**Original Research**

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# NETosis Induced by Serum of Patients with COVID-19 is Reduced with Reparixin or Antibodies Against DEK and IL-8

**Irfan Baki Kilic[1], Acelya Yasar[1], Irem Yalim Camci[1], Turkan Guzel[1], Aysegul Karahasan[2], Tamer Yagci[1], Naci Cine[3], Ayten Kandilci[1]**

[1]Gebze Technical University, Department of Molecular Biology and Genetics, Gebze, Kocaeli, Turkey
[2]Marmara University Faculty of Medicine, Department of Medical Microbiology, Istanbul, Turkey
[3]Kocaeli University Faculty of Medicine, Department of Medical Genetics, Kocaeli, Turkey

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**Corresponding Author:** Ayten Kandilci, Gebze Technical University, Department of Molecular Biology and Genetics, Gebze, Kocaeli, Turkey
**Phone:** +90 262 605 25 25 **E-mail:** akandilci@gtu.edu.tr **ORCID:** orcid.org/0000-0003-4209-4992

## Abstract

**Objective:** NETosis, a suicide mechanism of neutrophils, is an important immune defense mechanism against pathogens, and when uncontrolled, it contributes to severe tissue damage leading to acute lung injury in influenza and severe acute respiratory syndrome-coronavirus-2 infections. Here we aimed to determine whether blocking of C-X-C motif chemokine receptors 1/2 (CXCR1/2) by reparixin prevents *in vitro* NETosis induced by either recombinant DEK (rDEK), recombinant interleukin-8 (rIL-8) (both interacts with CXCR2 receptor) or the serum of patients with coronavirus disease-2019 (COVID-19).

**Materials and Methods:** Level of pro-inflammatory cytokines and NETosis markers in the serum of COVID-19 patients were determined by ELISA. NETosis was induced by treating neutrophils with either rDEK, rIL-8 or COVID-19 patients' serum in the presence or absence of reparixin, anti-DEK or anti-IL-8 antibodies. Subsequently, myeloperoxidase (MPO) activity, presence of extracellular neutrophil elastase, and morphology of the cells were analyzed to determine NETosis.

**Results:** IL-8, IL-6, IL-1β, MPO, and citrullinated histone H3 were increased whereas DEK was moderately decreased in the circulation of the COVID-19 patients. Reparixin and the antibodies against either DEK or IL-8 suppressed the NETosis induced by either the patients' serums or by the rDEK and rIL-8.

**Conclusion:** Our results show for the first time that DEK-CXCR2 interaction plays a role in the NETosis and support the use of reparixin as a potential therapeutic strategy in COVID-19.

**Keywords:** DEK, NETosis, COVID-19, CXCR1/2, reparixin, anti-DEK, anti-IL-8

## Introduction

NETosis is characterized by extracellular web-like structures called neutrophil extracellular traps (NETs) that are generated with cell-extruded DNA bound to histones and granule proteins (1). Microbial or viral components and pro-inflammatory cytokines may trigger NETosis. Neutrophils infected with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and cytokine storm caused by this infection induce NETosis in coronavirus disease-2019 (COVID-19), which may be fatal in 1-3% of patients who were not vaccinated (2-7). Among the cytokines, IL-8 is a potent inducer of NETosis, and patients with COVID-19 exhibit a high level of IL-8 in their serum and lung tissues (3,8,9).

Ubiquitously expressed oncoprotein DEK shares an ELR motive with IL-8, and it acts as a chemokine for neutrophils, $CD8^+$ T-cells, and NK cells when secreted (10,11). Including IL-8, all chemokines that bind CXCR1/CXCR2 receptors induce NETosis, and reparixin (an allosteric inhibitor of CXCR1/CXCR2) reduces NETs formation induced by these chemokines (8,9). Accumulating data point to DEK as a player in inflammation. A wide range of post-translational modifications influences multiple roles -of DEK, mainly via decreasing its DNA binding capacity

**ORCID:** I.B. Kilic 0000-0002-1225-9920, A. Yasar 0000-0002-6992-7061, I. Yalim Camci 0000-0002-2534-4155, T. Guzel 0000-0002-8196-8931, A. Karahasan 0000-0002-1560-2624, T. Yagci 0000-0003-2050-7477, N. Cine 0000-0001-9063-1073, A. Kandilci 0000-0003-4209-4992

and allowing its secretion (12). DEK is secreted passively from T-lymphocytes that undergo apoptosis and actively from immune cells and epithelial cells after IL-8 stimulation (10-13). Secreted DEK may interact with heparan sulfate peptidoglycan receptors leading to entrance into the cell (10,14), or it may interact with the CXCR2 receptor and via Gαi signaling suppresses hematopoietic progenitor cell proliferation (15). Patients with juvenile idiopathic arthritis (JIA) carry autoantibodies against DEK, and neutrophils from the Dek-knockout JIA mouse model have reduced capacity to form NETs after phorbol-12-myristate-13-acetate (PMA) or lipopolysaccharide (LPS) stimulation; however, they retrieve to NETosis when co-stimulated with rDEK and PMA (or LPS) *in vitro*. Importantly, DEK-targeting aptamers reduce NETosis of the neutrophils in the JIA-mouse model (16). In addition, human bronchial epithelial cells release DEK upon IL-8 stimulation, and supernatants of these cells, or rDEK alone, induce NETosis of human neutrophils *in vitro*, which is suppressed by overexpression of miR-181b-5p that directly targets and inhibits DEK mRNA expression (13).

