in families. Testing for COVID-19 disease includes RT-PCR testing in influenza-like-illness (ILI) and severe acute respiratory infection (SARI), as well as testing of results among all visitors to fever clinics [1–6]. There is no pre-existing immunity for COVID-19 because it is caused by new human pathogen. That is the main risk factor of infection. Furthermore, its transmission dynamics is very fast, particularly during the epidemic growth phase and in the post-control period [1–6].

Its symptoms are non-specific and the disease can range from no symptoms (asymptomatic) to severe pneumonia and death. Typical symptoms include: fever (87.9%), dry cough (67.7%), fatigue (38.1%), sputum production (33.4%), shortness of breath (18.6%), sore throat (13.9%), headache (13.6%), myalgia or arthralgia (14.8%), chills (11.4%), nausea or vomiting (5.0%), nasal congestion (4.8%), diarrhea (3.7%), and hemoptysis (0.9%), and conjunctival congestion (0.8%) [1–6].

Approximately 80% of laboratory-confirmed cases have mild to moderate disease, which includes non-pneumonia and pneumonia cases, 13.8% have a severe disease (dyspnea, respiratory frequency ≥30/minute, blood oxygen saturation ≤93%, PaO2/FiO2 ratio <300, and/or lung infiltrates >50% of the lung area within 24–48 hours, whereby 6.1% are critical (respiratory failure, septic shock, and/or multiple organ dysfunction/failure). Individuals at the highest risk for severe disease and death are people aged over 60 years and those with underlying conditions such as hypertension, diabetes, cardiovascular disease, chronic respiratory disease, and cancer. Disease in children appears to be relatively rare and mild with approximately 2.4% of the total reported cases amongst individuals aged less than 19 years. A very small proportion of those aged less than 19 years have developed severe (2.5%) or critical disease (0.2%). Mortality increases with age, with the highest mortality among people over 80 years of age (CFR 21.9%) [1–6].

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The CFR is higher among males compared to females (4.7% vs. 2.8%), while patients who reported comorbid conditions had much higher rates of COVID-19: 13.2% were with cardiovascular disease, 9.2% diabetes, 8.4% hypertension, 8.0% chronic respiratory disease, and 7.6% with cancer. This virus is highly contagious, can spread quickly, and must be considered capable of causing enormous health, economic and societal damage, on the global level. It is unique among human coronaviruses because it combines high transmissibility, substantial fatal outcomes in some high-risk groups, and the ability to cause huge societal and economic disruption [1–6].

Nano masks and respirators 95

After the appearance of COVID-19, the use of facemasks has become necessary. The surgical facemasks are widely used by medical workers for protection from infection in contact with patients with respiratory diseases. The used facemasks are often very low quality. In the last time, the best filter and masks among them, like N95 filters in respirators show very low protection efficiency against COVID 19. Besides, model simulations, using data relevant to COVID-19 dynamics in the USA, suggest that broad application of even so relatively ineffective facemasks may significantly reduce community transmission of COVID-19 and decrease patient hospitalizations and deaths. Medical masks (i.e., surgical masks and N95 respirators) have yielded more controversial results [7, 8, 9]. The traditional model for respiratory disease transmission infection via infectious droplets (generally 5–10 µm) that have a short lifetime in the air and infect the upper respiratory tract, or finer aerosols, which may remain in the air for many hours, was applied in the case of SARS-CoV-2 [10, 11, 12]. Although the N95 respirator vs. surgical mask offers better protection, great numbers of medical workers that use them are infected by COVID-19, because medical systems in different countries are generally jeopardized. It is well known that such masks may have only a limited effect (but still nontrivial, in terms of absolute lives saved) in more severe epidemics, such as the ongoing epidemic COVID-19.

Masks with HEPA filters are designed with a filter that can be inserted and replaced made of a double-layered HEPA air filter, such as the 3M Filtrete 2800 Ultrafine Filter. The efficacy of non-fit tested HEPA filter masks, such as the Totobobo mask, is still inferior compared to N95, and it should be used with great caution [9]. Additionally it has been noticed that 3M Filtrete MP 2800 filter out particles as small as 0.3 microns, while the size of some viruses, like SARS-CoV-2 is about 0.06–0.14 microns in size. Therefore, its relative efficacy to filter pathogens of the current pandemic is very suspicious. Besides, many researchers think that their efficiency significantly reduces after washing, even in the case of the polypropylene filters (similar to 3M Filtrates). A particular challenge is the maintenance of reusable filters on hospital premises and their alternative decontamination and sterilization with methods such as ultraviolet germicidal irradiation and autoclaving.

N-95 respirator has 95% filtration efficiency, while surgical mask has < 50% filtration ability for small particles (0.1–0.4 µm in diameter). Corresponding studies showed that airflow through the commercial N-95 respirator was very poor, and inhaled air from the enclosed space of the mask was quite hypoxic, with a fraction of inspired oxygen [FIO2], about 16.4%, that is why it is not safe for patients who have pulmonary or cardiovascular diseases or sepsis and who require a good oxygen supply [10].

Future evaluation of masks should include not only their filtration efficiency and safety of their use but also its efficiency in preventing bacteria and viruses infections. Improved masks are urgently needed in order to help to prevent communicable infectious diseases. Finally, although filters in N-95 respirator and surgical masks can partially prevent inhalation of the nano-metric and submicronic airborne particles, they cannot protect us from viruses, like SARS-CoV-2. This fact is shown in one brilliant way given in the text of meta-analysis, which details are described in the Appendix of this paper [12, 13].

