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HMGB-1 as a predictor of major bleeding requiring activation of a massive transfusion protocol in severe trauma

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Massive bleeding causes approximately 50% of deaths in patients with major trauma. Most patients die within 6 h of injury, which is preventable in at least 10% of cases. For these patients, early activation of the massive transfusion protocol (MTP) is a critical survival factor. With severe trauma, high-mobility group box 1 (HMGB-1, i.e., amphoterin) is released into the blood, and its levels correlate with the development of a systemic inflammatory response, traumatic coagulopathy, and fibrinolysis. Previous work has shown that higher levels of HMGB-1 are associated with a higher use of red blood cell transfusions. We conducted a retrospective analysis of previous prospective single-center study to assess the value of admission HMGB-1 levels in predicting activation of MTP in the emergency department. From July 11, 2019, to April 23, 2022, a total of 104 consecutive adult patients with severe trauma (injury severity score > 16) were enrolled. A blood sample was taken at admission, and HMGB-1 was measured. MTP activation in the emergency department was recorded in the study documentation. The total amount of blood products and fibrinogen administered to patients within 6 h of admission was monitored. Among those patients with massive bleeding requiring MTP activation, we found significantly higher levels of HMGB-1 compared to patients without MTP activation (median [interquartile range]: 84.3 µg/L [34.2–145.9] vs. 21.1 µg/L [15.7–30.4]; $p < 0.001$). HMGB-1 level showed good performance in predicting MTP activation, with an area under the receiver operating characteristic curve of 0.84 (95% CI 0.75–0.93) and a cut-off value of 30.55 µg/L. HMGB-1 levels correlated significantly with the number of red blood cell units (r_s [95% CI] 0.46 [0.28–0.61]; $p < 0.001$), units of fresh frozen plasma (r_s 0.46 [0.27–0.61]; $p < 0.001$), platelets (r_s 0.48 [0.30–0.62]; $p < 0.001$), and fibrinogen (r_s 0.48 [0.32–0.62]; $p < 0.001$) administered in the first 6 h after hospital admission. Admission HMGB-1 levels reliably predict severe bleeding requiring MTP activation in the emergency department and correlate with the amount of blood products and fibrinogen administered during the first 6 h of hemorrhagic shock resuscitation.

Trial registration: NCT03986736. Registration date: June 4, 2019.

Keywords Major trauma, Bleeding, Massive transfusion protocol, HMGB-1

Abbreviations

ABC	Assessment of blood consumption
AIS	Abbreviated Injury Scale
DCR	Damage–control resuscitation

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ED	Emergency department
ELISA	Enzyme–linked immunosorbent assay
FFP	Fresh frozen plasma
INR	International normalized ratio
ISS	Injury severity score
HMGB-1	High-mobility group box protein 1
MT	Massive transfusion
MTP	Massive transfusion protocol
RBC	Red blood cell
STROBE	STrengthening the Reporting of Observational studies in Epidemiology
TASH	Trauma Associated Severe Hemorrhage
TIC	Trauma induced coagulopathy

Severe trauma is the leading cause of death in patients aged 1–44 years, despite advances in prevention, resuscitation, and surgical treatment[1,2]. Uncontrolled bleeding is responsible for around 50% of all trauma-related deaths, and at least 10% of these deaths are potentially preventable[2,3]. Surgical control of bleeding and early activation of a massive transfusion protocol (MTP), along with other damage-control resuscitation (DCR) measures, improve outcomes for critically injured patients[4]. MTP is activated when massive blood loss is suspected based on the mechanism of injury, diagnosed anatomical injuries, patient physiology, and response to initial resuscitation efforts[5,6]. However, in clinical practice, early detection of life-threatening bleeding can be difficult in some patients. In previous years, based on retrospective studies, several predictive models have been developed to determine the risk of MT, such as the TASH (Trauma Associated Severe Hemorrhage)[7], ABC (i.e., assessment of blood consumption)[8], and ETS (i.e., emergency room transfusion score)[9]. These models incorporate vital signs, laboratory test results, and other variables to determine the likelihood of the need for a massive blood transfusion. However, they are based on the traditional definition of massive transfusion (MT), which is the need for ≥ 10 units of red blood cells (RBCs) in 24 h[10]. This definition is retrospective and excludes patients who die before 10 units are administered or before 24 h have elapsed (survivorship bias), thus representing less acute patients[11,12].

Patients with severe trauma develop a systemic inflammatory response almost immediately after injury. This response is triggered by complement and damage-associated molecular patterns (i.e., DAMPs), which are released from damaged tissue[13]. Among these molecules, high-mobility group box protein 1 (HMGB-1) is one of the most important mediators of the early phase of post-traumatic inflammation. HMGB-1 (also known as amphoterin) is a nuclear protein that acts as a DNA chaperone and is involved in the regulation of transcription, DNA replication, and a variety of other functions[14,15]. A previous study showed that amphoterin is released into the bloodstream within 45 min of severe trauma and that its levels correlate with the severity of injury, traumatic coagulopathy, and hyperfibrinolysis. In the same investigation, patients with high HMGB-1 levels had a greater blood loss and required more RBC transfusions compared to patients with low levels of the protein[14]. In the present study, we tested the hypothesis that HMGB-1 levels at admission can identify patients with severe bleeding requiring MTP activation. Most patients with fatal bleeding die within 3–6 h, so rapid and correct identification of these patients is essential[16].

