# Bacterial Translocation in Thoracic and/or Head Trauma

## Toraks ve/veya Kafa Travmasında Bakteriyel Translokasyon

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**ABSTRACT Objective:** Bacterial translocation (BT) is among the critical components in the progress of multiple organ failure. We performed this study to investigate whether BT with such serious complications occurs following isolated blunt thoracic trauma or multiple traumas including blunt thoracic and head trauma components. **Material and Methods:** In this study, 48 Wistar rats were randomly divided into four groups (n= 12) as control (C), thoracic trauma (TT), head trauma (HT) and head+thoracic trauma (HTT) groups. Following sacrifice at 24 hours after trauma multiple specimens from mesenteric lymph nodes, spleen, liver, lung, and ileum and blood samples were collected from all rats and were evaluated for bacterial growth after inoculation onto 5% blood agar. **Results:** When compared to other groups, the incidence of BT in the HTT group was significantly higher (p= 0.003). Although the rate of BT in the TT group seemed higher than C and the HT groups, the difference was not significant. **Conclusion:** TT concurrent with HT resulted in a significantly higher rate of BT. Further studies to clarifty the correlation between inflammatory responses following head and TTs and BT are important in the sense to reduce mortal consequences of this phenomenon significantly in the near future.

**Key Words:** Bacterial translocation; thoracic injuries; head injuries

**ÖZET Amaç:** Bakteriyel translokasyon (BT), çoklu organ yetmezliği gelişmesinde kritik komponentlerden biridir. Bu çalışmanın amacı, izole künt toraks travması ile künt toraks travması ve kafa travmasını da içeren multipl travmaları, bakteriyel translokasyon gelişmesi açısından karşılaştırmaktır. **Gereç ve Yöntemler:** Çalışmamızda 48 adet Wistar cinsi sıçan rastgele, kontrol (C), toraks travması (TT), kafa travması (HT), kafa + toraks Travması (HTT) olmak üzere 4 gruba ayrıldı. Tüm sıçanlar, travmadan sonraki 24. saatte öldürüldü; mezenterik lenf bezleri, dalak, karaciğer, akciğer ve kan örneklerinde bakteriyel üreme gelişip gelişmediği %5'lik kanlı agar besiyerine ekimleri yapılarak değerlendirildi. **Bulgular:** HTT grubu diğer gruplarla karşılaştırıldığında bakteriyel translokasyonun bu grupta anlamlı derecede daha fazla olduğu görülmüştür (p= 0.003). TT grubundaki üreme HT ve kontrol grubuna göre daha fazla olmakla birlikte,aradaki fark istatistiksel açıdan anlamı değildir. **Sonuç:** TT ile birlikte HT olduğunda BT'nin belirgin olarak daha yüksek oranda gerçekleştiği görülmüştür. Multipl travmalardan sonra gelişen yangısal yanıt ile BT arasındaki korelasyonun yapılacak çalışmalarla aydınlatılması sayesinde bu olguların ölümcül sonuçlarının yakın gelecekte ciddi oranda azaltılabileceğine inanıyoruz.

**Anahtar Kelimeler:** Bakteriyel translokasyon; torasik yaralanmalar; kafa travması

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One-third of patients admitted to hospitals following a blunt thoracic trauma may develop pulmonary contusion that can progress to pneumonia, Acute lung injury (ALI) or adult respiratory distress

syndrome (ARDS), which still have an overall mortality rate of 10-25%, despite considerable advances in intensive care management in recent years.[1,2]

Several previous studies have shown in detail that a serious inflammatory response develops in the first 24-48 hours following trauma, which normalizes by the end of the 7th day.[1,3,4] It has been reported that to clearly elucidate the pathogenesis of post-contusion pneumonia, ALI and ARDS, some other co-factors which may contribute to this inflammatory response need to be investigated as well.[5,6]

The passage of intestinal bacteria and/or their endotoxins across the intestinal barrier is referred to as "bacterial translocation (BT)".[7] The phenomenon of BT can occur following many conditions such as abdominal trauma, abdominal compartment syndrome, malnutrition, malignancies, burns, hemorrhagic shock and exposure to radiation.[8-11]

Thus, regarding trauma as a possible cause of mortalities such as sepsis and multiple organ failure (MOF), we designed this study to assess whether BT occured following isolated TT and/or HT including TT.

