Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

References

1. Admiraal M, Hermanides J, Meinsma SL, et al. Current multidisciplinary approaches to preventing chronic postoperative pain. *Br J Anaesth* 2021; **127**: 331–5
2. Haroutiunian S, Nikolajsen L, Finnerup NB, Jensen TS. The neuropathic component in persistent postsurgical pain: a systematic literature review. *Pain* 2013; **154**: 95–102
3. Gartner R, Jensen MB, Nielsen J, Ewertz M, Kroman N, Kehlet H. Prevalence of and factors associated with persistent pain following breast cancer surgery. *JAMA* 2009; **302**: 1985–92
4. Schreiber KL, Zinboonyahgoon N, Flowers KM, et al. Prediction of persistent pain severity and impact 12 months after breast surgery using comprehensive preoperative assessment of biopsychosocial pain modulators. *Ann Surg Oncol* 2021; **28**: 5015–38
5. Bendixen M, Jørgensen OD, Kronborg C, Andersen C, Licht PB. Postoperative pain and quality of life after lobectomy via video-assisted thoracoscopic surgery or anterolateral thoracotomy for early stage lung cancer: a randomised controlled trial. *Lancet Oncol* 2016; **17**: 836–44
6. Aasvang EK, Gmaehle E, Hansen JB, et al. Predictive risk factors for persistent postherniotomy pain. *Anesthesiology* 2010; **112**: 957–69

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Dexmedetomidine does not directly inhibit neutrophil extracellular trap production

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Editor—Dexmedetomidine is a highly selective α_2-adrenergic receptor agonist widely used in clinical anaesthesia as a sedative and analgesic agent.[1] It has also been associated with numerous anti-inflammatory effects in preclinical models, including protection against leucocyte-mediated acute lung injury (ALI) after caecal ligation puncture,[2] reducing pulmonary oedema in lipopolysaccharide-induced ALI,[3] and attenuating cell injury in experimental severe acute pancreatitis via the cholinergic anti-inflammatory pathway.[4] In a recent case report, clinical improvement upon dexmedetomidine treatment was suggested to have spared a patient with COVID-19 with worsening hypoxaemia from mechanical ventilation.[5] Indeed, there are ongoing clinical trials registered to examine dexmedetomidine in palliative sedation for severe COVID-19 (NCT04350086) and to evaluate its immunomodulatory profile in patients recovering from COVID-19-related acute respiratory distress syndrome (ARDS) (NCT04413864).

Neutrophil extracellular trap formation (NETosis) is a specialised cell death process in which release of chromatin components such as DNA and histones provides a framework for trapping and killing invading microbes.[6] However, when dysregulated, NETosis can also aggravate harmful inflammatory responses, including those driving the pathogenesis and thrombosis of severe COVID-19 in lungs and other major organs.[7,8]

In 2020, Jain and collegaues[9] hypothesised that 'given the anti-inflammatory effects of dexmedetomidine, it too may inhibit NETosis and be beneficial in COVID-19 patients'. They went on to provide a detailed schematic illustration of the many feedforward mechanisms potentiating NETosis during COVID-19 and the molecular pathways through which they predicted dexmedetomidine could act to inhibit NET activation.

Our research group has a longstanding interest in the biology and pathobiology of NETs in animal models of infectious diseases such as necrotising fasciitis[10] and bacterial

pneumonia,[11] and recently we studied NET phenotypes in critically ill patients with COVID-19.[12] In parallel, we have examined how NETosis is modulated by common medications including statins,[13] tamoxifen,[14] desferoxamine,[15] and propofol.[16] With this background, we tested the hypothesis that dexmedetomidine inhibits human NETosis.

