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Aerosols Contamination in the Dental Practice Following Everyday Procedures

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Abstract

Objectives: The purpose of present observational study was to evaluate the bacterial load in the air following various dental procedures.

Materials and methods: Air contamination following 7 dental procedures resulting in aerosols generation was assessed. The air volume was sampled by means of a wet cyclone collector for 10 minutes during 10 different sessions of each of the following dental procedures: a) air-polishing b) ultrasonic instrumentation c) manual instrumentation d) rubber cup e) cavity preparation with the 1:5 red contra-angle f) cavity preparation with turbine and Low Volume Evacuator (LVE) g) cavity preparation with turbine and High Volume Evacuator (HVE). Baseline air samples were analysed as well. Contamination of the sampled solution was determined using ATP (Adenosine TriPhosphate) quantification of the viable bacterial count.

Results: By far the highest increase in air contamination was observed after the use of turbine with LVE. The use of turbine with HVE and the use of the red hand-piece both resulted in elevated bacterial counts, while the application of air polishing, ultrasonic instrumentation, hand instrumentation and rubber cups did not result in higher bacterial count than baseline.

Conclusion: Routine professional oral hygiene procedures do not increase the air contamination produced by aerosols. However, cavity excavation creates significantly higher bacterial count in the air. The highest contamination was seen after the use of turbine with LVE.

Keywords: Aerosol • Contamination • Dental procedures • Prevention • Infection control

Introduction

The COVID-19 pandemic has boosted the interest in airborne diseases and appropriate infection control measures [1]. Different transmission routes in the dental setting have to be considered. Among those, the most important aspect resulting in contamination in the dental office may be the direct or indirect splatter of aerosols produced during dental routine procedures [1,2]. As present day dental medicine practices apply a number of techniques that utilize ultrasonics, air polishing and abundant spay with drilling instruments, it is obvious that contamination of saliva and its spread in the generated aerosols provides an entry door for pathogens [3].

Splatter is described as a mixture of water and/or solid substances in the form of droplets with a diameter higher than 50 mmicrons. Given these dimensions, splatter does not spread far away from the area of its production and precipitates quickly on the surrounding surfaces [4]. On the other hand, aerosol particles are smaller than 50 microns and may remain suspended in the air for a long time [5]. Bio-aerosols may have a highly heterogeneous composition depending on their source of origin. Hence, they pose a serious risk of inhalation and infection with contained viable micro-organisms [6,7].

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Guidelines for the management of the first SARS-CoV virus strongly recommended performing dental procedures that produced lower amount of aerosols if possible [8]. Moreover, personal protection through proper Personal Protective Equipment (PPE) was strongly recommended [8].

It has been demonstrated that aerosols produced by ultrasonic scalers, air polishing devices or even rotary drilling with air coolant can be reduced by e.g. High Volume Evacuator (HVE) [9]. Moreover, the splatter of aerosols into the immediate environment around the dental chair will be affected by applying HVE [9]. Supplementary methods to minimize the impact of aerosols include pre-treatment rinses with disinfectant mouthwashes and the use of rubber dam [1,9-11].

Airborne contamination maybe evaluated in experimental and clinical settings applying various techniques:

- Aerosol sampling by passively allowing microorganisms to settle on surfaces like agar plates. This may be useful to evaluate an aerosol settling rate.
- Active sampling of defined air for a set period of time [12].
- Active sampling applying wet-cyclones to allow air-to-liquid transferal of the particles contained in the sampled air volume [12]. The liquid content may then be analysed.

Quantification of Adenosine TriPhosphate (ATP) content of the collected liquid is a well-known analytical method for viable microorganisms. ATP is an energy-carrying molecule found in all living prokaryotic cells. A special bioluminescence assay using the luciferin-luciferase reactions is able to determine the quantity of ATP in a solution, i.e. the quantity of viable microorganisms. ATP assays are considered to be simple, rapid and sensitive methods for monitoring surface contamination in hospital settings, waterlines and dental settings [13,14]. Cyclone systems are proven to have an excellent sampling performance and - together with ATP enzymatic reactions - represent an excellent solution for monitoring the indoor and outdoor environments [15].

The aim of the present study was to evaluate the microbial load in the aerosol generated during various every-day dental procedures.