Even though the current data suggest that DEK contributes to NETosis via regulating the chromatin architecture (16), the role of DEK-CXCR2 interaction in NETs formation is not elucidated yet. Here we aimed to determine whether the interaction of rDEK with the CXCR2 receptor affects NETosis *in vitro*. Given that NETosis is an important contributor to COVID-19 pathogenesis, we also analyzed and compared the amount of DEK and the selected cytokines elevated in the circulation of patients with COVID-19. Furthermore, neutrophils treated with serum samples of patients with COVID-19 develop NETosis (7) and we tested the inhibitory effect of reparixin, anti-DEK, and anti-IL-8 antibodies on the NETosis induced by COVID-19 patients' serums.

## Materials and Methods

### Human Samples

Serum samples of COVID-19-patients (n=67; obtained from Marmara University Pendik Training and Research Hospital) or healthy volunteers (n=47; from Kocaeli University Faculty of Medicine) were collected, aliquoted, and stored at -20°C. Patients' samples were grouped as acute phase (AF) [(n=23; average age= 51.2 ± 21 [mean ± standard deviation (SD); 12 males/11 females; serums were obtained 24-48 hours after reverse transcription-polymerase chain reaction (RT-PCR) positivity)] and convalescent phase (CF) samples [n=44, average age= 53.5 ± 12.2 (mean ± SD); 22 males/22 females]. All patients were tested positive for SARS-CoV-2 at the hospital. Briefly, viral RNA was extracted from nasopharyngeal swab samples by usingBio-speedy® viral nucleic acid buffer (Bioexen LTD, Istanbul, Turkey) and RT-PCR was performed with Bio-speedy® COVID-19 qPCR detection kit, Version 2 (Bioexen LTD) using primers and probes targeting the RNA-dependent RNA polymerase (RdRp) gene fragment in a LightCycler® 96 System (Roche, Switzerland). Healthy donors' samples were confirmed negative by using a COVID-19 IgG/IgM lateral flow antibody test (UNSCIENCE, Wuhan, China).

### ELISA Test

The level of the DEK , IL-6 (both from Cusabio, Houston, TX, USA), IL-1β, myeloperoxidase (MPO), IL-8 (all three from Abcam, Cambridge, UK) and Cit-H3 (Cayman, Michigan, USA) in the serum samples were detected by using ELISA kits and applying manufacturers' protocol. Serums were diluted 1:5 for DEK, IL-1β, IL-6, Cit-H3, and 1:8 for IL-8 and MPO before using.

### Neutrophil Isolation and NETosis Assays

In all experiments, neutrophils of healthy volunteers were used. Neutrophils were isolated by following the protocol published by Brinkmann et al. (17). $2x10^5$ neutrophils/well were seeded into a poly-l-lysine (100 µg/mL, Sigma Aldrich, Missouri, USA) coated 8-well IBIDI slide (IBIDI, Germany) or a 96-well plate in culture medium (RPMI 1640 containing 1% human serum). After addition of recombinant human proteins (DEK, MyBioSource, California, San Diego, USA; IL-8, R&D) or in some experiments patients' serum [5% of the total volume of the culture medium (7)], cells were treated for an additional 1 hour (for recombinant proteins) or for 2 hours (for patients' serum). When necessary, the cells were pre-treated with the indicated antibodies or reparixin (Med Chem, NJ, USA) for indicated times in the legends. Then, the cell-impermeable Sytox (Thermo Fisher Scientific, Waltham, MA, USA) (at final concentration of 500 nM) was added to the cells for additional 15 minutes. Cells were fixed with 4% PFA (Sigma Aldrich) in the presence of Sytox, washed with PBS and the images were captured by using confocal microscope (Carl Zeiss, LSM880 Airyscan) or ZOE Fluorescence Cell Imager (Biorad). Five different areas per well were imaged in the IBIDI or 96-well plates. Anti-DEK antibodies were obtained from BD Biosciences (NJ, USA) and Bethyl Laboratories (Waltham, MA, USA) whereas anti-IL-8 was from R&D. All chemicals were first tested for the optimal dose (data not shown) and the neutrophils were treated with each corresponding vehicle (or with 5% healthy serum in the patients' serum-induced NETosis assays) as a negative control in all experiments.

For the quantification of NETosis, Image J (National Institutes of Health) and DANA I (DNA Area and NETosis Analysis) software was used (18). In this analysis, NETs were quantified by comparing and counting the cell areas to the control cells with a normal morphology (19).