## MATERIAL AND METHODS

All experiments were performed in adherence to the "National Institutes of Health Guidelines on the Care and Use of Laboratory Animals". The approval of the Ethics Committee was obtained before the onset of the study.

In this study, forty-eight albino Wistar rats weighing between 300-350 g were randomized into four groups as C (n= 12), TT; n= 12, HT; n= 12 and HTT; n= 12. The animals were given a water suspension including 80000 cfu/mL *Escherichia coli* in addition to standard rat chow 24 hours prior to trauma. In all groups, anaesthesia was induced with 25 mg/kg$^{-1}$ thiopenthal sodium (Pental, İE Ulagay, Turkey) before the trauma. Group C received only anaesthesia without any trauma. Group TT was exposed to TT with a force of 2.7 J using the mechanism published by Raghavendran et al followed by anaesthesia.[3] In the HT group, sequential thoracic and head traumas were created in the same manner as mentioned above in group HTT (Figure 1).[12]

Twenty-four hours after the trauma, the rats were anaesthetized, and the chest cavities were opened. The animals were euthanized by cardiac blood aspiration. Blood samples (1 mL each) were incubated aerobically for 48 hours at 37$^0$ C in 5 mL of triptych soy broth and were plated on triptych soy agar with 5% blood. Liver, spleen, mesenteric lymph nodes and lung were extracted, were weighed separately and were placed in a sterile grinding tube. The samples were homogenized with 1 mL of triptych soy broth using sterile ground glass stoppers. After grinding (Pottere S, Biolab, Melcungen-Germany), 500 µL of homogenate was transferred into a tube containing 4.5 mL of 0.9% NaCl and was used to perform four serial dilutions; the final dilution was $10^{-4}$. From this dilution, 100-µL aliquots were inoculated onto two different plates, tyriptic soy agar with 5% blood and EMB agar. Quantitative culture results were determined by the number of colony-forming units (CFU) per

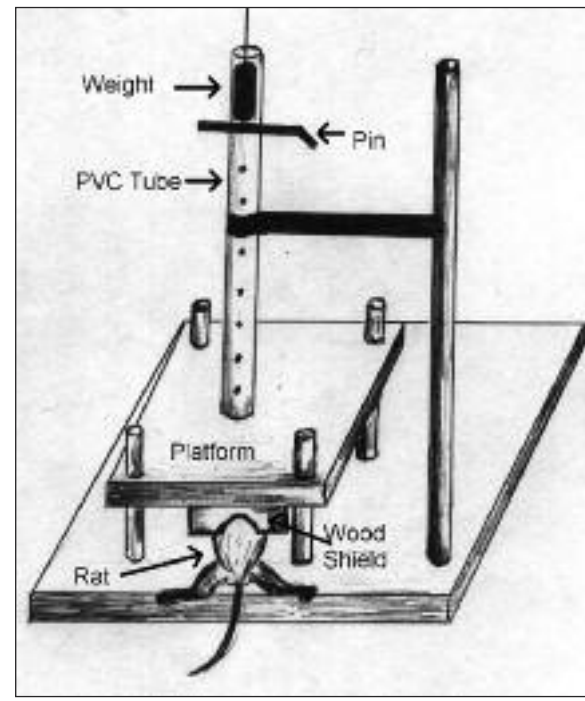



**FIGURE 1:** Schematic representation of the isolated bilateral pulmonary contusion model: The precordial wood shield allowed the induction of bilateral pulmonary contusion but spared the mediastinum and associated structures.

