

Comparison of the previous and current traumarelated shock classifications – The more the better? – A retrospective cohort study from a level I trauma

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Abstract

Background: The aim was to compare the predictive performance of the current, extended (VS+BD) Advanced Trauma Life Support (ATLS) classification for hypovolaemic shock over the previous, vital sign (VS)-based classification with respect to mortality outcomes. We also studied the prognostic values of heart rate (HR), systolic blood pressure (SBP), Glasgow Coma Scale (GCS) and base deficit (BD).

Methods: The present study is a retrospective analysis at a level I trauma centre between 11 July 2014 and 11 September 2019. Trauma patients (inclusion criteria: trauma team activation, transport directly from scene, no need for resuscitation on scene, precise and detailed medical documentation, age \geq 16, 30-day follow-up, complete dataset for HR, SBP, GCS and BD) were allocated to shock classes (I–IV) based on the VS and VS+BD criteria. The predictive values for the classifications were compared with a two-proportion Z-test, while individual parameters were examined with receiver operating characteristic (ROC) analyses.

Results: A total of 156 patients met the inclusion criteria out of 60,037 trauma admissions. Both the VS and VS+BD classifications have shown a strong relation to mortality (P=0.0001 vs. P=0.000009). There was no significant difference in their predictive performance. According to the statistical analysis, GCS, BD and SBP showed significant prognostic values (AUC_{GCS}=0.799 [CI: 0.722, 0.875]; AUC_{BD}=0.683, [CI: 0.576, 0.790]; AUC_{SBP}=0.633, [CI: 0.521, 0.744]). HR was found ineffective in predicting mortality.

Conclusions: The current ATLS classification for hypovolaemic shock did not appear to be superior to the previous, VS-based classification in our study setting. GCS, BD and SBP were proven to be useful parameters in prognosticating outcome. The role of HR should be reconsidered, since it does not seem to reflect the clinical condition accurately.

Background

Injuries accompanied by massive blood loss represent a leading cause of death among young adults [1] [2]. The mortality rate for hypovolaemic shock, which is the second main cause of mortality in trauma patients [3] [4], could be improved significantly through early recognition and appropriate guidelines for intravenous fluid resuscitation and blood transfusions [5]. Advanced Trauma Life Support (ATLS) provides guidelines for the early assessment and initial management of patients with major trauma by allocating them to different shock classes (I–IV) [6] [7]. Until recently, the guidelines used vital signs, including heart rate (HR), systolic blood pressure (SBP) and the Glasgow Coma Score (GCS), to estimate blood loss [8] [9]. However, the predictive value of the vital sign (VS)-based classification has been called into doubt. In 2013, data analysis of international trauma registries conducted by Mutschler et al. indicated that the VS classification does not reflect the clinical condition accurately and recommended the use of the base deficit (BD) as a sole parameter in the classification [10] [11]. BD is a metabolic marker and reflects the acid-base status of the patient. Due to the rapid availability of blood gases, BD is commonly used to assess haemorrhage and its consequences [12] [13] [14]. Several studies have

documented its ability to predict outcome in trauma and highlighted its role in patient classification [11] [15] [16] [17] [18] [19]. As a consequence, the latest ATLS (ATLS Student Course Manual, 10th edition) recommendation expanded the assessment criteria with the BD value [6]. However, the specificity of BD for hypovolaemia is reduced by many factors: BD is raised not only by metabolic acidosis, but also by therapeutic resuscitation, such as fluid loading with crystalloids (lactate Ringer or saline) [18]. Previous alcohol or drug use, both being common in trauma patients, may also impair its predictive accuracy [20] [21]. In addition, patients over 55 may have significant injuries and mortality risk without manifesting a BD out of the normal range [22].

The primary goal of this study was to compare the prognostic potential of the current ATLS classification for hypovolaemic shock (VS + BD) with the previous, VS-based one. For this purpose, we conducted a retrospective cohort analysis on patients at a level I trauma centre to compare the VS + BD and VS classifications in terms of their ability to predict mortality to confirm the superiority of the current ATLS guidance. Our secondary goal was to determine which parameters are the strongest prognostic factors for mortality in the early assessment of the injured. We calculated the individual predictive values of HR, SBP, GCS and BD to be able to set a power ranking.

Materials And Methods

Study design

The present study is a retrospective cohort analysis at a single, level I trauma centre.