Materials and Methods

The present study represents a single-blinded, observational study of an environmental parameter in a private practice in Brescia (Italy). The dental treatments were provided to patients referred for professional oral hygiene or treatment of tooth decay. All participants were informed about the environmental measurements and analysis, and signed a consent form prior to their inclusion in the study. As no patient data were recorded and only environmental data from the dental clinic were evaluated, no approval by an ethical committee was required.

Clinical procedures

Baseline measurements were obtained by sampling the air in the clinical room of the dental office for 10 minutes during a conversation between the two operators, both wearing a FFP2 facial mask. Prior to the baseline measurement the room had been disinfected and exposed to the fresh air for 15 minutes.

As was customary in the dental office, a pre-treatment rinse with 0.1% chlorhexidine digluconate and cetylpiridinium chloride (0.05%) was administered (BACTER X PRO®, EMS®, Nyon, Switzerland). Either Low Volume Evacuator (LVE) only or a combination of LVE and HVE were applied, according to the clinicians' preference. The operators were wearing full PPE equipment during each treatment.

Aerosol collection was performed during the following procedures performed by two professionals:

- Air-polishing (Airflow Prophylaxis Master®, EMS®, Nyon, Switzerland) with Erythritol powder (PLUS®, EMS®, Nyon, Switzerland). The power was set at 5/10 and the water supply at 10/10. HVE and LVE were both applied
- Ultrasonic Instrumentation with a perio-slim tip (Piezon® PS, EMS, Nyon, Switzerland). The power was set at 3/10 and water supply at 10/10. LVE was applied
- Polishing with rubber cup (1241RA, Edenta®, Switzerland) and prophylaxis paste (NUPRO®, Dentsply Sirona, York, Pennsylvania, U.S.A). A regular 1:1 contra-angle hand-piece (blue band) was used. LVE was applied
- Manual instrumentation with Gracey curettes 7/8, 11/12 and 13/14 (LM-Dental™, Finland). LVE was applied
- Cavity preparation with a Turbine with water cooling (KaVo® Kerr Dental). LVE was applied
- Cavity preparation with a Turbine with water cooling (KaVo® Kerr Dental). HVE and LVE were both applied
- Cavity preparation with 1:5 red contra-angle with water cooling (Sirona® Multiplier, Dentsply Sirona). HVE and LVE were both applied.

In order to minimize external water contamination, the Airflow Prophylaxis Master® was used with an independent bi-distilled and demineralized water supply. The dental chair was also supplied with the same water through a bottle system. Bi-distilled demineralized water was produced through a water distiller (Mophorn®, U.S.A.).

10 different procedures for each of the treatment modality above were sampled, for a total of 70 measurements. The clinical room was cleaned and disinfected following standard infection-control procedures, and the window was opened for 15 minutes between each treatment.

Aerosol sample collection

Sampling was performed using a wet-cyclone sampler system (PRELECT®, Medentex, Germany). The cyclone system consisted of two components: the upper part containing the cyclonic structure and the lower part acting as a water collector and container. The air containing the aerosol was sucked into the cyclone container. Aerosol droplets were then accelerated and centrifuged in the vortex created so that they were pushed into contact with the moving pre-filled water in the collector. The aerosol bacterial and viral debris were transferred to the collecting water, preventing desiccation. The system accumulated the living material in the collector. The collector was filled with 120 mL of 0.45-micron syringe-filtrated water. To maximize the air collection, a suction funnel with 100 mm external diameter was added to guide the aerosol into the system. All the suction power was generated by an independent suction system (H-POWER 700®, Hoover, U.S.A) connected to the cyclone exit tube with a 30 mm tube to avoid pressure loss due to the tubing.

To obtain a best estimation of the air volume collected, the suction flow rate was measured as follows: a standard plastic bag (110 L trash bag) was fixed to the suction system with a tape. The exact air-filled bag volume in this setup was measured by filling it previously with water and resulted in 115 L. Time was then recorded to fill up the bag with air until the pressure in the bag started increasing. A flow of c.a. 900 L/min was confirmed.

To control and limit system contamination during the setup preparation the following protocol was applied before each procedure:

- The upper part of the system was rinsed twice with 30 mL of 0.45 micron syringe-filtrated water;
- The collector was also rinsed twice with 60 mL of 0.45 micron syringe filtrated water;
- Both parts were dried with compressed medical grade air;
- A second rinse was performed for both parts;
- The cyclone was then set-up, and 120 mL of 0.45 micron syringe filtrated water was inserted into the system through the exit channel.

