

## **Supplemental appendix**

### **Patients' exclusion criteria**

Exclusion criteria were as follows: systemic steroid therapy, chronic lung diseases (including chronic obstructive or restrictive pulmonary diseases), left ventricular dysfunction (left ventricular ejection fraction <50%), and the need for vasopressor or inotropic agents before surgery.

### **Cytometry and gating strategies**

Whole blood or bone marrow were labeled using a multicolor antibody panel: CD66b FITC (Beckman Coulter, Miami, FL), CD35 PE, CD11b PerCP5.5, CD63 PC7, CD64 APC-H7, CD45 BV510, HLA-DR BUV395, CD14 BUV805 (Becton Dickinson, San Jose, CA, USA), CD88 APC, CD16 Alexa Fluor 700, CD15 Pacific Blue (Biolegend, San Diego, CA, USA). For the study of endothelium adhesion, a multicolor panel containing CD11a FITC, CD11b BV605, and CD18 APC (Biolegend, San Diego, CA), was used on purified neutrophils.

For the migration assay, purified neutrophils were labeled using CD66b BV421 (Becton Dickinson, San Jose, CA, USA) and the number of migrated neutrophils was calculated using Precision Count Beads (Biolegend, San Diego, CA, USA).

For the phagocytosis assay, purified neutrophils were labeled using CD66b BV421 (Becton Dickinson, San Jose, CA, USA) and *Staphylococcus aureus* were stained using FITC Sigma-

Aldrich, St Louis, MO, USA). The number of double positive cells were determined by flow cytometry.

Samples were run on a Fortessa X-20 flow cytometer (Becton Dickinson, San Jose, CA, USA) and data were analyzed using Kaluza 2.0 software (Beckman Coulter, Miami, FL, USA). Gating strategies are detailed in the supplementary appendix

### **Determination of the neutrophil to *Staphylococcus aureus* ratio for each experiment**

For the NETs production experiment, we compare different neutrophils to *Staphylococcus aureus* (PMN/SA) ratio to LPS stimulation. As detailed on the supplemental figure 3A, using a ratio of 1 PMN to 10 SA, the NETs production was at its peak. As for the ROS production experiment, we also compared the ratio to LPS stimulation. The results detailed in supplemental figure 3B showed that the data could not be analyzed using a 1 PMN to 100 SA ratio or a 1 PMN to 50 SA ratio due to a loss of signal. Finally, we decided to use a 1 PMN to 20 SA ratio as previously described<sup>1</sup>. As shown in supplemental figure 3C, the phagocytosis rate was approximately at 50% when using a 2 PMN to 1 SA ratio. Finally, for the bacteria killing assay, we compared different ratio as detailed in supplemental figure 3D and we decided to use the 1 PMN to 20 SA ratio as previously described<sup>1</sup>.

### **Supplemental Material and methods**

#### **Counting alive neutrophils**

Circulating neutrophils were purified as previously described<sup>1</sup>. Then, they were cultured for 24 hours in RPMI 7% FCS 1% Penicillin Streptomycin. For the apoptosis assay using neutrophils from healthy volunteers, plasma from patients under CBP was added for a total of 10% of the culture media. Cells were then stained with an anti CD66b antibody (Becton Dickinson, San diago, CA, USA), AnnexinV (Tau technologies, Kattendijke, The Netherlands) and Dapi (Sigma-Aldrich, St Louis, MO, USA). Alive neutrophils were determined using flow cytometry as follow: CD66b<sup>pos</sup>-DAPI<sup>neg</sup>-AnnexinV<sup>neg</sup>. The gating strategy is detailed in supplemental appendix.