Data collection

Data were collected on trauma patients between 11 July 2014 and 11 September 2019 from the electronic database (MedSolution) at the University of Szeged Emergency Department. In the past decades, there were several important modifications in emergency trauma guidelines, such as limitation of the amount of crystalloids and the use of tranexamic acid. Taking this into account, we have chosen to analyze data only from the past 5 years. The protocols of emergency trauma care including massive transfusion protocols in our institute have complied with the principles of ATLS during the whole study period. Accordingly, base deficit was included in the initial assessment of the severely injured from 2018. In our 5 year study period, there were no other significant changes in the management protocol.

Inclusion criteria

Inclusion criteria consisted of trauma team activation, transport directly from scene, an age of 12 years or greater and a detailed documentation with a complete dataset for HR, SBP, GCS and BD recorded at presentation, Abbreviated Injury Scale (AIS), Injury Severity Score (ISS) and accurate mechanism of injury. Trauma team activation is based on anatomical and physiological criteria and the mechanism of injury. The clinical handover between paramedics and emergency department staff follows the MIST and AMPLE templates (MIST: M – Mechanism of Injury; I – Injuries Sustained; S – Signs; T – Treatment and

Trends in the Vital Signs. AMPLE: A – Allergies; M – Medications; P – Past Medical History; L – Last Ate; E – Events). The age limit of 16 years was selected based on the fact that normal values of HR and SBP by adolescents of that age do not differ largely compared to adults [23] [24]. The recorded variables included the mechanism of injury, the International Statistical Classification of Diseases and Related Health Problems (ICD) codes, vital parameters measured by the trauma team (HR, SBP and GCS), BD and 30-day survival. It is important to note that prehospital treatment might have influenced the parameters. Paramedics use a unified protocol including guidance regarding the prehospital fluid resuscitation, administration of vasopressors and opioid analgesics also.

Patients who received cardiopulmonal resuscitation on scene or primary survey in an other institute were excluded. Patients with imprecise documentation or missing variables were also excluded.

Patient groups

The ATLS does not explicitly declare whether the worst parameter or a combination of all the parameters should determine the shock class of the patients. Most trauma patients cannot be allocated correctly to the four ATLS shock classes (I–IV) when a combination of vital parameters is assessed [8] [10]. Therefore, participants in our study were allocated based on their worst parameter within the VS and VS+BD criteria. Since the current ATLS classification for hypovolaemic shock does not describe exact values for HR, SBP and GCS, we adopted HR values from the previous ATLS classification and SBP and GCS values from the study by Dunham et al. to make the classification criteria objective [19]. (Table 1)

Criteria	Class I	Class II	Class III	Class IV
HR* (bpm)	<100	100-119	120-139	≥140
BP* (mmHg)	≥110	100-109	90-99	<90
GCS*	15	15	12-14	<12
D** (mEq/L)	<2.0	2.0-5.9	6-9.9	≥10

Table 1. Simplified version of the previous and current, extended ATLS classification criteria

HR=heart rate, SBP=systolic blood pressure, GCS=Glasgow Coma Scale, BD=base deficit, bpm=beats per minute. Due to missing information, respiratory rate and urinary output are not included.

*The current ATLS classification for hypovolaemic shock only offers exact values for BD. The values for HR were adopted from the previous ATLS classification, while we adopted the values used by Dunham et al. for SBP and GCS [19].

**A negative base excess (BE) is called a base deficit (BD) and indicates metabolic acidosis.

Outcomes

As a primary outcome, we compared the VS and VS+BD classifications with respect to mortality outcomes in order to confirm the superiority of the latest ATLS classification of hypovolaemic shock over the previous one. We also studied the prognostic potential for the individual parameters (HR, SBP, GCS and BD) to be able to determine the strongest and weakest predictive factors in the initial assessment.

Statistical analysis

Continuous data were expressed as mean±standard deviation. Categorical data were expressed as frequency or relative frequency (percentages).

Chi-square tests for independence were performed to test the reationship between VS+BD classification result and outcome of mortality. The assumption of chi-square test for independence was slightly violated in the crosstabulation of VS classification result and outcome of mortality, therefore Fisher's exact test was used to test the relationship between VS classification result and outcome of mortality.

Two-proportion Z-test was performed to compare the predictive power of the VS and VS+BD classifications.

