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Research Article

JOURNAL OF WOMENS HEALTH AND DEVELOPMENT

ISSN: 2644-2884

In Vitro Pro-Inflammatory Cytokine Inhibition by Exogenously Generated Nitric Oxide; Potential Complimentary Clinical Application to Bacterial Rhinosinusitis Management

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Abstract

Background: Recurrent acute rhinosinusitis (RARS) is characterized by episodic inflammation of the nasal and paranasal sinus mucosa and is frequently triggered by viral infection, with secondary bacterial involvement contributing to symptom persistence and antimicrobial use. Nitric oxide (NO) exhibits antimicrobial and immunomodulatory properties that may be therapeutically advantageous in this setting. This study evaluated the *in vitro* anti-inflammatory effects of NO generated from a nitric oxide–releasing solution (NORS) and explored the clinical efficacy and safety of a nitric oxide nasal spray (NONS) in adults with RARS.

Methods: *In vitro* experiments assessed NO-mediated cytokine modulation in primary human monocytes and THP-1 cells following lipopolysaccharide stimulation, measuring TNF-α, IL-1β, IL-6, and IL-8 secretion and cytotoxicity across graded NO exposures. Clinically, a multicenter, randomized, double-blind, placebo-controlled phase 2 trial compared the NO generated from NONS to a saline placebo nasal spray in adults with a history of RARS. Participants (68.4% of which were women) initiated a two week (5 doses/day) treatment regimen at the onset of symptoms and were followed for 4 weeks. The primary endpoint was clinical success (cured or much improved) at Day 8; secondary outcomes included quality of life (SNOT-22), intranasal corticosteroid use, antibiotic rescue and safety.

Results: *In vitro*, low NO exposures consistently inhibited TNF-α, IL-1β, and IL-6 secretions from baseline by approximately 40–60%, while IL-8 inhibition was not observed at tolerated non-cytotoxic concentrations. Clinically, 53.6% of NONS-treated participants achieved clinical success at Day 8 compared with 46.3% receiving saline (absolute difference 7.3%; p=0.391), with trends favoring NONS for improved quality of life, lower intranasal corticosteroid use and reduced antibiotic rescue. NONS was well tolerated with adverse events comparable to saline, with no treatment-related serious adverse events observed.

Conclusions: NO generated from NORS demonstrates biologically plausible anti-inflammatory activity in monocytic models, and when delivered as NONS shows favorable efficacy trends and good tolerability in RARS. Although statistical significance was not achieved, likely due to the limited evaluable sample size, these findings support further adequately powered trials to define the clinical role of NO-based nasal therapy in RARS management. NONS may have the potential to reduce the duration of each RARS episode, decrease the severity of symptoms of each episode and decrease the number of episodes/year.

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Citation: K Moore, C Miller, J Martins, J Leemhuis, A Rausch, A Javer. In Vitro Pro-Inflammatory Cytokine Inhibition by Exogenously Generated Nitric Oxide; Potential Complimentary Clinical Application to Bacterial Rhinosinusitis Management. Journal of Women's Health and Development. 9 (2026): 26-34.

Received: March 12, 2026
Accepted: March 16, 2026
Published: April 16, 2026

Keywords: Anti-inflammatory; Cytokine inhibition; *In vitro* investigation; IL; LPS; Monocyte; Nitric oxide; Nitric oxide-donor / generator; NONS; Phase 2 clinical trial; THP-1 cell; TNF; Women

Introduction

Recurrent acute rhinosinusitis (RARS) are short-term (<4 weeks in duration), recurring episodes (≥4/year) of moderate-to-severe sinus and nasal signs and symptoms. Mucous membrane inflammation is simultaneously observed in the nasal and paranasal sinus cavities (*Payne 2025*).

Clinical management guidelines for rhinosinusitis emphasize initial symptom relief primarily using intranasal saline irrigations, intranasal corticosteroids and oral analgesics, especially if considered virus induced. Rhinovirus, parainfluenza/influenza virus, and respiratory syncytial virus exposure often trigger rhinosinusitis (*Lee 2022*).

Empirical oral antimicrobial therapy is initiated if no improvement of severe symptoms occurs within 3-5 days, e.g., watchful waiting, for presumed bacterial episodes. Antibiotics are initiated sooner if symptoms worsen or are accompanied by fever >39°C (>102°F), significant facial pain, or persistent purulent nasal discharge (*Payne 2025*). *Streptococcus* pneumonia, *Hemophilus* influenza and *Moraxella* catarrhalis are the most commonly implicated bacterial pathogens linked to the non-abatement of bacterial sinusitis (*Benninger 2006*).

