Editorial

Stressing the endoplasmic reticulum response as a diagnostic tool for sepsis

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Sepsis, the life threatening organ dysfunction due to a dysregulated host response to infection (1,2), is the leading cause of death in critically ill patients in intensive care units (3,4). Despite the high mortality rate, effective therapies beyond the standard of care and supportive therapy are limited (5). Identification of at-risk patients and early diagnosis of sepsis is an unmet need. While several biomarkers have been proposed, the heterogeneity of critically ill patient populations hinder their diagnostic and prognostic utility (6). Delineating the biological process involved in the pathogenesis of sepsis may reveal strategies to initiate timely intervention and improve outcomes in septic patients. Advances in endoplasmic reticulum (ER) stress signalling have recently revealed novel interactions between sepsis and ER stress associated cell death (7,8). Targeting ER stress with pharmacological or gene therapy strategies has proven successful in reducing pathological features in various experimental models of inflammatory diseases (7,9,10).

Glucose-regulated protein 78 (GRP78) is a key molecular chaperone responsible for protein folding in the ER. An accumulation of misfolded proteins which exceeds the protein-folding capacity in the ER results in ER stress (11). ER stress can be mitigated through the activation of the unfolded protein response (UPR), where GRP78 dissociates from three transmembrane proteins, such as protein kinase RNA-like endoplasmic reticulum kinase (PERK), activating transcription factor-6 (ATF-6), and inositol-requiring kinase 1 alpha (IRE1α). These molecules decrease protein translation as well as increase chaperone expression to alleviate ER stress (11). However, prolonged ER stress leads to the activation of downstream PERK mediators including activating transcription factor-4 (ATF-4) as well as C/EBP homologous protein (CHOP), which is a known transcriptional inducer of apoptotic cell death by direct activation of several caspases (12). CHOP has been identified as a driver of ER stress-induced apoptosis (13-16). Recent evidence also implicates CHOP as a mediator of sepsis-related inflammation. Increased H_2S induced CHOP expression was observed in a mouse model of sepsis, where inhibiting CHOP expression improved survival rate (7). Suppressing ER-mediated apoptosis is a viable treatment strategy to reduce sepsis-induced lymphocyte apoptosis in animal models (8,17). Notably, increased ER stress results in aberrant lymphocyte apoptosis in the cecal ligation and puncture model of sepsis in mice (8,17). These findings further demonstrate an essential role for ER stress-induced CHOP in the pathogenesis of septic injury in animal models as depicted in *Figure 1*.

In this publication Li *et al.* (18) test whether GRP78 and CHOP protein expression serve as potential biomarkers for sepsis diagnosis. This appears to be a follow-up study

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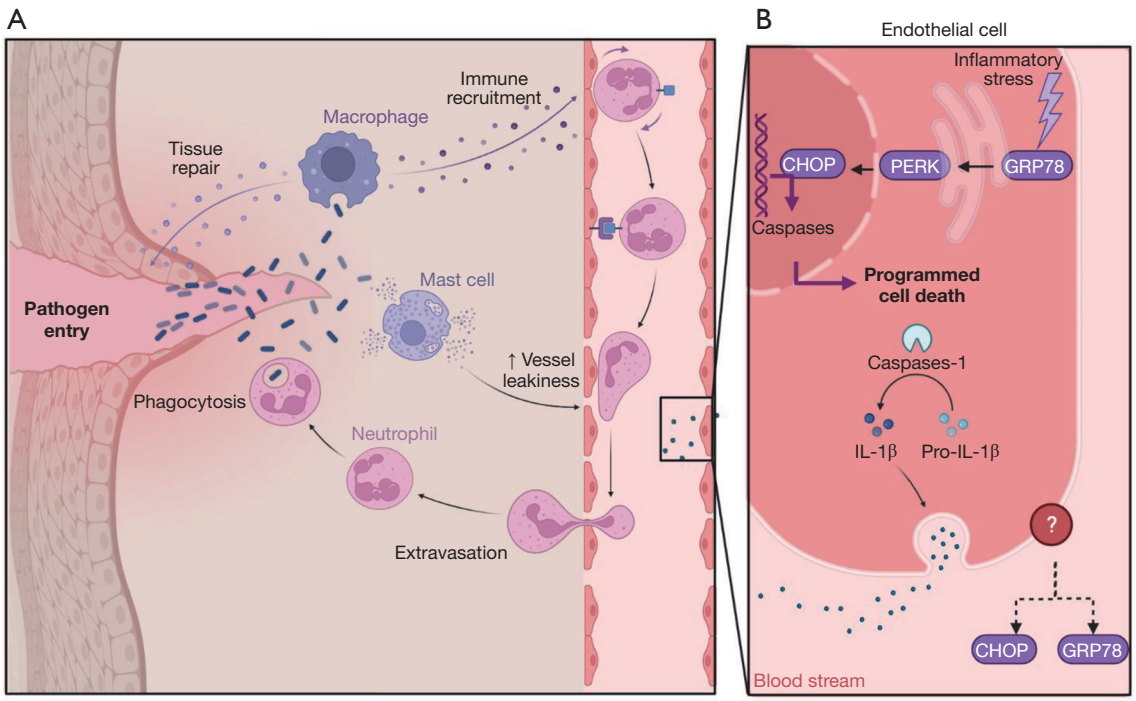

