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1 **Neutrophil function and bactericidal activity against *Staphylococcus aureus* after**  
2 **cardiac surgery with cardiopulmonary bypass**

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14  
15 Summary sentence: Cardiopulmonary bypass does not immediately affect neutrophils  
16 functions.

17  
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19  
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23  
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- 3 -Introduction: 253 words
- 4 -Results and Discussion: 1424 words
- 5 -1 table
- 6 -4 figures
- 7

1 **LIST OF ABBREVIATION**

2

3 CBP: Cardiopulmonary Bypass

4 CFU: Colony Forming Units

5 G-CSF: Granulocyte-Colony Stimulating Factor

6 IL: Interleukin

7 NET: Neutrophils Extracellular Traps

8 ROS: Reactive Oxygen Species

9 TNF- $\alpha$ : Tumor Necrosis Factor  $\alpha$

1 **ABSTRACT**

2

3 *Staphylococcus aureus* is the main bacterial pathogen encountered in mediastinitis after  
4 cardiac surgical procedures that remains a devastating complication carrying a high mortality.  
5 As neutrophils have a primordial role in the defense against staphylococcus infection and  
6 cardiopulmonary bypass (CPB) is known to induce immunosuppression, the aim of this study  
7 was to investigate CPB impact on neutrophil functions. Patients without known  
8 immunosuppression scheduled for cardiac surgery with CPB were included. Bone marrow  
9 and blood samples were harvested before, during and after surgery. Neutrophil phenotypic  
10 maturation and functions (migration, adhesion, Neutrophil Extracellular Trap (NET) release,  
11 Reactive Oxygen Species (ROS) production, phagocytosis and bacteria killing) were  
12 investigated. Two types of *Staphylococcus aureus* strains (one from asymptomatic nasal  
13 carriage and another from mediastinitis infected tissues) were used to assess in vitro bacterial  
14 direct impact on neutrophils. We found that CPB induced a systemic inflammation with an  
15 increase of circulating mature neutrophils after surgery. Bone marrow sample analysis did not  
16 reveal any modification of neutrophil maturation during CPB. Neutrophil lifespan was  
17 significantly increased and functions such as NET release and ROS production were enhanced  
18 after CPB whereas bacteria killing and phagocytosis were not impacted. Results were similar  
19 with the two different isolates of *Staphylococcus aureus*. These data suggest that CPB induces  
20 a recruitment of mature neutrophils via a demargination process rather than impacting their  
21 maturation in the bone marrow. In addition, neutrophils are fully efficient after CPB and do  
22 not contribute to postoperative immunosuppression.

23

24

25

# 1 INTRODUCTION

2

3 Cardiac surgery with cardiopulmonary bypass (CPB) induces important immunological  
4 changes identical to those observed in critically ill septic patients, such as lymphopenia and  
5 decrease of HLA-DR expression on monocytes, therefore exposing patients to postoperative  
6 infections and increased mortality rate<sup>1-5</sup>

7 Observational studies have demonstrated that 15 to 20% of patients undergoing cardiac  
8 surgery with CPB will experience postoperative infections which dramatically affect survival  
9 and readmissions<sup>6</sup>. Amid these infections, sternal wound infections and post-sternotomy  
10 mediastinitis are frequent, requiring antibiotic and additional surgery with high mortality  
11 rate<sup>7</sup>. Among bacterial pathogens responsible for post-sternotomy mediastinitis,  
12 *Staphylococcus aureus* is the most commonly encountered despite effective prevention  
13 strategies with intranasal and topical decolonization<sup>8,9</sup>.

14 The leading role of staphylococcus infections after cardiac surgery could be the translation of  
15 acquired postoperative neutrophils dysfunction<sup>10</sup> since neutrophils play a critical role in host  
16 defense against infections specially against *Staphylococcus aureus*<sup>11-13</sup>. However, there are  
17 few data about neutrophil function after CPB with conflicting results<sup>14</sup>. Furthermore, recent  
18 data have highlighted that neutrophils actively orchestrate resolution of inflammation and  
19 contribute to tissue repair, and should not be limited to bactericidal cells<sup>15</sup>.

20 We hypothesized that cardiac surgery with CBP affects neutrophil functions which could  
21 expose patients to postoperative infections due to *Staphylococcus aureus*. Thus, the aim of  
22 this study was to assess whether cardiac surgery with CPB affects neutrophil ability to kill  
23 *Staphylococcus aureus*. Furthermore, because *Staphylococcus aureus* may develop different  
24 strategies to avoid killing from neutrophils, we exposed neutrophils to two different strains,  
25 one from asymptomatic nasal carriage and one responsible for mediastinitis<sup>16</sup>.

## 1 MATERIAL AND METHODS

2

### 3 Patients

4 Patients without known immunosuppression scheduled for cardiac surgery using CPB were  
5 prospectively enrolled in the University Hospital of Rennes (France). Exclusion criteria are  
6 listed in the supplementary appendix. The study protocol was approved by the local ethic  
7 committee (n°15-97, n°01/06-567, n°13-8 and n°15.44-2) and informed consents were  
8 obtained.

9 Four timepoints have been assessed for this study. “Day 0” and “Day 1” were respectively  
10 after induction of anesthesia and 24 hours after intervention. “Time 0” and “Time 1” were  
11 during surgery, respectively after the sternotomy and before sternum closure.

12

### 13 Cytokine quantification

14 Interleukine (IL)-6, IL-8, IL-10, TNF- $\alpha$ , and G-CSF were quantified in plasma by ELISA  
15 DuoSET (R&D System).

16

### 17 Cytometry

18 Whole blood or bone marrow samples were labeled using a multicolor antibody panel detailed  
19 in supplemental appendix (for example, circulating immatures and matures neutrophils have  
20 been determined using a CD11b and CD16 staining). Samples were run on a Fortessa X-20  
21 flow cytometer within 1 hour after harvestment (Becton Dickinson) and data were analyzed  
22 using Kaluza 2.0 software (Beckman Coulter).