Exogenous nitric oxide (NO) gas application in multiple animal inhalation models has been shown to down-regulate cytokine induced leukocyte and mast-cell inflammation (*Guidot 1995; Gaboury 1996*). NO gas generation has been previously shown to inhibit inflammatory cytokine production by human alveolar macrophages (*Thomassen, 1997*). The authors set out to demonstrate the NO gas generated during the actuation of nitric oxide releasing solutions (NORS) from a nitric oxide nasal spray (NONS) inhibits monocytic pro-inflammatory cytokines with its concurrent bactericidal and virucidal actions against nasal and respiratory pathogens. Preliminary clinical trial efficacy results are presented suggesting a positive trend for NONS in RARS deserving further clinical investigations.

NO gas in liquid generated from NORS has been previously shown to be bactericidal in multiple *in vitro* (*Ghaffari 2006*) and *in vivo (Regev-Shoshani 2013, Regev-Shoshani 2017)* models, in addition to being virucidal for nasal/respiratory pathogens, i.e., SARS-COV-2, Influenza, HRV and RSV (*Figure 1; Martins, 2026*). Phase 2 and 3 clinical trials have previously demonstrated the benefits of NONS treatment in quickening clinical resolution of COVID-19 infections (*Winchester 2021, Tandon 2022*).

Pro-Inflammatory Cytokine Inhibition with Nitric Oxide Releasing Solution (NORS)

Non-clinical laboratory investigations were conducted to document the degree of anti-inflammatory efficacy of nitric oxide generated from NORS/NONS in human monocytes. The first objective was to identify the optimal NO exposure/incubation time in inflammatory cell cultures (primary human monocytes & THP-1 cell line) to study. Then analyze the cytotoxicity potential and NO-directed cytokine modulation generated from NORS/NONS.

Materials & Procedures: Biomaterials included primary human monocytes obtained from 2 healthy donors, concurrently with purchased THP-1 cell lines. Cell cultures were either stimulated with LPS (100 ng/mL; serotype 0127:B8) or remained unstimulated. Nitric oxide was generated from NO-releasing solution (NORS, a proprietary donor-activator system), or directly from NORS in a commercial nasal spray (NONS). NO exposure occurred for 2.5, 5, 10, 20 and 30 minutes of duration (timepoints). Normal saline (0.9% NaCl) was used as a treatment control (*Table 1*).

Each investigation spanned three days. On Day 1, NO treatment occurred for the indicated time-points via low binding matrix extracellular protein-protein interactions (ePPIs, @25°C). RPMI medium 1640, supplemented with 3% fetal bovine serum was added to stop NO release (via elevating pH >6), with cells centrifuged to wash away NO. Treated cells were resuspended in fresh media and plated in 5 x10^5 cells/well (96 well plate) and incubated overnight at 37°C.

LPS stimulation or unstimulated controls began on Day 2 for a total of 24 hours. Supernatants were collected on Day 3. The degree of inhibition of TNFα, IL-1β, IL-6, IL-8 was measured by Mesoscale (ELISA) with cytotoxicity assessed via the CellTiter-Glo® test. All timepoints were performed in triplicate.

Results: Cell viability (cytotoxicity) was dependent primarily on the NO exposure generated from the nitric oxide–releasing products. The NO gas concentration cytotoxicity is expressed as a function of the area under the curve (AUC, *Figure 2*). In monocytic cells, NO cytotoxicity was induced by lower concentrations than expected. Primary monocytes were less sensitive to increasing concentrations of NO compared to THP-1 cells. NO produced from NONS (nasal spray) was cytotoxic to both primary monocytes and THP-1 cell line at tested concentration of >0.065ppm*min NO.

Upon stimulation with LPS, primary human monocytes as well as monocytic THP-1 cells secreted high levels of pro-inflammatory cytokines TNFα, IL-6, IL-1β and IL-8 compared to non-stimulated cells. The NO produced by NORS inhibited the pro-inflammatory cytokines consistently

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DOI:10.26502/fjwhd.2644-288400148

over all timepoints (*Table 2*). A mild-to-moderate (40-60%) inhibition of the pro-inflammatory cytokines TNFα, IL-1β and IL-6 occurred when human monocytes are exposed to bacterial lipopolysaccharide (LPS) stimulation from baseline in the presence of a low exposure of nitric oxide (*Figure 3*; 2.5 and 20 minutes of NO exposure depicted). NO inhibition was approximately 15-30% greater when compared to the application of 0.9% NaCl. In human monocytes the extent of inhibition was comparable to NaCl by 30 minutes. IL-8 is not inhibited at these exposures studied.