gram calculated from the dilutions of tissue homogenate cultures with the following formula; [(number of CFU x reciprocal of dilution x10) / weight of tissue].[13] Finally, the terminal ileal loop was excised to determine indigenous bacteria and was placed in a tube of thioglycollate broth. To prevent contamination from the environment, the cultures were prepared as the last step of the procedure. All agar plates and thiglycollate broth tubes were incubated aerobically in 5% $CO_2$ for 24 hours at $37^0$C. We did not study obligate anaerobes because they are rare causes of translocation.[13] The Gram negative enteric organisms were identified by their biochemical profiles; *Staphylococcus* was identified by the catalase test and the tube coagulase test, and *Enterococcus* was identified by esculin hydrolysis in the presence of bile and grown in broth containing 6.5% NaCl.

Chi-square analysis in SPSS 11.5 was used to evaluate the difference between the groups.

## RESULTS

Bilateral ventral and dorsal marked hemorrhage and pulmonary contusion was observed in all rats exposed to TT (Figure 2). Within the first 30 minutes following trauma 3 rats in the HT group and another 3 rats in the TT group died and were excluded from the study. Of the 3 rats which died in the HT group, 2 had epistaxis, while the remaining one had no marked macroscopic signs. An evaluation of the 3 rats which died in the TT group revealed massive hemothorax. Therefore, 3 rats were added to each of these groups and corresponding traumas were repeated to maintain the standard "n" number of 12. All rats, which were subjected to trauma, developed serious dyspnea and remained motionless for an hour following the trauma. Dyspnea regressed after the first six hours, while motor movements remained mostly unchanged. At 12 hours, the rats were observed to be motile in their cages, and at 24 hours they showed a marked increase in motility and decrease in dyspnea. No treatment or oxygen support was given to the rats in this observation process. All rats that were exposed to TT were found to have macroscopic contusion. When abdomens of the rats were opened to obtain tissue samples, no secondary signs to trauma were found in the abdominal organs of traumatized rats.

**FIGURE 2:** The appearance of the lungs after the trauma. Bilateral upper lobes of the lungs have to be seen contused.

Any bacterial growth in solid organs (liver, spleen, lymph nodes) without any growth in lung was considered a positive sign of BT which occurred in 9 rats of the HTT group (75%), 5 rats of the TT group (41.6%) and 2 rats each in the C and the HT groups (17% each). Blood cultures remained sterile in all rats. In only one rat in the HT group, bacterial growth in lung tissue was observed. The distribution of bacterial growth among groups and harvested tissues were demonstrated in Table 1.

The bacteria isolated were *E. coli* (90%), enterococci (7%) and staphylococci (3%). As the indigenous flora of the intestines also includes enterococci and staphylococci besides *E. coli*, their growth in harvested tissue cultures was considered a sign for BT, as well.

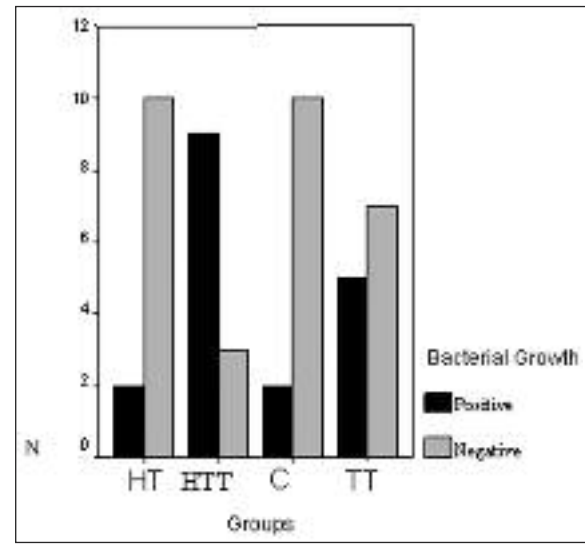
When compared to other groups, the incidence of BT in the HTT group was significantly higher