### Quantification of NET-associated MPO Activity

MPO activity was detected by following the protocol published by Zuo et al. (7). $1x10^5$ neutrophils were seeded into the 96-well plate and stimulated for NETosis as indicated above. After stimulation, medium was discarded. Cells were treated 5 U/mL Micrococcal Nuclease (Thermo Fisher) in 1XPBS for 10 minutes at 37°C in an incubator with 5% $CO_2$. Reaction was stopped by adding 10 mM EDTA (final concentration 0.5 mM) (Invitrogen), and the PBS containing NETs was transferred into a V-shaped 96-well plate. After centrifugation at 400g for 5 minutes, supernatant was transferred into a new F-bottom 96-well plate, and the same volume of TMB substrate (Thermo Fisher) was added to each well. The plate was incubated at room temperature for 10 minutes and the reaction was stopped by adding the same volume of 2N sulfuric acid. Absorbance was measured at 450 nm by using a MultiSkan Fc (Thermo Fisher). The obtained absorbance values were normalized by dividing the values of the treated samples by their untreated-sample and the fold changes were depicted in the graphs.

### Immunofluorescence Staining

Neutrophils were seeded into the 8 well IBIDI µ-slides as $2x10^5$ cells per well. NETosis was stimulated as described above with a final 15 minutes incubation in the presence of Sytox. After adding an equal amount of 8% PFA (final concentration 4%) (17) and fixing the cells overnight at 4°C, cells were washed with PBS, permeabilized with 0.5% Triton-X-100 (Sigma Aldrich) for 1 minute and blocked with 2% BSA (SigmaAldrich) prepared in PBS for 20 minutes. Subsequently, cells were stained with a 1:50 diluted rabbit anti-human DEK antibody (Bethyl Laboratories) for 1 hour. Then, the cells were washed and incubated for an additional 1 hour with a 1:500 diluted secondary anti-rabbit Alexa Fluor 647 antibody (Cell Signaling Technologies). Then, the same cells were washed, fixed again with 4% PFA, blocked with 2% BSA, and stained with a 1:50 diluted mouse anti-human neutrophil elastase antibody (Thermo Fisher) for overnight at 4°C. After staining for 1 hour with a 1:500 diluted anti-mouse Alexa Fluor-555 secondary antibody (Cell Signaling Technologies), cells were directly imaged by using confocal microscopy (Carl Zeiss, LSM880 Airyscan).

### Statistical Analysis

All statistical analyses were performed by using GraphPad Prism (Version 8.0, GraphPad Software Inc). Significance was evaluated by the Mann-Whitney U test, Pearson and unpaired t-tests. A p-value of 0.05 or less was considered statistically significant.

## Results

### Recombinant DEK induces NETosis via CXCR2 interaction

To determine whether NETosis induced via rDEK also depends on CXCR2 interaction, we induced the primary neutrophils with either rDEK or rIL-8 as a control and then analyzed the NETs associated markers including the morphological changes of the cells, the presence of extracellular neutrophil elastase, and the activity of NETs-associated MPO. We found that both rDEK [Figure 1A, 1C (p=0.0592), 1D (p=0.0331)] and rIL-8 [Figure 1A, 1C (p=0.0477), 1D (p=0.0424)] stimulate NETs formation and pre-incubation of the cells with antibodies against DEK or IL-8 suppress this cellular process [Figure 1B, 1C (p=0.0081, p=0.014), 1D (p=0.0186, p=0.0503)]. Interestingly, pre-treatment with reparixin also reduced DEK-induced NETosis indicating that DEK-CXCR2 interaction plays a role in NETs formation [Figure 1B, 1C (p=0.0398, p=0.0057), 1D (p=0.0315, p=0.1711)].

### The Level of DEK is Lower in the Serum of Patients with COVID-19

Next, we analyzed whether the level of DEK in the circulation of patients was affected along with cytokines (IL-8, IL-6, IL-1β) and NETosis markers (MPO, Cit-H3) by using ELISA. We found that the DEK were significantly lower whereas all other analyzed markers were significantly higher in the serums of patients (n=67) compared to those of the healthy controls (n=38) (Figure 2A, p=0.0006). When we grouped patients, we observed that the level of DEK was lower at the early stage [24 to 48 hours after the positive PCR-test result; (n=23)] when compared to the late stage of the disease [21 days after the initial positive PCR-test; CF (n=44)] (Figure 2B, p=0.0005 and p=0.009). Contrary to the DEK, the levels of IL-8 (Figure 2C p=0.0023), IL-6 (Figure 2D p=0.0001), IL-1β (Figure 2E p=0.01238), MPO (Figure 2F p=0.0007), and Cit-H3 (Figure 2G p=0.0474) were lower in healthy individuals compared to the AF and CF of the disease. Pearson correlation analysis indicated a statistically significant negative correlation only between the level of DEK and IL-8 in healthy individuals (Figure 2H, r=-0.3308, p=0.0425) and between the level of DEK and MPO in the AF of COVID-19 (Figure 2I, r=-0.4817, p=0.0232).