Binary logistic regression was applied for further analysis between VS+BD classification

result (group 1,2 vs group 3,4) and outcome of mortality, odds-ratio and 95% confidence interval for oddsratio were calculated.

The predictive performance of individual variables was assessed using receiver operating characteristic (ROC) analysis. Area under ROC curve was calculated for each individual variables (candidate predictors: GCS, HR, SBP, BD). Hypothesis tests for AUC ROC were performed and 95% confidence bounds for AUC ROC were calculated with nonparametric method. A P-value *P*<0.05 was considered to be statistically significant. All data were analyzed by using statistical software IBM SPSS 25.0 (IBM Corporation, Chicago, IL, USA).

Results

In total, 60,037 trauma admissions were identified for further analysis from the database at the University of Szeged Emergency Department between 11 July 2014 and 11 September 2019. The trauma team was activated in 542 cases. A total of 156 patients met the inclusion criteria. The flowchart for patient enrolment is shown below (Fig. 1).

The mean age of the participants was 49.4. ± 20.7 years, and only 26.7% of the patients were female. The most common mechanisms of injury that required trauma team activation were road traffic accidents (56.41%) and falls (29.49%). The most affected body regions were the head and neck (74.36%), thorax (53.85%), and extremities (48.08%). Due to the fact, that most patients suffered a high energy trauma, multiple body regions were involved in most cases. The characteristics of the patient population and the

different shock classes are shown in Table 2. *(Table 2 is presented at the end of the manuscript)* The distribution of injury mechanisms and affected body regions are demonstrated on Table 3.

Table 3. Severity and mechanisms of injury, affected body regions

jury severity	Patient population (n=156)
ISS median (IQR)	29 (20-34)
$AIS_{head, neck} \ge 3 n (\%)$	47 (30.13)
$AIS_{face} \ge 3 n \ (\%)$	6 (3.85)
$AIS_{chest} \ge 3 n \ (\%)$	44 (28.21)
$AIS_{abdomen, pelvic contents} \ge 3 n (\%)$	24 (15.38)
$AIS_{extremities, pelvic girdle \ge 3 n$ (%)	51 (32.69)
$AIS_{external} \ge 3 n (\%)$	3 (1.92)
$AIS \ge 3 \text{ in } 2 \text{ or more regions } n (\%)$	19 (12.18)
Lechanism of injury n (%)	00 (EC 41)
Road traffic accidents	88 (56.41)
Pedestrian	18 (20.45)
Bicicle	<i>16 (18.18)</i> <i>17 (10.22)</i>
<i>Motorcycle Automobile</i>	17 (19.32)
Falls	37 (42.05)
Fails Assault	46 (29.49) 6 (3.85)
Autoaggression	3 (1.92)
Other	13 (8.33)
ffected body regions n (%)	15 (0.55)
Head	116 (74.36)
<i>Fracture of the skull</i>	24 (20.69)
Intracranial haemorrhage	47 (40.52)
Concussion	14 (26.92)
Subdural bleeding	21 (40.38)
Epidural bleeding	7 (13.46)
Subarachnoid haemorrhage	19 (36.54)
Thorax	84 (53.85)
Pneumothorax	37 (44.05)
Haemothorax	9 (10.72)
Lung contusion	4 (4.76)
Abdomen and pelvis	49 (31.41)
Inraabdominal organ injury	18 (37.50)
Injury of the spleen	13 (72.22)
Injury of the liver	5 (27.78)
Pelvic or retroperitoneal organ injury	6 (12.24)
Kidney injury	6 (100)
Pelvic or sacral fracture	25 (51.02)
Extremities	75 (48.08)
Shoulder or upper arm	23 (30.66)
Elbow or forearm	19 (25.33)
Wrist or hand	14 (18.66)
Hip or thigh	26 (34.66)
Knee or leg	35 (46.66)
Ankle or feet	6 (8.00)
AIIME OF ICCL	
Spine	
Spine	40 (25.64)
	40 (25.64) <i>4 (10.00)</i> <i>11 (27.50)</i>

ISS=Injury Severity Score, AIS=Abbreviated Injury Scale

Road traffic accidents and falls were the most common mechanisms that required the activation of the trauma team. The regions of the head and neck, thorax, and extremities were involved in a high number of cases. Several patients acquired injuries in more than one body regions.

