Brain, Behavior, & Immunity - Health 1 (2020) 100007

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Splenectomy does not affect the development of pneumonia following severe traumatic brain injury

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ARTICLE INFO

Keywords:
Traumatic brain injury
Pneumonia
Immunosuppression
Splenectomy
Cholinergic anti-inflammatory pathway

ABSTRACT

The cholinergic anti-inflammatory pathway offers a proposed mechanism to describe the increased risk of pneumonia following severe traumatic brain injury (sTBI). Vagal activity transmitted to the spleen results in decreased inflammatory cytokine production and immunosuppression. However, no clinical evidence exists. We sought to compare pneumonia rates among patients with TBI and splenectomy using a retrospective analysis of all trauma patients with splenic injury requiring splenectomy or TBI admitted to an ACS verified level one trauma center from 2011 to 2016. Admission Glasgow Coma Score (GCS) ≤ 8 was used to identify sTBI. Pneumonia was defined by respiratory culture obtained by bronchoalveolar lavage. Analysis included χ^2 and one-way analysis of variance followed by multivariate logistic regression to determine the association of sTBI and splenectomy of development of pneumonia. Four hundred and twenty-seven patients were included for primary analysis, 247 with sTBI, 180 with splenectomy, and 14 with both sTBI and splenectomy. Rates of pneumonia were increased, although not significant among patients with sTBI and splenectomy and both sTBI alone (71.4 vs. 49.4%, $p = 0.11$). On multivariate regression, the risk of pneumonia was increased with both splenectomy and sTBI (OR 3.18; 95% CI, 0.75–13.45) and sTBI alone, although significant in the latter only (OR 3.56; 95% CI, 2.12–5.97). Based on these results, splenectomy does not appear to influence the development of pulmonary immunosuppression and pneumonia following sTBI.

1. Introduction

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality following injury worldwide, with over 10 million new cases occurring annually (Peeters et al., 2015). TBI is responsible for one-third of injury related deaths in the US (Centers for Disease, 2015; Pfeifer et al., 2016). While previously thought to exist in isolation due to the blood brain barrier (BBB), severe TBI is now recognized to carry significant systemic complications with potential to induce organ dysfunction (Zygun et al., 2005). Along with local tissue destruction during the initial injury, alterations of normal inflammatory and immunologic pathways may exacerbate morbidity and mortality (Lim and Smith, 2007).

Pulmonary complications occur with increased frequency following severe TBI (Piek et al., 1992). Rates of ventilator associated pneumonia (VAP) are significantly elevated in comparison to other patterns of traumatic injury, with a reported incidence of 40–60% (Helling et al., 1988; Hsieh et al., 1992; Woratyla et al., 1995). These findings were initially believed to be a result of loss of oropharyngeal control and increased aspiration risk.

However, patients with neuromuscular disorders and similar oropharyngeal dysfunction have significantly lower rates of infection and pneumonia (Pelosi et al., 2011). Furthermore, less than half of patients with these injuries actually aspirate, suggesting that an alternative mechanism is chiefly responsible for an increased risk of respiratory infection (Meisel et al., 2005).

Numerous mechanisms have been proposed to explain the correlation between TBI and respiratory infection. The cholinergic anti-inflammatory pathway has been identified as a possible explanation for the observed pulmonary immunosuppression (Tracey, 2002). Autonomic dysfunction secondary to intracranial hypertension may have widespread effects on multiple sites including the spleen, a known repository of immunologic activity (Hall et al., 2014). Aberrations in autonomic

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https://doi.org/10.1016/j.bbih.2019.100007
Received 8 October 2019; Accepted 10 October 2019
Available online 14 November 2019

activity transmitted to the spleen may result in improper inhibition of normal splenic macrophage inflammatory function, resulting in decreased levels of circulating inflammatory.

Cytokines such as IL-1 and TNF- α (Kox et al., 2008). When coupled with the common requirement for mechanical ventilation in these patients, lack of a functional immune response via this mechanism may be responsible for the elevated pneumonia rates (Hall et al., 2014).

While the mechanism is not yet fully understood, the cholinergic anti-inflammatory pathway involves inappropriate vagal stimulation, activation of the α7-nicotinic acetylcholine receptor, and stimulation of splenic macrophages to suppress production and circulation of inflammatory cytokines such as IL-1, HMGB-1, and TNF-α (Fig. 1) (Martelli et al., 2014). Evidence to support the pathway exists *in vitro* and in animal models. Vagotomy in murine models results in increased pulmonary neutrophil activation while vagal stimulation limits pulmonary inflammation (Bregeon et al., 2011; dos Santos et al., 2011)Mikulski et al., 2010. Vagal nerve stimulation following splenectomy or in α −7 knockout models fails to prevent similar inflammatory cytokine production, suggesting the necessity of both the spleen and α −7 receptor (Vida et al., 2011; Huston et al., 2006, 2008). Despite the intriguing translational work, direct clinical support of this phenomenon remains lacking. To date, no direct comparisons of patients with TBI have been performed to determine if splenectomy influences rates of respiratory infection. As such, we sought to provide a review of patients with severe TBI to determine if splenectomy decreases the incidence of pneumonia in this population. Given that immunosuppression facilitated by splenic macrophages is thought to result in increased susceptibility to pneumonia, we hypothesize that patients with severe TBI concurrently requiring splenectomy should demonstrate a decrease in the incidence of pulmonary infection.