**TABLE 1:** The distribution of bacterial growth among groups.

| Groups | Lymph Node | Liver | Spleen | Lung | Group BT / Total BT* | BT in Multiple Organs |
|---|---|---|---|---|---|---|
| HTT (n= 12) | 5 /12 (42%) | 7/12 (58%) | 4/12 (33%) | - | 16/27 (59%) | 5/12 (42%) |
| TT (n= 12) | 5/12 (42%) | - | 1/12 (8%) | - | 6/27 (22%) | 1/12 (8%) |
| HT (n= 12) | 2/12 (17%) | - | - | 1/12 (8%) | 3/27 (11%) | 1/12 (8%) |
| C (n= 12) | 2/12 (17%) | - | - | - | 2/27 (8%) | |

* (Group BT / Total BT): Group BT; the number of BTs in each group, Total BT; the sum of number of BTs in all groups.
HTT; Head +Thoracic trauma, TT; Thoracic trauma, HT; Head trauma, C; Control, BT; Bacterial translocation.

(p= 0.003). Although the rate of BT in the TT group seemed than the rate in the C and the HT groups, the difference was not significant (Figure 1).




**GRAPHIC 1:** The distribution of bacterial growth among groups.
(C; Control, HT; Head Trauma, TT; Thoracic Trauma, HTT; Head + Thoracic Trauma).

## DISCUSSION

BT is the spread of bacteria from the intestinal tract to other systemic organs through lymphatic channels secondary to immune failure, proliferation of bacteria in the small intestines, injury to intestinal mucosa and increase in vascular permeability. It is believed that sepsis resulting from bacterial location plays an important role in MOF, which is among the most significant post-traumatic complications.[9,14] It is natural that as the number of tissues and organs involved in the trauma and/or the degree of involvement increases, the resulting complications increase as well.[15] In this study, we demonstrated that BT developed in the rats in which TT and HT were induced together as a multiple trauma. Although the rate of bacterial translocation in the mesenteric lymph nodes of rats with only TT was 41.6%, this was not significant when compared to the control group.

Blunt thoracic traumas rate high among problems experienced in traumatic patients presenting in the emergency units. A study involving patients with a major trauma found a high rate of major traumas associated with thorax, including TT at a rate of 68% and pulmonary contusion at a rate of 55%.[15] Of all trauma patients, 30% develop systemic inflammatory response syndrome (SIRS) and MOF triggered by trauma and almost 20% of the patients are lost due to post-traumatic MOF.[14] Although the mortality rate associated with MOF has decreased from 80% to 25-36% in the last decade due to the changes in the treatment protocols based on results of studies aiming to clarify this pathologic process, MOF remains an important issue in traumas.[16,17]

Systemic diffusion of inflammatory mediators after trauma was reported to have a role in the onset of MOF. Pathologies that most likely developed after pulmonary contusions like ALI and ARDS had a central role in the development of MOF.[14] Previous studies demonstrated that efforts to prevent post-traumatic pulmonary pathologies reduced the incidence of MOF.[18-21] Pulmonary contusion that develops after blunt thoracic trauma is a risk factor for pneumonia, ALI and ARDS, and results in death in 10 to 25% of adults. The fundamental pathophysiology of contusion was defined as ventilation/perfusion mismatch, increased intra-

pulmonary shunt, enhanced pulmonary fluid, segmental pulmonary injury and reduced compliance.[3]

Previous studies reported that the respiratory system was the first organ that underwent failure in MOF. Lungs could be the pacemaker of MOF; ARDS played a major role in MOF and prevention of ARDS could depend on decreasing the MOF incidence.[15-17] Lung-preserving treatment approaches, particularly in patients with mechanical ventilation, brings about a decline in MOF incidence.[22,23] These studies were carried out on patients with MOF; the facus infection could be identified in only 1/3 of patients groups who were clinically considered septic. Bacterial translocation was reported to be a potential significant source of infection.[24] Rapid impairment of the mucosal barrier function due to intestinal hypoperfusion, and the associated spread of bacteria in the intestinal flora through translocation had a significant role in the development of SIRS and MOF. The main foundation of this hypothesis is the development of pneumonia and other infections in patients due to bacteremia of the translocated intestinal bacteria.[25]