### Reparixin or Anti-DEK and Anti-IL-8 Antibodies Suppress NETosis Stimulated with the Serum of Patients

We further examined the inhibitory effects of reparixin, anti-DEK, and anti-IL-8 antibodies on NETs formation induced by the patients' serums. We showed that neutrophils treated with the patients' serums form NETs (Figures 3A and 3B). Immunofluorescence staining indicated that both DEK

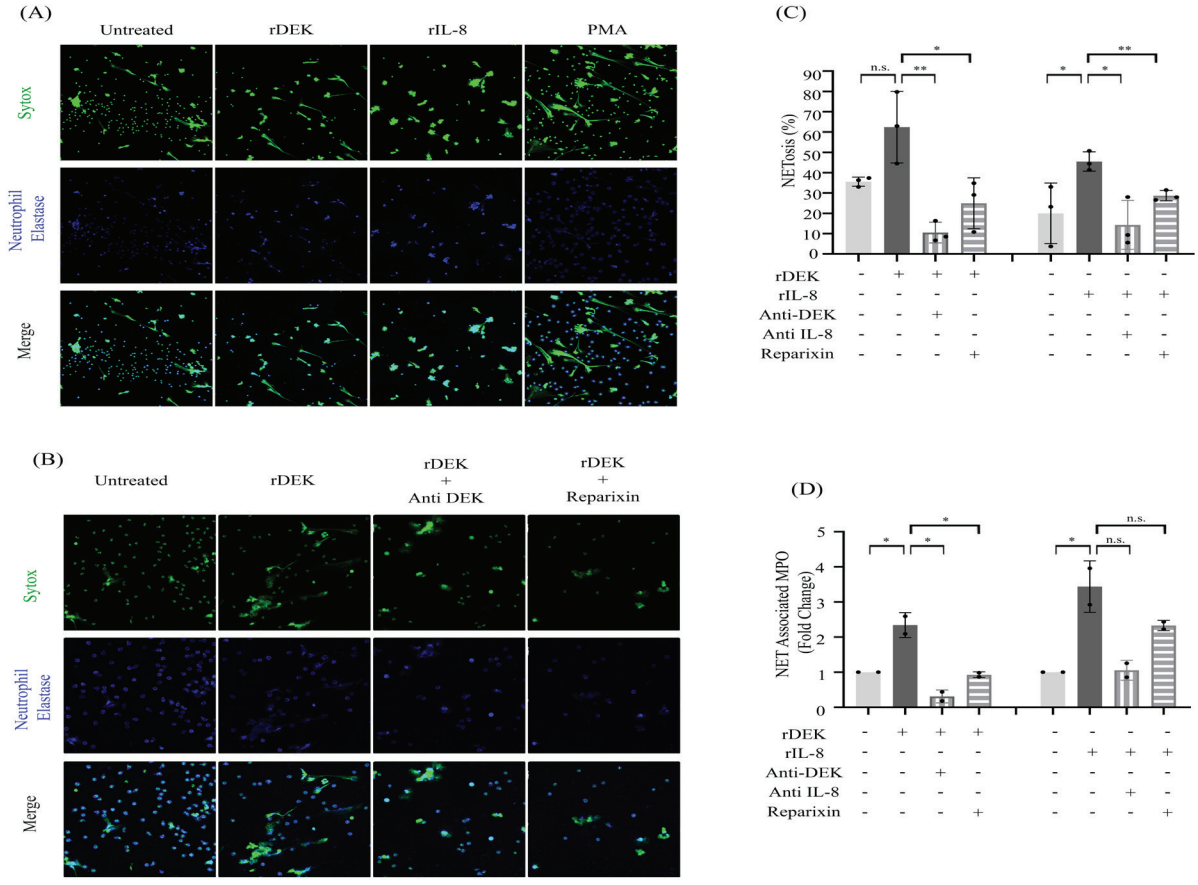


**Figure 1.** Reparixin reduces DEK-induced NETosis. Primary neutrophils were induced with rDEK [5 µg/mL (A) or 3.5 µg/mL (B-D) or rIL-8 (100 ng/mL)] for 1 hour; Sytox was added and the cells were incubated for an additional 15 minutes. For the confocal microscopy images (200X) in (A) and (B), cells were fixed and further stained with an anti-NE (neutrophil elastase) antibody (blue). In a parallel experimental group, cells were first pretreated with anti-DEK (5 µg/mL in B; 2.5 µg/mL in C and D), anti-IL-8 (2.5 µg/mL), or reparixin (400 nM in B; 200 nM in C and D) for 15 minutes. (C) Confocal images captured in 5 different areas were analyzed using Image J and DANA I software and the percentage of cells showing NETs morphology was shown in the graph. Black circles in the graphs indicate the results of three independent assays. (D) Cells were treated similarly with recombinant proteins or chemical agents and NETs-associated myeloperoxidase activity were measured. The normalized value of each sample by its untreated sample was shown as a fold change. Black circles in the graphs indicate the results of two independent assays. Data were analyzed by parametric unpaired t-test exact p-values indicated in the text. Error bars: ± standard deviation.