2. Methods

A retrospective analysis of all patients at our institution with either traumatic brain injury or splenic injury requiring splenectomy from January 2012 to January 2016 was performed, following approval by the institutional review board. The University of Alabama at Birmingham is an American College of Surgeons verified level one trauma center and serves as a tertiary referral center for the state of Alabama. The trauma service maintains an institutional registry of all patients requiring trauma activation and all patients requiring admission to the service.

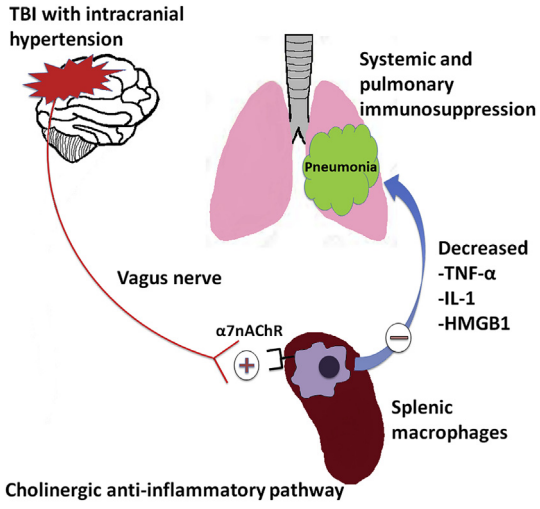

Fig. 1. Summary of Proposed Mechanism of Cholinergic Anti-Inflammatory Pathway. Traumatic brain injury with intra-cranial hypertension causes inappropriate vagal stimulation. Neural stimulation of the α-7-nicotinic acetylcholine receptor is relayed to splenic macrophages resulting in suppression of inflammatory cytokines such as TNF- α, IL-1, and HMGB-1. Diminished circulating pro-inflammatory molecules causes systemic and pulmonary immunosuppression.

Individual patients were identified using ICD-9 codes. Data was obtained from the trauma registry and the electronic medical record. Inclusion criteria consisted of traumatic injury with TBI or splenic injury requiring splenectomy upon admission. Patients less than 18 years of age or who died within 48 h of admission were excluded from analysis. The primary outcome of interest was pneumonia rate following splenectomy in the setting of severe TBI.

Ventilator associated pneumonia (VAP) was defined as 1×10^5 colony forming units of bacterial growth following respiratory culture obtained via bronchoalveolar lavage (BAL) at least 48 h post admission in patients requiring mechanical ventilation. Similar growth within 48 h of admission was classified as community acquired pneumonia (CAP). Length of time in days for development of pneumonia was recorded from admission to the date of BAL culture.

Glasgow coma scale score (GCS) at both admission and 24 h following admission were recorded. Severity of TBI was defined using initial GCS, with a score less than or equal to 8 being categorized as severe and 9–15 as non-severe. Type of brain injury was also specified, with the presence of subarachnoid hemorrhage (SAH), subdural hemorrhage (SDH), epidural hemorrhage (EDH), intraparenchymal hemorrhage (IPH), and diffuse axonal injury (DAI) separately identified following review of the patient's available imaging by the trauma attending and attending radiologist.

Splenic injury, if present, was identified and graded either intra-operatively or by CT. Grading of splenic injury was performed according to standard grading as described by the American Association for the Surgery of Trauma. Splenic injury grades were confirmed using pathology reports following splenectomy or available imaging for measurement.

Following patient identification, patients were placed into cohorts consisting of patients with either severe TBI not requiring splenectomy, splenic injury requiring splenectomy without TBI, or severe TBI with concurrent splenic injury requiring splenectomy. Hospital free, ICU, and mechanical ventilation days, were calculated and censored at 30 days. Data analysis was performed using Pearson's χ^2 and one-way analysis of variance (ANOVA) or Student's *t*-test for categorical and continuous variables, respectively. Post-hoc analyses with pairwise χ^2 or Tukey's test were performed in the event of significance to identify differences between cohorts.

Multivariate logistic regression analysis was performed to determine the association of the injury patterns of concern with the development of pneumonia. Adjustment was performed for potential confounding covariates, including age, gender, mechanism of injury, injury severity score (ISS), and length of mechanical ventilation. An *a priori* p value of 0.05 was considered statistically significant.

3. Results

Seven hundred and eleven patients were identified from the trauma registry. Forty-eight were excluded after identification of splenic injury initially treated non-operatively, but ultimately requiring delayed splenectomy. Another 152 were excluded secondary to mortality within 48 h of admission. Five hundred and ten patients met the study criteria and were included for analysis. TBI was identified in 330 (64.7%) patients; 247 (48.4%) were identified as severe and 83 as mild/moderate (16.3%). One hundred and eighty (35.3%) patients required splenectomy upon admission due to splenic trauma, with 14 (2.7%) suffering concurrent severe TBI.

Patients were demographically matched among the three primary cohorts of interest, those being severe TBI not requiring splenectomy, splenic injury requiring splenectomy without TBI, and severe TBI with concurrent splenectomy. Patients were predominantly male, middle age, and suffered from blunt mechanisms of injury. ISS was significantly increased among patients with severe TBI and splenectomy compared to

the isolated TBI or splenectomy cohorts. Among patients with TBI, there were no significant differences in GCS among those with or without concurrent splenectomy on admission or at 24 h (Table 1).