Methods

In this study, we retrospectively analyzed data from a previous prospective study conducted at our center[17]. Following Institutional Review Board approval (April 25, 2019; reference number:424/2019) and registration (June 4, 2019; NCT03986736), 121 consecutive patients were enrolled during the study period from July 11, 2019, to April 23, 2022. We included adults (age > 18 years) with major trauma (Injury Severity Score [ISS] > 16), admitted to the emergency department (ED) of the Level I trauma center directly from the scene. Each patient enrolled in the study signed an informed consent form. In cases in which the patient's medical condition did not allow consent, it was obtained from the legal guardians; however, efforts were made to obtain consent from these patients retrospectively. Patients with injuries that are incompatible with life and with an expected survival time of < 24 h, or circulatory arrest at the scene or during transport were excluded from the study. Patients were also excluded if they had known severe kidney disease or were pregnant. This study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (i.e., STROBE) guidelines[18] and closely followed the principles of the Declaration of Helsinki.

Outcome measures

The objectives of the study were predefined. The main objective was to determine whether HMGB-1 levels at admission could identify patients with life-threatening bleeding requiring MTP activation in the ED. Another aim of the study was to analyze the relationship between HMGB-1 and the use of blood products and fibrinogen in the first 6 h after hospital admission.

Blood sampling

During the first 10 min after admission, 2.6 ml of blood was drawn into a tube (Monovette EDTA K3E 2.6 ml; Sarstedt, Germany) with EDTA as an anticoagulant agent. The sample was immediately centrifuged (2500 g for 10 min at 4 °C) in a central laboratory, snap-frozen in liquid nitrogen at -80 °C, and stored. Samples were analyzed by a single investigator blinded to patient data and using the HMGB-1 enzyme-linked immunosorbent assay (ELISA; Biovendor LM; Brno, Czech Republic). All study blood samples were analyzed with two of these kits, and measurements were taken according to the manufacturer's instructions. The analysis showed satisfactory intra- and inter-assay stability, with coefficients of variation < 5% and < 15%, respectively[19].

Data collection

We prospectively recorded baseline demographic data, vital signs, mechanism of injury, and length of time spent in pre-hospital care. The severity of the injury was assessed according to the ISS[20]. When the research sample was drawn, arterial blood also was taken for basic laboratory tests as part of standard management of a patient with severe trauma. The presence of tissue hypoperfusion (shock) was determined based on the methodology of a previous study using an admission base deficit > 6 mmol/L[14]. To identify trauma-induced coagulopathy (TIC), we used a conventional coagulation test with an international normalized ratio (INR) > 1.2 and > 1.5, representing values associated with worse outcomes in severely injured patients[21].

MTP consisted of four units of RBCs, fresh frozen plasma (FFP), and platelets (1:1:1 ratio) and was immediately available in the emergency room. Activation of the MTP was entirely at the discretion of the attending physician, who was not part of the research team, and was based on the patient's clinical condition, available laboratory results and estimated blood loss in accordance with the principles of DCR. The activation of MTP was recorded in the trial documentation. In addition, a TASH score was calculated (using the mdcalc tool, available at www.mdcalc.com) to facilitate the decision to activate MTP as soon as all variables were known[7].

Patients were followed for 6 h from admission to the ED. During this time, it was possible for early TIC to develop, characterized by an absence of hemostasis, which can lead to uncontrollable bleeding[22]. Total RBC, FFP, platelet, and fibrinogen requirements were recorded in the study protocol by an independent staff member using a case report form. HMGB-1 levels were measured after study recruitment to ensure complete blinding of the entire trauma team. The management of patients with severe trauma followed the principles of DCR. Patients were transferred from the ED to the operating room for damage control surgery or directly to the intensive care unit.

Statistical analysis

Data were analyzed using R (version 4.3.1, www.r-project.org). Categorical data are presented as absolute and relative frequencies (%). Numerical variables are expressed as medians with interquartile ranges [IQRs]. Differences between groups were assessed with the Mann–Whitney U test, the chi-squared test of independence for contingency tables, or the Fisher's exact test, as appropriate. Analysis of diagnostic accuracy was performed using the area under the receiver operating characteristic curve (AUC), sensitivity, specificity, positive and negative predictive value and positive and negative likelihood ratio, with all parameters presented with 95% confidence intervals (95% CIs). The point on the receiver operating characteristic curve closest to the upper left corner with coordinates (0,1) was used to determine the optimal cut-off. Spearman's correlation coefficient (r_s) was used for correlation analysis. The significance level was set to 0.05.

Results

The study included 104 consecutive patients with severe trauma admitted during a 33-month period. The study flowchart is shown in Fig. 1. The duration of the study was unexpectedly extended because of the COVID-19 pandemic, which significantly reduced the number of patients with severe trauma admitted to the study center. The characteristics of the patients and their injuries are shown in Table 1. The cohort was predominantly middle-aged men, with a median age of 41 years (IQR 31–57 years). At admission to the ED, 33 patients were in hemorrhagic shock, and 10 had laboratory evidence of TIC. Four patients died within the first 6 h of admission, three because of uncontrolled hemorrhage and one who developed malignant cerebral edema confirmed by CT scan (with subsequent transition to palliative care). These patients were not excluded from the study because their blood samples were taken, and in each case, death occurred in the last hour of study observation after all monitored parameters were already recorded.

MTP was activated in 31 patients with major trauma hemorrhage. In these patients, the median HMGB-1 level at admission was significantly higher than levels among patients without MTP activation (median [IQR]: 84.3 µg/L [34.2–145.9] vs. 21.1 µg/L [15.7–30.4]; $p < 0.001$; Fig. 2). Clinical (hypotension, tachycardia) and laboratory (base deficit > 6 and lactate > 4.4 mmol/l) signs of tissue hypoperfusion and shock were more common in patients in the MTP group on admission to the emergency department than in patients who did not require MTP activation. Table 2 gives a comparison of patient data between the MTP and non-MTP groups. HMGB-1 level showed good performance in predicting MTP activation (AUC 0.84; Fig. 3). In our cohort, HMGB-1 had 80.6% sensitivity and 75.3% specificity for predicting the need for MTP, with a cut-off value of 30.55 µg/L. Further measures of the diagnostic accuracy of HMGB-1 for predicting MTP are shown in Table 3.