In order to prevent water projections into the exit channel, the vortex movement of the liquid was progressively created. The aspiration flow rate was increased from 70 m bar to 210 m bar in 10 seconds. Treatments began at this stage. At the end of the treatment the same protocol was applied in reverse to obtain a smooth switch.

For each procedure, air collection was performed for 10 minutes, with the cyclone system placed at about 20 cm from the patient's mouth. A total of 9 m^3 of air was sampled per treatment.

Sample preparation for ATP analysis

The bio-contamination of the aerosol was assessed by means of ATP (Adenosine TriPhosphate) quantification. Measurements were performed using an ATP bioluminescence assay based on luciferine-luciferase reactions (Institut Clinident®, France). The enzymatic reaction of bioluminescence between luciferin (2 MIN REAGENT®, Institut Clinident, France) and firefly luciferase (Test Tube, Institut Clinident®, France) causes ATP to release energy in the form of light. The emitted light was measured with a luminometer (Lumitester Smart®, Kikkoman Biochemifa Company, Japan). This measurement is strongly dependent on environmental temperature. Therefore, after every sample measurement, a referenced ATP amount (Standard 1000, Institut Clinident®, France) was added to the same sample and the emitted light was measured again to obtain system calibration.

In order to concentrate bacteria floating in the solution and to increase the sensitivity of the system, all solutions collected from the cyclone were filtrated on a sterile syringe filter (0.45 microns PES, Merk Millipore, Germany). Once the solutions were filtrated, 160 µL of enzymatic luciferin agent was sucked into the filter with the syringe, forming the luciferin-ATP complex in the bacteria concentrate on the filter. The solution was then expelled through the filter into the test tube containing the luciferase, followed immediately by the light measurements (result=R1). Immediately after this first measurement, the calibration ATP STANDARD 1000 was added into the tube (40 µL) and the light measurement was taken again (results=R2).

The amount of light measured in RLU (Relative Light Unit) can be directly transformed in ATP amount using the two equations below:

Correction factor = (R2-R1)/1000

With R1 being the result obtained in [RLU] for the sample, and R2 the result obtained in [RLU] for the sample + STANDARD 1000

[ATP]=R1/(Correction factor × V)

With [ATP] the concentration of ATP in [pgATP/mL], and V the volume of solution filtered in [mL].

The conversion of ATP to the total bacteria amount was made according to the equation below:

1 picogram ATP ≈ 1000 bacteria

Therefore, the ATP measurement provided directly results in CFU/mL (Colony Forming Unit):

Bacteria = 1000 × [ATP]

With "Bacteria" the concentration of bacteria in the collected water in [CFU/mL]

To ensure maximal sensitivity for these tests, the entire water volume (V) was used to collect the aerosol (approximatively 100 mL).

Microbial load calculation

To obtain the microbial aerosol load, the total amount of collected CFU/mL was divided by the total amount of collected air in order to get bacteria concentration per unit of air volume:

Bacteriaairload=(Bacteria × V_water)/(Q air × Time)

With “Bacteria air load” being the quantity of bacteria suspended in the air in [CFU/lair], “Bacteria” the concentration of bacteria in the collected water in [CFU/ml], Vwater the total amount of water collected, Qair the air suction capacity in [NL/min] (= 900 NL/min], and “Time” the collection time in [min], which is 10 for all the tests applied.

Statistical analysis

Bacterial air load was modeled using a generalized linear model with Gamma family and identity link. This allows toaccount for a substantial skewness in the data distribution. Results were reported as estimated averages and 95% confidence intervals. All tests were two-sided and assumed a 5% significance level. All analyses were performed using R (version 4.1.0).

Results

A total of 70 air samples were collected following various dental procedures. These represented 7 different treatment modalities that all generated various amount of aerosols. Each of the modalities was tested with 10 samples. The mean bacterial load in the air adjacent to the dental chair is summarized in Table 1 and Figure 1.

Pre-treatment baseline assessment of the room contamination showed an average of 1.45 (0.85-2.04) CFUs/L of air. Very similar results were seen after the use of Airflow® with HVE+LVE, the manual instrumentation with LVE, Piezon® instrumentation with LVE and the rubber cup application with LVE.