Severe TBI was associated with significantly worse outcomes than either mild/moderate TBI or splenectomy without TBI. Compared with patients requiring splenectomy alone, patients with severe TBI suffered significantly higher 30-day mortality, increased lengths of stay, and increased rates of pneumonia (Table 1). When compared against those with only mild/moderate TBI, the results were again significantly worse among those with severe TBI (Table 2).

The addition of splenic injury requiring splenectomy did not improve outcomes among patients with severe TBI. Patients demonstrated increased rates of pneumonia and rates of 30-day mortality, although not significant. There were no differences in hospital length of stay ICU free days, or ventilator free days among the two cohorts (Table 3). On multivariate regression analysis, severe TBI was significantly associated with an increased risk of development of pneumonia whereas the risk with severe TBI was elevated, although not significantly (Table 4).

Comparing patients with pneumonia to those without, patients were significantly more likely to be male and suffer blunt mechanisms of injury. Patients were significantly more likely to have severe TBI and less likely to have required splenectomy. Development of pneumonia was associated with increased lengths of stay, although this was not associated with increased rates of 30-day mortality (Table 5).

Table 1
Comparison of clinical characteristics among patients with traumatic brain injury and splenectomy.

	Severe TBI (n = 247)	Required Splenectomy (n = 166)	Severe TBI and Splenectomy (n = 14)	p-value
DEMOGRAPHICS				
Age (Years)	38.7 ± 17.5	41.7 ± 16.0	45.2 ± 16.7	0.11
Gender (%)				
Male	178 (73.0)	120 (72.3)	10 (71.4)	0.98
Female	66 (27.0)	46 (27.7)	4 (28.6)	
CLINICAL				
Injury Severity Score	27.6 ± 10.4ˆ	27.3 ± 11.8ǂ	46.8 ± 9.5ǂ	<0.001
Admission GCS	5.3 ± 1.7ˆ	13.7 ± 3.2ǂ	4.1 ± 2.8ǂ	<0.001
GCS 24-Hours After Admission	6.8 ± 2.1ˆ	11.3 ± 3.8ǂ	5.6 ± 1.8ǂ	<0.001
Mechanism of Injury (%)				
Blunt	227 (93.0)	136 (81.9)	14 (100)	0.001
Penetrating	17 (7.0)	30 (18.1)	0	
TBI (%)				
Subarachnoid	135 (55.1)	7 (4.2)	10 (71.4)	<0.001
Subdural	124 (50.6)	6 (3.6)	5 (35.7)	<0.001
Epidural	23 (9.4)	0	1 (7.1)	<0.001
Intraparenchymal	100 (41.0)	4 (2.4)	7 (50.0)	<0.001
Diffuse Axonal Injury	64 (26.1)	2 (1.2)	4 (28.6)	<0.001
Splenic Injury Grade (%) <0.001				
1	7 (2.8)	2 (1.2)	0	
2	4 (1.6)	17 (10.2)	5 (35.7)	
3	1 (0.4)	59 (35.5)	4 (28.6)	
4	0	46 (27.7)	3 (21.4)	
5	0	35 (21.1)	2 (14.3)	
OUTCOMES				
Death Within 30 Days	36 (14.6)ˆ	5 (3.0)ˆǂ	3 (21.4) ǂ	<0.001
Length of Stay (Days)	26.0 ± 23.0ˆ	15.0 ± 14.3ǂ	34.3 ± 30.6ǂ	<0.001
ICU Free Days**	7.6 ± 13.5ˆ	5.0 ± 3.7ˆ	5.0 ± 5.0	0.046
Ventilator Free Days**	11.8 ± 16.1ˆ	7.5 ± 4.9ˆ	10.0 ± 10.4	0.004
ARDS	24 (9.7)	13 (7.8)	2 (14.3)	0.64
Pneumonia (%)	122 (49.4)ˆ	29 (17.5)ˆǂ	10 (71.4) ǂ	<0.001
Community acquired	9 (3.6)	7 (4.2)	1 (7.1)	0.79
Ventilator associated	121 (49.0)ˆ	25 (15.1)ˆǂ	10 (71.4) ǂ	<0.001
Episodes of Pneumonia (%)				
One	121 (49.0)	29 (17.5)	10 (71.4)	<0.001
Two	34 (13.8)	5 (3.0)	2 (14.3)	0.001
Three	4 (1.6)	1 (0.6)	1 (7.1)	0.035
Timing of Pneumonia (Days)				
First Episode	7.0 ± 5.8	8.2 ± 7.3	9.0 ± 7.2	0.45
Second Episode	16.3 ± 7.8	21.8 ± 15.4	24.5 ± 10.6	0.24
Third Episode	31.5 ± 21.1	–	40.0	0.44
Disposition (%)				
Death	48 (19.8)	7 (4.2)	3 (21.4)	<0.001
Home	32 (13.2)	123 (74.1)	2 (14.3)	
Long Term Acute Care Facility	20 (8.3)	5 (3.0)	0	
Inpatient Rehab	134 (55.4)	24 (14.5)	7 (50.0)	
Skilled Nursing Facility	8 (3.3)	7 (4.2)	2 (14.3)	

*Data presented as mean ± SD unless otherwise noted. Estimates from Pearson χ^2 and one-way ANOVA for categorical and continuous variables, respectively. Patients with death within 48 h excluded.
ˆ ǂ Significant on posthoc pairwise comparisons with χ^2 or Tukey's test for categorical or continuous variables.
**Free days within 30 days.