We found significantly higher median levels of HMGB-1 in patients whose blood loss required the administration of massive transfusion, defined as the administration of 5 or more units of red blood cells over 4 h (referred to as MT1 in this study) and the administration of more than 4 any blood products over 2 h (MT2), compared with the group of patients who did not require MT (83.9 [29.7–148.2] vs. 21.6 [15.9–35.6]; $p < 0.001$, and 56.7 [29.3–130.9] vs. 20.4 [15.6–27.8]; $p < 0.001$)-—Table 4. HMGB-1 satisfactorily predicts the need for MT administration (AUC of 0.78 for MT1 and AUC of 0.79 for MT2)—Fig. 4. Other parameters of the diagnostic accuracy of HMGB-1 in the prediction of the need for MT are shown in Table 5.

HMGB-1 levels correlated significantly with RBC units (r_s [95% CI] 0.46 [0.28–0.61], $p < 0.001$), FFP units (0.46 [0.27–0.61], $p < 0.001$), platelets (0.48 [0.30–0.62], $p < 0.001$), and fibrinogen (0.48 [0.32–0.62], $p < 0.001$) administered in the first 6 h after hospital admission. Significantly higher medians of HMGB-1 levels were also found in patients with severe tissue hypoperfusion (BD > 6) and trauma-induced coagulopathy (INR > 1.2 and 1.5)-—Table 4.

We found no significant correlation between HMGB-1 level at admission and TASH score (r_s [95% CI] 0.07 [– 0.31 to 0.42], $p = 0.708$). Among patients requiring MTP activation, 83.9% (26 of 31) had a TASH score < 18, indicating a predicted < 50% risk for needing MTP (Fig. 5).

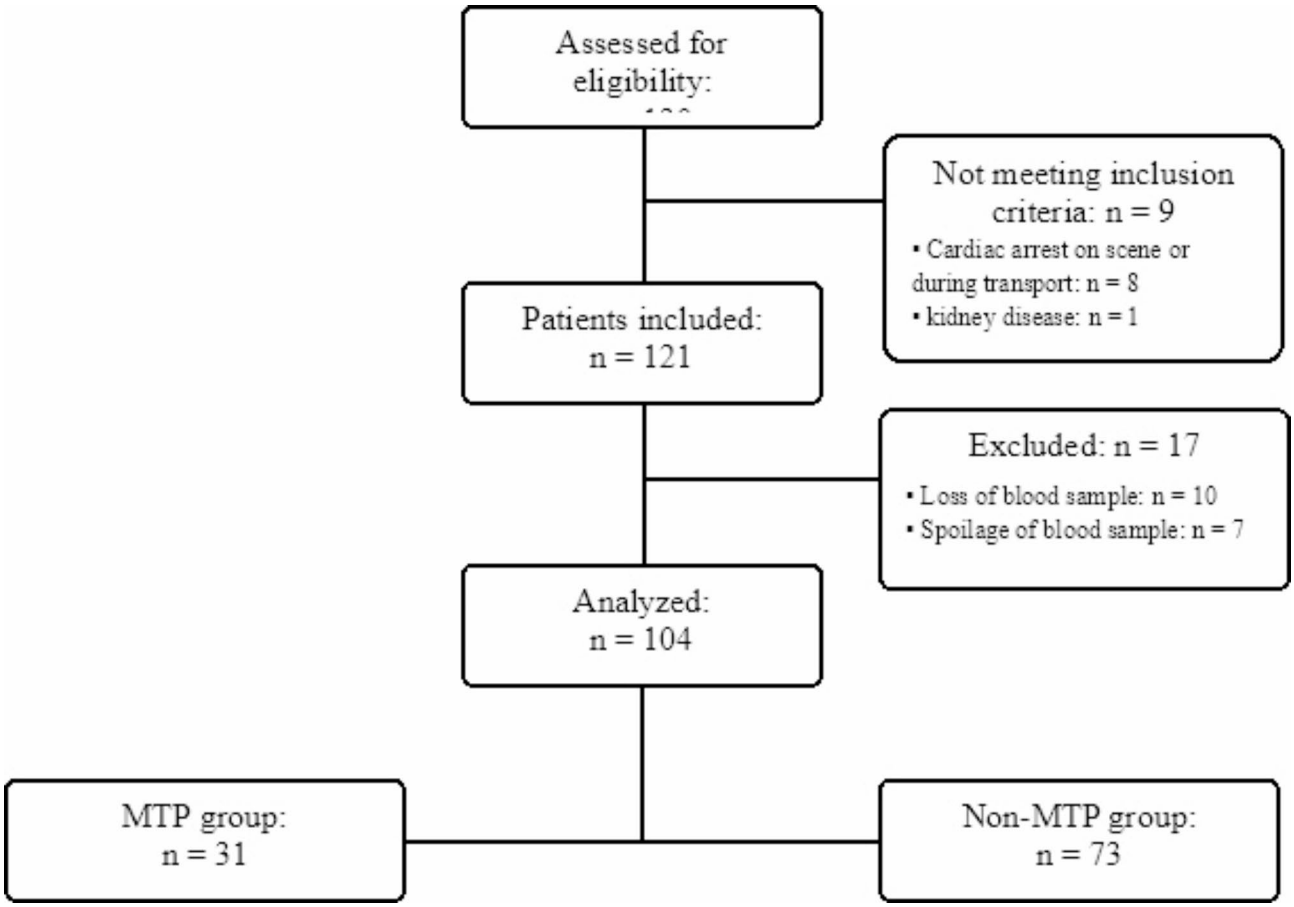

Fig. 1. Flowchart of the study. *MTP* massive transfusion protocol.