Preliminary Clinical Management Results of RARS with Nitric Oxide Nasal Spray

Nitric oxide releasing solution (NORS) therapy administered as NONS, has a potential to shorten clinically meaningful RARS symptoms duration and more quickly resolve the negative quality of life (QoL) associated with the condition. NONS therapy may reduce the concomitant use of intranasal corticosteroids (INCS) and oral ATBs used to manage RARS.

Methodology

A multicenter, randomized, double-blind placebo-controlled, two-arm, parallel group, phase 2 clinical trial was conducted to determine the efficacy and safety of NONS to treat RARS (#281493, NCT06264141). Adults (18 years or older) presenting with a history of mild to severe RARS symptoms (i.e., nasal obstruction (congestion), purulent nasal discharge, postnasal drip, headache, facial pain) were eligible. Radiographic findings consistent with acute sinusitis were not necessary at baseline. Participants were screened and checked regarding inclusion and exclusion criteria after informed consent obtained. Vital signs, urine pregnancy test, clinical safety laboratories (chemistry/hematology) and physical examinations were conducted during screening and at the end of the trial.

Nasal, sinus, head and facial symptoms were recorded and assessed using major rhinosinusitis symptoms score (MRSS); twice daily during treatment, once daily thereafter. MRSS is a 4-point scale (0=no symptoms, 1=mild symptoms, 2=moderate symptoms, 3=severe symptoms) to evaluate manifestations of acute rhinosinusitis [(anterior (nasal) and posterior (postnasal drip) secretions, nasal obstruction (congestion), headache and facial pain/pressure, maximum MRSS score 15]). Participants were required to have a typical RARS episodic history MRSS score of 5 to 12 (with two being at least moderate in severity of the five symptoms) at screening.

Suitable candidates were randomized to a 2-week course of nitric oxide nasal spray (NONS) compared to saline placebo nasal spray (1:1 ratio), administered as five doses daily, begun at the onset of their sinusitis episode post-randomization. INCS (fluticasone) was dispensed with the study medication; Antibiotics (ATBs) were prescribed separately (as needed). Participants were contacted by the site weekly (text, phone call or email) as a reminder to begin study procedures/medication with the onset of sinusitis. Participants were instructed to inform the site (text or email) when they had begun study medication/procedures, e.g., after having RARS symptoms for at least 3 hours (Day 1).

Nasal/oral decongestants, nasal/oral antihistamines and oral mucolytics were not permitted for the first 7 days of study medication treatment. Oral or parenteral corticosteroids and immune-suppressive medications were not permitted. Participants were allowed to continue the use of inhaled corticosteroids (if asthmatic).

Participants recorded their RARS symptoms in a standardized ePRO diary twice daily, which was also used to assess study medication compliance during the 2-week treatment phase, followed by once daily recording to the end of the trial. Participants were asked to rate their RARS symptoms using the MRSS score and when clinical success had been achieved, i.e., on a 6-point Likert scale participants report being 'cured' or 'much improved'; other options include 'somewhat improved', no change', 'somewhat worse' and 'much worse'.

If participants' condition had not improved by Day 5 (or significantly worsened before Day 5); once daily INCS could be initiated. If the participant did not improve by Day 8 (or significantly worsened before Day 8), standardized ATB therapy could be added to the participant's management. A preferred first-line ATB (azithromycin), second-line ATB and alternatives for those allergic to the first-line class of ATBs were provided.

Clinical success percentage of participants (% proportions [ITT]) at Day 8 was defined as those achieving being 'cured or much improved of their sinusitis' (primary endpoint). The sample size was based on this assessment with a desired per group sample size of n=93. The expectations were a greater than 20% treatment difference between arms, with an NNT <10. Sinusitis quality of life was measured using the Sinonasal Outcome Test-22 (SNOT-22) at Screening, Baseline, Day 8 and Day 15. The questionnaire lists symptoms and social/emotional consequences of the participants' nasal disorder (6-point scale; 0=no problem to 5=bad as it can be).

Disposition

One hundred eighty-six participants were to be enrolled, with a total of 93 participants per treatment arm planned. The study evaluation duration was four weeks, which included the assessment sinusitis episode onset (Day 1), 2 weeks (14 days) of treatment, and 14 days of follow-up. The trial was conducted at six Canadian sites (BC, Ontario and Quebec) from February 2024 to February 2025.

One hundred sixty-two of the 186 planned participants were enrolled and randomized (*Table 3*). Only 136 participants were evaluable (ITT population), but the groups

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DOI:10.26502/fjwhd.2644-288400148

were comparable in prior medical histories and concurrent medications. Thirty-one participants terminated early; 26 did not have another sinusitis episode while on trial and did not receive treatment. The Per Protocol (PP) population was five less than the intent to treat (ITT) population.