23

### 24 Cell isolation and culture

25 Neutrophils were purified as previously described<sup>17</sup>.

1 **Counting alive neutrophils**

2 Using AnnexinV (Tau technologies) and Dapi (Sigma-Aldrich) after a 24 hours culture,  
3 double negative neutrophils were determined by flow cytometry.

4

5 **Migration assay**

6 Using transwell chambers with 5µm pore filters with purified neutrophils in the upper  
7 compartment and recombinant IL-8 (50ng/mL, R&D System) or 50% of defrosted plasma  
8 from patients under CBP, the absolute number of CD66b<sup>pos</sup> neutrophils was quantified using  
9 Precision Count Beads (Biolegend) in the lower chamber after 1 hour of migration.

10

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

12 Neutrophils were labeled with 5 µM Sytox blue (Invitrogen) and stimulated with a ratio of 1  
13 neutrophil for 10 *Staphylococcus aureus* for 3 hours at 37°C. The release was quantified by  
14 measuring fluorescence with a microplate fluorescence reader (Varioskan, ThermoFisher  
15 Scientific). In order to assess the free-floating DNA, controls using DNase have been used.  
16 Results were expressed as the ratio of fluorescence between Day 0 and Day 1.

17

18 **ROS production**

19 Neutrophils incubated with H2DCFDA probes (10 mM; Thermo Fisher Scientific) for 30  
20 minutes and then were stimulated with a ratio of 1 neutrophil for 20 SA for 3 hours at 37°C.  
21 Quantification of ROS production was determined quantified by measuring fluorescence with  
22 a microplate fluorescence reader (Varioskan, ThermoFisher Scientific) kinetically every 10  
23 minutes during the 3-hour stimulation. Results were expressed as the ratio of fluorescence  
24 between Day 0 and Day 1.

25



1 **Phagocytosis assay**

2 Labeled neutrophils with an anti-CD66b BV421 antibody (Becton Dickinson) were incubated  
3 with a ratio of 2 neutrophils for 1 *Staphylococcus aureus* previously labeled with FITC  
4 (Sigma-Aldrich) for 15 minutes at 37°C. Phagocytosis rate was determined by flow cytometry  
5 as the percentage of double positive cells. Results were expressed by the ratio of phagocytosis  
6 rate between Day 0 and Day 1.

7

8 **Neutrophil bacterial killing assay**

9 Neutrophils were incubated with a ratio of 1 neutrophil for 20 *Staphylococcus aureus* for 16  
10 hours at 37°C. Cells were lysed with Triton X-100 (0.1%, Sigma Aldrich), and were  
11 incubated on agar plates overnight at 37°C. The amounts of viable bacteria colonies were  
12 calculated as colony-forming units (CFU). Results were expressed as the ratio of CFU  
13 between Day 0 and Day 1.

14

15 ***Staphylococcus aureus* strains**

16 Two different strains of *Staphylococcus aureus* were used: one from asymptomatic nasal  
17 carriage (healthy carrier strain) and one from post-operative mediastinitis with bacteremia  
18 (mediastinitis strain). The genome analysis is detailed in the supplementary appendix  
19 (supplementary Table 1) and showed that the “Healthy carrier” strain is ST398 and the  
20 “Mediastinitis” strain is ST30.

21

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

23 In order to determine the best ratio between neutrophils and SA for each experiment, multiple  
24 ratios have been tested compared to LPS stimulation (see supplemental appendix).

25

1 **Statistical analysis**

2 All of the quantitative variables were expressed as median (interquartile range, IQR) when  
3 indicated, and qualitative variables as number and were compared using the nonparametric  
4 Mann-Whitney *U* test, Kruskal-Wallis test or Wilcoxon test for matched pairs as appropriate  
5 with GraphPad Prism 6.2 (GraphPad Software). A two-tailed testing has been used for each  
6 hypothesis and  $p < 0.05$  have been used for statistical significance.

## 1 **RESULTS AND DISCUSSION**

2

### 3 **Patients and surgery characteristics.**

4 From January 2017 to June 2017 (n = 27) and from June 2021 to July 2021 (n = 6), 33  
5 patients undergoing cardiac surgery with CPB were included in the study. Characteristics of  
6 patients undergoing surgery are summarized in the table 1. Of note, no patient presented any  
7 nosocomial infection during their hospital stay.

8

### 9 **CPB induces systemic inflammation and cytokine release.**

10 As expected, automated hemocytometry showed that cardiac surgery with CPB induced  
11 changes in total and differential leukocyte count with a significant increase in circulating  
12 neutrophils and monocytes after CBP whereas lymphocytes decreased after cardiac surgery  
13 (Figure 1A). Furthermore, IL-6 and TNF- $\alpha$ , two pro-inflammatory cytokines were  
14 significantly increased, as well as the anti-inflammatory IL-10<sup>18</sup>. CPB induced the secretion  
15 of G-CSF, which stimulates survival, proliferation, differentiation, and functions of neutrophil  
16 precursors and mature neutrophils<sup>19</sup> (Figure 1B). Lastly, IL8, a major chemoattractant  
17 molecule that has been shown to be responsible for guiding neutrophils to reach the injured  
18 tissue was increased after CPB<sup>20</sup>.

