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L-FABP and NGAL are novel biomarkers for detection of abdominal injury and hemorrhagic shock

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ABSTRACT

Introduction: Delayed diagnosis of abdominal injuries and hemorrhagic shock leads to secondary complications and high late mortality in severely traumatized patients. The liver fatty acid-binding protein (L-FABP) is expressed in intestine, liver and kidney; the neutrophil gelatinase-associated lipocalin (NGAL) in colon and kidney. We hypothesized that L-FABP is an early biomarker for abdominal injury and hemorrhagic shock and that L-FABP and NGAL are specific markers for detection of liver and/or kidney injuries.
Patients and Methods: Traumatized patients with an age ≥18 years and an abdominal injury (AIS_{abd}≥2), independently from Injury Severity Score (ISS), were prospectively included from 04/2018 to 05/2021. 68 patients had an abdominal injury ("Abd") and 10 patients had an abdominal injury with hemorrhagic shock ("HS Abd"). 41 patients without abdominal injury and hemorrhagic shock but with an ISS ≥ 25 ("noAbd") were included as control group. Four abdominal subgroups with isolated organ injuries were defined. Plasma L-FABP and NGAL levels were measured at admission (ER) and up to two days post-trauma.
Results: All patient groups had a median ISS≥25. In ER, median L-FABP levels were significantly higher in "HS Abd" group (1209.2 ng/ml [IQR=575.2–1780.3]) compared to "noAbd" group (36.4 ng/ml [IQR=14.8–88.5]), and to "Abd" group (41.4 ng/ml [IQR=18.0–235.5]), $p<0.001$. In matched-pair-analysis L-FABP levels in the group "Abd" were significantly higher (108.3 ng/ml [IQR=31.4–540.9]) compared to "noAbd" (26.4 ng/ml [IQR=15.5–88.8]), $p = 0.0016$. L-FABP correlated significantly with clinical parameters of hemorrhagic shock; the optimal cut-off level of L-FABP for detection was 334.3 ng/ml (sensitivity: 90%, specificity: 78%). Median L-FABP-levels were significantly higher in patients with isolated liver or kidney injuries and correlated significantly with AST, ALT and creatinine value. Median NGAL levels in the ER were significantly higher in "HS Abd" group (115.9 ng/ml [IQR=90.6–163.8]) compared to "noAbd" group (58.5 ng/ml [IQR=41.0–89.6],$p<0.001$) and "Abd" group (70.5 ng/ml [IQR=53.3–115.5], $p<0.05$). The group "Abd" showed significant higher median NGAL levels compared to "noAbd", $p = 0.019$. NGAL levels correlated significantly with clinical parameters of hemorrhagic shock.
Conclusion: :L-FABP and NGAL are novel biomarkers for detection of abdominal trauma and hemorrhagic shock. L-FABP may be a useful and promising parameter in diagnosis of liver and kidney injuries, NGAL failed to achieve the same.

Introduction

Among the 25% of severely traumatized patients with an abdominal injury, forty percent suffered a high 24h-mortality caused by massive bleeding. [1] Due to the high mortality rate, which is significantly associated with missed abdominal injuries, early

Abbreviations: Abd, Patients with abdominal injury; AIS, Abbreviated Injury Scale; ALT, aspartate transferase; AST, alanine transferase; AUC, Area under the curve; CI, Confidence interval; ER, Emergency Room; FFP, Fresh frozen plasma; H-FABP, Heart-type fatty acid binding protein; Hb, Hemoglobin; HS Abd, Patients with hemorrhagic shock and abdominal injury; Intestinal, isolated injuries of the intestine; ICU, Intensive care unit; IQR, Interquartile Range; IL-6, interleukin-6; INR, International normalized ratio; ISS, Injury Severity Score; Kidney, isolated injuries of the kidney; L-FABP, Liver fatty acid-binding protein; Liver, isolated injuries of the liver; MAP, Mean arterial pressure; MODS, Multiple organ dysfunction syndrome; NGAL, neutrophil gelatinase-associated lipocalin; noAbd, Patients with hemorrhagic shock and without abdominal injury; PLT, Platelets; NPV, Negative predictive value; PPV, Positive predictive value; PRBC, Packed red blood cells; PTT, Partial thromboplastin time; ROC, Receiver operator characteristic curves; SBP, Systolic blood pressure; SI, Shock index; SIRS, Systemic inflammatory distress syndrome; Spleen, isolated injuries of the spleen.