Blood was collected from healthy adults under a protocol approved by the University of California San Diego Institutional Review Board (IRB), and neutrophils were isolated using the PolyMorphPrep™ Kit (Fresenius Kabi, Oslo, Norway) per the manufacturer's instructions. The effective sedative concentration of dexmedetomidine in plasma has been estimated to be 0.2–3.2 ng ml^{-1} (~1–16 nM).[17] We stimulated neutrophils to produce NETs by exposure to live methicillin-resistant *Staphylococcus aureus* (MRSA) or to the classical NET inducer phorbol myristate acetate (PMA) at 25 nM, in the presence or absence of dexmedetomidine at final concentrations of 0.5, 5, 50, and 500 ng ml^{-1}. For all dexmedetomidine exposures, no

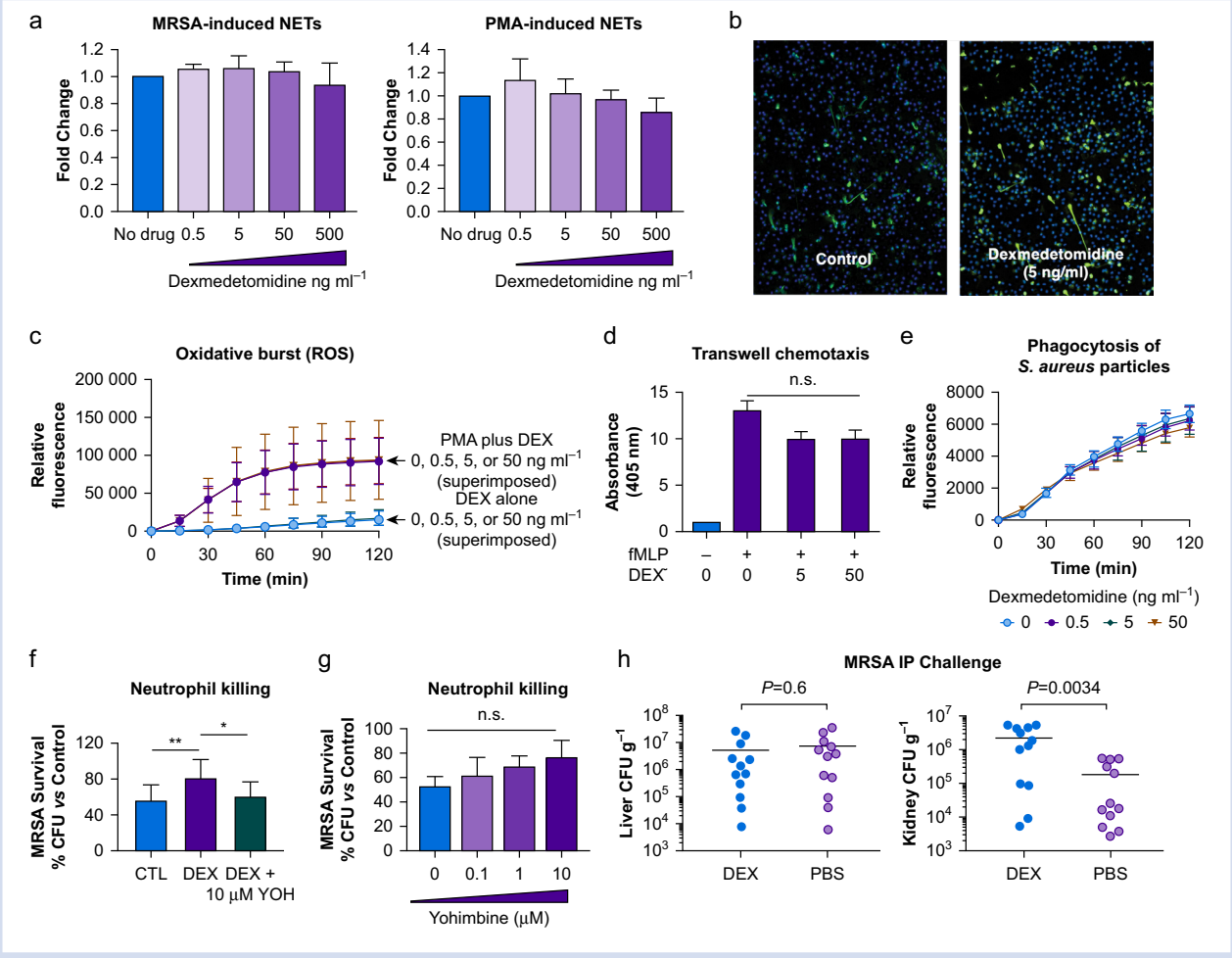

Fig 1. (a) Fold change in NETosis triggered by either methicillin-resistant *Staphylococcus aureus* (MRSA; stationary phase, MOI 10, n=4) or phorbol myristate acetate (PMA; 25 nM, n=5) in the presence of increasing concentrations of dexmedetomidine. NET production (extracellular DNA) was quantified using PicoGreen dye. (b) Immunocytochemical analysis of NETosis in response to PMA (25 nM) in the presence or absence of 5 ng/mL (25 nM) dexmedetomidine. Green: myeloperoxidase (staining NETs); blue: DAPI (staining nuclei). (c) Time course of reactive oxygen species (ROS) production by human neutrophils (measured at indicated time points using H_2DCFDA, n=3) in the presence or absence of dexmedetomidine either alone or with PMA. (d) Chemotaxis of human neutrophils in response to 100 nM N-formylmethionyl-leucyl phenylalanine (fMLP) in the presence or absence of several concentrations of dexmedetomidine (assessed using Transwell inserts with a 3 µm pore size as described previously, n=5). (e) Phagocytosis time course of *S. aureus* bioparticles in the presence and absence of several concentrations of dexmedetomidine, n=4. (f) MRSA killing by human neutrophils (MOI 10) in the presence of dexmedetomidine at 5 ng/mL (25 nM) and dexmedetomidine and yohimbine at indicated concentration as compared with the respective control without cells expressed as % colony-forming units (CFU) ml^{-1}, n=8. (g) MRSA killing by human neutrophils (MOI 10) in the presence of several concentrations of yohimbine as compared with the respective control without cells expressed as % CFU ml^{-1}, n=3. (h) amount of MRSA per g of either liver or kidney