Discussion

In the present study, we found that patients with major bleeding requiring MTP activation had higher HMGB-1 levels at hospital admission compared with patients who did not require MTP activation. In addition, patients with high HMGB-1 levels had higher use of blood transfusion products and fibrinogen in the first 6 h after admission compared to patients with low HMGB-1 levels. We propose two possible explanations for these findings. First, HMGB-1 level correlates with the extent of injuries[14,23] and may be an indirect indicator of risk for major blood loss. Second, the release of HMGB-1 from damaged tissues into the bloodstream is associated with the development of early TIC[24], leading to uncontrolled bleeding[22]. Although the role of HMGB-1 in TIC is not fully understood, a negative effect of this protein on primary hemostasis has recently been reported[24]. Platelets are essential for a rapid hemostatic response, and platelet dysfunction early after injury leads to increased blood loss[25,26]. Although local release of HMGB-1 is important for proper platelet function[27], its systemic release from trauma-injured tissues leads to excessive platelet activation, a decrease in platelet number, and decreased platelet aggregation. In their work with a mouse model, Sloos et al. found that monoclonal antibody inhibition of HMGB-1 activity led to a significant improvement in clot formation and clot strength, as measured by rotational thromboelastometry[24]. These findings indicate an important role for HMGB-1 in the development of coagulopathy, especially given that the human HMGB-1 amino acid sequence shares 99% identity with the murine sequence[23]. In addition, once released into the blood, HMGB-1 triggers an inflammatory response via the receptor for advanced glycation end products (i.e., RAGE) and Toll-like receptors 2 and 4[28]. Inflammation and coagulation are highly interrelated processes that influence each other[22]. Experimental data showed a strong positive correlation between the increase in pro-inflammatory cytokines (TNF-α and IL-6) and the decrease in platelets[29].

Our results are consistent with those of Cohen et al., who reported that patients receiving ≥ 2 units of RBCs had higher levels of HMGB-1 compared with patients receiving < 2 units[14]. Our results not only confirm this previous finding but also extend it by showing a positive correlation between HMGB-1 levels and the administration of FFP, platelets, and fibrinogen. Bleeding is the leading preventable cause of death in critically injured patients and is responsible for 1 million deaths worldwide each year[30]. Most of these patients die within the first 6 h of admission, making early detection and MTP activation critical to their survival[16]. Every minute of delay in transfusion leads to a 5% increase in mortality. The decision to activate MTP is usually based on clinical assessment, decision tools, and response to treatment[31]. Our results suggest that HMGB-1 may be a reliable

Characteristics	Whole study cohort ($N = 104$)
Demographic data	
Male gender	77 (74.0)
Age, years	41 [31–57]
Physiological variables at admission	
Heart rate > 100 beats/min	41 (39.4)
Systolic blood pressure < 100 mmHg	35 (33.7)
Base deficit > 6 mmol/L	33 (31.7)
Lactate > 4.4 mmol/L	35 (33.7)
Hemoglobin, g/L	124 [107–138]
Platelet count, $\times 10^9$/L	224 [193–266]
Fibrinogen < 2 g/L	33 (31.7)
INR > 1.2	29 (27.9)
INR > 1.5	10 (9.6)
Injury characteristics	
ISS	32 [25–41]
Severe head injury (AIS > 3)	19 (18.3)
Blunt trauma	100 (96.2)
Motor vehicle accident	30 (30.0)
Motorbike	12 (12.0)
Fall	34 (34.0)
Pedestrian, bicycle	22 (22.0)
Other	2 (2.0)
Penetrating injury	4 (3.8)
Stab wounds	3 (75.0)
Gunshot wounds	1 (25.0)
TASH	5 [4–10]
Length of prehospital care, min	41 [35–47]
24-h mortality	5 (4.8)

Table 1. Characteristics of the patients and their injuries. Values are medians with interquartile ranges or absolute and relative frequencies (%). *AIS* Abbreviated Injury Scale, *INR* international normalized ratio, *ISS* Injury Severity Score, *TASH* Trauma Associated Severe Hemorrhage.

biomarker for identifying patients with major bleeding and provide decision support for activation of MTP. An HMGB-1 level > 30.55 µg/L predicts the need for MTP with satisfactory diagnostic accuracy.

Of note, we identified several patients who required MTP activation despite low HMGB-1 levels. In all of these patients, the source of the life-threatening bleeding was a lower limb semi-amputation with no significant damage to other tissues, explaining the lack of significantly increased HMGB-1 levels. These patients also were treated in prehospital care with a tourniquet, which prevented HMGB-1 release from the injured limb.

We emphasise that HMGB-1 identified patients requiring MTP activation despite a low TASH score and despite the fact that this score uses a number of predictors to estimate blood loss (hemoglobin concentration, presence of tachycardia and hypotension, etc.) which (as expected) were significantly different compared to the non-MTP group. This finding is not surprising because the TASH score is based on the classic definition of MT, i.e., administration of ≥ 10 RBC units over 24 h[7]. However, because of advances in DCR, the incidence of MT defined in this way is low. Therefore, other definitions for MT, such as > 5 RBC units over 4 h, are now increasingly used[11]. A recent Delphi study provided a new consensus definition of MT in severely injured adult patients, namely the need for > 4 units of any blood component administered within 2 h of injury[32]. Our findings indicate that even with this new definition, HMGB-1 can be a predictor of the need for MT.

ELISA is the most commonly used method for measuring HMGB-1 levels in clinical practice. The technique is time-consuming, however, requiring ~ 3 h, and is therefore not suitable for predicting risk of massive bleeding in the ED. However, measurement time can be significantly reduced by using an electrochemical immunosensor, which tracks changes in electrical impedance at the electrodes during the formation of the immunocomplex (HMGB-1 and capture antibody). In this way, HMGB-1 levels can be determined in < 20 min. In addition, the measurement can be accelerated by using a single impedance value obtained from a single frequency value[33]. In the future, with the introduction of new and faster methods to measure HMGB-1 levels, this biomarker could help guide the management of patients with severe bleeding.