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detection of these injuries is of the utmost clinical importance. A delayed diagnosis may cause secondary complications such as peritonitis, abdominal compartment syndrome, sepsis, acute kidney failure and subsequent mortality. [1–3] Furthermore, hemorrhagic shock results in a circulatory dysfunction and leads to bacteria translocation in the intestine [4–6], resulting in hyperinflammatory response and secondary complications such as systemic inflammatory distress syndrome (SIRS), sepsis and multi-organ dysfunction syndrome (MODS) [7–9], which are the main causes of late mortality among severely injured patients [10,11].

Currently, ultrasound examination and computed tomography are routinely used for the detection of abdomal injury. Ultrasound is very sensitive, but its scope of detection is limited to active bleeding and it depends on the experience of its operator. [12,13] The CT scan is also a very sensitive method, but its disadvantages include high costs and radiation exposure for the patients. [13–15]

FABPs (fatty acid-binding proteins) are small intracellularly or within the plasma membrane localized proteins and are released into the extracellular space in their soluble extracellularly form early after cell or tissue damage. [16] Therefore, they are analyzed as plasma and urine markers for tissue-specific injuries. [17] Nine organ-specific isoforms of FABP are known [18] and they can be measured within hours by ELISA. Liver-type (L-) FABP is expressed in several tissues, such as the liver, intestine, kidney and pancreas. [19,20] It has been studied as a biomarker for intestine ischemia, such as necrotizing enterocolitis in humans [21], as a sensitive marker for hepatocellular damage in liver transplantation [22,23] and it has been suggested as a urinary marker for kidney injury in several studies [24–26]. In our previous studies, we have demonstrated that L-FABP is a novel biomarker for the detection of abdominal injury [27,28].

Neutrophil gelatinase-associated lipocalin (NGAL) is a small molecule belonging to the superfamily of proteins called lipocalins. [29] This protein was initially described in neutrophils and it is also normally expressed at very low levels in the epithelial cells of several human tissues (proximal tubule of the kidney, lungs, stomach and colon). [30] NGAL expression is markedly induced in injured epithelia. [31,32] In animal models it is one of the most upregulated genes and proteins in the kidney very early after acute injury [33] or after ischemic [34]. NGAL has already been studied as a biomarker for acute kidney injury after cardiac surgery or in critical ill patients. [35,36]

In this study, we hypothesized that L-FABP, as a marker for intestinal damage, is not only an early marker for abdominal injury but also for hemorrhagic shock, which leads to intestinal hypoperfusion and damage. Furthermore, because of its occurrence in the kidney and liver, we hypothesized that L-FABP is a marker for the specific detection of an injury to the kidney and the liver. Additionally, we hypothesized that NGAL is a marker for the early detection of an injury to the kidney in traumatized patients.

Patients and methods

Study design

This study was performed at the University Hospital Frankfurt of the Goethe University with the approval of the Institutional Ethics Committee (408/16, in accordance with the Declaration of Helsinki and reported following the Strengthening the Reporting of OBservational studies in Epidemiology, STROBE guidelines). [37] Written informed consent was obtained from all enrolled subjects or their nominated legally authorized representatives on behalf of the participants in accordance with the ethical standards.

Patients

Injured patients ≥ 18 years of age, independently from ISS (Injury Severity Score) [38], were included at admission to the emergency room (ER) and sequential blood measurement over 3 days was achieved.

The study period was 04/2018 to 05/2021. Further inclusion criteria consisted of a history of acute blunt or penetrating trauma.

Patients with burns, concomitant acute myocardial infarction, chronic diseases, and lethal injuries were excluded.

Overall, 119 patients were included in this study. Three patient groups were defined: patients with abdominal injury but without hemorrhagic shock (“Abd”); patients with abdominal injury and with hemorrhagic shock (“HS Abd”); and patients without abdominal injuries and without the presence of hemorrhagic shock, with an ISS≥25, for better comparability with the “HS Abd” group, as the control group (“noAbd”). For further analysis the “Abd” group was divided into four subgroups according to the isolated injured visceral organ: “Spleen”, “Liver”, “Kidney/urogenital”, “Intestinal”.

Data collection

Upon arrival to the ER, the vital parameters of all patients were recorded. Each injury was assigned an Abbreviated Injury Scale (AIS) by a trained physician at hospital discharge and the ISS was calculated.

Abdominal injury was defined as an injury of the kidney, liver, spleen, pancreas, bladder, ureter and urethra, abdominal blood vessels and intestine with an AIS abdomen ≥ 2 points. The patient’s characteristics were obtained from the patientś digital files. Abdominal injury was further defined as the presence of injury on CT scan or by intra-operative findings.