Table 2
Comparison of clinical characteristics and outcomes among patients with severe versus mild/moderate traumatic brain injury without splenectomy.

	Severe TBI (n = 247)	Mild/Moderate TBI (n = 83)	p-value
DEMOGRAPHICS			
Age (Years)	38.8 ± 17.5	43.4 ± 18.8	0.041
Gender (%)			
Male	178 (73.0)	56 (67.5)	0.34
Female	66 (27.0)	27 (32.5)	
CLINICAL			
Injury Severity Score	27.6 ± 10.4	23.5 ± 10.7	0.002
Admission GCS	5.3 ± 1.7	10.7 ± 1.8	<0.001
GCS 24-Hours After Admission	6.8 ± 2.1	10.5 ± 2.5	<0.001
Mechanism of Injury (%)			
Blunt	227 (93.0)	80 (96.4)	0.27
Penetrating	17 (7.0)	3 (3.6)	
TBI (%)			
Subarachnoid	135 (55.1)	46 (55.4)	0.96
Subdural	124 (50.6)	38 (45.8)	0.45
Epidural	23 (9.4)	8 (9.6)	0.96
Intraparenchymal	100 (41.0)	24 (28.9)	0.050
Diffuse Axonal Injury	64 (26.1)	4 (4.8)	<0.001
OUTCOMES			
Death Within 30 Days	36 (14.6)	4 (4.8)	0.02
Length of Stay (Days)	26.0 ± 23.0	14.5 ± 15.2	<0.001
ICU Free Days**	7.6 ± 13.5	4.8 ± 8.8	0.032
Ventilator Free Days**	11.8 ± 16.1	7.8 ± 9.9	0.009
ARDS	24 (9.7)	3 (3.6)	0.079
Pneumonia (%)	122 (49.4)	11 (13.2)	<0.001
Community acquired	9 (3.6)	3 (3.6)	0.99
Ventilator associated	121 (49.0)	8 (9.6)	<0.001
Episodes of Pneumonia (%)			
One	121 (49.0)	11 (13.2)	
Two	34 (13.8)	1 (1.2)	0.001
Three	4 (1.6)	0	0.24
Timing of Pneumonia (Days)			
First Episode	7.0 ± 5.8	8.1 ± 6.3	0.57
Second Episode	16.3 ± 7.8	13.0	0.68
Disposition (%)			
Death	48 (19.8)	5 (6.0)	<0.001
Home	32 (13.2)	36 (43.4)	
Long Term Acute Care Facility	20 (8.3)	7 (8.4)	
Inpatient Rehab	134 (55.4)	34 (41.0)	
Skilled Nursing Facility	8 (3.3)	1 (1.2)	

*Data presented as mean ± SD unless otherwise noted. Estimates from Pearson χ^2 and Student's *t*-test for categorical and continuous variables, respectively. Mortality within 48 h excluded.
**Free days within 30 days.

Table 3
Comparison of clinical characteristics among patients with traumatic brain injury and splenectomy.

	Severe TBI (n = 247)	Severe TBI and Splenectomy (n = 14)	p-value
CLINICAL			
Injury Severity Score	27.6 ± 10.4	46.8 ± 9.5	<0.001
Admission GCS	5.3 ± 1.7	4.1 ± 2.8	0.18
GCS 24-Hours After Admission	6.8 ± 2.1	5.6 ± 1.8	0.30
OUTCOMES			
Death Within 30 Days	36 (14.6)	3 (21.4)	0.48
Length of Stay (Days)	26.0 ± 23.0	34.3 ± 30.6	0.302
ICU Free Days**	7.6 ± 13.5	5.0 ± 5.0	0.64
Ventilator Free Days**	11.8 ± 16.1	10.0 ± 10.4	0.76
ARDS	24 (9.7)	2 (14.3)	0.58
Pneumonia (%)	122 (49.4)	10 (71.4)	0.11
Community acquired	9 (3.6)	1 (7.1)	0.51
Ventilator associated	121 (49.0)	10 (71.4)	0.10
Episodes of Pneumonia (%)			
One	121 (49.0)	10 (71.4)	0.58
Two	34 (13.8)	2 (14.3)	0.96
Three	4 (1.6)	1 (7.1)	0.14
Timing of Pneumonia (Days)			
First Episode	7.0 ± 5.8	9.0 ± 7.2	0.11
Second Episode	16.3 ± 7.8	24.5 ± 10.6	0.43
Third Episode	31.5 ± 21.1	40.0	0.44
Disposition (%)			
Death	48 (19.8)	3 (21.4)	0.25
Home	32 (13.2)	2 (14.3)	
Long Term Acute Care Facility	20 (8.3)	0	
Inpatient Rehab	134 (55.4)	7 (50.0)	
Skilled Nursing Facility	8 (3.3)	2 (14.3)	

*Data presented as mean ± SD unless otherwise noted. Estimates from Pearson χ^2 and one-way ANOVA followed by Tukey's test for categorical and continuous variables, respectively. Patients with death within 48 h excluded.
**Free days within 30 days.