Silver and silver compounds

In general, silver is the most efficient antimicrobial and antiviral metal, although some other metals such as zinc, copper, and cobalt have shown effective inhibition of microbes. It is very often used for preventing bacterial colonization of medical devices, as well as on other textile fabrics [12, 14]. It is believed that heavy metals react with proteins by combining the thiol (–SH) groups, which leads to protein inactivation. In the presence of moisture (e.g. from the air), metal ions are formed and inhibit microbial replication. Proposed antimicrobial mechanism is that metal ions destroy or pass through the cell membrane, and bond to the –SH group of cellular enzymes. The consequent critical decrease of enzymatic activity causes microorganism's metabolisms to undergo change and their growth to be inhibited, up to the death of the cell [14]. As it is known, metal ions catalyze the production of oxygen radicals that interact with the molecular structure of bacteria. Then, silver ions lead to protein denaturation influencing cell death by their reaction with nucleophilic amino acid residues in proteins, and their attachments to sulphydryl, amino, imidazole, phosphate and carboxyl groups of membrane or enzyme proteins. Silver also inhibits a number of oxidative enzymes such as yeast alcohol dehydrogenase, influencing the uptake of succinate by membrane vesicles and respiratory chain of *Escherichia coli*, consequently causing metabolite efflux and interference with DNA replication [14, 15]

The actual mechanisms by which antimicrobial substances control microbial growth vary and depend on the type of agent used. Generally, they prevent cell reproduction, damage cell walls or cell permeability, denature proteins, block enzymes and make cell survival impossible [16, 17], while polycationic antimicrobial compounds damage their cytoplasmic membranes following the mechanism adsorption and diffusion through the cell walls, binding to the cytoplasmic membrane and its disruption, releasing cytoplasmic constituents like K^+ ion, DNA and RNA, and finally causing the cells death. Antimicrobial agents act in two distinct ways: by contact and by diffusion [16, 17].

Surgical face ALBO – nanosliver mask

For the first time, innovative company ALBOS doo, following the procedure that will be patented soon, started producing new protection facemasks. This concept is completely different from the recently used concepts of protection against viruses. Nanosilver masks are very safe, due to its high activity against viruses and bacteria, as it was shown in the example of the *Staphylococcus aureus* bacteria. This experiment, made in Laboratory for control food and drugs, of Scientific Veterinary Institute of Serbia, using so-called horizontal method for quantification of the number of positive staphylococci, showed that the concentration of the total number and coagulase-positive *Staphylococcus* bacteria measured in CFU/ml is less than 1. For comparison both of these values measured for surgical masks (masks without nanosilver) were higher: 1,500,000 CFU/ml, while the total number of bacteria was 18,180,000 CFU/ml. From these data it is clear that these differences are incredibly high, showing the extraordinary efficiency for the concept with nanosilver in antimicrobial protection, not only for health workers but also for general population.

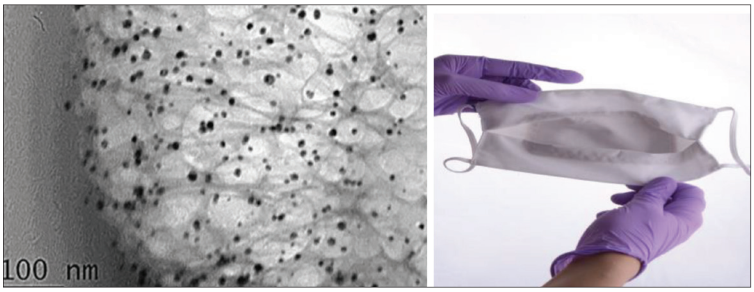

Figure 1. TEM: a) Cross section of thick cotton canvas of ALBO nanosilver mask: average sizes of nanosilver particles are 8–15 nm; b) Typical appearance of ALBO mask with nanosilver
Slika 1. TEM: a) Presek pamučnog dela nanosrebrne maske ALBO: srednja vrednost nanosrebrnih čestica je 8–15 nm; b) Tipičan izgled maske sa nanosrebrnim česticama

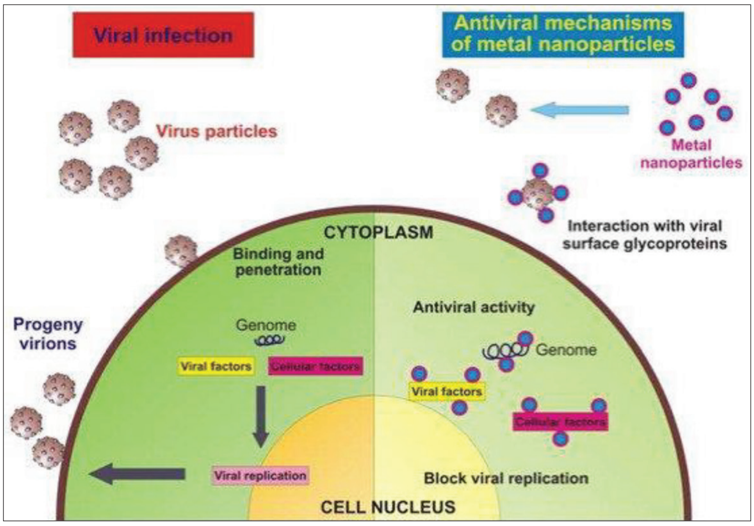

Figure 2. Proposed antiviral mechanisms of action of colloidal silver
Slika 2. Antivirusni mehanizam delovanja koloidalnog srebra

Important question is why *Staphylococcus aureus* was chosen for the experiment. Knowing that they are the leading cause of both healthcare- and community-associated bloodstream infections in the industrialized world influencing significant morbidity and mortality, it was natural to choose such kind of bacteria. Namely, *S. aureus* is a pathogen, which frequently attacks the cardiovascular system inducing its serious complications, such as infective endocarditis or thrombophlebitis, and causing organ failure and death [18–23]. Although similar investigation but on SARS-CoV-2 virus was not done yet, based on the results for *Staphylococcus aureus*, it is possible to extrapolate results. Knowing that virus size is of the order of 100 nm (HIV size is about 120 nm and SARS virus size is also about 100 nm), probably these sizes of NSPs of about 10 nm and less are the most effective in interacting with the virus because the NSPs are significantly smaller than the virus. In the case of the ALBO nanosilver mask, the suitable sizes of NSPs are within the range of 3–10 nm, which are probably the most effective range of NSPs for the killing of any kind of viruses (Figure 1).