and NE located in the granules of neutrophils (Figures 3A and 3B) and interestingly we did not observe DEK in the nucleus. In the cells stimulated with a patient's serum, cell-extruded DNA showed staining with both DNA-binding dye Sytox and neutrophil elastase, whereas the DEK was contained mainly in the granule-like structures and barely co-located with Sytox or neutrophil elastase (Figures 3A and 3B). Further analysis of available serum samples of patients (n=15) similarly indicated that COVID-19-patients' serum induced NETosis (Figure 4A, p=0.0001) and all three agents, reparixin (Figure 4A, p=0.0001, p=0.0001), anti-DEK (Figure 4A, p=0.0007 and p=0.0008), and anti-IL-8 (Figure 4A, p=0.0027 and p=0.0004), significantly reduced the NETs formation, as judged by both morphological analysis of the cells and NETs-associated MPO activity. We grouped the patients' samples based on the ELISA values of DEK and IL-8 (Supplementary Table 1). We observed that anti IL-8 antibody decreased NETosis stimulated by COVID-19 sera effectively in groups that DEK high/IL-8 high (Figure 4B, p=0.0079, p=0.0159), DEK high/IL-8 low (Figure 4C, p=0.0952, p=0.5476), DEK low/IL-8 high (Figure 4D, p=0.0079, p=0.0079). Antibody against DEK reduced the NETosis in each groups of DEK high/IL-8 high (Figure 4B, p=0.0079, p=0.0159), DEK high/IL-8 low (Figure 4C, p=0.0079, p=0.0317), DEK low/IL-8 high (Figure 4D, p=0.0317, p=0.4206). We noticed that anti-DEK or anti-IL-8 antibodies more effectively reduced the NETosis in the groups where their level was higher, whereas reparixin exhibited a better inhibition in all three groups [Figure 4B (p=0.0079, p=0.0079), 4C (p=0.0079, p=0.0317), 4D (p=0.0079, p=0.0079)].