Table 4
Multivariate logistic regression analysis of the association of injury patterns on development of pneumonia 95% Confidence Interval.

Variable	OR*	Lower	Upper
Severe TBI	3.56	2.12	5.97
Mild TBI	0.31	0.13	0.70
Splenectomy	0.51	0.29	0.89
Severe TBI and Splenectomy	3.18	0.75	13.45

*OR – Odds ratio.
**Adjusted for Age, Injury Severity Score, Gender, Mechanism of Injury, and Length of Mechanical Ventilation; 48-h mortality excluded.

Table 5
Comparison of clinical characteristics and outcomes among patients with and without development of pneumonia.

	Pneumonia (n = 171)	No Pneumonia (n = 339)	p-value
DEMOGRAPHICS			
Age (Years)	39.3 ± 16.3	41.4 ± 17.8	0.20
Gender (%)			
Male	135 (78.9)	229 (68.2)	0.012
Female	36 (21.1)	107 (31.8)	
CLINICAL			
Injury Severity Score	29.9 ± 11.2	26.1 ± 11.4	0.001
Admission GCS	6.6 ± 3.7	10.0 ± 4.4	<0.001
GCS 24-Hours After Admission	7.0 ± 2.6	9.8 ± 3.7	<0.001
Intubated on Arrival (%)	137 (80.1)	34 (19.9)	<0.001
Mechanism of Injury (%)			
Blunt	161 (94.2)	296 (88.1)	0.040
Penetrating	10 (5.8)	40 (11.9)	
TBI (%)			
Mild/moderate	10 (5.8)	73 (21.5)	<0.001
Severe	132 (77.2)	129 (38.1)	<0.001
TBI (%)			
Subarachnoid	81 (47.4)	117 (34.7)	0.007
Subdural	80 (46.8)	93 (27.6)	<0.001
Epidural	7 (4.1)	25 (7.4)	0.18
Intraparenchymal	57 (33.3)	78 (23.2)	0.019
Diffuse Axonal Injury	40 (23.4)	34 (10.1)	<0.001
Required Splenectomy (%)	39 (22.8)	141 (41.6)	<0.001
evere TBI and Splenectomy (%)	10 (5.8)	4 (1.2)	0.007
Splenic Injury Grade (%)			
1	5 (2.9)	5 (1.5)	0.039
2	9 (5.3)	18 (5.3)	
3	17 (9.9)	47 (13.9)	
4	9 (5.3)	40 (11.8)	
5	9 (5.3)	28 (8.3)	
OUTCOMES			
Death Within 30 Days	17 (9.9)	31 (9.1)	0.75
Length of Stay (Days)	33.6 ± 25.3	14.3 ± 13.6	<0.001
ICU Free Days**	8.8 ± 14.8	4.9 ± 6.8	<0.001
Ventilator Free Days**	13.7 ± 17.2	7.6 ± 8.3	<0.001
ARDS	29 (17.0)	13 (3.8)	<0.001
Pneumonia (%)			
Community acquired	20 (11.7)	–	
Ventilator associated	164 (95.9)	–	
Disposition (%)			
Death	22 (12.9)	41 (12.2)	<0.001
Home	27 (15.9)	166 (49.6)	
Long Term Acute Care Facility	19 (11.2)	13 (3.9)	
Inpatient Rehab	91 (53.5)	108 (32.2)	
Skilled Nursing Facility	11 (6.5)	7 (2.1)	

*Data presented as mean ± SD unless otherwise noted. Estimates from Pearson χ^2 and Student's *t*-test for categorical and continuous variables, respectively. Mortality within 48 h excluded.
**Free days within 30 days.

4. Discussion

The infectious and inflammatory derangements associated with severe injury are a topic of increasing interest and investigation. Mortality among trauma patients is typically considered to be either bi-modal or tri-modal, with immediate and early deaths occurring due to devastating neurologic injuries, hemorrhage, and cardiorespiratory failure secondary to hemothorax or pneumothorax and late deaths occurring days to weeks later due to infection or multi-system organ failure (Trunkey and Lim, 1974; Gunst et al., 2010). Sterile inflammation and the innate immune system are essential in the development of late morbidity and mortality following trauma. Previously, TBI was thought to exist outside of this model due to isolation behind the BBB. However, TBI, in particular severe TBI, is increasingly recognized as having potential for widespread systemic consequences, including dysautonomia, end organ dysfunction, and immunosuppression.

A variety of mechanisms may explain these effects (Ransohoff et al., 2003). Following cerebral injury and cell damage, astrocytes and microglia release inflammatory cytokines, such as IL-1 and TNF-α. Neutrophil recruitment and activation at the BBB results in enzymatic breakdown of the barrier with a subsequent increase in permeability. As a result, inflammatory cytokines and other damage associated molecular patterns (DAMPs) can enter the systemic circulation. Elevated levels of these inflammatory molecules have been identified in samples of jugular venous blood as compared to carotid blood in humans (McKeating et al., 1997). Additional cytokine circulation through novel systems, such as the glymphatic pathway, a CNS clearance pathway, are also believed to play a role via drainage from venous sinuses along the cribriform plate (Plog et al., 2015). Lastly, TBI may result in vagal stimulation and alter neural signaling with hypothesized potential to cause pulmonary and systemic immunosuppression (Hall et al., 2014; Cuoco et al., 2016).