Figure 1 Sepsis induced ER stress mediators of inflammation and cell death. (A) Once pathogens enter and infect the endothelium, leukocytes and monocytes become active and migrate to the site of infection. Leukocytes and monocytes adhere to the endothelium, roll, and enter the interstitial layer through extravasation. Within the interstitial layer, neutrophils will undergo phagocytosis to engulf pathogens. Immune cells such as, mast cells can enter and release antimicrobial peptides and cytokines. Simultaneously, monocytes will differentiate into macrophages and secrete chemokines to signal the migration of immune cells. Macrophages can also secrete chemokines to induce an inflammatory response as well as engulf pathogens. M2 macrophages are also involved in tissue repair. (B) Inflammation induces GRP78 detachment from the three arms of the UPR (PERK, IRE1α and ATF-6). GRP78 activation leads to PERK mediated CHOP induction. CHOP acts as a known transcriptional activator of several caspases and is a potent inducer of programmed cell death. Caspases promote the inflammatory response through cleavage and release of IL-1β from endothelial cells into the bloodstream. Li *et al.* suggest that serum CHOP and GRP78 can be detected in high levels in patients with sepsis, however, the exact mechanism by which they accumulate in the circulation is unknown. Identifying how these ER stress-response factors are released from injured endothelial cells and whether their increased release in the circulation correlate with elevated levels of autoantibodies, another potential biomarker, would further enhance these interesting findings by Li *et al.* (18). Diagram was made with BioRender by TY and HS. ER, endoplasmic reticulum; UPR, unfolded protein response.

in which Ma *et al.* (19) assessed the diagnostic utility of GRP78 and CHOP mRNA expression in serum of septic patients. In this single center cohort study, patients admitted to the ICU were grouped in either the sepsis group (group I) or non-septic but infected group (group II) based on the Sepsis-3 definition (2). Sequential blood samples were obtained from patients after 1-, 2-, 3-, and 7-day post-ICU admission. Levels of GRP78 and CHOP were assessed by enzyme-linked immunoassay from frozen samples. Septic patients at day 1 had higher levels of GRP78 (208.79±21.76 ng/mL) and CHOP (6.56±0.69 ng/mL) compared to group II GRP78 levels (190.55±46.87 ng/mL, P=0.021) and to group II CHOP levels (5.81±0.57 ng/mL, P<0.001), respectively. To assess the predictive potential of GRP78 and CHOP as diagnostic markers of sepsis, receiver operating characteristic (ROC) curve analysis was performed. Levels of GRP78 and CHOP in patients at day 2 displayed the greatest efficacy in diagnosing sepsis. The threshold value associated with GRP78 is 157.29 ng/mL, the specificity is 75%, sensitivity is 73.1% (P<0.05) and the area under the curve (AUC) is 0.771. This analysis suggests that GRP78 levels correlate with moderate sensitivity and specificity in the diagnosis of sepsis. For CHOP, the threshold value is 4.951 ng/mL, the specificity is 96.2%,

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and sensitivity is 57.7% (P<0.05). While CHOP showed a high specificity for sepsis, it scored rather low for sensitivity suggesting that it may not be able to accurately determine patients who are infected. Day 2 expression of GRP78 and CHOP was used in comparison to traditional indicators routinely employed in the clinical assessment of infections. In this comparison, procalcitonin (PCT) and C-reactive protein (CRP) exhibited superior AUC under ROC curve (AUROC) values in comparison to GRP78 and CHOP, however, GRP78 and CHOP indicated higher AUROC than that of interleukin-6 (IL-6) and lactic acid (LAC). The combination of GRP78 + CHOP yielded the best AUROC curve next to PCT which suggests that these markers are similarly expressed to the current clinically used markers; however, at 2 days into ICU admission this may be too late to be practical.

These observations increase our knowledge of sepsis-induced ER stress activation and reveal novel target strategies for the diagnosis and treatment of sepsis. The biological mechanisms related to the functional role of serum GRP78 and CHOP remain unclear. Although CHOP upregulation may induce cell death and contribute to vascular injury in various cell types, it is unclear how these molecules are released into circulation. During ER stress, GRP78 has been found to migrate to the cell surface, possibly secreted via exosomes or theoretically released along with other cellular contents by caspase-1 induced pyroptosis (4,20,21). GRP78 has antigenic qualities which can be targeted by immune cells to form anti-GRP78 autoantibodies (20). Anti-GRP78 autoantibodies are positively associated with prostate cancer progression and atherosclerotic lesion development (20,22). The upregulation of GRP78 in septic patients suggest a plausible role for anti-GRP78 autoantibodies in the progression of sepsis. To date, anti-GRP78 autoantibodies have not been investigated in relation to sepsis progression, however, the findings by Li *et al.* (18) warrant further investigation into this potential diagnostic marker. Measured changes of potential diagnostic markers serve the highest utility within the first 48 hours of hospital admission. Extending the study timepoints to one week did not result in prognostic value.

The search for a reliable and practical diagnostic biomarker for sepsis has become a seemingly unattainable goal. Sepsis is a heterogenous disease with variable organ dysfunction and clinical presentation. Exploring biomarkers that align with the known mechanisms that lead to the pathogenic inflammatory response including activation of coagulation and subsequent tissue injury are the most attractive (23). Most patients present to hospital and ICU with community acquired infections leading to sepsis so any potential diagnostic test should be readily available as early in the clinical presentation as possible.

Although standards are available for designing and reporting both diagnostic (24) and prognostic tests (25), most studies, similar to the current study, are limited to single centre studies, measure the marker too late in the presentation, often limit this to an ICU cohort and have a relatively small sample size. This highlights the need for larger observational study trials to examine both the diagnostic and prognostic potential of GRP78 and CHOP expression. Future research should address whether mitigating ER stress with chemical chaperones such as 4-phenylbutyrate can reduce the organ dysfunction associated with sepsis.

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