tissue after an 24 h *in vivo* intraperitoneal (i.p.) challenge of CD-1 mice that received either dexmedetomidine or phosphate-buffered saline (PBS) i.p. at time of infection and 1 h after infection, n=24, 12 in each group. One-way analysis of variance with *post hoc* analysis was used to assess significance for data shown here, with the exception of the MRSA i.p. challenge, where unpaired Student's *t*-test was used. *P<0.05; **P<0.01. CTL, control; DEX, dexmedetomidine; H_2DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; NETOsis, neutrophil extracellular trap formation; NETs, neutrophil extracellular traps; n.s., not significant; YOH, yohimbine.

inhibition of NET production by MRSA- or PMA-stimulated neutrophils was seen via PICO green quantification of extracellular DNA release (Fig. 1a) or immunocytochemistry using antibodies against myeloperoxidase (Fig. 1b).

Neutrophil oxidative burst/generation of reactive oxygen species (ROS) can promote NETosis.[18] We found that dexmedetomidine at final concentrations of 0.5, 5.0, and 50 ng ml^{-1} did not inhibit PMA-induced neutrophil ROS production as measured by a 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA; Sigma-Aldrich, St Louis, MO, USA) fluorescence assay (Fig. 1c). While examining broader neutrophil functions, we found that similar dexmedetomidine concentrations did not significantly affect neutrophil chemotaxis across a Transwell membrane toward *N*-formylmethionyl-leucyl-phenylalanine (fMLP) (Fig. 1d), nor did it influence the efficiency of neutrophil phagocytosis of *S. aureus*-coated particles (pHrodo™ Red *S. aureus* Bioparticles; Invitrogen Corp., Carlsbad, CA, USA; Fig. 1e). In an *ex vivo* bactericidal assay, dexmedetomidine (5 ng ml^{-1}) impaired neutrophil killing of MRSA, an effect that was reversed by the α_2-adrenergic receptor antagonist yohimbine (Fig. 1f), whereas yohimbine alone did not significantly affect killing (Fig. 1g). Finally, in a murine intraperitoneal MRSA infection model approved by the University of California San Diego Institutional Animal Care and Use Committee (IACUC), treatment with 166 μg kg^{-1} of dexmedetomidine i.p. at time of infection and again 1 h after bacterial challenge was associated with significantly increased recovery of bacterial colony-forming units (CFU) from kidneys 24 h later (Fig. 1h), although no change was seen in CFUs recovered from the liver.