According to the proposed cholinergic anti-inflammatory pathway, vagal stimulation of the splenic T-cells and macrophages result in decreased inflammatory cytokines and ultimately pulmonary

immunosuppression. Loss of splenic function should therefore prevent the immunosuppression proposed according to this pathway. However, in this retrospective review of trauma patients at a large, level 1 trauma center, we found no evidence of decreased pneumonia rates in brain injured patients whom underwent splenectomy when compared to other patients with severe TBI. Potentially, patients among the splenectomy and severe TBI cohort may have suffered increased complications secondary to increased disease severity, given the significant difference in ISS among these patients and those with only severe TBI. However, even when controlling for potential significant confounding covariates such as ISS and length and mechanical ventilation, patients with severe TBI with or without splenectomy were both associated with similar increases in the risk of development of pneumonia. These findings seem to contradict the proposed hypothesis of the cholinergic anti-inflammatory pathway.

What is apparent from our findings is that severe traumatic brain injury is associated with significant pulmonary complications. Examination of patients with pneumonia in our study revealed that those with pneumonia were significantly more likely to suffer severe TBI compared to those without pneumonia. Conversely, patients without pneumonia were significantly more likely to have suffered mild/moderate TBI or splenectomy without TBI at all. Patients in our study confirmed those found in other studies that identified significantly increased risk of pneumonia following severe TBI (Woratyla et al., 1995; Zygun et al., 2006; Jovanovic et al., 2015; Piek, 1995). Respiratory infection is significantly less common in the trauma population at large, similar to what we identified among patients with splenectomy without TBI in our study (Magnotti et al., 2004).

Multiple animal models have been developed to support the proposed cholinergic anti- inflammatory pathway. Bilateral vagotomy in a murine model has demonstrated decreased levels of serum TNF-α (Borovikova et al., 2000). Further, vagal stimulation causes decreased peripheral inflammatory cytokines. Additionally, splenectomy in murine sepsis models prevents activation of the anti- inflammatory pathway (Huston et al., 2006). However, clinical studies demonstrating the cholinergic anti- inflammatory pathway are lacking. To our knowledge, our study is currently the only study directly comparing rates of pneumonia among patients with TBI dependent on concurrent splenectomy.

Given the lack of clinical supportive evidence in our study and others, another mechanism is likely responsible for the increase in pulmonary infections. Respiratory infection following severe TBI may be related to direct vagal action within the lungs themselves. Vagal innervation of other visceral organs has been shown to cause local anti-inflammatory effects. Additionally, pulmonary macrophages are known to share the α-7-nicotinic acetylcholine receptor that is found on splenic macrophages (Mikulski et al., 2010). Local inhibition may pose a feasible explanation for the inhibition of inflammatory cytokine generation necessary to combat infection.

Evidence of systemic consequences related to dysfunctional neuronal signaling to individual organs following severe TBI is increasingly recognized. Among the most commonly identified is the sympathetic storm, which likely results from deranged autonomic signaling and imbalance in excitatory and inhibitory stimulation (Baguley, 2008). Myocardial dysfunction may be evidenced by abnormalities detected during electrocardiography or echocardiography and are often secondary to repolarization irregularities or from direct neuronal injury along sympathetic nerve distribution (Dujardin et al., 2001; Krishnamoorthy et al., 2014; Zaroff et al., 2000). Dysfunctional nervous signaling to the pulmonary vasculature may result in acute and transient, pulmonary edema (Theodore and Robin, 1976). Increased intracranial pressure has long been associated with gastric ulcer formation due to increased gastrin and gastric acid production from autonomic dysregulation or with gastroparesis and ileus (Kemp et al., 2015; Kao et al., 1998; Olsen et al., 2013).

Our study attempts to clinically evaluate the cholinergic anti-inflammatory pathway through evaluation of pneumonia rates comparing requirement for concurrent splenectomy in patients with severe TBI. However, given the retrospective nature of our analysis, our study has several limitations. To ensure uniform diagnosis of infection, respiratory culture data alone was used to define pneumonia. Tracheobronchial colonization may have been misidentified as a true peripheral lung infection. Furthermore, some definitions of VAP require clinical and subjective radiographic correlation. Attempts to qualify these clinical criteria through the existing medical record were not feasible. Further study is needed to better isolate the source of pulmonary immunosuppression and develop strategies to minimize the risk of pneumonia associated with TBI.

5. Conclusions

We present the first comparison of pneumonia rates among patients with TBI based on the concurrent performance of splenectomy as an examination of the cholinergic anti- inflammatory pathway. Severe TBI is a strong risk factor for the development of pneumonia. Splenectomy does not mitigate this risk. Pulmonary immunosuppression following TBI likely relies on a spleen independent mechanism and further investigation is warranted.

Authorship statement

PH: contributed significantly to study design, data acquisition, analysis and interpretation of data, and manuscript preparation.

RU: contributed significantly to study design, data acquisition, analysis and interpretation of data, and manuscript preparation.

