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Elevated serum levels of S100A8/A9 and HMGB1 at hospital admission are correlated with inferior clinical outcomes in COVID-19 patients

Liting Chen[1], Xiaolu Long[1], Qian Xu[1], Jiaqi Tan[2], Gaoxiang Wang[1], Yang Cao, Jia Wei[1], Hui Luo[1], Hui Zhu[3], Liang Huang[1], Fankai Meng[1], Lifang Huang[1], Na Wang[1], Xiaoxi Zhou[1], Lei Zhao[1], Xing Chen[1], Zekai Mao[1], Caixia Chen[1], Zhen Li[3], Ziyong Sun[4], Jianping Zhao[5], Daowen Wang[6], Gang Huang[7], Wei Wang[8] and Jianfeng Zhou[1]

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COVID-19 is a disease with heterogeneous clinical appearances. Most patients are asymptomatic or exhibit mild to moderate symptoms; approximately 15% progress to severe pneumonia and about 5% are eventually admitted to the intensive care unit (ICU) due to acute respiratory distress syndrome (ARDS), septic shock and/or multiple organ failure. ICU patients respond poorly to currently available treatments and exhibit a high mortality rate.[1–3] Inadequate identification of the determinants of fatal outcomes is one of the major obstacles to the improvement of the outcomes in severe COVID-19 patients. A previous study reported a scoring system (COVID-GRAM) which accurately predicted the occurrence of critical illness in hospitalized COVID-19 patients.[4] Damage-associated molecular patterns (DAMPs), or alarmins, are a number of molecules, released by stressed cells undergoing microbial infection or sterile injury, that act as danger signals to promote and exacerbate the inflammatory response.[5,6] Of note, the serum level of S100A8/A9 and HMGB1 was found to be correlated with both the severity of pathogen-associated tissue damage and excessive cytokine storm.[7] Despite the hypothesis that S100A8/A9 and HMGB1 are significantly involved in COVID-19, so far, no study has yet tried to substantiate the hypothesis. In this study, we aimed to define the role of S100A8/A9 and HMGB1 in progression to a fatal outcome and develop clinically relevant risk strata for COVID-19 patients.

A total of 121 patients were enrolled in this retrospective study, of which 40 patients were in ICU and 81 patients in general wards at enrollment (Table S1). ICU Patients had much higher COVID-GRAM risk scores in comparison to those in general wards. Complications, including ARDS, sepsis, septic shock, secondary infection, acute renal injury, acute cardiac injury or failure, were more frequent in CCOVID-19 patients admitted to ICU. As of the cutoff date of April 30, 2020, most of non-ICU patients (96.3%) had been discharged alive, while 82.5% of ICU patients had died in ICU.

COVID-19 patients treated in general wards had significantly elevated level of S100A8/A9 ($P = 0.033$) but not HMGB1 ($P > 0.9999$) as compared to healthy controls, suggesting that S100A8/A9 is a more sensitive alarmin than HMGB1 in response to SARS-CoV-2 infection. However, both S100A8/A9 and HMGB1 were significantly elevated extracellularly in ICU-admission patients compared to non-ICU patients, or in fatal outcomes patients compared to alive patients (Fig. 1a–d), indicating that significant elevation of S100A8/A9 and HMGB1 was associated with high mortality.

We further examined the Spearman's correlation between the serum S100A8/A9 or HMGB1 levels and clinical manifestations in COVID-19 patients. First of all, either serum levels of S100A8/A9 or HMGB1 at admission were positively correlated with peak CT score and oxygen demand, which is indicative of the severity of acute lung injury and ARDS (Fig. 1e, f and S1a, b). Moreover, the degree of organic impairment, as evaluated by the MCP classification, NT-proBNP level, cTn I level, and AKI stage were well correlated with the serum levels of S100A8/A9 or HMGB1 (Fig. S1c–j). The level of peak D-dimer significantly was elevated as the serum S100A8/A9 or HMGB1 increased (Fig. 1g, h). On the other hand, the ratio of neutrophils to lymphocytes was positively correlated with the serum S100A8/A9 but not with HMGB1, suggesting S100A8/A9 plays a more important role in the substantial reduction of the peripheral lymphocytes. The serum S100A8/A9 was strongly correlated with the qSOFA score,[8] a quick indicator of sepsis-related organ dysfunction, indicating that patients with higher S100A8/A9 or HMGB1 levels tended to suffer from more severe sepsis-related organ dysfunction (both $P < 0.0001$) (Fig. 1i–l).