We conclude that dexmedetomidine at therapeutically relevant concentrations and higher does not directly inhibit production of NETs by human neutrophils in response to commonly used NETosis inducers, nor does it significantly alter neutrophil behaviour in selected other common phenotypic assays including ROS generation, chemotaxis, and phagocytosis. Dexmedetomidine slightly but significantly (1) impaired human neutrophil killing of MRSA in an α_2-adrenergic receptor-dependent manner and (2) reduced kidney bacterial burden in a murine systemic infection model, but it is premature to conclude whether these modest phenotypes are related or clinically significant for humans. Of note, dexmedetomidine is mainly hepatically metabolised and can reach liver concentrations much higher than plasma, after which its metabolites are primarily excreted through the kidneys.[19]

Our study has several limitations. First, we describe *in vitro* studies with purified human neutrophils and *in vivo* studies using mice, both relatively distant from the clinical setting. Second, the stimuli used to trigger NETosis and other neutrophil effector functions, although commonly used in the field, are not of viral origin. Follow-up *ex vivo* studies using COVID-19 patient blood, along with *in vitro* studies using activators of viral origin, will be important.

Several anaesthetic drugs are known to possess important anti-inflammatory and immunomodulatory properties, including those acting on neutrophils,[20] that can influence their pharmacodynamics and clinical effectiveness. Of immediate impact, there is emerging clinical opinion that the immunomodulatory activities of dexmedetomidine might be harnessed to improve patient outcomes in severe COVID-19.[9,21] Our studies, with the stated limitations, suggest that the proposed benefits do not include direct inhibition of extracellular trap formation by human neutrophils.

Authors' contributions

Project concept: RC, VN, AM
Conduct of experiments: RC, BES, JOl, AM
Data analysis: RC, BES, AM
Data interpretation: VN, AM, RC
Editing of the manuscript: RC, AM, VC
Conduct of initial concept experiments: JOk, AM
Conduct of imaging experiment: AM, JMS
Writing of the manuscript: VN, AM

Declarations of interest

The authors declare that they have no conflicts of interest.

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References

1. Keating GM. Dexmedetomidine: a review of its use for sedation in the intensive care setting. *Drugs* 2015; **75**: 1119–30
2. Karabulut G, Bedirli N, Akyurek N, Bagriacik EU. Dose-related effects of dexmedetomidine on sepsis-initiated lung injury in rats. *Braz J Anesthesiol* 2021; **71**: 271–7
3. Jiang Y, Xia M, Xu JHuang Q, Dai Z, Zhang X. Dexmedetomidine alleviates pulmonary edema through the epithelial sodium channel (ENaC) via the PI3K/Akt/Nedd4-2 pathway in LPS-induced acute lung injury. *Immunol Res* 2021; **69**: 162–75
4. Huang DY, Li Q, Shi CY, Hou CQ, Miao Y, Shen HB. Dexmedetomidine attenuates inflammation and pancreatic injury in a rat model of experimental severe acute pancreatitis via cholinergic anti-inflammatory pathway. *Chin Med J (Engl)* 2020; **133**: 1073–9
5. Stockton J, Kyle-Sidell C. Dexmedetomidine and worsening hypoxemia in the setting of COVID-19: a case report. *Am J Emerg Med* 2020; **38**: 2247 e2241–7, e2242
6. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science* 2004; **303**: 1532–5
7. Iliadi V, Konstantinidou I, Aftzoglou K, Iliadis S, Konstantinidis TG, Tsigalou C. The emerging role of neutrophils in the pathogenesis of thrombosis in COVID-19. *Int J Mol Sci* 2021; **22**: 5368
8. Cahilog Z, Zhao H, Wu L. The role of neutrophil NETosis in organ injury: novel inflammatory cell death mechanisms. *Inflammation* 2020; **43**: 2021–32
9. Jain A, Lamperti M, Doyle DJ. Dexmedetomidine: another arrow in the quiver to fight COVID-19 in intensive care units. *Br J Anaesth* 2021; **126**: e35–8
10. Walker MJ, Hollands A, Sanderson-Smith ML, et al. DNase Sda1 provides selection pressure for a switch to invasive group A streptococcal infection. *Nat Med* 2007; **13**: 981–5
11. Berends ET, Horswill AR, Haste NM, Nizet M, von Kockritz-BLickwede M. Nuclease expression by Staphylococcus aureus facilitates escape from neutrophil extracellular traps. *J Innate Immun* 2010; **2**: 576–86