Besides, it is approved recently that NSPs of particle sizes between 3 nm – 7 nm are highly effective to suppress viral mechanisms of infection [18–23]. Since biological interactions viruses with human cells are generally multivalent, their attachment and entry into host cells induce strong connections between viral surface components and cell membrane receptors [20–25].

In the case of HIV-1, it is approved that silver nanoparticles inhibit the initial stages of the HIV-1 infection cycle by blocking viral entry, blocking particularly the gp120-CD4 interaction. Besides, silver nanoparticles inhibit also post-entry stages of the HIV-1 life cycle, due to form complexes between silver ions and its donor groups containing sulfur, oxygen, or nitrogen, inside thiols or phosphates in viruses' amino acids and nucleic acids. As a consequence, reverse transcription by direct binding to the RNA or DNA is reduced, as it is shown in Figure 2 [18–23]. Similar kind of interactions can be expected even in the case of SARS-CoV-2.

CONCLUSION

Numerous disadvantages of protective surgical facemasks and masks and respirators with nanofillers N95 are presented. Mata analyses showed no statistically significant differences between them. Besides, it is discussed that a new approach with ALBO filters and masks on the base of nanosilver is new very promising approach. The extraordinary efficiency of these gears on the example of *Staphylococcus aureus*, indicates efficiency of this product could be much better in case of SARS-CoV-2, comparing to any recently globally applied solution.

Appendix: Meta-analysis of the recently used surgical masks and N95 filters: unexpectedly poor protective efficiency against SARS-CoV-2

There are many conflicting and confusing recommendations for the severe acute respiratory syndrome (SARS) and pandemic influenza: the World Health Organization (WHO) recommends using masks in low risk situations and respirators in high risk situations, but the Centers for Disease Control and Prevention (CDC) recommends using respirators in both low and high risk situations. Besides, N95 respirators are frequently unaffordable [24, 25]. Additionally, previous Meta analyses concluded that real efficiency of N95 respirators is not scientifically and statistically confirmed. More rigorous studies comparing N95 respirators with surgical masks against influenza published in the past decade were not included in previous Meta analyses [26, 27]. However, systematic review and meta analysis on the effectiveness of filters N95 in respirators and surgical masks for prevention of` influenza are extraordinary significant.

One review that compared the efficiency of respirators and ordinary surgical masks was provided for the first time [24]. This review included: i) randomized controlled trial (RCT) study (including cluster randomized trial) and nonrandomized controlled study; ii) humans with influenza (including pandemic strains, seasonal influenza A or B viruses and zoonotic viruses such as swine or avian influenza), and other respiratory viral infections (as a proxy for influenza); iii) N95 respirators versus surgical masks, iv) primary outcome: laboratory confirmed influenza; v) secondary outcomes: laboratory confirmed respiratory viral infections, laboratory confirmed bacterial colonization, laboratory confirmed respiratory infection, and influenza-like illness; and vi) settings: hospital or community [24].

RCTs were selected due to the potential of high evidence level results. Search strategy included all corresponding topic data on PubMed, EMBASE, and The Cochrane Library databases from January 27, 2020; to identify all published papers with the subject related to evaluating the use of masks for preventing influenza. The strategy was adequately adjusted to use in other databases, which included all papers with this topic in the past five years from January 27, 2015, to January 27, 2020. A search also included ClinicalTrials.gov to obtain unpublished data, without publication status and language restrictions on selecting the studies [24–27].

Two reviewers were involved, who independently screened the articles based on the titles, abstracts, and full texts. Then, both of them independently extracted the data included in study like the first author, publication year, country, disease, details of study population and intervention, study design, sample size, settings, and results, while all disagreements were subjected to discussion. Both reviewers independently assessed the risk of bias of the selected RCTs using the Cochrane Risk of Bias tool, which included domains on random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, and selective reporting [24–27].

For each RCT, every domain was judged among 3 levels: high risk, unclear risk, and low risk. Disagreements were resolved by discussion. All statistical analyses were performed using Review Manager (RevMan) version 5.3. Comparable data from studies with similar interventions and outcomes were pooled using forest plots. Relative risk (RR) with 95% confidence intervals (CIs) for dichotomous data was used as the effect measure. Between studies heterogeneity was assessed using the I2 for each pooled estimate [28].

It was adopted a random effects model for heterogeneity $p < 0.10$ and performed a subgroup analysis based on the settings (hospital, community) due to the opportunity of clinical heterogeneity. A sensitivity analysis was done to evaluate the robustness of the results by excluding individual studies for each forest plot. A total of 9,171 participants in Canada, Australia, China, or America were included, and the number of participants in each RCT ranged from 435 to 5180 patients. The follow up duration varied from 2 to 15 weeks. Five studies included participants in hospitals and one in households. Because of different definitions of outcome in included studies, it was redefined the laboratory confirmed respiratory infection as respiratory influenza, other viruses, or bacteria infection. Five RCTs involving 8,444 participants reported laboratory confirmed influenza. Meta analysis with fixed effects model revealed no statistically significant differences in preventing influenza using N95 respirators and surgical masks (RR = 1.09, 95% CI 0.92–1.28, $p > 0.05$) [24, 29, 30].

The results of subgroup analyses were consistent regardless of the observed hospital or the community. The results of the sensitivity analysis were not changed after exclusion of any trial. Four RCTs, involving 3,264 participants reported laboratory confirmed respiratory viral infections. Meta analysis with a fixed effects model revealed no statistically significant differences in preventing respiratory viral infections using N95 respirators and surgical masks (RR = 0.89, 95% CI 0.70–1.11, $p > 0.05$). The results of subgroup analyses were also consistent regardless of the hospital or the community [24, 29, 30]. Two RCTs involving 6,621 participants with reported laboratory confirmed respiratory infection. Meta analysis with random effects model revealed no statistically significant differences in preventing respiratory infection using N95 respirators and surgical masks in hospitals (RR = 0.74, 95% CI 0.42–1.29, $p > 0.05$) [24, 29, 30].