19

### 20 **CPB induces significant increase in circulating mature neutrophil due to a** 21 **demargination process.**

22 As shown figure 2A, circulating neutrophils were mainly mature neutrophils in patients after  
23 CPB. This is worth noting since circulating immature neutrophils are known to be  
24 immunosuppressive with impaired ability of phagocytosis and bacteria killing<sup>17,21,22</sup>. To  
25 understand how matures neutrophils were recruited after CBP, flow cytometry analysis of

1 bone marrow samples and blood samples harvested at sternum opening (Time 0) and closing  
2 (Time 1) were performed. As shown figure 2C, CPB did not change neutrophil progenitor  
3 counts, although the number of blood circulating immatures neutrophils, as well as matures  
4 neutrophils, significantly expended just after CBP (Time 1) (Figure 2B). However, analyses  
5 of the blood compartment showed that immatures neutrophils accounted significantly less  
6 than matures neutrophils (respectively 114/mm<sup>3</sup> [IQR 54.6/mm<sup>3</sup> – 250/mm<sup>3</sup>] and 726/mm<sup>3</sup>  
7 [IQR 545.9/mm<sup>3</sup> – 857/mm<sup>3</sup>],  $p < 0.0001$ ). Therefore, it appears that recruitment of matures  
8 neutrophils after CBP is mainly due to a demargination process. Marginated neutrophils is a  
9 well-known phenomenon<sup>23</sup>, as neutrophils can infiltrate naïve tissues and then be recruited<sup>24</sup>.  
10 This demargination process is in accordance with the major increase of plasma IL-6 observed  
11 after surgery which has been found to be a strong inductor of the mobilization of neutrophils  
12 into the circulating pool from the marginated pool<sup>25</sup>. However, these results need to be  
13 tempered. In order to highlight changes induced by cardiac surgery with CPB on bone  
14 marrow progenitors, samples should have been harvested later after surgery rather than at  
15 time of sternum closure. Unfortunately, invasive nature of bone marrow aspiration did not  
16 allow harvesting 24 hours after surgery.

17

### 18 **Neutrophils exhibit activated and functional profile after CPB.**

19 Although we found that circulating neutrophils after CPB were mature neutrophils, their  
20 functions needed to be analyzed more thoroughly. Neutrophils directly control infectious  
21 agents through phagocytosis, degranulation, ROS and Neutrophil Extracellular Traps (NETs),  
22 and their loss of function could have dramatic consequences<sup>22</sup>. As shown figure 3A, using a  
23 Dapi/Annexin V staining, neutrophil lifespan was significantly increased after CPB, since  
24 the proportion of alive neutrophils after 24 hours in vitro culture was higher after than before  
25 CPB. Furthermore, bacteria's eradication by neutrophils is a well-known process, starting by

1 their migration towards the infected tissue. Dissemination of bacteria is associated with loss  
2 of neutrophil ability to reach the site of infection which has been described in sepsis<sup>26</sup>. We  
3 found that migratory ability of neutrophils was significantly increased after CPB (Figure 3B).  
4 Additionally, to reach the injured site, neutrophils in the bloodstream have to be slowed down  
5 near the tissue and to adhere to endothelial cells<sup>27</sup>. The  $\beta$ 2-integrin family members  
6 CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1) are involved in firm adhesion of  
7 neutrophils to endothelial cells<sup>20,27</sup>, and CD11b is also essential for neutrophils infiltration in  
8 muscle repairs after sterile injury<sup>28</sup>. As shown figure 3C, expression of CD11a, CD11b and  
9 CD18 remained unchanged after surgery. Moreover, CD88, a receptor for C5a which  
10 activates the neutrophils and considered as an important mediator of neutrophil dysfunction<sup>29</sup>,  
11 is not decreased after CPB (Figure 3D). Along these lines, expression of CD35, CD63 and  
12 CD66b, three degranulation markers respectively for secretory vesicles, gelatinase granules,  
13 and azurophilic granules, were increased (CD35, CD66b) or remained stable (CD63) after  
14 CPB<sup>12</sup> (Figure 3E). These degranulation markers could be a marker of activated neutrophils  
15 involved in wound healing has already shown in sterile liver injury<sup>30</sup>.

16 When neutrophils reach site of infection, they will develop different strategies to kill bacteria  
17 and control infection<sup>31</sup>. Among antimicrobial mediators, release of DNA into the extracellular  
18 milieu (NETs) and production of ROS are key neutrophilic antimicrobial mechanisms. As  
19 shown in figures 3F, cardiac surgery did not decrease ability of neutrophils to produce NETs.  
20 Moreover, the ROS production was increased after CBP when neutrophils were stimulated  
21 with “Mediastinitis” strain (Figure 3G). Finally, neutrophil ability to phagocyte and kill  
22 *Staphylococcus aureus* was not affected by cardiac surgery with CPB (Figures 3H and 3I).  
23 Our data suggest that CBP does not have immediate negative impact on neutrophils ability to  
24 kill bacteria. Conversely, neutrophils after CPB appeared fully functional to contribute to  
25 wound healing. Although the underlying mechanisms have not been fully elucidated, many

1 anesthetics suppress various aspects of human neutrophil function that could affect the results  
2 of our experiments, such as ROS production<sup>32</sup>. Finally, neutrophils functions (NETs release,  
3 *Staphylococcus aureus* phagocytosis and killing) was not impacted by the strain of  
4 *Staphylococcus aureus*.

5  
6 **Plasma from patient with CBP decrease healthy donors' neutrophils NETs release and**  
7 **ROS production.**

8 Since neutrophils are easily activated during the isolation procedure<sup>33</sup> and their function can  
9 be increased or diminished by anesthetic agents<sup>32</sup>, we decided to reproduce experiments using  
10 neutrophils from healthy donors and studied the impact of plasma from patients undergoing  
11 cardiac surgery with CPB to demonstrate that main neutrophils function could be impacted by  
12 ex-vivo conditions.