Demographics

Overall, 68.4% (93/136) of the evaluable ITT population participants were females, while 31.6% (43/136) were males, i.e., the incidence of RARS was greater in women (as expected). Women reported a higher subjective disease burden, including significantly worse baseline SNOT-22 scores compared to men. Seventy-eight (57.4%) of the participants were White, 19.1% were Asian, and 16.2% were Black (7.3% were 'Others'), 88.2% were not Hispano/nor Latino. The average age was 44.8 years (43yrs [median]; range: 18 to 77 yrs). Forty-three (31.6 %) participants reported having allergies to at least one allergen.

Efficacy Results

More NONS participants (53.6%) compared to SALINE participants (46.3%) achieved clinical success (Day 8, i.e., 'cured or much improved of their sinusitis'; *Table 4*). An absolute treatment difference of 7.3% favoring NONS (p=0.391) was observed, with a number needed to treat [NNT; 95% CI] of 13 (ITT). A Kaplan-Meier plot provides the separation between NONS & SALINE (*Figure 4*), with NONS success typically greater than SALINE on each day. In the PP population, 54.6% of the NONS group vs 46.2% of the SALINE group achieved clinical success; treatment difference 8.4%, NNT 95% CI of 11 (Day 8). Clinical success at Day 5 trended in favor of NONS, while by Day 15 the two treatment groups were comparable.

The other secondary efficacy endpoints revealed a similar trend, with slightly more NONS participants (16.4%) achieving a quality of life (SNOT-22) improvement vs SALINE participants (12.1%, p=0.66) at Day 8. A smaller proportion of participants receiving NONS (20.3%) required the use of INCS compared to SALINE (23.9%) by the end of the trial (Day 29). NONS participants were less likely to use their INCS early compared to the SALINE group (10 vs 7 days [median]). Slightly less NONS participants (11.6%) required additional antibiotic therapy vs SALINE participants (16.4%) by the end of the trial.

Safety Results

NONS was well-tolerated. Adverse events (AE), regardless of relationship, occurred in 31.9% of the NONS participants and 31.3% on SALINE therapy. Thirty-three AEs occurred in the NONS group, while 37 AEs occurred with SALINE.

There were no therapy-related SAEs. Early terminations (ETs) due to AEs were more common in the SALINE group. One severe AE/treatment emergent AE (TEAE) occurred in each group. Both the NONS and SALINE treatment groups had 9 TEAEs considered treatment related. Severity was comparable between groups, with 3-moderate AEs and 6-mild AEs in severity per treatment group. All but three TEAEs were AEs of interest, which included somnolence (mild) and hot flush (moderate) reported with NONS, and nausea (mild) reported with SALINE.

AEs of interest within the Respiratory System Classification (SOC), included 4 total NONS nasal TEAEs which included 4 nasal discomforts, (e.g., 3 burning & 1 tingling) with no epistaxis in 4 participants. AEs of interest within the SALINE group, included 7 total nasal TEAEs, with 5 nasal discomforts (1 dry throat, 1 nasal congestion, 1 nasal pain, 1 nasal dryness, 1 throat pain) and 2 epistaxis in 4 participants. Two headaches were reported in the NONS arm, and none in the SALINE arm. The overall low incidence of headaches reported (<3%) is likely due to their inclusion in the PRO data capture, i.e., nasal-sinusitis diary reporting.

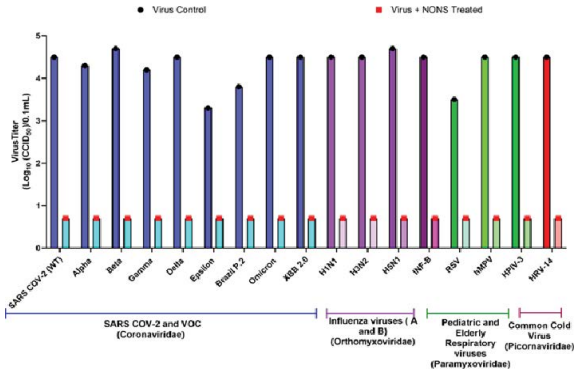

Figure 1: In Vitro NONS Antiviral Activity Across Multiple Respiratory Pathogens.