Five RCTs involving 8,444 participants reported influenza-like illness [24, 29, 30]. Meta analysis with the random effects model also revealed no statistically significant differences in preventing influenza-like illness using N95 respirators and surgical masks (RR = 0.61, 95% CI 0.33–1.14, $p > 0.05$). The sensitivity analysis showed results remained unchanged after excluding each trial [24, 29, 30]. This meta-analysis showed no statistically significant differences in preventing laboratory confirmed influenza, laboratory confirmed respiratory viral infections, laboratory confirmed respiratory infection, and influenza like illness using N95 respirators and surgical masks. In subgroup analysis, similar results could be found in the hospital and community for laboratory confirmed influenza and laboratory confirmed respiratory viral infections. However, sensitivity analysis showed unstable results for the prevention of laboratory confirmed respiratory viral infections and laboratory confirmed respiratory infection. Through the course of influenza pandemics, large numbers of facemasks may be required to use in long periods to protect people from infections [24, 31].

Using N95 respirators is likely to result in discomfort, for example, headaches. A previous study reported that there was an inverse relationship between the level of compliance with wearing N95 respirator and the risk of clinical respiratory illness. It is difficult to ensure high compliance due to discomfort of N95 respirators in all studies [24, 31]. The reason for the similar effects on preventing influenza with use of N95 respirators *versus* surgical masks may be related to low compliance to N95 respirators wear, which may lead to more frequent doffing compared to surgical masks [31]. Although N95 respirators in the routine use seem to be less acceptable than surgical masks due to more significant discomfort, it should be noted that surgical masks are primarily designed to protect the environment from the wearer, whereas the respirators are supposed to protect the wearer from the environment [24, 32].

In conclusion, the current meta analysis shows the use of N95 respirators compared to surgical masks is not associated with lower risk of laboratory confirmed influenza. It suggests that N95 respirators should not be recommended for the general public and medical staff that are not in close contact with influenza patients or suspected patients [24, 32].

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Novi pristup efikasnoj zaštiti protiv kovida 19 baziran na nanotehnologiji

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KRATAK SADRŽAJ

U ovom radu opisan je novi pristup zaštiti od bakterija i virusa, uključujući i SARS-CoV-2. Na konkretnom primeru *Staphyloccocus aureus* zaštitni filteri ALBO nanosrebrne maske pokazali su izuzetnu efikasnost. Dobijeni rezultati daleko prevazilaze efikasnost običnih hirurških maski. Pored toga, metaanaliza koja je analizirala hirurške maske i filtere za maske N95 pokazuje da nema statistički značajne razlike u njihovoj efikasnosti kada je u pitanju zaštita od virusa SARS-CoV-2. Na osnovu eksperimentalnih rezultata i metaanalize prikazane u ovom radu može se zaključiti da ALBO nanosrebrne maske imaju značajne prednosti nad svim sada korišćenim maskama i predstavljaju veoma perspektivan koncept razvoja nove zaštitne opreme.

Ključne reči: kovid 19; bakterije; virusi; ALBO maske sa nanosrebrom; maske N95; hirurške maske

O virusu SARS-CoV-2 koji izaziva bolest kovid 19

Krajem 2019. uzorci iz bronhijalnih alveola kod bolesnika sa pneumonijom nepoznate etiologije ispitivani su real-time PCR metodom, koja je pokazala prisustvo pan-beta koronavirusa SARS-CoV-2. Dalje, korišćenjem ilumina i nanopornog sekvenciranja određene su sve sekvence genoma virusa, čime je potvrđena sličnost između SARS i SARS-CoV-2, čiji je oblik krune i citopatogeni efekat potvrđen 96 sati posle inokulacije. Ispitivanja na transgenim miševima soja ACE2 i majmunima vrste Rhesus pokazala su multifokalnu pneumoniju sa intersticijalnom hiperplazijom [1]. Histološko istraživanje postmortem uzoraka 50-godišnjeg bolesnika iz Vuhana pokazalo je difuzno bilateralno oštećenje alveola sa ćelijskim fibromiksoidnim eksudatom. Pored toga, njegova pluća pokazala su akutni respiratorni distres sindrom, ćelijsku i fibromiksoidnu eksudaciju, deskvamaciju pneumocita i plućni edem, dok je intersticijalni mononuklearni infiltrat bio sačinjen pretežno od limfocita u oba plućna krila. Uz to, multinukelarne sincicijalne ćelije, sa atipičnim uvećanim pneumocitima karakterišu velika jedra, granulirana citoplazma i prominentni nukleolusi u intraalveolarnim prostorima, koji pokazuju citopatične promene [1–6].

Kao što je već poznato, SARS-CoV-2 se prenosi putem čestica u bliskom nezaštićenom kontaktu sa inficirane osobe na neinficiranu, dok prenos putem vazduha nije zabeležen. Njegov prenos sa osobe na osobu najčešće se odvija u okviru porodica. Testiranje za SARS-CoV-2 podrazumeva RT-PCR u okviru testiranja na bolesti slične gripu i akutnu respiratornu infekciju, kao i testiranje među svim posetiocima u infektivnoj klinici [1–6].

S obzirom na to da je SARS-CoV-2 novi patogen, ljudi ne poseduju imunitet na njega, što predstavlja glavni faktor rizika za infekciju. Dalje, njegova transmisija je jako brza, posebno u uzlaznoj fazi epidemije i postkontrolnom periodu [1–6].