13 Neutrophils' lifespan was increased when cultured with plasma after CBP (Figure 4B). As  
14 shown in Figure 4C, neutrophils migration is promoted by plasma after CBP. This result was  
15 expected as IL-8, at a higher concentration after CBP, promotes neutrophils' migration<sup>34</sup>.  
16 After a 1 hour incubation in plasma after CBP, neutrophils from healthy donors did not show  
17 a reduction of adhesion (CD11a, CD11b and CD18), activation (C5aR) and degranulation  
18 markers (CD35 and CD63) (Figure 4D, 4E and 4F). Moreover, CD66b was significantly  
19 increased by plasma after CBP (Figure 4F). Surprisingly, NETs formation was decrease by  
20 plasma after CBP (Figure 4G). This result was unexpected because neutrophils from patients  
21 under CBP had a preserved NETs' release function. This could be due to high concentration  
22 of IL-10 in the plasma of patients after CPB. In fact, Garley et al. have shown that IL-10  
23 delayed the NETs formation *in vitro*<sup>35</sup>. This NETs formation decrease is worth noticing since  
24 this mechanism is also involved in wound healing and neutrophil functions go well beyond  
25 the scope of immune defenses as recent studies have demonstrated their role in healing

1 process after sterile injury<sup>12,36,37</sup>. Along these lines, ROS production was decreased by CBP  
2 plasma (figure 4H). These findings highlighted the immunosuppressive role of the plasma  
3 after CBP. Finally, the ability to phagocyte and kill *Staphylococcus aureus* were not impacted  
4 by plasma from patient under CBP (Figures 4I, and 4J). Neutrophils appeared to be extremely  
5 effective and did not represent a potential target to restore immune function after cardiac  
6 surgery with CPB. Recent data have shown that lymphocyte dysfunction along to myeloid  
7 derived suppressed cells could mediate immunosuppression after CBP and exposed patients to  
8 postoperative infections<sup>38</sup>. In this study, Hubner et al. found that MDSC induce an  
9 immunosuppressive T cell phenotype by increasing serum arginine breakdown, which could  
10 explain the efficacy of L-arginine administration to reduce postoperative infections after  
11 cardiac surgery in patients at high risk of infection after cardiac surgery<sup>39</sup>. Furthermore, the  
12 strain of *Staphylococcus aureus* used did not impact neutrophils except from ROS production.  
13 Interestingly, the “Mediastinitis” strain enhanced the ROS production compare to the  
14 “Healthy carrier strain” (Figure 3G and 4H). However, our results have to be tempered since  
15 *Staphylococcus aureus* were not opsonized with human serum before any experiment. It is  
16 well known that human serum contains IgG and complements that are important for  
17 *Staphylococcus aureus* phagocytosis and killing by neutrophils although not essential<sup>40</sup>.  
18 Nevertheless, our study adds original data on host-pathogen interactions, and may contribute  
19 to better understanding of pathways towards postoperative infection.

20

21 In conclusion, our study brings new highlights on the impact of CBP on neutrophils. In fact,  
22 CBP induces the recruitment of mature neutrophils via a demargination process. Moreover,  
23 neutrophils keep intact *Staphylococcus aureus* killing ability and do not contribute to  
24 postoperative immune dysfunction after cardiac surgery with CPB.

25

1 Authorship:

2 M.L., M.G., C.P., V.C and E.D. performed the experiments;

3 M.L., M.G., A.G., M.R., P.T., K.T. and J.-M.T. wrote the report and designed the

4 experiments;

5 M.L., A.F., A.R., E.F., J.-P.V. included the patients;

6 M.L., M.G., A.G., M.R., P.T., K.T. and J.-M.T. analyzed and discussed the results and read

7 and discussed the report;

8 M.L., M.G. and A.G. performed the statistical analyses;

9 M.L. , M.G., A.G., V.C., A.F., C.P., E.D., M.R., P.T., A.R., J.-P.V., E.F., Y.L.T, K.T. and J.-

10 M.T read and approved the final report

11

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14

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16



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50

1 **Figures legends**

2

3 **Figure 1. CPB induces systemic inflammation and cytokines release.**

4 **A.** Number of circulating leukocytes, neutrophils, monocytes and lymphocytes determined  
5 after induction of anesthesia (Day 0) and 24 hours after intervention (Day 1) (n=27). **B.**  
6 Quantification of IL-6, IL-10, TNF- $\alpha$ , G-CSF and IL-8 by ELISA in plasma from patients at  
7 Day 0 and Day 1 (n=23). Median and IQR are represented by the box plots which are framed  
8 by bars which refer to the maximum and the minimum values.

9

10 **Figure 2. CBP induces a recruitment of mature neutrophils.**

11 **A.** Number of blood circulating immature and mature neutrophils after induction of anesthesia  
12 (Day 0) and 24 hours after surgery (Day 1) (n=10). **B.** Number of blood circulating immature  
13 and mature neutrophils after the sternotomy (Time 0) and just before sternum closure (Time  
14 1) (n=10). **C.** Neutrophil number at different stages into the sternal bone marrow of CPB  
15 patients immediately after the sternotomy (Time 0) and just before sternum closure (Time 1)  
16 (n=6). Median and IQR are represented by the box plots which are framed by bars which refer  
17 to the maximum and the minimum values.

18

19 **Figure 3. Neutrophils are activated after CPB and do not show alteration in their**  
20 **functions.**

21 **A.** Proportion of AnnexinV<sup>neg</sup>/DAPI<sup>neg</sup> cells among CD66b<sup>pos</sup> neutrophils after induction of  
22 anesthesia (Day 0) and 24 hours after surgery (Day 1) assessed by flow cytometry after 24  
23 hours *ex vivo* culture (n=8). **B.** Migration of neutrophils from CPB patients after induction of  
24 anesthesia (Day 0) and 24 hours after surgery (Day 1) in response to IL-8 (50ng/mL).  
25 Migration index was calculated as the ratio between the number of migrated neutrophils at