Under normal conditions, the intestinal wall serves as a barrier protecting against bacteria. Interest in BT has increased because bacteria in the intestinal flora increase mortality and morbidity by causing pneumonia, bacteremia, urinary infection and sepsis through BT, which was shown to develop in SIRS and MOF.[25] Literature studies demonstrated that BT occurred in various types of trauma, particularly in abdominal trauma, burns and hemorrhagic shock.[26,27] The organs primarily affected by the passage of organisms or their products are the mesenteric lymph nodes followed by the liver, the spleen and the general circulation. In the HTT model that we induced, BT occurred principally in mesenteric lymph nodes and liver, while septicemia or BT into the pulmonary tissue did not develop.

Additionally, as previous studies showed that the first 24 hours following trauma was the most marked period for BT, we looked for BT at 24 hours in our study.[25] One report demonstrated the development of BT in rats in with lower extremity fractures and HT. In the same study, BT did not occur in case of multiple fractures in the isolated lower extremity. In relation to the respiratory system, the literature showed development of BT only in rigid bronchoscopy and ARDS.[28,29]

Previous clinical studies reported that 35-40% of traumatized patients developed an infection. The most common infection foci were shown to be the respiratory tract (23-28%) and the urinary tract (17-24%). The most common microorganisms were *Staphylococcus aureus, E. coli,* enterococci and *Pseudomonas,* which, interestingly, are microorganisms detected in the BT. It was also noted that more than half of the patients who had infections developed SIRS, and had a high rate of mortality. The rate of ALI in such patients was 20%. Rate of infection in patients who had ALI and who required ventilator support was reported to be significantly higher and sepsis and ARDS developed more frequently in those patients. Sepsis was claimed to be responsible for mortality in 44% of patients with blunt trauma. Bacteria in the intestinal tract rank first among the causes of infections that develop in trauma patients and sepsis is the major cause of mortality in patients with blunt trauma.[16,17] According to previous studies, factors that increase the risk of infection in trauma cases include spinal cord injury, requirement for intubation and ventilation, central catheterizations, multiple transfusions and multiple surgical procedures. Salim et al emphasized in their study that complication rates and morbidity increased in cases with HT and concurrent ARDS or lung injury.[30] In our study, we similarly found that BT was less common in rats with isolated head trauma or isolated TT, whereas BT was more common in the HTT group, where HTT were concurrent.

In order to reduce complications and infections, patients should undergo surgical intervention, if necessary, in the early period. Early surgical intervention in cases that require elective surgery after trauma was reported to have a protective effect against development of infections. In an experimental study, Öztuna et al stated that use of early internal fixation in rats which developed bone fracture prevented BT.[31] In addition, avoidance of un-

necessary catheterizations is recommended, and development of sepsis and MOF can be significantly reduced by selection of pulmonary function-preserving ventilator strategies to refrain from ventilator-induced lung injury (VILI) in patients who require mechanical ventilation.[30-33]

Our study was designed to include blunt thoracic trauma and head and thoracic trauma, since there is no study to our knowledge on BT in such a model. We evaluated the coexistence of pulmonary contusion and BT, which were reported to play an active role in the development of post-traumatic MOF.[28,29] While BT does not occur in isolated blunt thoracic trauma or isolated HT, it develops in HTT, involving the head and thorax. Respiratory system is the main system that affects morbidity and mortality in patients who had a major trauma.

The results of our study suggest that the presence of TT in multiple trauma makes a significant difference in the development of BT. We think that calrifying demonstrating the relation between BT and TT, which is considered to serve as a pacemaker in the development of ARDS, sepsis and MOF can help prevent deaths resulting from post-traumatic complications, which are the most common causes of mortality among the young. The correlation between inflammatory response and BT that develops after HTT should be evaluated in further studies should be extensively investigated to prevent these processes, which have high mortality rates.

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