VP: contributed significantly to study design, data acquisition, analysis and interpretation of data, and manuscript preparation.

JK: contributed significantly to study design, analysis and interpretation of data, and manuscript preparation.

PB: contributed significantly to study design, analysis and interpretation of data, and manuscript preparation.

Declaration of competing interest

There are no conflicts of interest to disclose. There is no funding to disclose related to this paper. This paper has not been previously presented or published aside from presentation at the 2016 annual meeting American College of Surgeons.

References

Baguley, I.J., 2008. The excitatory:inhibitory ratio model (EIR model): an integrative explanation of acute autonomic overactivity syndromes. Med. Hypotheses 70 (1), 26–35. PubMed PMID: 17583440.

Borovikova, L.V., Ivanova, S., Zhang, M., et al., 2000 May 25. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405 (6785), 458–462. PubMed PMID: 10839541.

Bregeon, F., Xeridat, F., Andreotti, N., et al., 2011. Activation of nicotinic cholinergic receptors prevents ventilator-induced lung injury in rats. PLoS One 6 (8), e22386. PubMed PMID: 21857926. Pubmed Central PMCID: PMC3152549.

Centers for Disease, C., 2015. Report to congress on traumatic brain injury in the United States: epidemiology and rehabilitation. In: Centers for Disease C, Ed. Atlanta, GA: National Center for Injury Prevention and Control; Divsion of Unintentional Injury Prevention.

Cuoco, J.A., Fennie, C.N., Cheriyan, G.K., 2016 04/11/2016. The cholinergic anti-inflammatory pathway: a novel paradigm for translational research in neuroimmunology. J. Neurol. Neurosci. 7 (2:86), 1–7.

dos Santos, C.C., Shan, Y., Akram, A., et al., 2011 Feb 15. Neuroimmune regulation of ventilator-induced lung injury. Am. J. Respir. Crit. Care Med. 183 (4), 471–482. PubMed PMID: 20870758.

Dujardin, K.S., McCully, R.B., Wijdicks, E.F., et al., 2001 Mar. Myocardial dysfunction associated with brain death: clinical, echocardiographic, and pathologic features. J. Heart Lung Transplant. 20 (3), 350–357. PubMed PMID: 11257562.

Gunst, M., Ghaemmaghami, V., Gruszecki, A., et al., 2010 Oct. Changing epidemiology of trauma deaths leads to a bimodal distribution. SAVE Proc. 23 (4), 349–354. PubMed PMID: 20944754. Pubmed Central PMCID: PMC2943446.

Hall, S., Kumaria, A., Belli, A., 2014 Apr. The role of vagus nerve overactivity in the increased incidence of pneumonia following traumatic brain injury. Br. J. Neurosurg. 28 (2), 181–186. PubMed PMID: 24024980.

Helling, T.S., Evans, L.L., Fowler, D.L., et al., 1988 Nov. Infectious complications in patients with severe head injury. J. Trauma 28 (11), 1575–1577. PubMed PMID: 3141628.

Hsieh, A.H., Bishop, M.J., Kubilis, P.S., et al., 1992 Aug. Pneumonia following closed head injury. Am. Rev. Respir. Dis. 146 (2), 290–294. PubMed PMID: 1489114.

Huston, J.M., Ochani, M., Rosas-Ballina, M., et al., 2006 Jul 10. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. J. Exp. Med. 203 (7), 1623–1628. PubMed PMID: 16785311. Pubmed Central PMCID: PMC2118357.

Huston, J.M., Wang, H., Ochani, M., et al., 2008 Sep 01. Splenectomy protects against sepsis lethality and reduces serum HMGB1 levels. J. Immunol. 181 (5), 3535–3539. PubMed PMID: 18714026. Pubmed Central PMCID: PMC4533852.

Jovanovic, B., Milan, Z., Markovic-Denic, L., et al., 2015 Sep. Risk factors for ventilator-associated pneumonia in patients with severe traumatic brain injury in a Serbian trauma centre. Int. J. Infect. Dis. 38, 46–51. PubMed PMID: 26166697.

Kao, C.H., ChangLai, S.P., Chieng, P.U., Yen, T.C., 1998 Jul. Gastric emptying in head-injured patients. Am. J. Gastroenterol. 93 (7), 1108–1112. PubMed PMID: 9672339.

Kemp, W.J., Bashir, A., Dababneh, H., Cohen-Gadol, A.A., 2015 Apr-Jun. Cushing's ulcer: further reflections. Asian J Neurosurg 10 (2), 87–94. PubMed PMID: 25972936. Pubmed Central PMCID: PMC4421974.

Kox, M., Pompe, J.C., Pickkers, P., et al., 2008 Feb 05. Increased vagal tone accounts for the observed immune paralysis in patients with traumatic brain injury. Neurology 70 (6), 480–485. PubMed PMID: 18250293.

Krishnamoorthy, V., Prathep, S., Sharma, D., et al., 2014 Sep. Association between electrocardiographic findings and cardiac dysfunction in adult isolated traumatic brain injury. Indian J. Crit. Care Med. 18 (9), 570–574. PubMed PMID: 25249741. Pubmed Central PMCID: PMC4166872.