1 Day 1 compared to Day 0 (n=6). **C.** Ratio of rMFI between Day 0 and Day 1 (rrMFI) of  
2 adhesion molecules CD11a, CD11b and CD18 were compared by flow cytometry (n=8). **D.**  
3 rMFI of C5a receptor was compared by flow cytometry after induction of anesthesia (Day 0)  
4 and 24 hours after surgery (Day 1) (n=9). **E.** Ratio of rMFI between Day 0 and Day 1 (rrMFI)  
5 of degranulation surface markers (CD35, CD63, CD66b) were compared by flow cytometry  
6 (n=9). **F.** NET quantification by Sytox blue fluorescence (5 $\mu$ M) after incubation for 3 hours  
7 with *Staphylococcus aureus* from healthy carrier (n=8) or mediastinitis (n=7). Results are  
8 expressed as the ratio of NET quantification after induction of anesthesia (Day 0) and 24  
9 hours after surgery (Day 1) of neutrophils from CPB patients. **G.** Neutrophils purified from  
10 blood of CPB patients at Day 0 and Day 1 were loaded with H2 DCF-DA for 30 minutes.  
11 Then, cells were incubated with *Staphylococcus aureus* from healthy carrier (n=6) or  
12 mediastinitis (n=6) for 3 hours and ROS quantification was measured kinetically every 10  
13 minutes during the 3-hour stimulation. Results are expressed as the ratio of ROS production  
14 after induction of anesthesia (Day 0) and 24 hours after surgery (Day 1) of neutrophils from  
15 CPB patients. **H.** Phagocytosis rate of neutrophils from cardiac surgical patients after  
16 induction of anesthesia (Day 0) and 24 hours after surgery (Day 1) labeled with an anti-  
17 CD66b BV421 antibody was determined after 15 minutes of incubation with *Staphylococcus*  
18 *aureus* from healthy carrier (n=9) or mediastinitis (n=9) labeled with FITC. Phagocytosis rate  
19 was assessed by flow cytometry and determined as the percentage of double positive cells.  
20 Results are expressed by the ratio of phagocytosis rate between Day 1 and Day 0. **I.**  
21 Neutrophils from CPB patients after induction of anesthesia (Day 0) and 24 hours after  
22 surgery (Day 1) were incubated with *Staphylococcus aureus* from healthy carrier (n=24) or  
23 mediastinitis (n=16) for 16 hours and the number of viable bacteria was determined with a  
24 CFU assay. Results are expressed by the ratio of CFU between Day 1 and Day 0. Median and

1 IQR are represented by the box plots which are framed by bars which refer to the maximum  
2 and the minimum values.

3 **Figure 4. Plasma from CBP patients decreases ROS and NETs production**

4 **A.** Graphical abstract of the following experiments: neutrophils from healthy donors were  
5 isolated and cultured during 1 hour with plasma from CBP patients after induction of  
6 anesthesia (Day 0) and 24 hours after surgery (Day 1) before assessing their phenotype and  
7 functions. **B.** Proportion of AnnexinV<sup>neg</sup>/DAPI<sup>neg</sup> cells among CD66b<sup>pos</sup> neutrophils from  
8 healthy donors assessed by flow cytometry after 24 hours *ex vivo* culture with plasma from  
9 CBP patients after induction of anesthesia (Day 0) and 24 hours after surgery (Day 1) (n=10).  
10 **C.** Migration of neutrophils from healthy donors in response to plasma from CBP patients  
11 after induction of anesthesia (Day 0) and 24 hours after surgery (Day 1). Migration index was  
12 calculated as the ratio between the number of neutrophils migrated in response to plasma at  
13 Day 1 compared to Day 0 (n=10). **D.** Ratio of rMFI between Day 0 and Day 1 (rrMFI) of  
14 adhesion molecules CD11a, CD11b and CD18 were compared by flow cytometry on  
15 neutrophils from healthy donors after a 1 hour incubation with plasma from form CBP  
16 patients after induction of anesthesia (Day 0) and 24 hours after surgery (Day 1) (n=10). **E.**  
17 rMFI of C5a receptor was compared by flow cytometry on neutrophils from healthy donors  
18 after a 1 hour incubation with plasma from form CBP patients after induction of anesthesia  
19 (Day 0) and 24 hours after surgery (Day 1) (n=10). **F.** Ratio of rMFI between Day 0 and Day  
20 1 (rrMFI) of degranulation surface markers (CD35, CD63, CD66b) were compared by flow  
21 cytometry on neutrophils from healthy donors after a 1 hour incubation with plasma from  
22 form CBP patients after induction of anesthesia (Day 0) and 24 hours after surgery (Day 1)  
23 (n=10). **G.** NET quantification by Sytox blue fluorescence (5µM) of neutrophils from healthy  
24 donors after incubation for 3 hours with *Staphylococcus aureus* from healthy carrier (n=10) or  
25 mediastinitis (n=10) and plasma from CBP patients after induction of anesthesia (Day 0) and

1 24 hours after surgery (Day 1). Results are expressed by the ratio of NET quantification  
2 between Day 0 and Day 1. **H.** Neutrophils purified from blood of healthy donors were loaded  
3 with H2 DCF-DA for 30 minutes. Then, cells were incubated with *Staphylococcus aureus*  
4 from healthy carrier (n=10) or mediastinitis (n=10) and plasma from CBP patients after  
5 induction of anesthesia (Day 0) and 24 hours after surgery (Day 1) for 3 hours and ROS  
6 quantification was measured kinetically every 10 minutes during the 3-hour stimulation. ROS  
7 quantification has been determined as the ratio of ROS production between Day 0 and Day 1.  
8 **I.** Phagocytosis rate of neutrophils from healthy donors labeled with an anti-CD66b BV421  
9 antibody was determined after 15 minutes of incubation with *Staphylococcus aureus* from  
10 healthy carrier (n=10) or mediastinitis (n=10) labeled with FITC and plasma from CBP  
11 patients after induction of anesthesia (Day 0) and 24 hours after surgery (Day 1).  
12 Phagocytosis rate was assessed by flow cytometry and determined as the percentage of double  
13 positive cells. Results are expressed by the ratio of phagocytosis rate between Day 1 and Day  
14 0. **J.** Neutrophils from healthy donors were incubated with *Staphylococcus aureus* from  
15 healthy carrier (n=10) or mediastinitis (n=10) and plasma from CBP patients after induction  
16 of anesthesia (Day 0) and 24 hours after surgery (Day 1) for 16 hours and the number of  
17 viable bacteria was determined with a CFU assay. Results are expressed by the ratio of CFU  
18 between Day 1 and Day 0. Median and IQR are represented by the box plots which are  
19 framed by bars which refer to the maximum and the minimum values.