Simptomi kovida 19 su nespecifični i variraju od izostanka simptoma (asimptomatskih) do ozbiljnih pneumonija i smrti. Tipični simptomi statistički podrazumevaju: groznicu (87,9%), suvi kašalj (67,7%), umor (38,1%), stvaranje sputuma (33,4%), kratak dah (18,6%), zapaljenje grla (13,9%), glavobolju (13,6%), bol u mišićima ili zglobovima (14,8%), drhtavicu (11,4%), muku i povraćanje (5,0%), nazalnu kongestiju (4,8%), dijareju (3,7%), hemoptiziju (0,9%) i konjuktivalnu kongestiju (0,8%) [1–6].

Otprilike 80% laboratorijski potvrđenih slučajeva ima slabo ili umereno izraženo oboljenje koje podrazumeva slučajeve sa pneumonijom i bez nje, 13,8% ima ozbiljne simptome (dispneja – frekvenca disanja ≥ 30/minuta, saturacija kiseonikom ≤ 93%, PaO_2 / FiO_2 ratio < 300, plućni infiltrat infiltrira > 50% plućne regije u roku od 24–48 sati, dok je 6,1% pacijenata kritično (respiratorni zastoj, septični šok, i/ili disfunkcija ili zastoj više organa). Osobe koje su u najvećem riziku za ozbiljno oboljenje i smrt jesu osobe preko 60 godina i osobe sa već postojećim oboljenjima kao što su hipertenzija, dijabetes, kardiovaskularne bolesti, hronične respiratorne bolesti i maligna oboljenja. Oboljenje je kod dece relativno retko i blago, otprilike 2,4% svih obolelih jesu osobe ispod 19 godina. Veoma mali procenat osoba ispod 19 godina je razvio ozbiljno (2,5%) ili kritično oboljenje (0,2%). Mortalitet se povećava sa godinama, a najveći je kod osoba preko 80 godina (CFR 21,9%) [1–6].

Stopa smrtnosti je veća među muškarcima nego među ženama (4,7% vs. 2,8%), posebno kod bolesnika sa udruženim komorbiditetima: 13,2% su kod onih koji su imali kardiovaskularne bolesti, 9,2% dijabetes, 8,4% hipertenziju, 8,0% hronične respiratorne bolesti i 7,6% maligne bolesti. Ovaj virus je visoko kontagiozan, brzo se širi i može se smatrati jako opasnim jer izaziva zdravstvene, ekonomske i socijalne probleme na globalnom nivou. Jedinstven je među humanim koronavirusima jer kombinuje visoku prenosivost, česte fatalne ishode u visokorizičnim grupama, kao i velike socijalne i ekonomske promene [1–6].

Nanomaske i maske N95

Nakon što se pojavio virus SARS-CoV-2, korišćenje zaštitnih maski postaje obevezno. Zaštitne maske se najviše koriste među zdravstvenim radnicima kao zaštita od kapljica u kontaktu sa bolesnicima sa respiratornim infekcijama. Izbor maski je različit za različite zemlje, uključujući i korišćenje maski N95. Simulacije modela, korišćenjem relevantnih podataka o dinamici infekcije kovid 19 u USA, sugerišu da bi široka upotreba čak i relativno neefikasnih maski mogla smanjiti prenos kovida 19 i umanjiti broj hospitalizovanih bolesnika i smrtnih ishoda. Podaci govore da bi upotreba univerzalnih (80%) ili umereno efikasnih (50%) maski mogla da spreči 17–45% očekivanih smrtnih ishoda. Medicinske maske (hirurške maske i maske N95) ipak pokazuju kontroverzne rezultate [7, 8, 9]. Podsetimo da se kod SARS-CoV-2 najčešće primenjuje tradicionalni model prenosa respiratornih infekcija preko infektivnih čestica (najčešće 5–10 µm), koje imaju kratko preživljavanje u vazduhu i inficiraju gornji deo respiratornog trakta, ili sitnijih čestica koje mogu ostati u vazduhu [10, 11, 12].

Iako maske N95 u odnosu na hirurške maske nude bolju zaštitu, one imaju i mnogo mana, što pokazuje i veliki broj obolelih zdravstvenih radnika od infekcije kovid 19, jer su medicinski sistemi generalno preopterećeni u svim zemljama. Poznato je da maske imaju samo ograničen efekat (ali i dalje nije zanemarljiv, u kontekstu spašenih života) kod ovako ozbiljnih epidemija kao što je kovid 19.

Maske sa HEPA filterima su dizajnirane tako da imaju zamenjivi filter napravljen od duplog HEPA vazdušnog filtera, kao što je 3M 2800 ultrafini filter. Efikasnost maski sa HEPA filterom, kao što je maska Totobobo, pokazuje i dalje njihovu inferniornost u smislu filtrativne sposobnosti u odnosu na maske N95, zato bi zbog svoje izuzetno niske efikasnosti trebalo da se koriste veoma oprezno [9].

Takođe, primećeno je da filteri 3M MP 2800 filtriraju čestice veličine 0,3 mikrona, dok je veličina nekih virusa, npr. SARS-CoV-2, oko 0,06–0,14 mikrona. S tim u vezi, njihova relativna efikasnost u filtriranju patogena u slučaju trenutne pandemije potpuno je upitna. Uz to, mnogi istraživači misle da se efikasnost ovakvih maski značajno smanjuje posle pranja čak i u slučaju polipropilenskih filtera (sličnih 3M filteru). Poseban izazov je održavanje filtera za višekratnu upotrebu u bolničkim prostorijama i njihova dekontaminacija i sterilizacija uz pomoć germicidnih lampi i autoklava.