The subgroups of the abdominal injuries were named after the isolated injured visceral organ:

- “Spleen”: isolated injuries of the spleen
- “Liver”: isolated injuries of the liver
- “Kidney”: isolated injuries of the kidney and urogenital tract (bladder, ureter, urethra)
- “Intestinal”: isolated injuries to the small intestine, stomach and colon.

For the present study, hemorrhagic shock was defined using the following criteria:

- positive shock index (SI) (≥1) prehospital or in the ER and
- hemoglobin (Hb) <10 g/dl in the ER and
- lactate value ≥ 4 mmol/l in the ER and
- the need for a massive transfusion (≥ 10 packed red blood cells (PRBC) within the first 24 h.

Sample collection

Blood samples were obtained at admission to the ER (d0) and daily for two days (d1-d2) following trauma. Blood samples were collected in prechilled ethylenediaminetetraacetic acid tubes (BD Vacutainer, Bectom Dickinson Diagnostics, Aalst, Belgium) and kept on ice. Blood was centrifuged at 2000 x g for 15 min at 4 °C. The supernatant was stored at – 80 °C until the batch sample analysis was performed.

Blinded specimens were used for the measurement of L-FABP levels and NGAL levels. L-FABP levels were determined by the laboratory of the Department of Trauma, Hand and Reconstructive Surgery at the Hospital of the Goethe University Frankfurt using a highly specific commercially available ELISA (*Hycult Biotechnology, Uden, The Netherlands*) according to the manufacturer’s instructions. NGAL levels were determined by the laboratory of the

Table 1
Summary of patient's demographic, injury characteristics and in-hospital outcome in the three main patient groups.

Patient's characteristics	Abd (n = 68)	noAbd (n = 41)	HS Abd (n = 10)	p-value all groups	p-value (Abd *vs.* noAbd.)
Age	50 (27–53)	52 (34–63)	49 (38–62)	0.056	0.019
Sex (male, n,%)	57 (84%)	29 (71%)	7 (70%)	0.22	0.17
ISS	25.0 (13.0–34.0)	29.0 (26.0–41.0)	52.0 (46.3–58.5)	<0.0001	0.0023
AIS Head Face Chest	0 (0–2) 0 (0–0) 3 (0–3)	3 (0–5) 0 (0–1) 4	3 (0–3) 0 (0–0) 5	<0.0001 0.46 0.0002	<0.0001 0.36 0.0017
Abdominal Extremity	3 (3–4) 2 (0–3)	(3–5) 0 (0–0) 2 (0–2)	(4–5) 4 (3–4) 4 (2–5)	<0.0001 0.02	<0.0001 0.56
Injury pattern (n, blunt: penetrating)	56: 12	41: 0	10: 0	0.006	0.003
ICU stay (days)	7 (4–15)	9 (5–17)	15 (3–20)	0.33	0.27
Hospital stay (days)	13 (8–23)	15 (9–23)	17 (5–38)	0.73	0.72

Values are reported as median (interquartile range, IQR) and as percentages.
Abd: abdominal injury; AIS: Abbreviated Injury Scale Score; HS Abd: hemorrhagic shock and abdominal injury; ICU: Intensive care unit; ISS: Injury Severity Score; noAbd: without abdominal injury.

Department of Trauma, Hand and Reconstructive Surgery at the Hospital of the Goethe University Frankfurt using a highly specific commercially available ELISA (*R&D Systems, Minneapolis, USA*) according to the manufacturer's instructions.

Blood sampling for the measurement of I-FABP and NGAL was started in 2018. For the purpose of this study, and as previously described, 67 patients with abdominal injury and 10 patients with hemorrhagic shock and abdominal injury were identified and the L-FABP assays as well as the NGAL assays from the banked blood were performed in 2021. Furthermore, 41 severely injured patients (ISS≥25) without hemorrhagic shock and without abdominal injury were selected coincidentally as controls and assays were run likewise.

Data analysis

The Kolmogoroff-Smirnoff-Lillefords test showed that the plasma concentrations of L-FABP were not Gaussian distributed. Median L-FABP levels, median NGAL levels of the three main groups ("no Abd" *vs.* "Abd" *vs.* "HS Abd") and those of the four subgroups ("Spleen" *vs.* "Liver" *vs.* "Kidney/urogenital" *vs.* "Intestinal") were compared using the Kruskal-Wallis test. For comparison of two groups, the Mann-Whitney *U test* was applied. Furthermore, after frequency-matching according to an ISS ± 5 in the three main groups, the Wilcoxon-matched-pair-test was used for median L-FABP and NGAL levels.

For categorical variables, the chi-squared test with Yates' continuity correction was used for 2 × 2 tables, and Pearson's chi-squared was used for tables with larger dimensions.