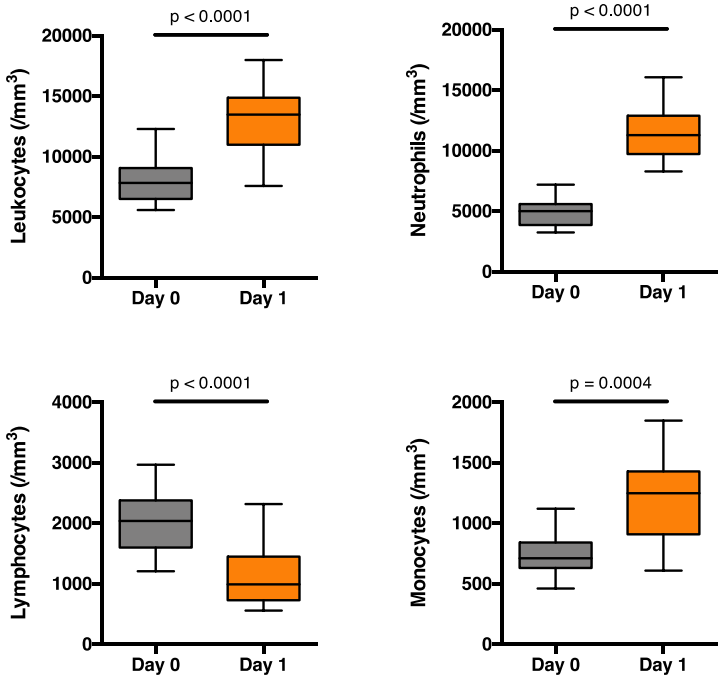
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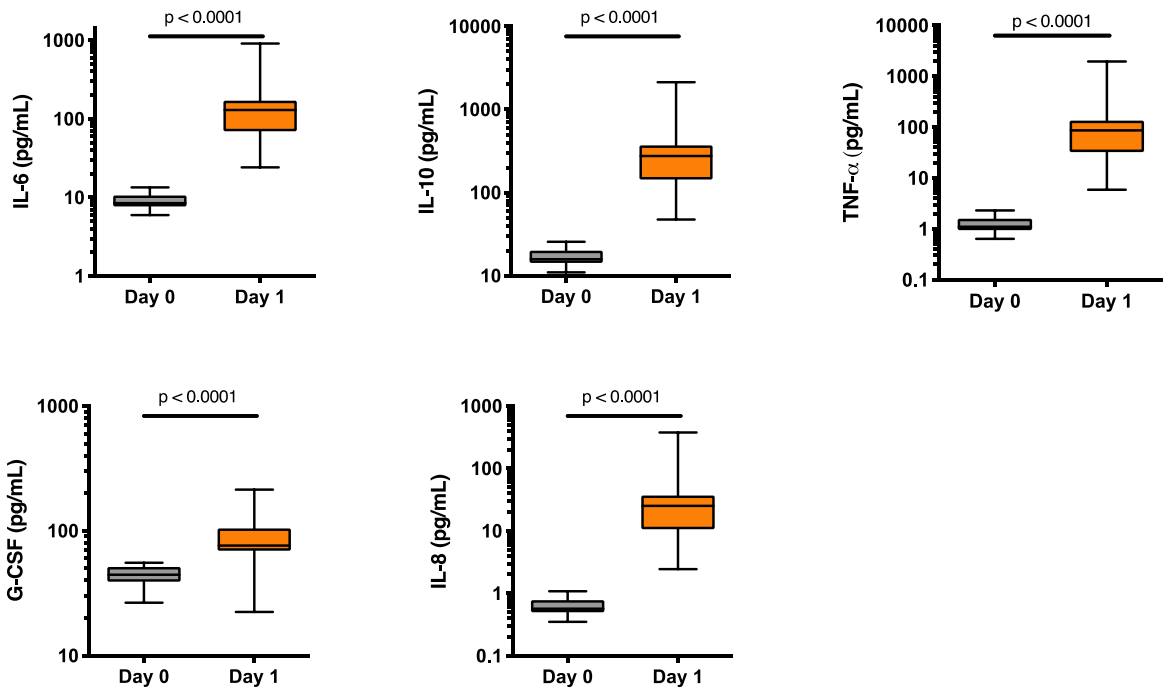
**Table 1. Patients characteristics**

N	33
Male (%)	23 (69.9%)
Age (years)	71 (63 – 79)
BMI (kg/m <sup>2</sup> )	24.4 (19.7 – 28.7)
HTA	24 (72.7%)
Diabetes	1 (3%)
Dyslipidemia	17 (51.5%)
Smoking	
Active	3 (9%)
Weaned	7 (21.2%)
None	23 (69.7%)
Creatinine (μmol/L)	89 (70 – 103)
Hemoglobin (g/dL)	13.8 (13 – 14.5)
Cardiopulmonary bypass duration (minutes)	78 (66 – 99)
Operative procedure	
Any cardiac valve	24 (72.7%)
Any coronary artery bypass	14 (42.4%)
Isolated cardiac valve	19 (57.5%)
Isolated coronary artery bypass	9 (27.2%)