According to VS, 31.41% of the patients were assigned to class I, 6.41% to class II, 13.46% to class III and 48.72% to class IV. Based on VS+BD criteria, 16.03% of the patients were reallocated to a higher shock class; however, this change affected mostly the low-risk classes (I and II). 34 patients died within the first 30 days, resulting in a mortality rate of 21.79%. The distribution of patients and mortality among the classes are shown in Fig. 2.

Both the VS and VS+BD classifications showed a strong relation to mortality in our chi-square and Fisher's exact tests (P_{VS} =0.0001 vs. P_{VS+BD} =0.000009). The results are shown in Tables 4 and 5. According to the two-proportion Z-test, there is no significant difference in their predictive performance of mortality (P=0.9808).

Variables	Shock classes	Survival	Exitus	Total	
HR; SBP; GCS	Ι	47	2	49	
	II	10	0	10	
	III	18	3	21	
	IV	47	29	76	
Total		122	34	156	
		P-value			
sher's exact test	0.000*				

Table 4. Fisher's exact test of VS and mortality

**P*<0.001

HR=heart rate, SBP=systolic blood pressure, GCS=Glasgow Coma Scale, df=degrees of freedom.

The results demonstrate a strong relation between mortality and VS classification. A p-value p<0.001 was considered to be statistically significant at 0.001 level

Table 5. Chi-square test of VS+BD and mortality

Variables	Shock classes	Survival	Exitus	Total	
R; SBP; GCS; BD	Ι	23	1	24	
	II	30	0	30	
	III	20	3	23	
	IV	49	30	79	
Total		122	34	156	
	df	P-value			
earson-Chi square	3	0.000*			

*P<0.001

HR=heart rate, SBP=systolic blood pressure, GCS=Glasgow Coma Scale, BD=base deficit, df=degrees of freedom

The results demonstrate a strong relation between mortality and VS+BD classification. A p-value p < 0.001 was considered to be statistically significant at 0.001 level.

Through a separate analysis of HR, SBP, GCS and BD, we found that GCS has the highest prognostic power (AUC_{GCS}=0.799, *P*<0.001; CI [0.722, 0.875]). Derangements in BD and SBP were significant but weak predictors of mortality (AUC_{BD}=0.683, *P*=0.001, CI [0.576, 0.790]; AUC_{SBP}=0.633, *P*=0.018, CI [0.521, 0.744]). HR was found ineffective in prognosticating outcome (AUC_{HR}= 0.595, *P*=0.090, CI [0.480, 0.710]). The results of the ROC analysis with the ROC curves for the variables are shown in Fig. 3.

Our binary logistic regression confirmed that the risk for mortality increases massively in the higher shock groups (III,IV) compared to the less severe ones (I,II). The results of the analysis are shown in Supplementary Table.

Discussion

The present study was designed to investigate the validity of the current ATLS classification and the prognostic power of each its parameters. As a primary outcome, we compared the predictive performance of the VS and VS+BD classifications with respect to mortality outcomes. Both classifications were found to be highly effective in predicting mortality, with no significant difference between their prognostic values. Therefore, the superiority of VS+BD over the VS classification could not be confirmed by our study.

It is also noteworthy that more than 90% of all deaths were distributed in classes III and IV in our study. This data underlines the importance of the threshold between classes II and III, where the first derangements in SBP, respiratory rate and urinary output, usually occur [6]. According to other studies, the threshold BD value between these two classes (6 mmol/L) shows a significant prognostic potential [16] [22]. Modifi et al. found the 6 mmol/L threshold of BD effective in predicting intra-abdominal injuries after blunt abdominal trauma [25]. It was also confirmed to be a useful tool for predicting mortality after thoracic injury in the study of Aukema et al. [26]. 6 mmol/L is also the point where the administration of blood products is recommended by ATLS [6]. The therapeutic and prognostic relevance of this point questions the reasonability of dividing trauma patients into four different groups during the primary survey. Because of the need for rapid decisions in the emergency trauma setting, the complexity and functionality of the ATLS classification have received criticism already before adding one more parameter, the base deficit to the criteria [27]. Based on our study, combining the less severe classes (I and II) and the severe classes (III and IV) could be a legitimate option to increase the practicality of the classification. Of course, a simplified classification like this will always be evaluated together with the adjuncts of the primary survey (e.g. extended Focused Assessment with Sonography for Trauma (eFAST) and pelvic X-ray). During the secondary survey, trauma patients could undergo a comprehensive, detailed assessment to estimate the extent of optimal fluid replacement.