The main strength of this study is that HMGB-1 levels were measured after patient recruitment had been completed, which completely eliminated the risk of bias associated with the trauma team. However, we acknowledge several study limitations. This study is retrospective, although all data were collected prospectively and recorded in the study registry. Given the broad variability in MTPs worldwide in terms of trigger and

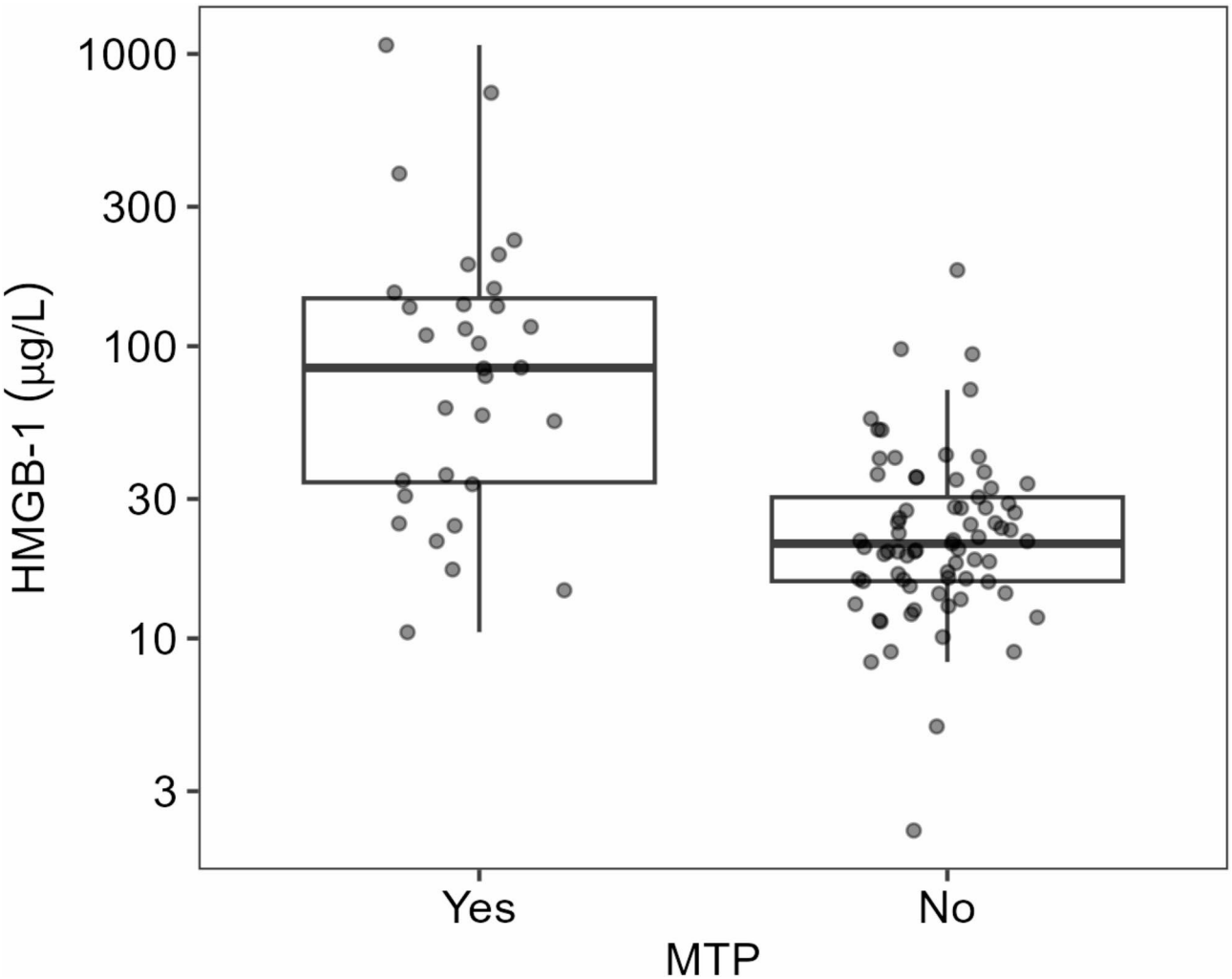

Fig. 2. High-mobility group box 1 (HMGB-1) levels in patients with versus without massive transfusion protocol (MTP) activation. Logarithmic scale used for better readability.

composition, our results may not be applicable across different systems of care for severely injured patients. In addition, the decision to activate the MTP was based in part on the clinical assessment of the patient, and therefore a degree of subjective assessment cannot be completely excluded. The HMGB-1 cut-off for MTP activation presented here relates to patients with a prehospital care time of approximately 40 min; with the short half-life of HMGB-1 from the time of an injury[23], the threshold for MTP activation may be different in trauma systems with longer hospital arrival times. Finally, most of the enrolled patients suffered blunt trauma, so that these results cannot be extrapolated to patients with penetrating injuries in whom the dynamics of HMGB-1 levels may differ.

Conclusion

HMGB-1 levels on admission provide a decision support for the activation of the MTP in severely injured patients in the emergency department. Amphoterin is a reliable biomarker of major bleeding and correlates with the amount of blood products and fibrinogen administered during the first 6 h of haemorrhagic shock resuscitation. Further studies will be necessary for confirmation of these results.