Lim, H.B., Smith, M., 2007 May. Systemic complications after head injury: a clinical review. Anaesthesia 62 (5), 474–482. PubMed PMID: 17448059.

Magnotti, L.J., Croce, M.A., Fabian, T.C., 2004 Fall. Is ventilator-associated pneumonia in trauma patients an epiphenomenon or a cause of death? Surg. Infect. 5 (3), 237–242. PubMed PMID: 15684794.

Martelli, D., McKinley, M.J., McAllen, R.M., 2014 May. The cholinergic anti-inflammatory pathway: a critical review. Auton. Neurosci. 182, 65–69. PubMed PMID: 24411268.

McKeating, E.G., Andrews, P.J., Signorini, D.F., Mascia, L., 1997 May. Transcranial cytokine gradients in patients requiring intensive care after acute brain injury. Br. J. Anaesth. 78 (5), 520–523. PubMed PMID: 9175965.

Meisel, C., Schwab, J.M., Prass, K., et al., 2005 Oct. Central nervous system injury-induced immune deficiency syndrome. Nat. Rev. Neurosci. 6 (10), 775–786. PubMed PMID: 16163382.

Mikulski, Z., Hartmann, P., Jositsch, G., et al., 2010 Sep 29. Nicotinic receptors on rat alveolar macrophages dampen ATP-induced increase in cytosolic calcium concentration. Respir. Res. 11:133. PubMed PMID: 20920278. Pubmed Central PMCID: PMC2955664.

Olsen, A.B., Hetz, R.A., Xue, H., et al., 2013 Jul. Effects of traumatic brain injury on intestinal contractility. Neuro Gastroenterol. Motil. 25 (7), 593-e463. PubMed PMID: 23551971. Pubmed Central PMCID: PMC3982791.

Peeters, W., van den Brande, R., Polinder, S., et al., 2015 Oct. Epidemiology of traumatic brain injury in Europe. Acta Neurochir. 157 (10), 1683–1696. PubMed PMID: 26269030. Pubmed Central PMCID: PMC4569652.

Pelosi, P., Ferguson, N.D., Frutos-Vivar, F., et al., 2011 Jun. Management and outcome of mechanically ventilated neurologic patients. Crit. Care Med. 39 (6), 1482–1492. PubMed PMID: 21378554.

Pfeifer, R., Teuben, M., Andruszkow, H., et al., 2016. Mortality patterns in patients with multiple trauma: a systematic review of autopsy studies. PLoS One 11 (2), e0148844. PubMed PMID: 26871937. Pubmed Central PMCID: PMC4752312.

Piek, J., 1995 Aug. Medical complications in severe head injury. New Horiz 3 (3), 534–538. PubMed PMID: 7496764.

Piek, J., Chesnut, R.M., Marshall, L.F., et al., 1992 Dec. Extracranial complications of severe head injury. J. Neurosurg. 77 (6), 901–907. PubMed PMID: 1432133.

Plog, B.A., Dashnaw, M.L., Hitomi, E., et al., 2015 Jan 14. Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. J. Neurosci. 35 (2), 518–526. PubMed PMID: 25589747. Pubmed Central PMCID: PMC4293408.

Ransohoff, R.M., Kivisakk, P., Kidd, G., 2003 Jul. Three or more routes for leukocyte migration into the central nervous system. Nat. Rev. Immunol. 3 (7), 569–581. PubMed PMID: 12876559.

Theodore, J., Robin, E.D., 1976 Apr. Speculations on neurogenic pulmonary edema (NPE). Am. Rev. Respir. Dis. 113 (4), 405–411. PubMed PMID: 178254.

Tracey, K.J., 2002 Dec 19-26. The inflammatory reflex. Nature, 420(6917):853-9. PubMed PMID: 12490958.

Trunkey, D.D., Lim, R.C., 1974. Analysis of 425 consecutive trauma fatalities. J. Am. Coll. Emerg. Physicians (Nov/Dec), 368–371.

Vida, G., Pena, G., Deitch, E.A., Ulloa, L., 2011 Apr 01. alpha7-cholinergic receptor mediates vagal induction of splenic norepinephrine. J. Immunol. 186 (7), 4340–4346. PubMed PMID: 21339364. Pubmed Central PMCID: PMC3083451.

Woratyla, S.P., Morgan, A.S., Mackay, L., et al., 1995 Nov. Factors associated with early onset pneumonia in the severely brain-injured patient. Conn. Med. 59 (11), 643–647. PubMed PMID: 8565507.

Zaroff, J.G., Rordorf, G.A., Ogilvy, C.S., Picard, M.H., 2000 Aug. Regional patterns of left ventricular systolic dysfunction after subarachnoid hemorrhage: evidence for neurally mediated cardiac injury. J. Am. Soc. Echocardiogr. 13 (8), 774–779. PubMed PMID: 10936822.

Zygun, D.A., Kortbeek, J.B., Fick, G.H., et al., 2005 Mar. Non-neurologic organ dysfunction in severe traumatic brain injury. Crit. Care Med. 33 (3), 654–660. PubMed PMID: 15753760.

Zygun, D.A., Zuege, D.J., Boiteau, P.J., et al., 2006. Ventilator-associated pneumonia in severe traumatic brain injury. Neurocritical Care 5 (2), 108–114. PubMed PMID: 17099256.