In order to study the inflammatory events, a murine model of poly-microbial sepsis was optimized using surgical method of cecal ligation and puncture (CLP). The precision in surgery was such that all mice died 34-36 hours post-surgery. Mice with sham and cecal ligated (CL) surgeries were taken as control which survived normally. CL mice showed a granuloma type necrotic growth at the site of cecum ligation, which regressed after 14-15 days of surgical intervention. The model was characterized in terms of various inflammation/ sepsis associated parameters (physiological, behavioral, immunological, histological and biochemical ones). The constant pro-inflammatory gene activation and/ or failure in anti-inflammatory gene activation was/were suspected to be the reason(s) for death observed in CLP mice. Such an alteration in gene expression could be attributed to chromatin states i.e., euchromatin and heterochromatin. Thus, the involvement of epigenetic mechanisms (specifically histone post-translational modifications) in inflammation/ sepsis was dissected out.

Considering the role of global histone modifications in tumor types, prognostic factors and patient outcomes, we also tried to observe global histone modification profiles of various histone site-specific antibodies in septic animals. Since continuous sampling of tissue for studying kinetics of histone modification from same mouse was not feasible, samples were collected from different mice at each time point. Such an experimental plan necessitated to analyze the individual-to-individual variation in terms of each histone modification used. We did not find any significant difference in mice from same colony with similar physiological status, under same environmental conditions. Though no direct, unidirectional correlation between any particular histone modification and sepsis could be traced, we did observe significant perturbations in histone modification status, indicating involvement of epigenetics in inflammatory/ septic events. The overall analysis indicates increase in acetylation at early phase of sepsis progression, which might be attributed to pro-inflammatory gene activation. Whereas inhibitory marks, as revealed by histone methylation, were observed during late sepsis. This might be involved in resolving the inflammatory process by down-regulating pro-inflammation but failed to do so, probably because of gravity of infection.
Expression level of TGF-β1 gene was analyzed using RT-PCR, to detect if it was the failure of anti-inflammatory branch to resolve the inflammation and restore homeostasis. TGF-β1 levels were found to be increasing during late sepsis, indicating along with its increased serum level that anti-inflammatory branch is functional. The status of TGF-β1 promoter was analyzed for histone modifications using chromatin immunoprecipitation. The transcription activation mark (histone H3 acetylation at lysine 9) was observed in late sepsis whereas transcription repression mark (histone H3 trimethylation at lysine 27) was not detected. Though the present study still has a number of lacunae, an attempt was made to understand epigenetic-inflammation link. For the first time, global histone modification status of inflamed/ septic liver was studied. Also, the promoter specific histone modification status of anti-inflammatory gene TGF-β1 was analyzed. The future studies must address cell- specific global histone modification status during sepsis, an elaborate kinetics of pro- anti-inflammatory mediators to envisage their roles in sepsis, and contribution of metabolomics in the epigenetic status of CLP induced sepsis. These studies would be of paramount importance to understand epigenetic-inflammation/ sepsis link.