Characteristics	MTP group ($n=31$)	Non-MTP group ($n=73$)	p
Male gender	24 (77.5)	53 (72.6)	0.789
Age, years	38 [30–48]	46 [34–60]	0.138
Heart rate > 100 beats/min	24 (77.4)	17 (23.3)	< 0.001
Systolic blood pressure < 100 mmHg	21 (67.7)	14 (19.2)	< 0.001
Base deficit > 6 mmol/L	19 (61.3)	14 (19.2)	< 0.001
Lactate > 4.4 mmol/L	19 (61.3)	16 (21.9)	< 0.001
Hemoglobin, g/L	113 [99–127]	127 [114–141]	0.001
Platelet count, $\times 10^9$/L	205 [173–249]	236 [198–285]	0.109
Fibrinogen < 2 g/L	18 (58.1)	15 (20.5)	< 0.001
INR > 1.2	26 (83.9)	3 (4.1)	< 0.001
INR > 1.5	10 (32.3)	0 (0.0)	< 0.001
ISS	41 [34–50]	27 [22–34]	< 0.001
Severe head injury (AIS > 3)	5 (16.1)	14 (19.2)	0.789
TASH score	11 [7–16]	5 [2–5]	< 0.001
Length of prehospital care, min	39 [34–47]	41 [37–46]	0.352
HMGB-1, µg/L	84.3 [34.2–145.9]	21.1 [15.7–30.4]	< 0.001
SOFA (24 h)	9 [8–11]	7 [6–9]	0.001
Number of RBC transfusions	7 [4–10]	1 [0–2]	< 0.001
Number of FFP transfusions	4 [4–7]	0 [0–1]	< 0.001
Number of platelet transfusions	1 [1–2]	0 [0–0]	< 0.001
Dose of fibrinogen, g	5 [4–8]	2 [0–4]	< 0.001
24 h mortality	3 (9.7)	2 (2.7)	0.155

Table 2. Comparison of the MTP and non-MTP groups. Values are medians with interquartile ranges or absolute and relative frequencies (%). The p value was obtained with the Mann–Whitney test, the chi-square test of independence for contingency tables, or the Fisher's exact test. *AIS* Abbreviated Injury Scale, *FFP* fresh frozen plasma, *HMGB-1* high-mobility group box 1, *INR* international normalized ratio, *ISS* Injury Severity Score, *MTP* massive transfusion protocol, *RBC* red blood cell, *SOFA* Sequential Organ Failure Assessment, *TASH* Trauma Associated Severe Hemorrhage.

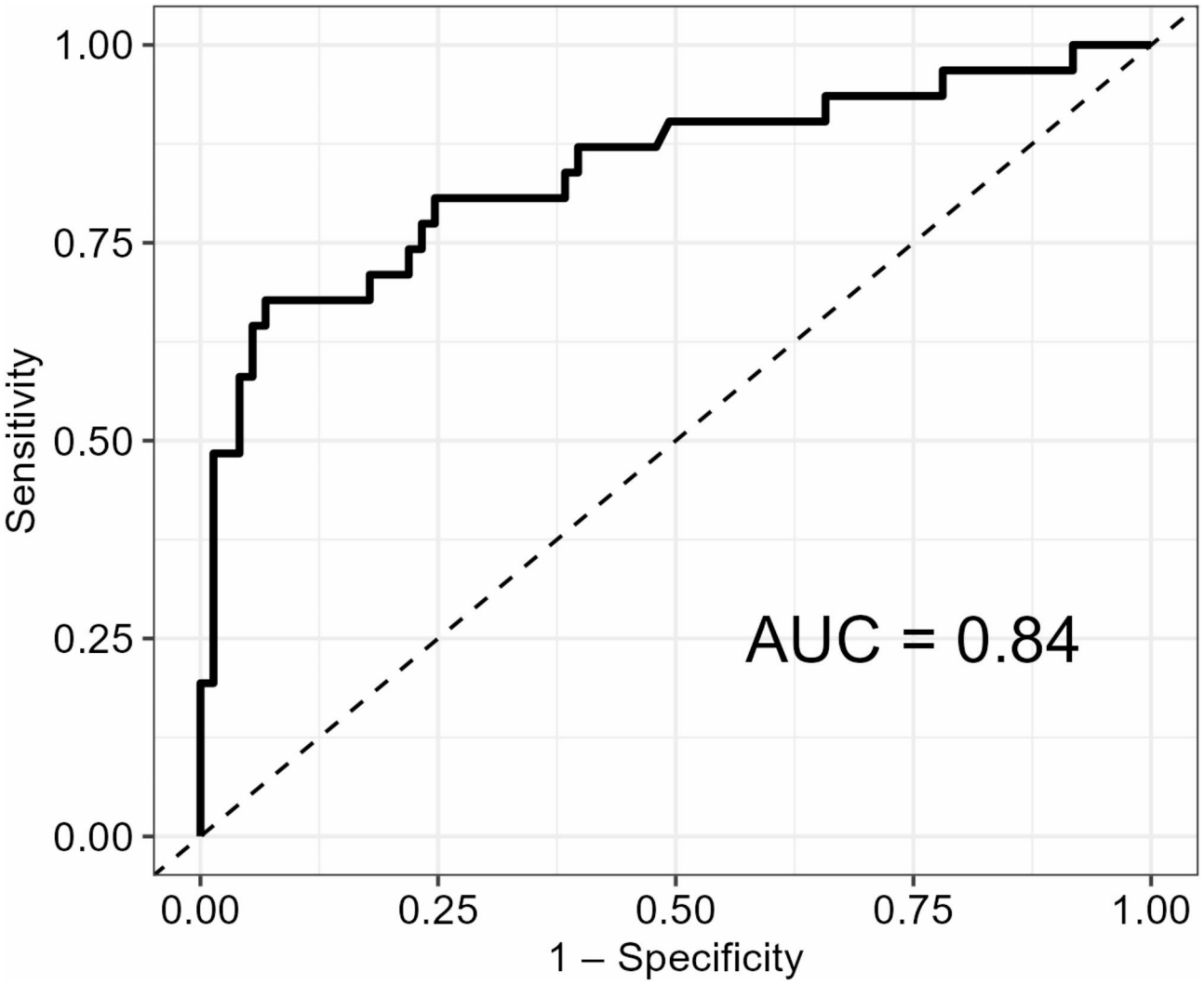

Fig. 3. Area under the receiver operating characteristic curve (AUC) of admission high-mobility group box 1 levels for prediction of massive transfusion protocol activation.