As a secondary outcome, the predictive values of the individual parameters were evaluated. GCS, BD and SBP showed a significant predictive performance. While GCS displayed a relatively strong relation to the outcome, the relation was weak for BD and SBP. In our study, BD and SBP alone do not appear to have a sufficiently high prognostic potential to be the foundation for early assessment. SBP is considered to have a poor reliability in the early assessment, since hypotension does not occur until the degree of shock is profound [3] [28]. GCS and respiratory rate may be strongly affected in the case of brain or chest injuries without the presence of haemorrhage and hypotension [29]. HR did not have a significant relation to mortality in our study. Numerous factors such as anxiety, pain, medication and spinal cord injury can lead to tachycardia, making the specificity of tachycardia for hypotension questionable [29] [30]. Increased HR may also be masked via beta blockers [31] [32] (particularly in combination with Ca-channel inhibitors and ACE inhibitors) or physiological bradycardia in athletes [33]. Multiple studies pointed out that HR tends to demonstrate a biphasic response for blood loss and that the patient becomes bradycardic as blood loss becomes profound after initial tachycardia [29] [34] [35]. In our study, the predictive values of the individual parameters showed the following ranking: GCS>BD>SBP>HR. The relevant differences in power between the variables suggest that weighing them and using their combination to allocate trauma patients could potentially increase the accuracy and specificity of the classification for haemorrhage. However, further research with a larger sample size is required to develop this method.

Limitations

Our study has several limitations. The retrospective nature of our analysis can be considered as a limitation in itself. The influence of prehospital medication and fluid replacement therapy could not be

ruled out from the study. Prehospital intubation affected GCS upon Emergency Department presentation in several cases. Although this is an important limitation, it might not have a strong influence on our study. With regard to GCS, prehospital intubation is only recommended for GCS < 8. GCS 8 already indicates an allocation to class IV, according to criteria in this study. Consequently, it might not make a significant difference as regards our results if the initial GCS of a patient was recorded as 7 or 3.

As shown on the flowchart, a large number of patients were excluded due to missing BD or mortality data. In some cases, BD was not recorded immediately after presentation, but some hours later. The therapy administered in this time interval could have a further influence on BD values.

Due to insufficient documentation for respiratory rate and urinary output, we could not include them in our classification criteria. Recently, a further prospective study has been warranted at our centre, where the limitation could be eliminated with accurate documentation of prehospital treatment and standardized blood gas protocols.

Conclusions

Despite the significant relationship between BD and mortality, the previous and current ATLS classifications yielded nearly equivalent predictive performances, thereby rendering the added value of BD to the classification questionable. The following ranking shows the power of each individual variable to predict mortality: GCS > BD > SBP > HR. The role of HR in the early assessment of trauma patients should be reconsidered, since it does not seem to reflect the clinical condition accurately.

Abbreviations

ATLS - Advanced Trauma Life Support

HR - heart rate

- SBP systolic blood pressure
- GCS Glasgow Coma Scale
- VS vital sign based classification of hypovolaemic shock based on ATLS guidance
- BD base deficit
- VS + BD the current, extended ATLS classification of hypovolaemic shock
- AIS Abbreviated Injury Scale
- ISS Injury Severity Score

MIST: M – Mechanism of Injury; I – Injuries Sustained; S – Signs; T – Treatment and Trends in the Vital Signs.

AMPLE: A - Allergies; M - Medications; P - Past Medical History; L - Last Ate; E - Events

ICD - International Statistical Classification of Diseases and Related Health Problems

ROC - receiver operating characteristic

AUC – area under curve

eFAST - extended Focused Assessment with Sonography for Trauma

Declarations

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and has been approved by the local medical ethics committee at the University of Szeged under reference number 182/2019-SZTE.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authorship contributions

PH and PJ elaborated the conception and design of the study. The acquisition of data was performed by PJ, EB and ECs. Data were analysed and interpreted by PH and PJ. Statistical analyses were performed by

FR. PJ drafted the manuscript. The manuscript was revised critically for important intellectual content by PH, EV and BB. All authors read and approved the final manuscript.