Maske N95 imaju 95% efikasnost filtracije, dok obične maske imaju 50% efikasnosti filtracije za male čestice (0,1–0,4 mikrona u dijametru). Studije su pokazale da je protok vazduha kroz komercijalnu masku N95 loš. Uz to, primećeno je da je inhalirani vazduh iz maske hipoksičan, sa frakcijom udahnutog kiseonika FIO_2 oko 16,4%, zbog čega nije bezbedan za bolesnike koji imaju plućne i kardiovaskularne bolesti ili sepsu, i koji zahtevaju dobro snabdevanje kiseonikom [10].

Evaluacija maski trebalo bi da uključuje ne samo efikasnost filtracije i bezbednost njihove primene već i efikasnost prevencije da bakterije i virusi prežive pri prolasku kroz filter. Sve navedeno ukazuje na to da su hitno potrebne maske koje mogu mnogo bolje da zaštite posebno medicinske radnike od infektivnih bolesti, od virusa SARS-CoV-2 [12, 13].

Srebro i srebrne legure

Srebro je, generalno, najefikasniji materijal kao antimikrobni i antivirusni metal, iako su neki drugi metali, kao što su cink, bakar i kobalt, takođe pokazali efikasnu inhibiciju mikroba. Najčešće se koristi za prevenciju bakterijske kolonizacije na medicinskim sredstvima, kao i na raznim tekstilnim materijalima [12, 14].

Veruje se da teški metali reaguju sa proteinima interreagujući sa tiol (-SH) grupom, što dovodi do njegove inaktivacije. U prisustvu tečnosti, npr. iz vazduha, oslobađaju se metalni joni koji inhibiraju replikaciju mikroba. Kratko objašnjenje njegovog antimikrobnog mehanizma je sledeće: metalni joni srebra uništavaju ili prolaze kroz ćelijsku membranu i vezuju se za -SH grupe ćelijskih enzima. Oni tako dovode do kritičnog smanjenja enzimske aktivnosti, koja dovodi do promene metabolizma mikroorganizama i inhibicije njihovog rasta, prouzrokujući na kraju ćelijsku smrt [14].

Metalni joni takođe proizvode kiseonične radikale, koji oksidišu molekularnu strukturu bakterija. Joni srebra mogu da dovedu i do denaturacije proteina i ćelijske smrti zbog njihove reakcije sa nuklofilnim reziduama aminokiselina u proteinu i njihovim sulfidnim vezama u amino, imidazol, ili fosfatnim i karboksilnim grupama membrane ili enzimskih proteina. Srebro takođe smanjuje koncentraciju oksidativnih enzima dehidrogenaze, utiče na preuzimanje sukcinata od strane vezikula i respiratornog lanca Ešerihije koli, što dovodi do metaboličkog efluksa i inhibira replikaciju DNK [14, 15].

Pravi mehanizmi kojima antimikrobne supstance kontrolišu mikrobni rast su ekstremno promenljive i zavise od korišćenog agensa. Generalno, oni sprečavaju ćelijsku reprodukciju, oštećuju zid ili ćelijsku propustljivost, denaturišu proteine, blokiraju enzime i onemogućavaju ćelijsko preživljavanje [16, 17], dok polikatjonske antimikrobne smeše oštećuju ćelijske citoplazmatske membrane mikroba kroz adsorpciju na površini ćelijske membrane mikroba, difuziju kroz ćelijski zid, vezivanje za citoplazmatsku membranu, oštećenje citoplazmatske membrane, otpuštanje citoplazmatskih konstituenata kao što su K^+ joni, oštećenja DNK i RNK i smrt ćelije [16, 17].

Hirurške maske sa nanosrebrom – ALBO

Prvi put, ove maske se proizvode od strane inovativne kompanije ALBOS doo, Beograd, procedurom koja će ubrzo biti patentirana. Ovaj koncept je potpuno drugačiji od dosadašnjih koncepata za proizvodnju zaštitne opreme, koji su kroz pomenutu metaanalizu pokazali značajne negativne strane, čak i u slučaju filtera N95 u nanomaskama N95. S druge strane, ove maske su veoma aktivne, kao što je pokazano u primeru njihove interakcije sa bakterijom *Staphylococcus Aureus*. U ovom eksperimentu, koji je sproveden u Laboratoriji za kontrolu hrane i lekova Veterinarskog fakulteta u Beogradu, korišćenjem horizontalne metode kvantifikacije broja pozitivnih stafilokoka pokazano je da je konačni broj bakterija i koagulaza-pozitivnih stafilokoka izmerenih u CFU/ml manji od 1. Radi poređenja, broj koagulaza-pozitivnih stafilokoka za masku bez nanosrebra iznosio je 1.500.000 CFU/ml, dok je ukupan broj bakterija iznosio 18.180.000 CFU/ml. Ove razlike su zaista izuzetno visoke, i pokazuju neverovatnu efikasnost ovog koncepta zaštite, ne samo za zdravstvene radnike već i za obične građane.

Suštinsko pitanje je zašto je *Staphylococcus Aureus* izabran za takav eksperiment. Znajući da su ove bakterije jedan od glavnih uzroka infekcije i u zdravstvu i među stanovništvom u industrijalizovanom svetu, sa visokim stepenom morbiditeta i mortaliteta, bilo je logično izabrati ovakvu bakteriju. Naime, *S. Aureus* je oportunistički veoma opasan patogen koji posle ulaska u kardiovaskularni sistem može dovesti do ozbiljnih komplikacija, kao što su infektivni endokarditis i tromboflebitis, rezultirajući otkazivanjem organa i smrtnim ishodom [18–23].

Iako još uvek nije bilo šanse da se slično istraživanje organizuje za SARS-CoV-2, na osnovu rezultata sa *S. Aureus* može se pretpostaviti da će rezultati biti i za SARS-CoV-2 mnogo bolji nego u slučaju sada dostupnih zaštitnih maski. S obzirom na to da su čestice virusa veličine 100 nm (HIV je oko 120 nm i SARS virus oko 100 nm), verovatno su veličine nanočestica srebra (NSP) od oko 10 nm i manje najefikasnije u interakciji sa virusom jer su takve čestice značajno manje od virusa. U slučaju nanosilver maske ALBO odgovarajuće čestice nanosrebra su u rangu 7–15 nm, što je verovatno veoma efikasna veličina u ubijanju bilo koje vrste virusa (Slika 1).