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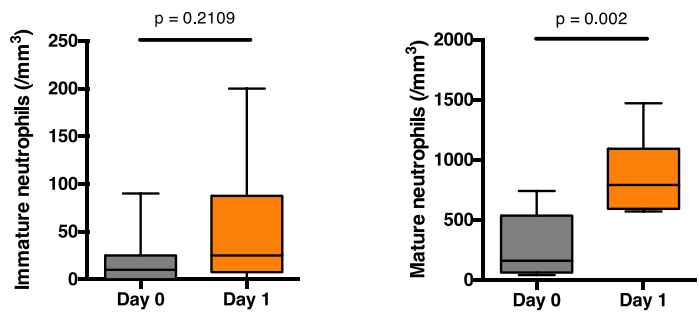


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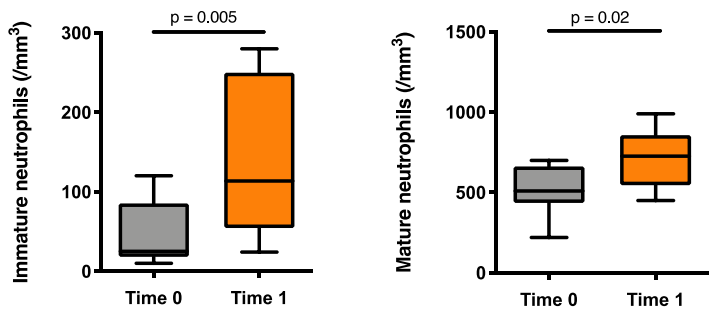




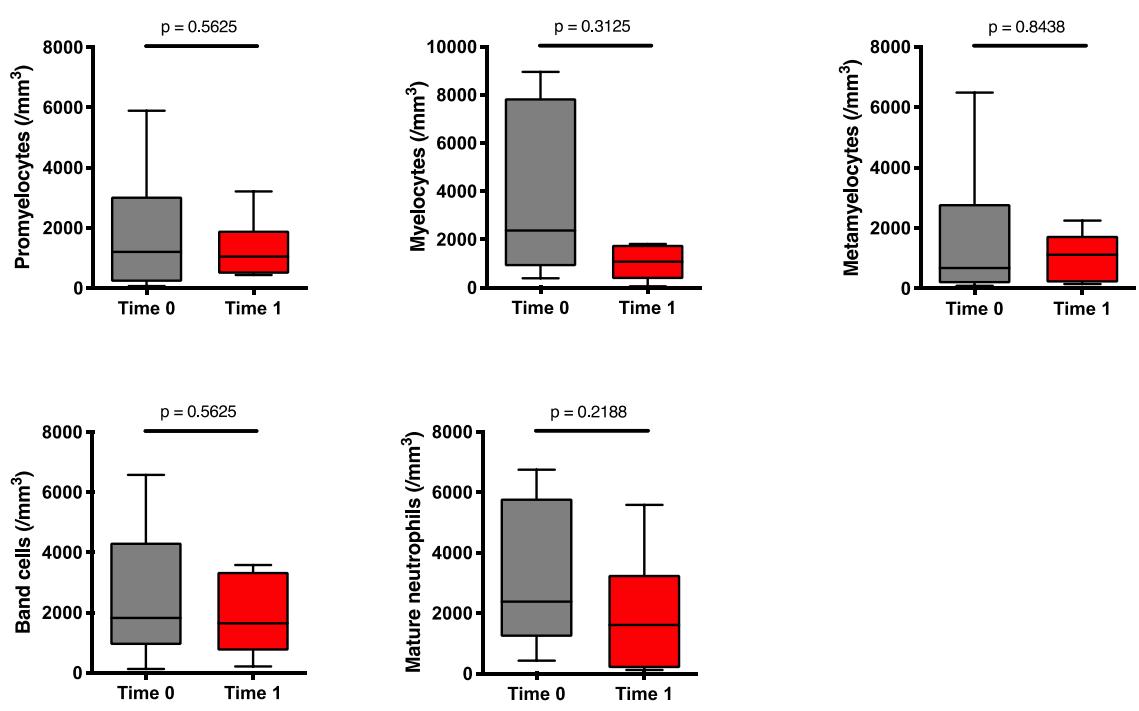
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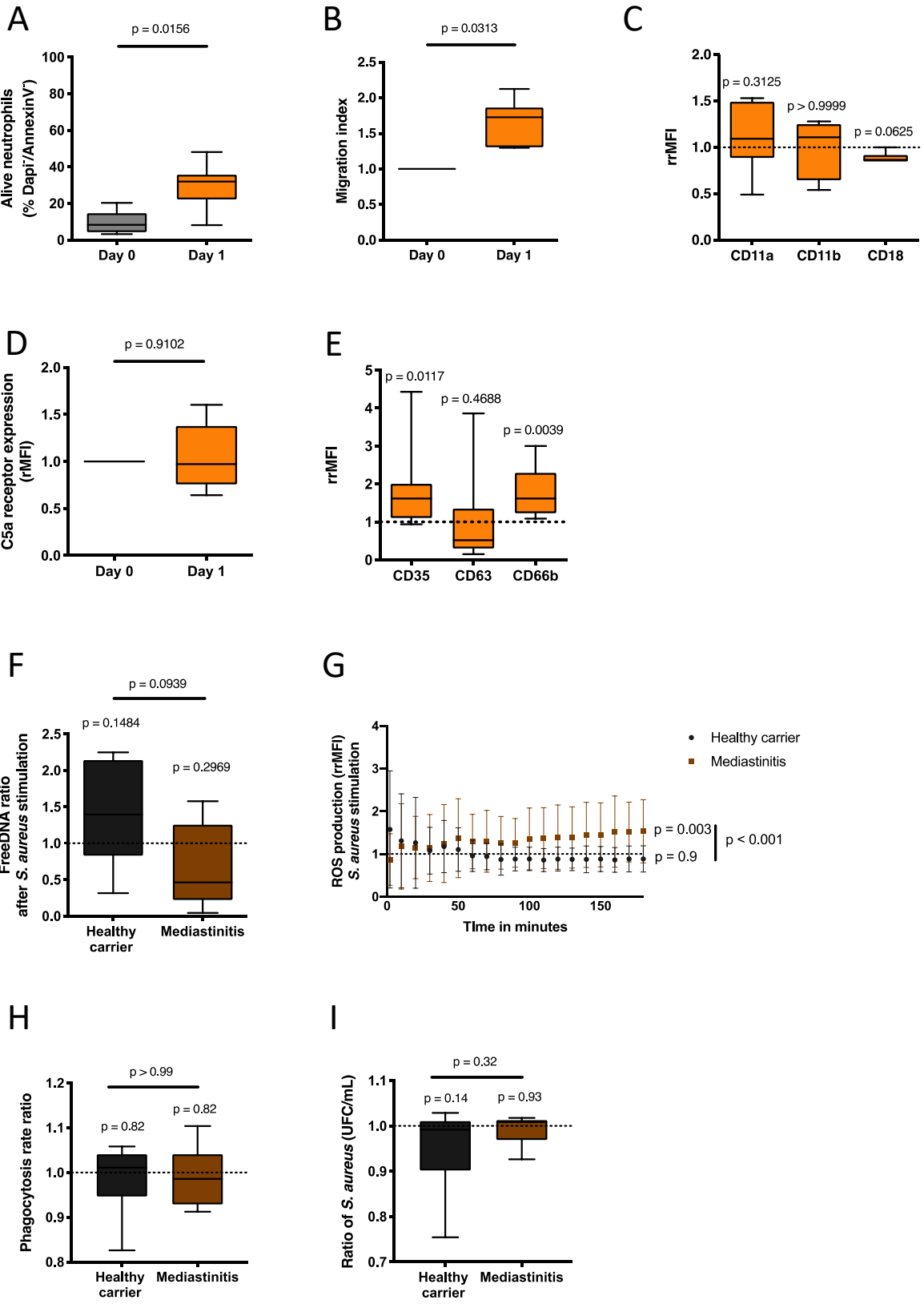