On the other hand, following the use of the 1:5 red contra-angle and the turbine with HVE+LVE the air contamination was clearly above the baseline. The highest count was observed following the application of the turbine with LVE with an average of 7,38 (3.87-10.89) CFUs per litre of air.

Table 2 analysed the difference between the baseline contamination and the air contamination following the various treatments. Only the difference between the bacterial load after the use of the turbine and baseline contamination reached statistical significance *(p<0.01)*. None of the air samples following all the other procedures yielded statistically higher concentration of bacteria when compared to baseline.

As various methods are used in a clinical setting, combination of procedures were analysed with regards to the aerosol contamination (Table 3). No statistically significant differences were noted between the contamination of the air adjacent to the chair following the application of Airflow® combined with Piezon® instrumentation and the usage of Piezon® instrumentation and rubber cup polishing. The same applies to the combination of Airflow® and ultrasonic instrumentation compared to the combination of manual instrumentation with rubber cups. As opposed to these comparisons, cavity preparation with turbine and LVE led to a statistically higher air contamination compared with preparation of the cavity with red 1:5 contra-angle with HVE + LVE *(p= 0.017)*. However, if the air contamination following the use of the turbine with HVE was compared with the red contra-angle with HVE no statistically significant differences were observed. As expected, cavity preparation with the turbine and LVE led to a

Table 1. Mean bacteria air load (CFU/Lair) and confidence interval per each procedure performed.

Group	Estimated concentration (95% CI)
Baseline	1.45 (0.85-2.04)
Air-polishing HVE	1.44 (0.57-2.32)
Ultrasonic Inst.	1.44 (0.49-2.40)
Rubber Cup	1.10 (0.30-1.89)
Manual Inst.	1.13 (0.32-1.95)
Turbine	7.38 (3.87-10.89)
Turbine HVE	2.98 (1.34-4.63)
1:5 contra-angle HVE	2.70 (0.18-4.22)

CI: Confidence Interval.

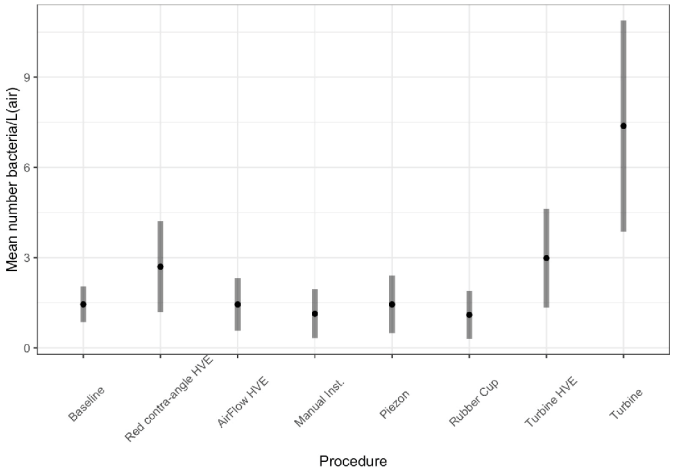

Figure 1. Mean bacteria air load (CFU/Lair).

Table 2. Difference in bacteria air load (CFU/Lair) between baseline sample and aerosol sample of 10 minutes of treatment.

Contrast	Estimate (95% CI)	p-value
1:5 contra-angle HVE *vs.* Baseline	1.25 (-0.38; 2.88)	0.133
Air-polishing HVE *vs.* baseline	-0.00 (-1.06; 1.05)	0.995
Manual Inst. *vs.* baseline	-0.31 (-1.32; 0.69)	0.543
Ultrasonic Inst. *vs.* baseline	-0.00 (-1.13; 1.13)	0.997
Rubber Cup *vs.* baseline	-0.35 (-1.34; 0.64)	0.490
Turbine HVE *vs.* baseline	1.54 (-0.21; 3.28)	0.085
Turbine *vs.* baseline	5.93 (2.37; 9.50)	<0.01

CI: Confidence Interval

Table 3. Comparison of bacteria air load (CFU/Lair) produced by different dental procedures and combinations of instruments.