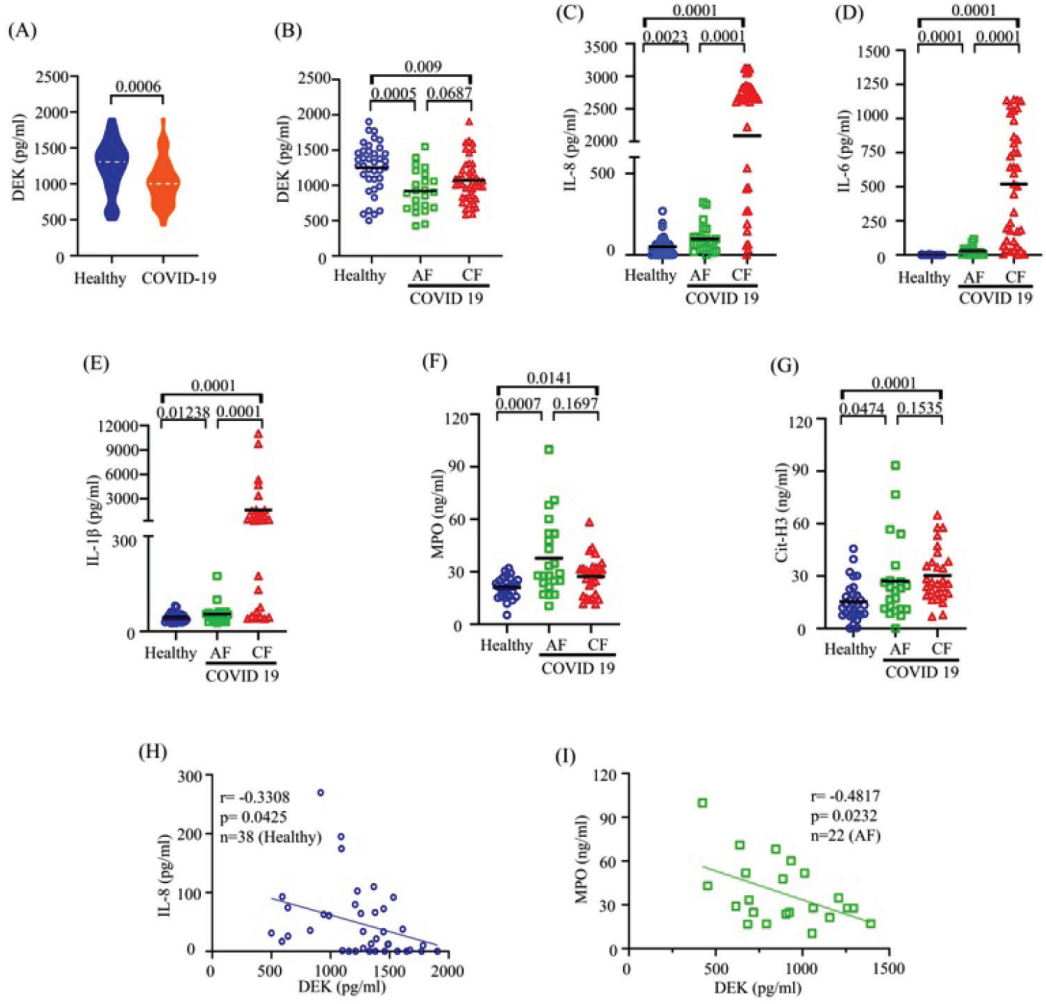


**Figure 2.** Patients with coronavirus disease-2019 (COVID-19) exhibit a lower level of DEK in the serum. (A-G) Level of DEK, IL-8 (healthy n=47; AF n=23; CF n=44), IL-6 (healthy n=47; AF n=16; CF n=39), IL-1β, myeloperoxidase (MPO) and citrullinated H3 (Cit-H3) (healthy n=30; AF n=22; CF n=28) were detected by using ELISA kits. (H-I) Graphs show Pearson correlation between the level of DEK and IL-8 in healthy individuals (H) and in patients with COVID-19 (at the AF of the disease). Lines indicate mean values. Data were analyzed by the Mann-Whitney test and Pearson Correlation, exact p values indicated on bars.

## Discussion

NETosis is correlated with the progression and severity of COVID-19, and the search for new drugs or clinical trials of existing drugs targeting NETosis is ongoing (20,21). Treatment modalities such as direct inhibition of cytokines IL-1β, IL-6, and IL-8 or blocking the CXCR1/CXCR2 receptors with drugs like reparixin offer promising results (21-25). Although the role of the secreted DEK in autoimmune disease-related NETosis was explored, to our knowledge, there are no available data on whether the same relationship applies to COVID-19.

Here, we found that *in vitro* NETosis induced by rDEK partially depends on the DEK-CXCR2 interaction, and reparixin reduces the DEK-induced NETosis. Our analyses showed that the amount of DEK in the serum of COVID-19 patients was lower than in healthy individuals, which was more obvious at the beginning of the disease. Unfortunately, we were not able to follow the disease outcome in the patients who participated in our study; therefore, we could not comment on whether the severity of the disease may also affect the level of DEK. It is not surprising that anti-DEK antibody suppressed the NETosis induced by patients' serums given that stimulation of the cells with IL-8, which was increased in the COVID-19 patients' serum, induces secretion of DEK *in vitro* (10,11). Previous reports focused on the role of DEK on the chromatin architecture and suggested that binding of DEK to the chromatin was required for NETosis in the JIA mouse model (16). Interestingly, we showed that DEK mainly located in the granules but not in the nucleus of the human neutrophils (Figures 3A and 3B). Moreover, blockage of CXCR1/2 receptors via reparixin reduced the NETosis induced by rDEK, suggesting that binding of DEK