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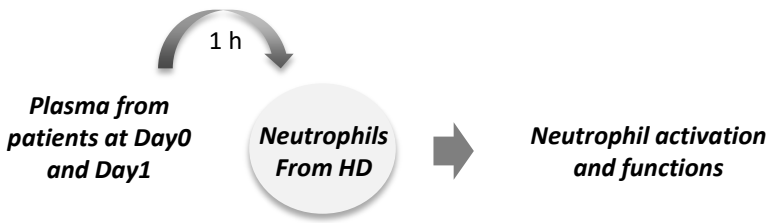


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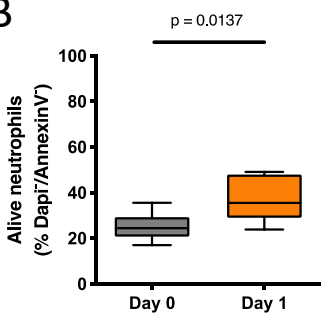




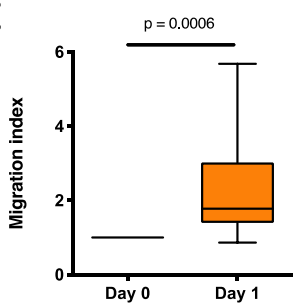
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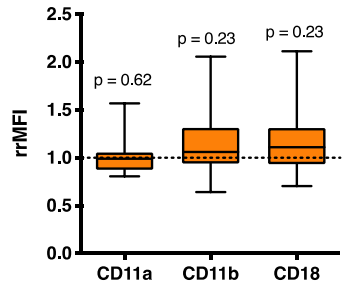
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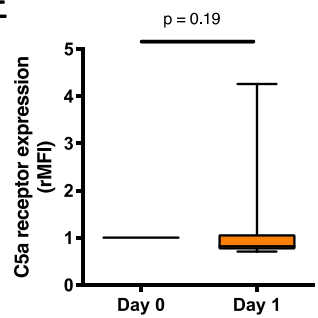
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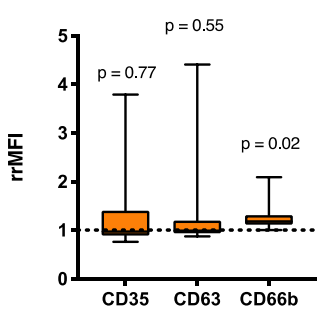
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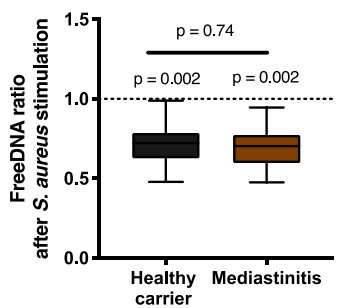
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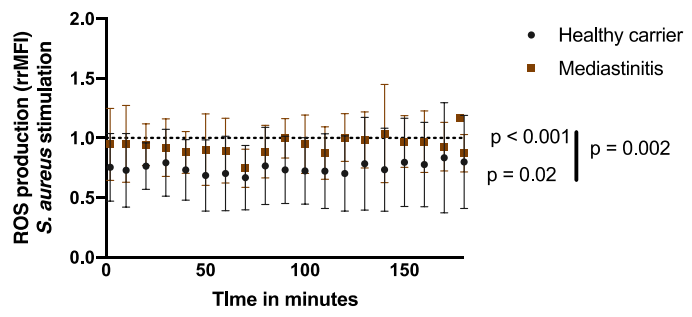
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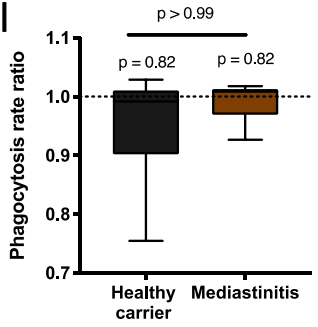
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