Pored toga, dokazano je da su nanočestice srebra veličine 7–15 nm visokoefikasne u supresiji virusnih mehanizama infekcije [18–23]. Uzimajući u obzir da su biološke interakcije virusa i humane ćelije generalno viševalentne, vezivanje i ulazak virusa u ćeliju domaćina predstavlja sjajan primer viševalentne interakcije između površine virusa i ćelijske membrane receptora [18–23].

U slučaju HIV-1 dokazano je da nanočestice inhibiraju inicijalni stadijum HIV1 infektivnog ciklusa, blokirajući posebno interakciju gp120-CD4. Pored toga, nanočestice srebra inhibiraju takođe stadijume životnog ciklusa, ulaska virusa HIV1 u ćeliju jer formiraju komplekse sa ćelijskim elektronskim donorskim grupama koje sadrže sumpor, kiseonik ili azot, koji su normalno prisutni u tiolima i fosfatima aminokiselina i nukleinskih kiselina. To sve ometa reverznu transkripciju direktnim vezivanjem za RNK i DNK molekule, čiji je mehanizam prikazan na slici (Slika 2) [18–23]. Sličan način interakcije se očekuje u slučaju SARS-CoV-2, što će veoma brzo biti provereno, posle prvog istraživanja zaštitnih svojstava nanorebrne maske ALBO, koje se očekuje u bliskoj budućnosti.

ZAKLJUČAK

U radu su prikazane brojne negativne strane zaštitne opreme, hirurških maski i maski N95. Metaanaliza je pokazala da ne postoji statistički značajna razlika između njih sa stanovišta njihove efikasnosti u zaštiti od virusa, uključujući i SARS-CoV-2. Pored toga, predstavljen je novi pristup zaštite sa filterima ALBO na bazi nanosrebra koji pokazuju neuporedivo veću efikasnost zaštite. Neverovatna efikasnost ovakvih maski na primeru bakterije *Staphylococcus Aureus* pokazuje da će očekivana efikasnost ovog proizvoda biti mnogo bolja i za SARS-CoV-2 od bilo kog do sada, bilo gde u svetu, primenjenog rešenja.

Apendiks: Metaanaliza – hirurške maske i filteri N95: neočekivano loša zaštita od kovida 19

Postoji mnogo konfliktnih stavova u vezi sa virusom SARS i pandemijskim gripom – Svetska zdravstvena organizacija (WHO) predlaže korišćenje maski u situacijama niskog rizika, a u situacijama visokog rizika predlažu se maske N95, dok Centar za kontrolu bolesti i prevenciju (CDC) predlaže korišćenje maski N95 u situacijama i niskog i visokog rizika. Pored toga, maske N95 su vrlo često finansijski nepristupačne [24, 25].

Dodatno, prethodna metaanaliza je dokazala da prava vrednost maski N95 nije naučno i statistički dokazana. Rigoroznija klinička testiranja i poređenja efikasnosti hirurških i maski N95 koja se odnose na prošlu deceniju nisu bila uključena u prethodnu metaanalizu [26, 27].

Zbog toga je povećan broj istraživanja na temu korišćenja maski za zaštitu protiv gripa, kao i broj preglednih radova i metaanaliza sa ciljem da se uporedi efikasnost hirurških i maski N95.

U jednom izuzetnom istraživanju na ovu temu dat je prvi pregled efikasnosti hirurških i maski N95. Ovaj rad je obuhvatio: 1) randomizovanu kliničku studiju (koja uključuje klaster-randomizovanu kliničku studiju) i nerandomizovanu kontrolisanu studiju, 2) osobe sa gripom (uključujući pandemijske vrste, sezonski grip A i B, svinjski i ptičji grip), 3) intervenciju i poređenje: maske N95 vs. hirurške maske, 4) primarni ishod: laboratorijski potvrđen grip, 5) sekundarni ishod: laboratorijski potvrđenu virusnu infekciju, laboratorijski potvrđenu respiratornu infekciju i oboljenje slično gripu i 6) okruženje: bolnicu ili lokalnu zajednicu [24].

Kliničke studije odabrane su zahvaljujući mogućnosti dobijanja kvalitetnih naučnih informacija. Strategija pretraživanja podešena je tako da uključi sve podatke u vezi sa tom temom u bazama PubMed, EMBASE i Cochraine Library od 27. januara 2020. kako bi se identifikovali svi objavljeni radovi na temu evaluacije maski u zaštiti od influence. Strategija je adekvatno prilagođena da bi mogla da koristi podatke i u drugim bazama podataka, što je omogućilo da se u ovu analizu uključe svi radovi na ovu temu u proteklih pet godina, od 27. januara 2015. do 27. januara 2020. Pretraga je takođe obuhvatila www.clinicaltrials.gov, da bi se u analizu uključili i neobjavljeni rezultati, bez statusa publikacije i jezičke restrikcije [24–27]. Istraživanje su radila dva istraživača, koja su nezavisno pretraživala radove na osnovu naslova, apstrakta i teksta. Zatim su oba istraživača nezavisno izdvojila podatke kao što je prvi autor, godina publikacije, država, oboljenje, detalji o populaciji i intervenciji, dizajn studije, veličina uzorka, mesto i rezultati, dok su sva neslaganja bila podložna diskusiji. Oba istraživača nezavisno su uključila u sebe i rizik od nezavisnosti odabranih kliničkih studija koristeći *Cochraine Risk of Bias tool* [26–29]. Za svaku kliničku studiju svaki domen je procenjivan na tri nivoa: visok rizik, nejasan rizik i nizak rizik. Neslaganja su rešena diskusijom. Sve statističke analize su sprovedene korišćenjem softvera Review Manager, verzija 5.3. Upoređeni su podaci iz studija sa sličnim intervencijama i svi njihovi rezultati su korišćeni u odgovarajućim bazama podataka. Relativni rizik sa 95% pouzdanosti za dihotomne rezultate je korišćen za procenu efikasnosti maski. Procena heterogenosti podataka je izvedena procenom heterogenosti svakog pojedinačnog seta podataka [28].