Virus titers (log_{10} $CCID_{50}$/0.1 mL) were determined for untreated virus controls and NONS-treated (2 min) samples across representative virus families, including *SARS-CoV-2* and variants of concern (Coronaviridae), influenza A and B viruses (Orthomyxoviridae), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), and human parainfluenza virus type 3 (HPIV-3) (Paramyxoviridae), and human rhinovirus 14 (HRV- 14) (Picornaviridae). NONS treatment resulted in significant titer reductions compared with untreated controls. Update data since the Tandon 2022 publication; Martins 2026.

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DOI:10.26502/fjwhd.2644-288400148

Table 1: *In Vitro* Procedures for Monocyte/THP-1 cells Treatments with Nitric Oxide vs SALINE.

	Day 1	Day 2	Day 3
CELLS Human Monocytes (2 donors) & THP-1 line	**Nitric Oxide (NO)** @ 0.065 to 1.65ppm*min or 0.9% **NaCl** (control) Treatments (for 2.5, 5, 10, 20 or 30 minutes)	**LPS Stimulation** begun on CELLS Total of 24 hours	CELL **Supernatants** collected **TNFα, IL-1β, IL-6, IL-8 measured** (in triplicate*)
	All CELLS resuspended in Fresh Media & Incubated Over-night @37°C	**Unstimulated Controls** Total of 24 hours	CELL **Supernatants** collected **TNFα, IL-1β, IL-6, IL-8 measured** (in triplicate*)
*Degree of inhibition of TNFα, IL-1β, IL-6, IL-8 measured by Mesoscale (ELISA®); cytotoxicity assessed via CellTiter-Glo® test.			

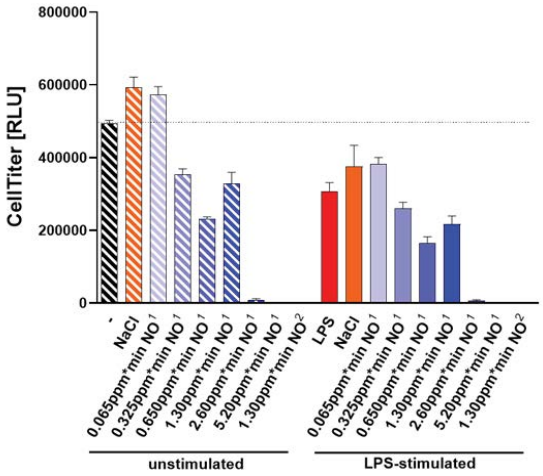

Figure 2: Assessment of NORS/NONS-Induced Cytotoxicity in Human Primary Monocytes.
Primary human monocyte cell viability following cumulative NO exposure post NORS or NONS administration depicted at 2.5 minutes. Comparable results were observed at all other time points.

[1]NORS, [2]NONS

Table 2: Pro-Inflammatory Cytokine Inhibition by Nitric Oxide (NO gas in liquid) Generated from Nitric Oxide Releasing Solution (NORS) or Nasal Spray (NONS).

CYTOKINE (pro-inflammatory)^	**MONOCYTES** (Human Primary Donors)#			**THP-1`** (Cell Line)		
	Time (min)	**[NO] (ppm*min)**	**Extent of Inhibition***	**Time (min)**	**[NO] (ppm*min)**	**Extent of Inhibition***
Tumor Necrosis Factor (TNF)-α	2.5	0.065	30% (62%)	2.5	0.065	22% (22%)
	5	0.11	44% (57%)	5	0.11	30% (34%)
	10	0.16	16% (49%)	10	0.16	15% (19%)
	20	0.26	31% (60%)[1]	20	0.26	14% (31%)
	30	0.32	3% (42%)	30	0.32	33% (41%)
Interleukin (IL)-6	2.5	0.065	27% (52%)	2.5	0.065	22% (25%)
	5	0.11	21% (36%)	5	0.11	24% (31%)[2]
	10	0.16	18% (46%)	10	0.16	24% (26%)
	20	0.26	24% (63%)[2]	20	0.26	12% (25%)
	30	0.32	<1% (32%)	30	0.32	29% (29%)
Interleukin (IL)-1β	2.5	0.065	16% (41%)	2.5	0.065	17% (18%)
	5	0.11	24% (32%)	5	0.11	21% (21%)
	10	0.16	7% (33%)	10	0.16	21% (13%)
	20	0.26	<1% (41%)	20	0.26	6% (11%)
	30	0.32	<1% (22%)	30	0.32	23% (21%)
Interleukin (IL)-8	No CFB			No CFB		