Data are presented as the median and interquartile range (IQR) unless stated otherwise. A p-value of < 0.05 was considered to be statistically significant.

Spearman's correlation coefficients were calculated to determine the correlations between L-FABP levels, NGAL levels and other variables.

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated and presented with 95% confidence intervals (CI). Receiver operator characteristic curves (ROC) were generated to analyze the optimal cut-off level of L-FABP and NGAL. The optimal cut-off level is determined from the contact point of a 45° rising tangent with the ROC curve by using Bias 11.04.

Bias 11.04 (Epsilon Verlag GbR 1989–2022, Germany) and GraphPad Prism 3.02 (GraphPad Software Inc. San Diego, CA) were used to perform the statistical analysis and computations.

Results

Patient characteristics of the three main groups

119 patients were enrolled in this prospective study.

During the study period 78 patients presented with an abdominal injury, according to the definition outlined herein and according to our inclusion and exclusion criteria; 68 patients presented with an abdominal injury but without hemorrhagic shock ("Abd"); and 10 patients with an abdominal injury and with hemorrhagic shock ("HS Abd"). Furthermore, 41 severely injured patients (ISS≥25) were coincidentally selected as control patients, who had no abdominal injuries and no hemorrhagic shock ("noAbd").

Table 1 depicts the patients demographic and injury characteristics. No significant differences were found between the three groups in terms of age, gender, hospital and intensive care unit (ICU) length of stay and mortality. By definition, the AIS abdomen score was higher in the "Abd" group compared to "noAbd".

Because of the inclusion criteria, which stated that every patient with an abdominal injury independently from ISS was included in this study, and patients with no abdominal injury had to have an ISS≥25, the ISS of the group "noAbd" (29.0 [IQR=26.0–41.0]) was significantly higher than in the group "Abd" (25.0 [IQR=13.0–34.0]), $p = 0.0023$. Furthermore, the ISS of the group "HS Abd" (52.0 [IQR=46.3–58.5]) was significantly higher than in both of the other patient groups ($p<0.0001$).

Table 2 outlines the physiological characteristics of the three patient groups.

With regard to the physiological characteristics in the groups "Abd" and "noAbd", there were no significant differences.

By definition, the group "HS Abd" was found to be different to group "Abd" and group "noAbd" concerning the presence of hemorrhagic shock.

L-FABP levels as a marker for abdominal injury and hemorrhagic shock

The median concentrations of L-FABP at admission to the ER were significantly higher in the "HS Abd" group (1209.2 ng/ml [IQR=575.2–1780.3]) compared to the "noAbd" group (36.4 ng/ml [IQR=14.8–88.5]) and to the "Abd" group (41.4 ng/ml [IQR=18.0–235.5]) (Fig. 1), $p<0.001$. The median L-FABP level was not significantly different in the "Abd" group compared to the "noAbd" group (Fig. 1a, $p>0.05$).

By using matched-pair-analysis with the criterion of an ISS±5, the group "Abd" showed significant higher L-FABP levels (n = 38; 108.3 ng/ml [IQR=31.4–540.9]) compared to "noAbd" (n = 38; 26.4 ng/ml [IQR=15.5–88.8]), $p = 0.0016$, Fig. 1b.

L-FABP in the two days post-trauma

Fig. 1c demonstrates the two-day timeline of I-FABP for the three patient groups. Significantly higher L-FABP levels were found in the "HS Abd" group on the day of admission (d0) and on day 1 (d1) compared to the other two patient groups (d0: $p<0.001$ and

Table 2
Summary of the physiologic characteristics in the three main patient groups.