Contrast	Estimate (95% CI)	p-value
Air-Polishing HVE + ultrasonic Inst. *Vs.* rubber cup + Ultrasonic Inst.	-0.00 (-1.30, 1.30)	0.999
Air-polishing HVE + ultrasonic Inst. *vs.* rubber cup + manual inst.	0.33 (-0.71, 1.37)	0.538
Turbine *vs.* turbine HVE	4.40 (0.52, 8.28)	0.026
1:5 contra-angle HVE *vs.* turbine HVE	-0.29 (-2.52, 1.95)	0.802
1:5 contra-angle HVE *vs.* turbine	-4.68 (-8.51, - 0.85)	0.017
Air-polishing HVE + ultrasonic Inst. *vs.* turbine HVE	-5.94 (-9.56, -2.32)	<0.01

statistically higher air contamination compared with the cavity preparation with Turbine and HVE + LVE *(p=0.026)*. Furthermore, the use of the turbine even with HVE led to a highly statistically significant contamination compared to the combination of Airflow® with HVE and Piezon® instrumentation ($p < 0.01$).

Discussion

The aim of the present study was to quantify the level of environmental contamination with aerosol produced during common dental procedures. Sampling through a wet-cyclone system was performed to measure the microbial contamination in the air adjacent to the dental chair. Bacterial load was determined by means of an ATP bioluminescence assay.

To determine the environmental air contamination, baseline assessments of the room air after disinfection and air refreshment were made. This assessment resulted in a baseline value of 1.45 CFU/lair. This value was used for comparison with the contamination resulting from various dental procedures. It is evident that most professional hygiene modalities did not trigger increased contamination values. In essence, the use of the Airflow® device, the Piezon® device as well as the use of hand instruments and prophylaxis rubber cup did not result in any additional contamination besides the background evaluated by the baseline assessment. This, in turn, means that professional hygiene devices may be applied without any additional risk for air contamination in the dental office. It is especially important to realize that such essential instruments do not seem to negatively affect the health of patients and operators in this time of pandemic. It is also important to highlight the relevance of proper use of HVE in conjunction with Airflow®, as recommended by the manufactures.

Substantially higher aerosol contamination than that noticed for the professional hygiene devices was observed after the use of rotary drilling instruments used for cavity preparation. When the preparation was performed with a red 1:5 contra-angle hand-piece with HVE, a moderate increase in air contamination was seen when compared to the prophylactic instruments. Apparently, the air contamination trigged by the water spray with the red contra- angle can be rather limited, provided that a HVE is simultaneously applied.

In many dental offices, turbines with abundant water cooling are in use with or without the application of HVE. In the latter case, the air contamination was significantly and substantially elevated compared to all other methods tested. This four-fold increase in air contamination renders the use of a turbine with Low Volume Evacuator (LVE) redundant. Consequently, it should be banned from being used in times of the COVID 19 pandemic.

On the other hand, air contamination following the use of a turbine but with the HVE yielded similar outcomes as after the use of the red contra-angle hand-piece. It is, therefore, imperative to recommend abundant cooling combined with HVE for restorative procedures.

It is interesting to notice that those procedures traditionally considered at much higher risk for aerosol production (air-polishing, ultrasonic scaling) did not seem to increase the environmental contamination significantly more than other supposedly safer procedures like caries excavation, or even simply polishing with rubber cup and manual instrumentation.

The traditional professional oral hygiene procedure for general patients involve the use of ultrasonic instrumentation followed by polishing with rubber cup and abrasive paste, or air-polishing in case of tough stains. If the patient presents periodontal involvement, normally manual instrumentation is applied as well. A novel minimally-invasive protocol has been introduced, involving the prominent use of Airflow® followed by Piezon® instrumentation, known by the name of GBT (Guided Biofilm Therapy) [16]. The results of the present study, suggest that Airflow® and Piezon® instrumentation do not increase air contamination significantly compared to manual scaling and polishing with rubber cups and abrasive paste.

Regardless of the procedures applied, it is well known that pre-procedural rinse [10] and the application of HVE play an important role in the control and removal of contaminated aerosol. Moreover when comparing AirFLow® and Piezon® PS instrumentation with more traditional methods, such as rubber cup and Piezon® PS no differences were found in terms of aerosol contamination.