Nitric oxide (NO)-releasing solution (NORS): a proprietary donor-activator system in 0.9% saline producing NO with cell exposure for 2.5, 5, 10, 20 & 30 minutes; NO-termination solution: RPMI1640 media (+ 3% FCS).
^Cytokine concentrations quantified via Mesoscale multiplex Electrochemiluminescence Immunoassay (ECLIA) methodology.
#Data of one independent and representative donor provided (n=2 healthy donors [in triplicates]).
`THP-1=Purchased human monocytic cell line derived form an acute monocytic leukemia (AML) patient; post treatment used as a model for human monocytes and stimulated in the undifferentiated state (2 runs [in triplicates].
*Upon stimulation with LPS, primary human monocytes and monocytic THP-1 cells secreted high levels of pro- inflammatory cytokines TNFα, IL-6, IL-1β and IL-8 compared to non-stimulated cells; normalized to 100% Vital Cells; percentages reflect NO compared to NaCl vs (Lipopolysaccharide, LPS; 100ng/ml, serotype: 0127:B8) stimulated for 24 hours.
[1]p< 0.001; [2]p< 0.05
CFB=Change from Baseline.

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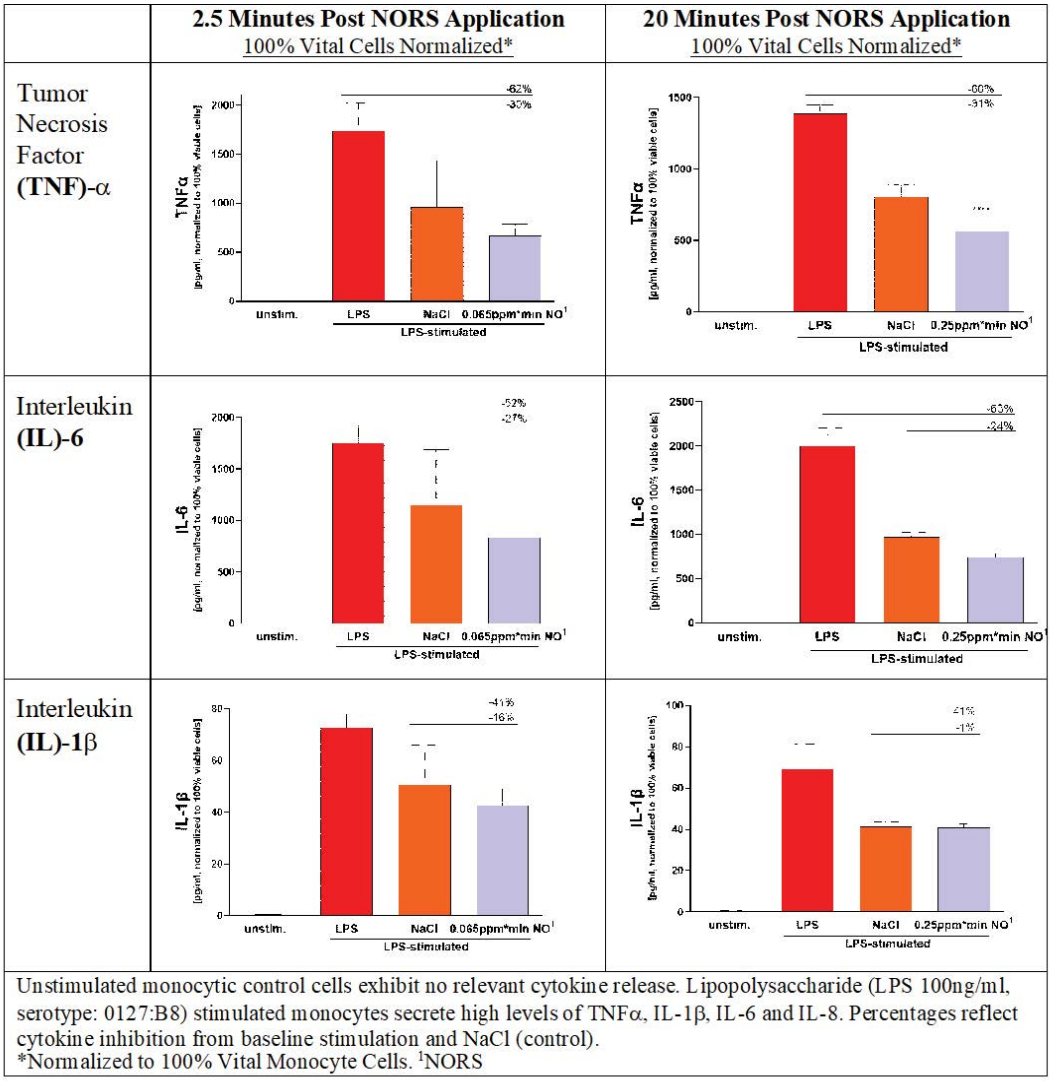

Figure 3: Cytokine Modulation in Primary Human Monocytes Following 2.5 and 20 Minutes of NO Exposure from NORS Pretreatment Prior to LPS-Stimulation

Table 3. Overall RARS Study Disposition.