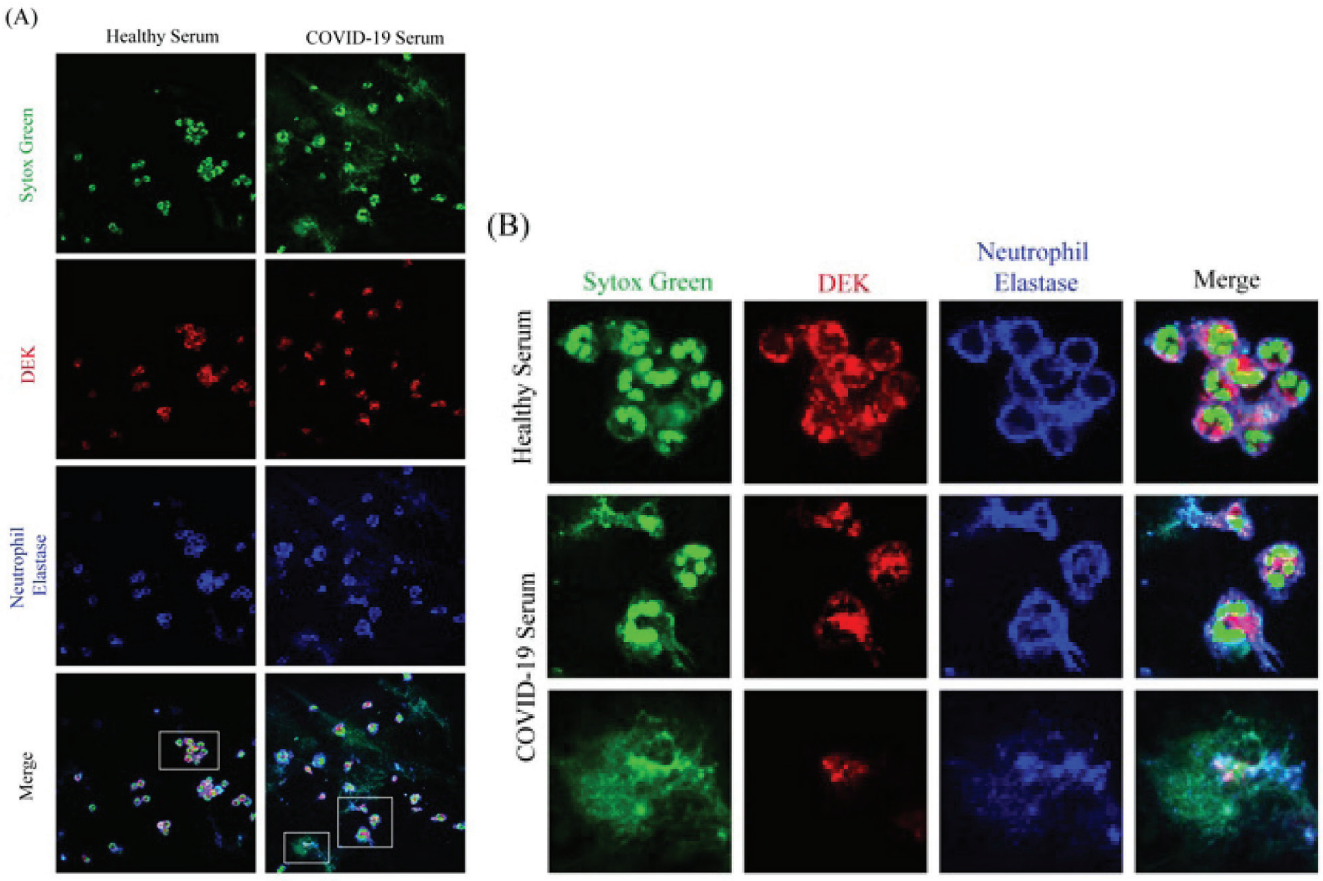


**Figure 3.** Immunofluorescence staining of neutrophils treated with healthy or COVID-19 serum samples. (A, B) Primary neutrophils were treated with 5% serum (healthy or COVID-19 samples) for two hours then the cells were treated for an additional 15 minutes in the presence of the Sytox (green). Fixed cells were double-stained with rabbit anti-DEK (Bethyl Laboratories) (red) and mouse anti-NE (neutrophil elastase) (blue) antibodies. Images were taken by using a confocal microscope (630X, with oil).
(B) Magnified images of the areas framed in (A) were shown.

to CXCR2 receptor also contributes NETosis. Our results also showed that DEK-CXCR2 interaction causes different outcomes in different types of hematopoietic cells, such as suppression of proliferation in hematopoietic progenitor cells (15) and induction of NETosis in neutrophils.

## Conclusion

In summary, our results showed that DEK and IL-8 play a role in COVID-19-induced NETosis and the blockage of CXCR1/CXCR2 receptors might be a potential therapeutic approach for the disease.

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### Ethics

**Ethics Committee Approval:** All samples were obtained from patients in accordance with protocols approved by Ethics Committee of Kocaeli University Medical Faculty (GOKAEK-2020/11.14 and GOKAEK-2021/15.13).