	Measure of diagnostic accuracy [95% CI]
Area under the curve	0.84 [0.75–0.93]
Optimal cut-off, µg/L	30.55
Sensitivity, %	80.6 [62.5–92.5]
Specificity, %	75.3 [63.9–84.5]
Positive predictive value, %	58.1 [42.1–73.0]
Negative predictive value, %	90.2 [79.8–96.3]
Positive likelihood ratio	3.27 [2.11–5.06]
Negative likelihood ratio	0.26 [0.12–0.53]

Table 3. Measures of diagnostic accuracy of HMGB-1 for prediction of MTP activation using the optimal cut-off estimated from the receiver operating characteristic curve. *HMGB-1* high-mobility group box 1, *MTP* massive transfusion protocol.

	Median HMGB-1 (IQR), µg/L
Base deficit > 6 mmol/L	
Yes	35.6 [24.7–102.1]
No	21.6 [15.8–36.4]
p	0.002
INR > 1.2	
Yes	84.3 [36.3–152.9]
No	21.1 [15.7–29.7]
p	< 0.001
INR > 1.5	
Yes	137.9 [110.5–212.2]
No	23.7 [16.0–40.3]
p	< 0.001
MT1	
Yes	83.9 [29.7–148.2]
No	21.6 [15.9–35.6]
p	< 0.001
MT2	
Yes	56.7 [29.3–130.9]
No	20.4 [15.6–27.8]
p	< 0.001

Table 4. Analysis of the association between HMGB-1 and the incidence of tissue hypoperfusion, coagulopathy and the need for massive transfusion. Values are medians with interquartile ranges of HMGB-1 (µg/L). The p-value was obtained with the Mann–Whitney test, *HMGB-1* high-mobility group box 1, *INR* international normalized ratio, *MT1* massive transfusion > 5 RBC units over 4 h, *MT2* massive transfusion > 4 units of any blood component administered over 2 h.

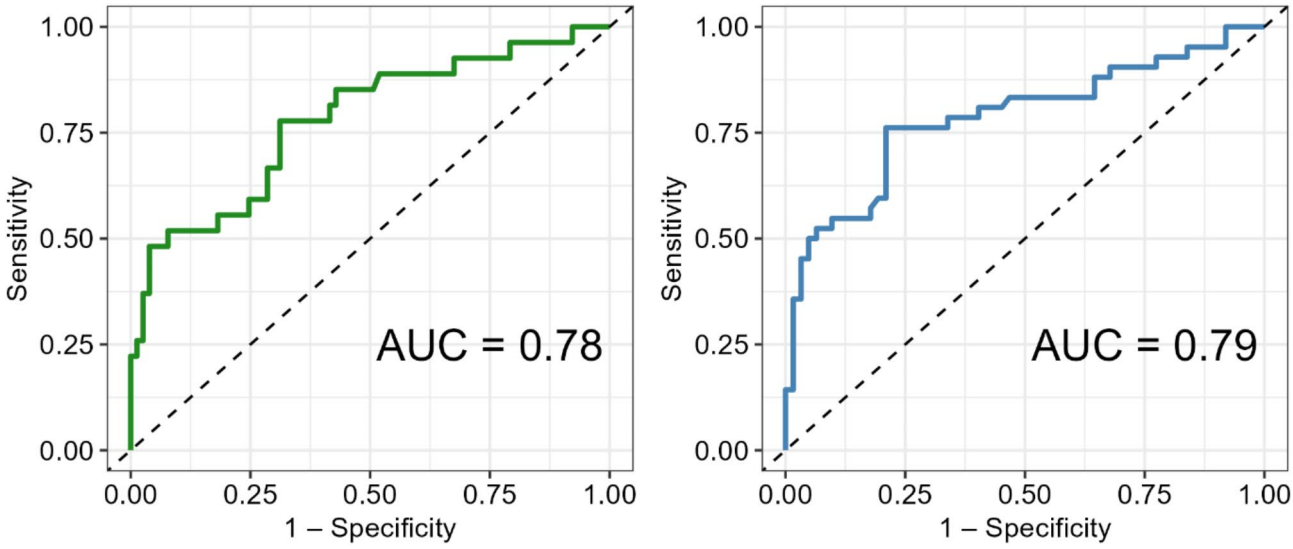

Fig. 4. Area under the receiver operating characteristic curve (AUC) of admission high-mobility group box 1 levels for prediction of MT1 (green) and MT2 (blue).

	Measure of diagnostic accuracy [95% CI]	
	MT1	MT2
Area under the curve	0.78 [0.67–0.89]	0.79 [0.69–0.88]
Optimal cut-off, µg/L	28.50	28.50
Sensitivity, %	77.8 [57.7–91.4]	76.2 [60.5–87.9]
Specificity, %	68.8 [57.3–78.9]	79.0 [66.8–88.3]
Positive predictive value, %	46.7 [31.7–62.1]	71.1 [55.7–83.6]
Negative predictive value, %	89.8 [79.2–96.2]	83.1 [71.0–91.6]
Positive likelihood ratio	2.50 [1.69–3.68]	3.63 [2.18–6.06]
Negative likelihood ratio	0.32 [0.16–0.66]	0.30 [0.17–0.53]

Table 5. Measures of diagnostic accuracy of HMGB-1 for prediction of MT1 and MT2 using the optimal cut-off estimated from the receiver operating characteristic curve. *HMGB-1* high-mobility group box 1, *MT1* massive transfusion > 5 RBC units over 4 h, *MT2* massive transfusion > 4 units of any blood component administered over 2 h.