	NONS N=83	Placebo N=79	ALL N=162
Randomized (N)	83	79	162
Early Terminations [ET] (N)	16	15	**31***
ITT population (N)	69	67	136
PP population (N)**	66	65	131
Completed Full Trial (N)	67	64	131
`Due to the study not having enough participants beginning therapy with their next sinusitis episode, trial is likely undersized/underpowered, i.e., potentially lacking power to detect a meaningful difference, even if one existed.			
*Includes 26 participants who did not achieve baseline, i.e., having another episode of RARS after enrollment.			
**2 participants in each group took <80% of total doses, 1 participant in NONS took a prohibited med (ATB); i.e., PP population had 5 less participants than the ITT population.			

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Table 4: RARS NONS vs SALINE Overall Trial Efficacy Results/Comparisons.

	Treatment Difference (Absolute Delta [Δ], favored shown)						Treatment Differences (Actual Values)	
Efficacy Endpoint [ITT Population]	NONS (N=69)			|	SALINE (N=67)		NONS | SALINE	P-Value
1. Clinical Success (%)-Day 5		◊		|			33.3 vs 28.4%	0.5
Clinical Success (%)-Day 8 (primary)	◊			|			53.6 vs 46.3% +	0.39
Clinical Success (%)-Day 15			◊	|			81.2 vs 80.6%	0.93
2. SNOT-22 ≥ 9-Point Improvement (%)-Day 8		◊		|			16.4 vs 12.1%	0.66
3. INCS Use (proportion; %)-Day 29		◊		|			20.3 vs 23.9%	*
Day commenced (median)		◊		|			10 vs 7	0.58
4. Antibiotic Rescue (%)-Day 29		◊		|			11.6 vs 16.4%	*
	7.5	5	2.5	0	2.5	5		
	Favors NONS				Favors Placebo			
+ Pre Protocol 54.6% vs 46.2% (Absolute Δ +8.4% for NONS, p=0.34)								
*Also, not statistically significant per CI, apparent value differences, or saline response better than NONS								

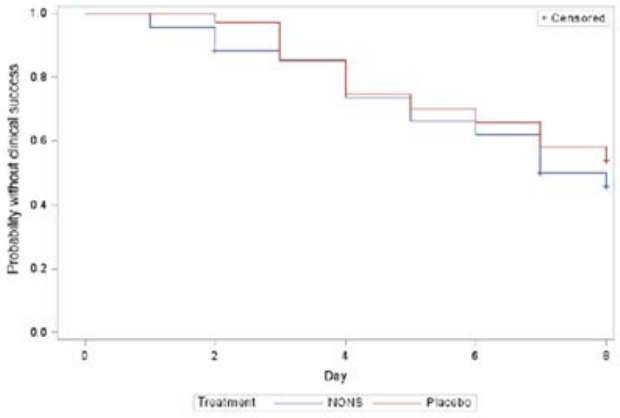

Figure 4: Clinical Success Achieved with NONS vs SALINE (Day 8, ITT).

Discussion

Bacterial rhinosinusitis is associated with a simultaneous infection and inflammation of the mucous membranes of the nasal cavity and paranasal sinuses. A therapeutic agent with anti-inflammatory, antibacterial and antiviral attributes may be advantageous in the management of RARS. Nitric oxide (NO) gas in liquid generated during the actuation of nitric oxide nasal spray (NONS) is bactericidal and virucidal for nasal and respiratory pathogens.

Intrinsic NO and nitric oxide donating drugs have been shown to modulate inflammatory processes; via down-regulation of neutrophil aggregation, release of histamine, platelet-activating factor (PAF) and TNF from mast cells, decreased production of immunomodulatory cytokines (IL-1, IL-12) from macrophages, and less aggregation and adherence of platelets (*Wallace, 2005*). The complimentary anti-inflammatory activity of NO gas produced by our nitric oxide releasing solution (NORS) platform was demonstrated *in vitro*.

The NO gas produced by NORS inhibited pro-inflammatory cytokines consistently over all time points (*2.5 to 30 minutes of NO application*). The degree of inhibition of TNFα, IL-1β and IL-6 was 40-60%, when human monocytes were exposed to bacterial lipopolysaccharide (LPS) stimulation in the presence of a low exposure of nitric oxide. The lack of IL-8 inhibition is consistent with previous reports indicating that IL-8 expression in human monocytes is regulated through signaling pathways that are less sensitive to nitric oxide–mediated modulation. Other pro-inflammatory cytokines (like TNFα), involve a greater contribution of NF-κB–independent regulatory components, p38 MAPK activity, and post-transcriptional mRNA stabilization (*Ma 2004*).