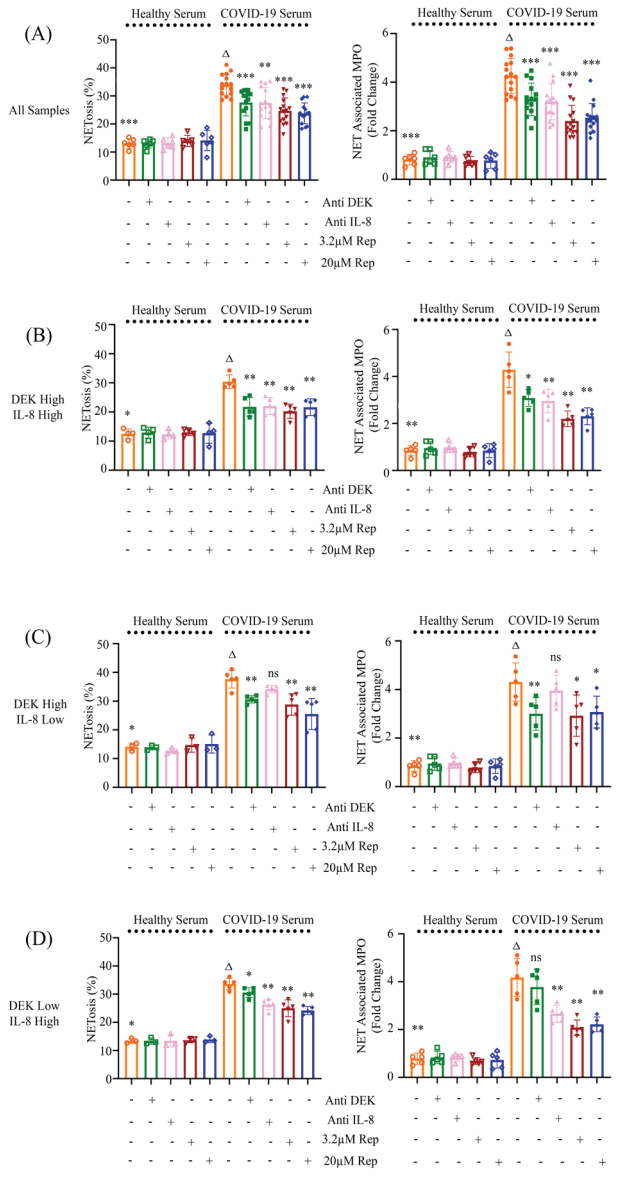


**Figure 4.** Reparixin or antibodies against DEK and IL-8 reduce NETosis induced by coronavirus disease-2019 (COVID-19)-patients'serum. (A-D) Neutrophils were treated with control (5% healthy serum) or COVID-19 serum (5%) samples for two hours then the cells were treated for an additional 15 minutes in the presence of the Sytox. In an additional setting, cells were pretreated for two hours with either reparixin (Rep), anti-DEK (Bethyl Laboratories), or anti-IL-8 antibodies before the addition of the 5% serum. Percent- NETosis analysis of the images taken from five different areas was performed by using Image J and DANA I software. For myeloperoxidase (MPO) analysis, cells were cultured similarly without the addition of Sytox as described in the text. Graphs indicate the percentage of NETosis (left panels) and the fold changes of NETs associated MPO (right panels) in all of the analyzed samples, which were obtained by dividing the values of COVID-19 treated samples by their untreated healthy serum samples. COVID-19 serum (n=15); healthy serum (n=4; two of the serums was used twice in the control experiments) in (A), or in the different types of groups based on the level of both the DEK and IL-8 in (B), (C) and (D). Statistical comparison performed against the group treated only with the COVID-19 patients' serum marked with triangle. Data were analyzed by the Mann-Whitney test and exact p-values indicated on bars. Error bars: ± standard deviation.

**Informed Consent:** Informed consent for all procedures in this study was obtained from all patients.

**Peer-review:** Externally and internally peer-reviewed.

### Authorship Contributions

Concept: A.Kan., Design: I.B.K., T.Y., A.Kan., Data Collection or Processing: I.B.K., A.Y., A.K., N.C., A.Kan., Analysis or Interpretation: I.B.K., A.Y., I.Y.C., T.G., T.Y., A.Kan., Literature Search: I.B.K., A.Kan., Writing: I.B.K., T.Y., A.Kan.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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**Supplementary Table 1.** Level of analyzed cytokines in patient samples

| Group | Sample | DEK (ng/mL) | IL-8 (ng/mL) | IL-6 (ng/mL) | IL-1β (ng/mL) | MPO (ng/mL) | Cit-H3 (pg/mL) |
|---|---|---|---|---|---|---|---|
| DEK High - IL-8 High (COVID-19) | CF 1 | 1297 | 2606.792 | 750.79 | 650.325 | 14959.04 | 17.60535 |
| | CF 2 | 1610 | 3119.752 | 1036.57 | 1601.27 | 25840.24 | 12.25895 |
| | CF 3 | 1596 | 3111.64 | 1134.02 | 1616.385 | 17197.76 | 4.49936 |
| | CF 4 | 1633 | 2628.376 | 723.56 | 348.5555 | 40413.12 | 28.9707 |
| | CF 5 | 1459 | 2816.528 | 637.819 | n.d. | n.d. | n.d. |
| DEK High - IL-8 Low (COVID-19) | CF 6 | 1905 | 31.05672 | 0 | 75.6515 | 31709.76 | 25.2099 |
| | AF 1 | 1393 | 110.8304 | 31.5899 | 44.4808 | 17022.64 | 0 |
| | AF 2 | 1294 | 65.24976 | n.d. | 27.1709 | 27773.36 | 15.6637 |
| | AF 3 | 1208 | 108.2256 | 95.9132 | 29.7685 | 34795.12 | 8.35485 |
| | AF 4 | 1156 | 23.64288 | 4.97047 | 31.2923 | 21321.52 | 18.2433 |
| DEK Low - IL-8 High (COVID-19) | CF 7 | 780 | 3115.168 | 846.138 | 3367.705 | 42009.28 | 38.457 |
| | CF 8 | 581 | 3117.8 | 865.372 | 319.516 | 31416.48 | 31.44635 |
| | CF 9 | 657 | 2797.32 | 643.04 | n.d. | n.d. | n.d. |
| | CF 10 | 592 | 2845.192 | 444.435 | n.d. | n.d. | n.d. |
| | CF 11 | 685 | 2749.504 | 193.546 | n.d. | n.d. | n.d. |
| Healthy (control) | H 1 | 1093 | 174.5056 | 5.17473 | 52.142 | 21529.12 | 12.5544 |
| | H 2 | 1344 | 12.68496 | 0 | 52.338 | 15758.64 | 7.87825 |
| | H 3 | n.d | 60.70472 | 0.003673 | 29.09425 | 21479.52 | 5.7961 |
| | H 4 | 828 | 35.63936 | 0.057 | 77.219 | 23253.36 | 8.913 |

n.d.: Not determined, MPO: Myeloperoxidase, COVID-19: Coronavirus disease-2019, IL: Interleukin