Hereby, all authors certify that they have participated sufficiently in the work to take public responsibility for the content. Additionally, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine*.

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Tables

Table 2. Patient characteristics

HR=heart rate, SBP=systolic blood pressure, GCS=Glasgow Coma Scale, BD=base deficit. Slightly declining tendency in mean SBPs, increasing in mean BDs. A large decrease between mean GCS rates of classes II, III and IV, suggesting that GCS might have had the strongest influence on patient allocation. Need for vasopressors mainly in Classes III-IV.

Characteristic	All	Class I		Class II		Class III		Class IV	
	classes	VS	VS + BD						
Mean Age (y) (mean ± SD)	49.4 ± 20.7	48.0 ± 18.7	46.4 ± 15.2	39.7 ± 14.8	48.2 ± 19.9	47.0 ± 24.3	44.2 ± 23.9	52.1 ± 21.2	52.2 ± 21.3
Female (%) Male (%) Mean HR (bpm)	26.9 73.1 82.3 ±	30.6 69.4 78.0 ±	33.3 66.6 79.3 ±	20.0 80.0 90.7 ±	23.3 76.7 81.5 ±	23.8 76.2 88.0 ±	26.1 73.9 84.4 ±	26.3 73.7 82.4 ±	26.6 73.4 82.9 ±
(mean ± SD)	21.4	11.1	9.4	21.3	15.5	18.5	20.5	26.4	26.0
Mean SBP (Hgmm) (mean ± SD)	125.7 ± 33.5	142.4 ± 22.5	144.2 ± 22.1	119.5 ± 16.2	137.0 ± 23.9	130.0 ± 27.8	127.4 ± 22.7	114.5 ± 37.9	115.3 ± 38.3
Mean GCS (mean ± SD)	9.8 ± 5.5	15.0 ± 0.0	15.0 ± 0.0	15.0 ± 0.0	15.0 ± 0.0	13.7 ± 1.0	14.0 ± 1.0	4.7 ± 3.3	5.0 ± 3.6
Mean BD (mmol/L) (mean ± SD)	4.1 ± 4.9	2.2 ± 2.1	0.6 ± 1.0	3.3 ± 3.6	3.0 ± 1.7	4.7 ± 5.6	3.9 ± 3.2	5.3 ± 5.6	5.7 ± 6.0
Vasopressor need n (%)	36 (23.1%)	2 (4.1%)	0 (0%)	0 (0%)	1 (3.3)	5 (23.8)	5 (21.7)	29 (38.2)	30 (38.0)

Figures

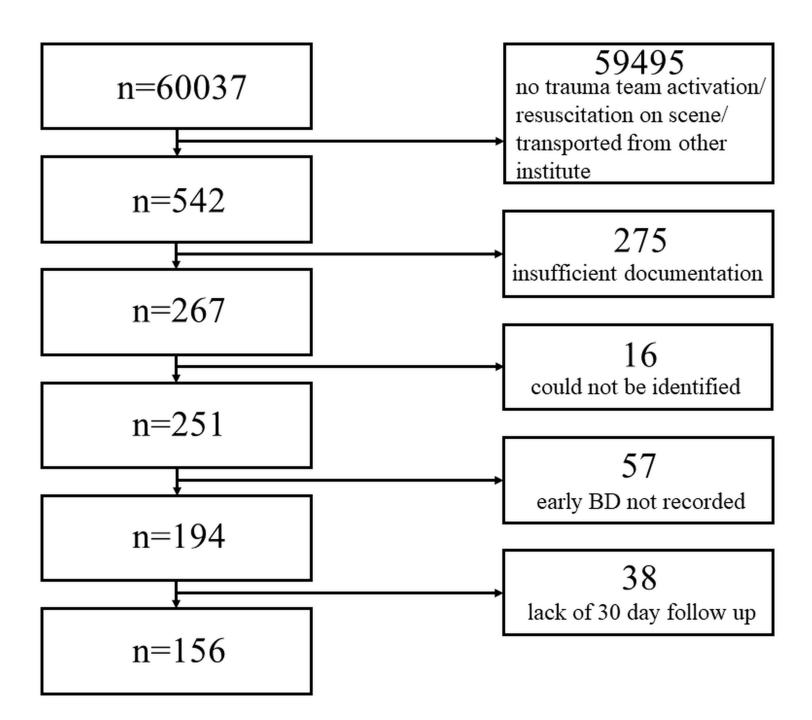


Figure 1

Study flowchart. The study flowchart illustrates that 60,037 trauma admissions occurred during the reported period. After excluding patients with no trauma team activation and who received resuscitation on scene or primary survey in an other institute, there were 542 trauma team activations left. The medical documentation was not comprehensive enough for our study in 275 cases. 16 patients with detailed medical record could not be identified due to the lack of personal data. Further 57 people were excluded due to missing early BD and 38 were also omitted because of the lack of 30-day follow-up. Ultimately, 156 patients were enrolled in the final analysis.