Pre-procedural rinses with chlorhexidine and cetylpyridinium chloride mouthwashes are recommended to decrease the oral microbial load [10] that may become aerosols [17]. Various combinations of LVE and HVE are commonly used in private practice, and were tested in the present study. As a consequence, HVE is strongly recommended, as it collects larger volumes of aerosols [9]. Unfortunately, HVE does not seem to be used as much. In a survey of American hygienists, King TB, et al. [18] reported that, even if most of the surveyed hygienists think that is very important to minimize dental aerosols, very few used HVE with air-polishers and ultrasonic scalers. Aurangjeb AM, et al. [19] surveyed Indian dental surgeons and found that only 3.8% of surgeons used HVE routinely. Yuzbasioglu E, et al. [20] showed that 41.6% Turkish dentists used HVE. However, chances are that after the COVID-19 outbreak, these numbers may have increased dramatically, together with the awareness of the importance of aerosol control. In the present study, HVE was used with those procedures that are at highest risk for creating aerosols. However, cavity preparations with Turbine + LVE and Piezon® PS instrumentation + LVE were also tested, due to the results of the aforementioned surveys.

The results of the present study are in agreement with an historical study by Micik RE, et al. [6]. These authors investigated the amount of aerosol produced during various dental procedures by placing agar plates in the test room, and measuring the contamination via the number of CFU per minute of dental procedure. Activities such as breathing, speaking, shouting, coughing and sneezing were analysed too. CFU counts are very useful because they account only for the viable microorganisms in the sampled aerosol. However, there are sensitivity limitations due to the fact that many bacterial species cannot grow on standard agar plates, and viruses are not detected at all [9]. Regardless, CFU counts may be used as a good index of airborne contamination. In agreement with the present study, cavity preparation was amongst the procedures causing the biggest amount of contamination, and the application of HVE significantly reduced it. Interestingly, coughing also seemed to produce a considerable amount of aerosols [21].

A recent study by Matys J and Grzech-Leśniak K, et al. [11] investigated the aerosol production during different dental procedures and with different suction devices. The colleagues came to the same conclusion that HVE allow a significant control over the produced aerosol when a high-speed hand piece is used for caries removal. Moreover, still in accordance with the present study, ultrasonic scaling did not seem to produce more aerosol than caries removal. However, a major difference between this study and Matys J and Grzech-Leśniak K, et al. [11] comes from the fact that the colleagues used manikin models instead of real patients. Also, measurements were taken with a laser particle counter. As the laser particle counter cannot distinguish between biological and non-biological materials, no information about the actual pathogenic potential of the aerosol in that study can be determined.

Whilst bacterial air contamination is important, most of the recent focus has been around aerosol contamination with viral particles, due to the COVID-19 pandemic. Adenosine triphosphate (ATP) is present in the cells of all living micro-organisms (bacteria, fungi and protozoa), but viruses cannot generate or store energy in the form of ATP. Therefore, one might argue that the present or the aforementioned studies cannot assess fully aerosol contamination. However, a study from Sifuentes Y, et al. [22] demonstrated that ATP measurements could be useful for evaluating the effectiveness of hygiene interventions aimed at preventing viral spread in the workplace. In their study, reduction in ATP reflected reduction in viral concentration.

A limitation of the present study was the fact that the area was sampled for only 10 minutes during treatment. Professional oral hygiene sessions or decay removal may often last longer possibly create more contamination. Moreover, this study did not take into account potential surface contamination by splatter. Given the bigger particle size of splatter (>50 μm), it settles on surfaces quite fast and it is able to contaminate only a small area around the dental chair [4]. Splatter control is performed through suction, proper Personal Protective Equipment (PPE), clear demarcation between dirty and clean areas and surface disinfection after each and every patient [8].

In conclusion, the results of the present study indicate that common dental and hygiene procedures do not trigger air contamination via aerosol spreading provided that proper suction devices and pre-procedural disinfection mouth rinses are applied. However, if LVE is used in combination with drilling procedures an increased concentration of contaminants has to be expected. The highest contamination was observed during caries excavation with turbine and LVE.

Conflict of Interest

M.M. reports grants, consulting fees and non- financial support from EMS-Electro Medical Systems outside the submitted work; E.S. reports grants, consulting fees and non-financial support from EMS-Electro Medical Systems outside the submitted work; A.S. reports consulting fees from EMS-Electro Medical Systems outside the submitted work; S.C. reports no conflict of interest. L.M reports no conflict of interest. S.M. reports no conflict of interest. N.L. reports no conflict of interest, and received consulting fees from EMS-Electro Medical Systems outside the submitted work.

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