### **Migration assay**

Purified neutrophils were added in RPMI 0.1% human serum albumin (HSA) to the upper compartment of transwell chambers with 5µm pore filters. Lower chambers contained recombinant IL-8 (50ng/mL, R&D System, Abindon, UK) when neutrophils from CBP patients were used and 50% of defrosted plasma from patients under CBP when neutrophils from healthy volunteers were used. Control have been performed using only RPMI 0.1% HSA in the lower chamber to determine the non-specific migration. After a 1 hour of migration, cells from the lower chamber were stained with an anti CD66b antibody (Becton Dickinson, San Jose, CA, USA) and the absolute number of CD66b<sup>pos</sup> neutrophils was quantified using Precision Count Beads (Biolegend, San Diego, CA, USA) by flow cytometry. The gating strategy is detailed in the supplementary appendix. Results are expressed as the migration index which is the ratio between the number of migrated neutrophils using one condition and the number of non-specific migrated neutrophils.

### **Neutrophil Extracellular Trap (NETs release quantification)**

Purified neutrophils were labeled with 5  $\mu$ M Sytox blue (Invitrogen, Carlsbad, CA, USA) for 15 minutes and stimulated with a ratio of 1 neutrophil for 10 *Staphylococcus aureus* for 3 hours at 37°C in RPMI 0.5% FCS in Costar 96-well black plates (Corning Costar Corporation, Cambridge, MA, USA). When purified neutrophils from healthy volunteers were used, plasma from CBP patients was added for a total of 10% of the culture media. The NETs release was quantified by measuring fluorescence with a microplate fluorescence reader (Varioskan, ThermoFisher Scientific, Waltham, MA, USA). In order to assess the extracellular free-floating DNA, controls using DNase (200UI/mL, Roche, Switzerland) have been used. The NETs release was calculated as the ratio between the fluorescence with and without DNase. Results were expressed as the ratio of fluorescence between Day 0 and Day 1.

### **ROS production**

Purified neutrophils were incubated with H2DCFDA probes (10  $\mu$ M; Thermo Fisher Scientific, Waltham, MA, USA) for 30 minutes in a PBS media. Then, neutrophils were stimulated with a ratio of 1 neutrophil for 20 *Staphylococcus aureus* at 37°C in Costar 96-well black plates (Corning Costar Corporation, Cambridge, MA, USA). When neutrophils from healthy volunteers were used, plasma from CB patients was added for a total of 10% of the culture media. Quantification of ROS production was determined measuring fluorescence with a microplate fluorescence reader (Varioskan, ThermoFisher Scientific, Waltham, MA, USA) every 10 minutes for 180 minutes. The results were expressed by the ratio of fluorescence between Day 0 and Day 1.

### **Phagocytosis assay**

Purified neutrophils were labeled with an anti-CD66b antibody (Becton Dickinson, San Jose, CA, USA) and incubated with a ratio of 2 neutrophils for 1 *Staphylococcus aureus* previously labeled with FITC (Sigma-Aldrich, St Louis, MO, USA) for 15 minutes at 37°C. Neutrophils phagocytosis was synchronized using a 5 minutes centrifugation. After phagocytosis, cells and bacteria were fixed with PFA 4% for 5 minutes at room temperature and in the dark before washing in PBS. Phagocytosis rate was determined by flow cytometry as the percentage of double positive cells (CD66b<sup>pos</sup> – FITC<sup>pos</sup>). The gating strategy is detailed in the supplementary appendix. Results were expressed by the ratio of phagocytosis rate between Day 0 and Day 1.

#### **Neutrophil bacterial killing assay**

Purified neutrophils were incubated with a ratio of 1 neutrophil for 20 *Staphylococcus aureus* for 16 hours at 37°C in RPMI 7% FCS. When neutrophils from healthy volunteers were used, plasma from CBP patients was added for a total of 10% of the culture media. Cells were lysed with Triton X-100 (0.1%, Sigma Aldrich, St Louis, MO, USA), and the remained *Staphylococcus aureus* were incubated on agar plates overnight at 37°C. The amounts of viable bacteria colonies were calculated as colony-forming units (CFU). Results were expressed as the ratio of CFU between Day 0 and Day 1.

1. Grégoire M, Tadié J-M, Uhel F, et al. Frontline Science: HMGB1 induces neutrophil dysfunction in experimental sepsis and in patients who survive septic shock. *J Leukoc Biol.* 2017;101:1281–1287.