We then evaluated the potential correlation between levels of S100A8/A9 or HMGB1 and COVID-GRAM risk scores. Either levels of S100A8/A9 ($P < 0.0001$) or HMGB1 ($P = 0.030$) significantly correlated with the COVID-GRAM risk scores (Fig. 1m, n). Serum levels of S100A8/

[1]Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095 Jiefang Avenue, Wuhan, Hubei 430030, China; [2]Department of Pediatrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095 Jiefang Avenue, Wuhan, Hubei 430030, China; [3]Department of Medical Imageology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095 Jiefang Avenue, Wuhan, Hubei 430030, China; [4]Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095 Jiefang Avenue, Wuhan 430030, China; [5]Department of Respiratory and Critical Care Medicine, Key Laboratory of Pulmonary Diseases of Health Ministry, Key Cite of National Clinical Research Center for Respiratory Disease, Tongji Hospital, Tongji Medical College Huazhong University of Science and Technology, No. 1095 Jie Fang Avenue, Wuhan 430030, China; [6]Division of Cardiology, Department of Internal Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China; [7]Divisions of Pathology and Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Room S7.224, Cincinnati, OH, USA and [8]Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095 Jiefang Avenue, Wuhan 430030 Hubei, China
Correspondence: Gang Huang (Gang.Huang@cchmc.org) or Wei Wang (wwang@vip.126.com) or Jianfeng Zhou (jfzhou@tjh.tjmu.edu.cn)
These authors contributed equally: Liting Chen, Xiaolu Long, Qian Xu, Jiaqi Tan