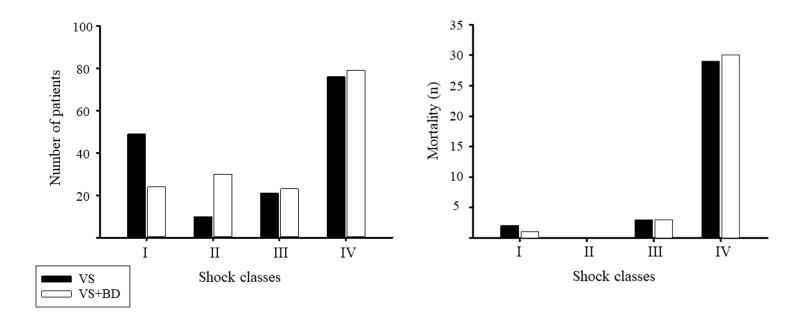


Figure 2

Distribution of patients (A) and mortality (B) among the shock classes based on VS and VS+BD. The difference in patient allocation mostly occurred in low-risk groups (I and II). Diagram A suggests that BD was not a key parameter in determining the shock class. Diagram B shows that the vast majority of mortality is located in class IV.

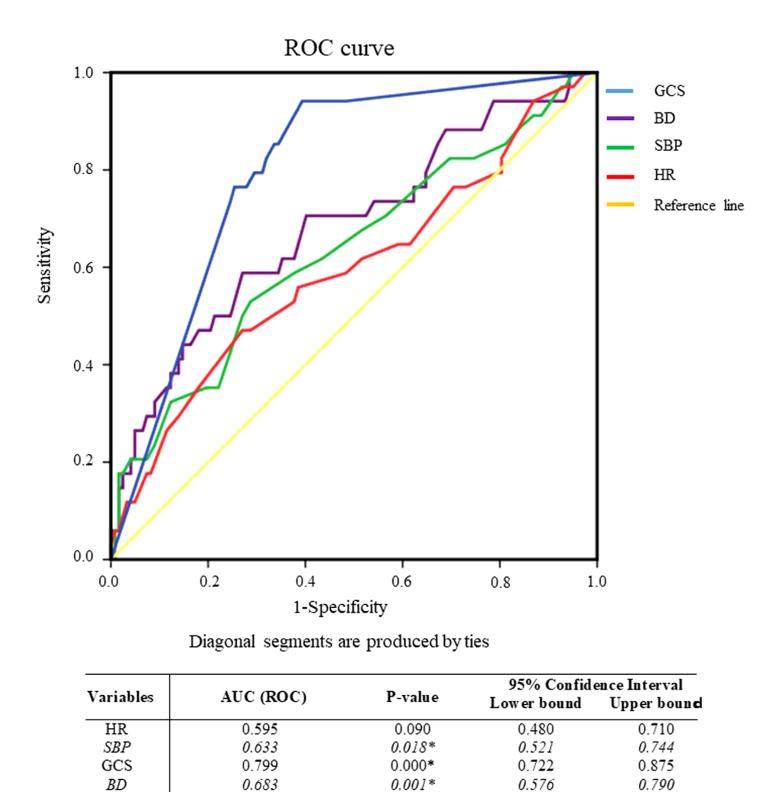


Figure 3

ROC analysis of the individual variables. HR=heart rate, SBP=systolic blood pressure, GCS=Glasgow Coma Scale, BD=base deficit, AUROC=area under the receiver operating characteristic curve ROC curves for the individual parameters. GCS has the largest AUCROC, showing the superiority of its predictive value over other parameters. A p-value p < 0.05 was considered to be statistically significant. *P<0.05

Supplementary Files

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• supplementarytable.docx