In the exploratory Phase 2 randomized placebo-controlled blinded clinical trial, with five times daily NONS therapy for 2 weeks, statistical significance was not achieved. An explanation may be the study did not have enough participants beginning therapy with a sinusitis episode post-randomization, i.e., potentially lacking power to detect a meaningful difference, even if one existed. The sample size was based achieving clinical success at Day 8 (primary endpoint) with a desired per group sample size of n=93, however less than 70 participants per group were evaluable.

A treatment difference favoring NONS over SALINE was demonstrated, with 53.6% of NONS participants achieving clinical success compared to 46.3% of SALINE participants after one week of therapy. The treatment difference per the COX regression mode suggests participants in the NONS group had a 1.6-times greater likelihood of being cured or much improved than those in the SALINE group at Day 8 (HR=1.57, P=0.1226).

Other efficacy endpoints suggested those receiving NONS were more likely to quickly resolve the negative quality of life (QoL, per SNOT-22) domains associated with their symptoms after one week of therapy. The use of NONS therapy may reduce the overall concomitant use of intranasal corticosteroids (INCS) and oral ATBs used to manage a RARS episode. NONS was well-tolerated with comparable adverse events to SALINE nasal spray.

Clinically, the use of NONS may have the potential to reduce the duration of each RARS episode, decrease the severity of symptoms of each episode and decrease the number of episodes/year. The anti-inflammatory activity of NONS may assist improving RARS overall, especially in those with allergies or an allergic rhinitis component. Although the trial's design attempted to mirror real world conditions, limitations include using a limited number of clinical sites, limiting enrollment to one country, and relying on participants to identify and begin therapy at their first post-randomization sinusitis episode.

Highlights (Key Take-Aways)

Recurrent acute rhinosinusitis (RARS) are short-term (<4 weeks in duration), recurring episodes (≥4/year) of moderate-to-severe sinus with nasal signs and symptoms, typically necessitating oral antibiotic (ATB) therapy if symptoms do not subside after 3-5 days.

- *Streptococcus* pneumonia, *Hemophilus* influenza and *Moraxella* catarrhalis are the most commonly implicated pathogens linked to the non-abatement of sinusitis.
- Rhinosinusitis is associated with a simultaneous inflammation of the mucous membranes of the nasal cavity and paranasal sinuses.

Nitric oxide (NO) gas in liquid generated during the actuation of nitric oxide nasal spray (NONS) is bactericidal and virucidal for nasal and respiratory pathogens. The NO gas produced by NONS is also anti-inflammatory, demonstrated by an *in vitro* laboratory investigation.

- A mild-to-moderate (40-60%) inhibition of the pro-inflammatory cytokines TNFα, IL-1β and IL-6 occurs when monocytes are exposed to bacterial lipopolysaccharide (LPS) stimulation in the presence of a low nitric oxide concentration (0.065ppm*min @2.5min).
- IL-8 is not inhibited at the low NO concentrations studied.

Clinically, RARS episode durations may be decreased with the short-term administration of NONS, begun at the onset of symptoms, secondary to the combination of NO-produced antibacterial, antiviral and anti-inflammatory actions. In an exploratory Phase 2 randomized placebo-controlled blinded clinical trial, daily NONS therapy for 2 weeks was investigated to demonstrate a quickened clinical success, defined as a patient reported outcome (PRO) of a cure or much improvement in sinusitis symptoms by Day 8.

- Although, the treatment difference for the primary endpoint was not statistically achieved, there was a trend favoring NONS, i.e., 53.6% of NONS participants achieved success compared to saline spray (46.3%) participants (p=0.391), with a number needed to treat [NNT] 95% CI of 13 (Day 8, ITT).
- Other significant efficacy endpoints favoring NONS at Day 8 of therapy were a SNOT-22 ≥9-Point improvement of 16.4% for NONS participants vs 12.1% for saline, less NONS participants (20.3%) required the use of INCS compared to saline (23.9%), with a lower antibiotic rescue required for NONS participants (11.6%) vs 16.4% for saline.

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Citation: K Moore, C Miller, J Martins, J Leemhuis, A Rausch, A Javer. In Vitro Pro-Inflammatory Cytokine Inhibition by Exogenously Generated Nitric Oxide; Potential Complimentary Clinical Application to Bacterial Rhinosinusitis Management. Journal of Women's Health and Development. 9 (2026): 26-34.