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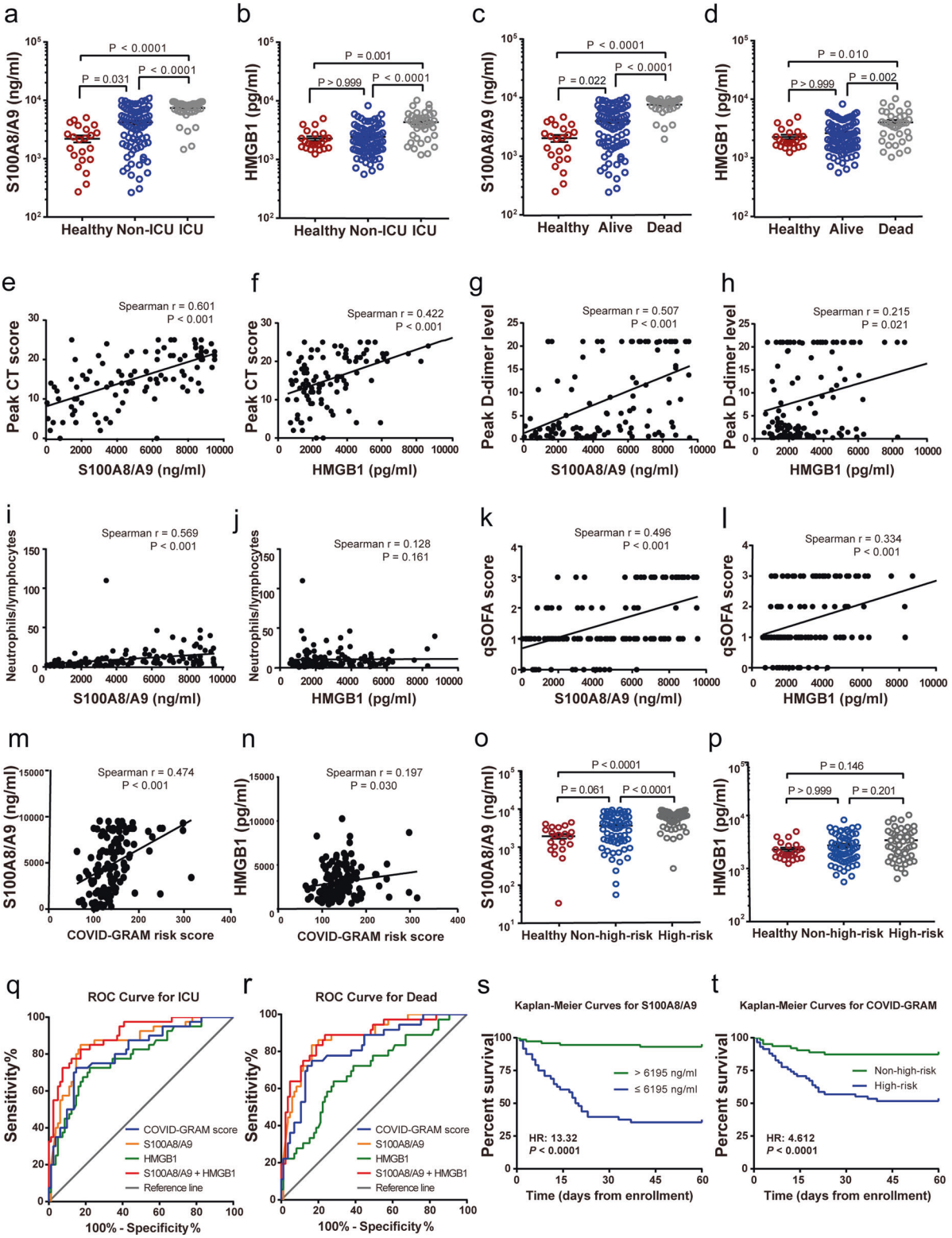

Fig. 1 Serum levels of S100A8/A9 and HMGB1 were strongly correlated with the severity of clinical manifestations and with great predictive power for the risk for ICU admission and death. **a**, **b** Comparison of S100A8/A9 and HMGB1 levels between healthy people, non-ICU patients, and ICU patients of COVID-19. **c**, **d** Comparison of S100A8/A9 and HMGB1 levels between healthy people, alive patients and dead patients of COVID-19. Spearman's correlation analyses between S100A8/A9 or HMGB1 levels and peak CT score (**e**, **f**), D-dimer level (**g**, **h**), neutrophil/lymphocyte ratio (**i**, **j**), and quick Sequential Organ Failure Assessment (qSOFA) scores (**k**, **l**). **m**, **n** Spearman's correlation analyses of S100A8/A9 or HMGB1 and COVID-GRAM risk score. **o**, **p** Comparison of S100A8/A9 or HMGB1 between healthy people and COVID-19 patients of different risk groups (divided according to COVID-GRAM risk score). **q**, **r** Receiver operating characteristic (ROC) curve evaluation of the performance of S100A8/A9, HMGB1, combined S100A8/A9 and HMGB1, and COVID-GRAM risk score in distinguish COVID-19 patients with ICU admission or subsequent death. Sixty days survival is shown for patients with different circulating S100A8/A9 levels (**s**) and different COVID-GRAM risks (**t**) by Kaplan–Meier curves

A9 but not HMGB1 were significantly increased in patient with high risk of COVID-GRAM risk scores which were defined according to the online calculator (http://118.126.104.170)[4] (Fig. 1o, p), highlighting that S100A8/A9 is a better prognostic indicator than HMGB1.