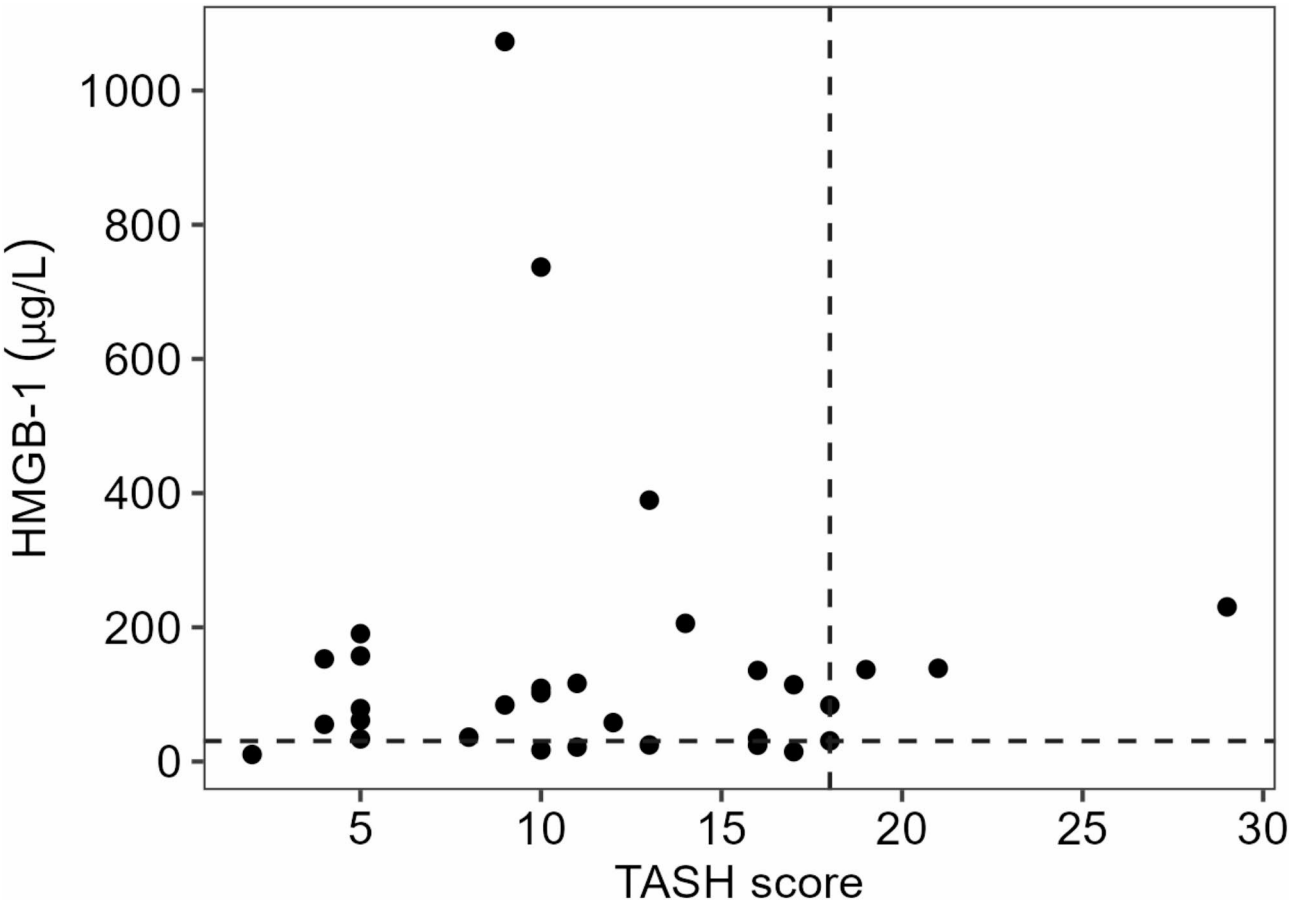

Fig. 5. Correlogram of high-mobility group box 1 (HMGB-1) levels and Trauma Associated Severe Hemorrhage (TASH) score in patients with massive transfusion protocol activation. Dashed lines represent cut-off values for TASH score and HMGB-1, i.e., TASH score equal to 18 and HMGB-1 equal to 30.55 µg/L.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Received: 26 September 2024; Accepted: 3 February 2025
Published online: 07 February 2025

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Acknowledgements

The authors are grateful to Zuzana Mučková for help with patient enrollment in the study and study administrative tasks.

Author contributions

M.F., M.B., J.P., F.B., and P.S. contributed to all aspects of this manuscript, including study conception and design, analysis, interpretation of data, and drafting of the article. V.V., O.J., M.B. and T.R. contributed to data acquisition and data curation. A.K. contributed to study design and data analysis. Z.Š. analyzed the blood samples. All authors read and approved the final manuscript.

Funding
This study was supported by the Ministry of Health, Czech Republic- Conceptual Development of Research Organization (FNOs/2019).

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and informed consent to participate
This study was approved by the Ethics Committee of the University Hospital Ostrava on April 25, 2019, with reference number 424/2019. All patients signed an informed consent form for inclusion in the study. Informed consent was obtained from the legal guardians for patients whose medical condition did not allow their giving consent. In these cases, every effort was made to obtain consent retrospectively.

Informed consent for publication
Patients in the study signed an informed consent form and consented to the publication of the results.

Additional information
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