12. Masso-Silva JA, Moshensky A, Lam MTY, et al. Increased peripheral blood neutrophil activation phenotypes and NETosis in critically ill COVID-19 patients: a case series and review of the literature. *Clin Infect Dis* 2021: ciab437. https://doi.org/10.1093/cid/ciab437
13. Chow OA, von Kockritz-BLickwede M, Bright AT, et al. Statins enhance formation of phagocyte extracellular traps. *Cell Host Microbe* 2010; **8**: 445–54
14. Corriden R, Hollands A, Olson J, et al. Tamoxifen augments the innate immune function of neutrophils through modulation of intracellular ceramide. *Nat Commun* 2015; **6**: 8369
15. Vollger L, Akong-Moore K, Cox L, et al. Iron-chelating agent desferrioxamine stimulates formation of neutrophil extracellular traps (NETs) in human blood-derived neutrophils. *Biosci Rep* 2016; **36**, e00333
16. Meier A, Chien J, Hobohm L, Patras KA, Nizet V, Corriden R. Inhibition of human neutrophil extracellular trap (NET) production by propofol and lipid emulsion. *Front Pharmacol* 2019; **10**: 323
17. Ebert TJ, Hall JE, Barney JA, Uhrich TD, Colinco MD. The effects of increasing plasma concentrations of dexmedetomidine in humans. *Anesthesiology* 2000; **93**: 382–94
18. Kirchner T, Moller S, Klinger M, Solbach W, Laskay T, Behnen M. The impact of various reactive oxygen species on the formation of neutrophil extracellular traps. *Mediators Inflamm* 2012; **2012**: 849136
19. Weerink MAS, Struys MMRF, Hannivoort LN, Barends CRM, Colin P. Clinical pharmacokinetics and pharmacodynamics of dexmedetomidine. *Clin Pharmacokinet* 2017; **56**: 893–913
20. Meier A, Nizet V. Impact of anesthetics on human neutrophil function. *Anesth Analg* 2019; **128**: 569–74
21. Zhao H, Davies R, Ma D. Potential therapeutic value of dexmedetomidine in COVID-19 patients admitted to ICU. *Br J Anaesth* 2021; **126**: e33–5

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High plasma dipeptidyl peptidase 3 levels are associated with mortality and organ failure in shock: results from the international, prospective and observational FROG-ICU cohort

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Editor—Shock is a common condition associated with high morbidity and mortality in the ICU.[1] Rapid and accurate stratification of the patient in shock could improve referral to an appropriate care centre, management, and prognosis. Few biomarkers have proved their value in the stratification of shocked patients. Circulating dipeptidyl peptidase 3 (cDPP3) is a metallo-peptidase involved in the metabolism of cardiovascular peptides.[2] Recent studies demonstrated the ability of cDPP3 to predict poor outcomes in septic or cardiogenic shock.[3,4] However, the prognostic properties of cDPP3 in haemorrhagic shock remain unknown. Thus, the aim of the present study was to assess the ability of cDPP3 to predict outcome in septic, cardiogenic, and haemorrhagic shock in a substudy of the FROG-ICU study.

This study is an ancillary analysis of the FROG-ICU study (NCT01367093) which has been described.[5] The purpose of this study was to assess the incidence of mortality in the year after ICU discharge. Patients were enrolled from August 2011 to June 2013. Haemorrhagic shock was defined as a hypovolaemic shock requiring catecholamines secondary to severe blood loss.[6] Septic shock was defined according to the Third International Consensus definition for sepsis and septic shock.[7] Cardiogenic shock was defined as cardiac impairment that results in reduced systolic BP (SBP) <90 mm Hg or inotrope use