The prognostic values of S100A8/A9, HMGB1 or COVID-GRAM risk scores were further evaluated by ROC analysis and their areas under curve were calculated (Fig. 1q, r). For the prediction of ICU admission, the AUCs for COVID-GRAM risk scores with S100A8/A9, HMGB1 and S100A8/A9 and HMGB1 in combination were 0.810, 0.860, 0.781 and 0.901, respectively (Table S2). For the prediction of subsequent death, the AUCs for COVID-GRAM risk scores, S100A8/A9, HMGB1 and S100A8/A9 and HMGB1 in combination were 0.818, 0.875, 0.694 and 0.881, respectively (Table S2). The sensitivity and specificity of S100A8/A9 and HMGB1 of the ROC curves illustrated in Fig. 1q, r were listed in Table S2. The combination of S100A8/A9 with HMGB1 increased the prediction power compared to S100A8/A9 or HMGB1 alone although no significant differences were observed statistically (Table S2).

Moreover, higher S100A8/A9 level ($P < 0.0001$) or higher COVID-GRAM risk score ($P < 0.0001$) resulted in significant worse overall survival (Fig. 1s, t). The COVID-19 patients were classified into low or high level groups according to the concentrations of S100A8/A9 at a cutoff of 6195 ng/ml, which was set by maximizing Youden's index according to the ROC curves (Fig. 1s). Meanwhile, the COVID-19 patients were also classified into high-risk or non-high-risk groups according to COVID-GRAM risk scores (Fig. 1t). The hazard ratio of high S100A8/A9 level was 13.32, which was greater than that of COVID-GRAM risk score (HR = 4.612). The concentrations of S100A8/A9 measured at hospital admission showed better predictive power than COVID-GRAM risk scores for subsequent death in COVID-19 patients.

To explore the possible correlation between S100A8/A9 or HMGB1 and cytokine storm in COVID-19, the serum levels of S100A8/A9 or HMGB1 were analyzed by Spearman's correlation tests to know their correlation with of each of the 48 cytokine concentrations in individual patients (Table S3). While the serum levels of S100A8/A9 or HMGB1 correlated with the concentrations of a different spectrum of pro-inflammatory cytokines, 12 cytokines, including IL-8, MCP-3, MCP-1, IL-1ra, β-NGF, IL-7, IL-10, RANTES, G-CSF, IL-1α, CTACK and IL-17A, simultaneously correlated with both the S100A8/A9 and HMGB1. Interestingly, 3 myeloid chemokines, IL-8, MCP-3 and MCP-1, were among the most significant cytokines simultaneously correlated with both the S100A8/A9 and HMGB1 and showed the lowest P value ($P < 0.0001$), indicating that the overproduced S100A8/A9 and HMGB1 in serum were associated with distinct signatures for cytokine storm in patients with COVID-19.[9,10]

In conclusion, this study identified two alarmins, especially S100A8/A9, could accurately identify patients who were subsequently admitted to ICU wards or died with a predictive precision similar to or better than COVID-GRAM risk score. Taking S100A8/A9 as a predictor for COVID-19 might offer some advantages over other clinical or laboratory indicators, since it is easy to measure and the result is easy to interpret. As a single parameter alone, to our knowledge, S100A8/S100A9 is the only one helpful for an early identification of COVID-19 patients who may be admitted to ICU admission or facing death. Using S100A8/A9 to distinguish COVID-19 patients with fatal outcomes is of great clinical significance.

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AUTHOR CONTRIBUTIONS

Conception and design: Jianfeng Z, WW and GH. Acquisition, analysis, or interpretation of data: LC, XL, QX, JT, GW, YC, JW, HL, HZ, CC. Patient care and clinical information provision: YC, Liang H, FM, Lifang H, NW, XZ, LZ, XC, ZM, Manuscript drafting: Jianfeng Z, LC, GW, YC. Critical revision of the manuscript for important intellectual contents: Jianfeng Z. Statistical analysis: LC, XL, JT, YC. Financial support: Jianfeng Z and WW. Administrative and technical support: Jianfeng Z, ZS, ZL, Jianping Z, DW, GH, WW. Supervision: Jianfeng Z, WW.

ADDITIONAL INFORMATION

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