Usvojen je random model heterogenosti $p < 0{,}10$ i sprovedena je analiza podgrupa na osnovu mesta ispitivanja (bolnica, lokalna zajednica) zbog kliničke heterogenosti. Urađena je analiza senzitivnosti zbog evaluacije usklađenosti rezultata za svaku posebnu bazu podataka. U studiju je uključeno ukupno 9171 učesnika u Kanadi, Australiji, Kini, dok je broj učesnika u pojedinačnim studijama bio između 435 i 5180 pacijenata. Period praćenja iznosio je od dve do 15 nedelja. Pet studija obuhvatilo je učesnike u bolnicama, i jedna u domaćinstvu. Zbog različitih definicija ishoda u uključenim studijama laboratorijski potvrđena respiratorna infekcija redefinisana je kao respiratorna influenca, ili neka druga virusna ili bakterijska infekcija. Pet kliničkih studija sa 8444 učesnika su potvrdile laboratorijski potvrđeni grip. Metaanaliza sa modelom fiksnih efekata otkrila je da ne postoji statistički značajna razlika u zaštiti od gripa između hirurških maski i maski N95 (RR = 1,09, 95% CI 0,92–1,28, $p > 0{,}05$) [24, 29, 30].

Metaanaliza primenom modela fiksnih efekata otkrila je da ne postoji statistički značajna razlika u prevenciji od SARS Cov 2 ili nekog drugog virusa i bakterije sa hirurškom maskom i maskom N95 (RR = 1,09, 95% CI 0,92–1,28, $p > 0{,}05$).

Rezultati analize podgrupe bili su konzistenti bez obzira na to da li je posmatrana bolnica ili neka lokalna zajednica. Rezultati testa senzitivnosti nisu se promenili posle isključenja bilo koje kliničke studije. Četiri studije koje uključuju 3264 učesnika saopštile su laboratorijski potvrđenu respiratornu virusnu infekciju. Metaanaliza primenom modela fiksnih efekata pokazala je da nema statistički značajnih razlika u prevenciji respiratorne virusne infekcije sa hirurškom maskom i maskom N95 (RR = 0,89, 95% CI 0,70–1,11, p > 0,05). Rezultati analize podgrupe su bili konzistentni bez obzira na to da li je u pitanju bolnica ili neka lokalna zajednica [24, 29, 30].

Dve studije obuhvatile su 6621 učesnika sa laboratorijski potvrđenom respiratornom infekcijom. Metaanaliza primenom modela fiksnih efekata pokazala je da nema statistički značajnih razlika u prevenciji respiratorne virusne infekcije sa hirurškom maskom i maskom N95 u bolnici (RR = 0,74, 95% CI 0,42–1,29, p > 0,05) [24, 29, 30].

Pet kliničkih studija sa 8444 učesnika prijavile su oboljenje slično gripu. Metaanaliza primenom modela fiksnih efekata pokazala je da nema statistički značajnih razlika u prevenciji oboljenja sličnom gripu sa hirurškom maskom i maskom N95 u bolnicama (RR = 0,61, 95% CI 0,33–1,14, p > 0,05). Senzitivnost analize pokazala je da rezultati ostaju isti posle isključivanja bilo koje kliničke studije.

Metaanalize su pokazale da ne postoje statistički značajne razlike kod prevencije laboratorijski potvrđenog gripa, laboratorijski potvrđene respiratorne virusne infekcije, laboratorijski potvrđene respiratorne infekcije i oboljenja sličnom gripu, uključujući i kovid 19, korišćenjem maske N95 i hirurške maske. U analizama podgrupa dobijaju se slični rezultati u bolnicama ili lokalnim zajednicama za laboratorijski potvrđen grip i laboratorijski potvrđenu respiratornu virusnu infekciju. Za vreme pandemije gripa potreban je jako veliki broj zaštitnih maski u dužem periodu kako bi se ljudi zaštitili od infekcije [24, 31].

Korišćenje maski N95, pored toga što su niske efikasnosti, najčešće je udruženo sa poteškoćama disanja, koje mogu dovesti do glavobolje. Prethodna studija pokazala je da postoji obrnuta veza između nivoa saradnje ispitanika pri nošenju maske N95 i rizika za kliničko respiratorno oboljenje. Teško je obezbediti visok nivo saradnje pri nošenju maski N95 u svim studijama, jer je ona izrazito nekomforna za pacijente i bolničko osoblje [26–32].

Razlog za slične efekte u zaštiti od gripa kod maski N95 i hirurških maski može biti loša saradnja pri nošenju maski N95. Zbog toga dolazi i do češćeg skidanja maski N95 u poređenju sa hirurškim [31].

Maske N95 u rutinskoj upotrebi izgledaju manje prihvatljive zbog manjeg komfora pri nošenju. Ovde treba napomenuti da su takve maske primarno dizajnirane da zaštite okolinu od osobe koja je nosi, dok su maske N95 napravljene da zaštite osobu koja je nosi od okoline [24, 32].

Ova metaanaliza je pokazala da korišćenje maski N95 u poređenju sa hirurškim maskama nema za posledicu manji rizik od laboratorijski potvrđenog gripa. Sugeriše se da maske N95 ne bi trebalo da se preporučuju stanovništvu i medicinskom osoblju ukoliko nisu u bliskom kontaktu sa pacijentima koji imaju grip ili sa suspektnim pacijentima [24, 32].