Physiologic charcteristics	Abd (n = 68)	noAbd (n = 41)	HS Abd (n = 10)	p-value all groups	p-value (Abd *vs.* noAbd.)
L-FABP (ng/ml, ER)	41.4 (18.0–235.5)	36.4 (14.8–88.5)	1209.2 (575.2–1780.3)	0.0001	0.24
NGAL (ng/ml, ER)	70.5 (53.3–115.5)	58.5 (41.0–89.6)	115.9 (90.6–163.8)	0.0006	0.02
SBP (mm Hg, pre-hospital)	126 (104–150)	128 (110–147)	65 (56–80)	0.015	0.64
SBP (mm Hg, ER)	146 (115–166)	140 (117–164)	48 (38–61)	0.0008	0.52
Heart rate (beats/min, pre-hospital)	100 (83–118)	98 (76–130)	96 (85–120)	0.99	0.92
Heart rate (beats/min, ER)	106 (89–114)	86 (77–124)	133 (106–140)	0.019	0.17
PRBC transfusion within 24 h (Units)	0 (0–1)	0 (0–0)	11 (10–18)	<0.0001	0.67
PRBC transfusion total (Units)	0 (0–3)	1 (0–3)	15 (13–23)	<0.0001	0.42
FFP transfusion within 24 h (Units)	0 (0–0)	0 (0–0)	8 (6–11)	<0.0001	0.63
FFP transfusion total (Units)	0 (0–0)	0(0–0)	8 (6–15)	<0.0001	0.62
Hemoglobin (g/dl, ER)	13.1 (10.9–14.3)	13.4 (10.8–14.4)	7.7 (7.0–8.4)	<0.0001	0.81
INR (ER)	1.1 (1.0–1.2)	1.1 (1.0–1.2)	1.5 (1.1–1.5)	0.026	0.79
Fibrinogen (mg/dl, ER)	236 (191–294)	231 (184–261)	111 (102–159)	0.0002	0.68
PLT count (x 10^3/µl, ER)	219 (180–263)	207 (170–258)	137 (94–195)	0.008	0.32
Lactate (mg/dl, ER)	24 (16–36)	22 (15–31)	60 (58–78)	<0.0001	0.58
Leukocytes (U/nl, ER)	12.2 (9.3–18.3)	12.9 (9.7–16.7)	10.5 (9.7–13.3)	0.68	0.98
Interleukin-6 (pg/ml, ER)	85.7 (17.0–186.5)	90.6 (27.2–190.0)	831.1 (198.8–1426.3)	0.004	0.68
AST (U/l, ER)	47 (31–116)	46 (33–69)	240 (92–438)	0.0015	0.34
ALT (U/l, ER)	43 (24–94)	32 (21–49)	98 (71–390)	0.001	0.04
Creatinine (mg/dl, ER)	1.0 (0.9–1.2)	0.9 (0.8–1.1)	1.2 (1.1–1.3)	0.015	0.17

Values are reported as median (interquartile range, IQR).
Abd: abdominal injury; AST: alanine transferase; ALT: aspartate transferase; ER: emergency room; FFP: fresh frozen plasma; HS Abd: hemorrhagic shock with abdominal injury; INR: International normalized ratio; L-FABP: liver fatty acid binding protein; NGAL: neutrophil gelatinase-associated lipocalin; noAbd: without abdominal injury; PLT: Platelets; PRBC: packed red blood cells; PTT: partial thromboplastin time; SBP: systolic blood pressure.

d1: $p<0.01$). Following the first peak at admission (ER), L-FABP levels decreased in all three patient groups over the observed time course. On day 2, no significant differences in L-FABP levels were noticed among the three patient groups.

L-FABP correlates with clinical parameters for hemorrhagic shock

The I-FABP levels at admission to the ER significantly correlated with the following clinical hemorrhagic shock parameters: base deficit ($R=-0.39$, $p<0.0001$), lactate value ($R = 0.36$, $p<0.0001$), SI ER ($R = 0.31$, $p = 0.0009$), SBP ER ($R=-0.31$, $p = 0.0007$), Hb value ($R=-0.35$, $p = 0.0001$), pH value ($R=-0.42$, $p<0.0001$), the amount of PRBC and FFP units transfused within the first 24 h ($R = 0.54$, $p<0.0001$ and $R = 0.46$, $p<0.0001$, respectively), International normalized ratio (INR, $R = 0.19$, $p = 0.0387$), PTT levels ($R = 0.27$, $p = 0.0039$) and fibrinogen ($R=-0.21$, $p = 0.0243$).

There were no significant correlations between I-FABP levels and platelet counts, temperature and leukocyte counts.

ROC analysis for optimal cut-off level of L-fabp in hemorrhagic shock

Comparing the groups “Abd” and “HSAbd”, receiver operating characteristic curve analysis shows an optimal cut-off level of L-FABP of 334.3 ng/ml for detecting hemorrhagic shock, with 90% sensitivity (CI: 56% - 100%) and 78% specificity (CI: 66%–87%). The area under the curve (AUC) is = 0.89 (CI: 0.75 - 0.95) (Fig. 2). The positive predictive value (PPV) and the negative predictive value (NPV) for L-FABP for the detection of hemorrhagic shock were 38% (CI: 19% - 59%) and 98% (CI: 90% - 100%), respectively.

NGAL levels as a marker for abdominal injury and hemorrhagic shock

The median concentrations of NGAL at admission to the ER were significantly higher in the “HS Abd” group (115.9 ng/ml [IQR=90.6–163.8]) compared to the “noAbd” group (58.5 ng/ml [IQR=41.0–89.6], $p<0.001$) and to the “Abd” group (70.5 ng/ml [IQR=53.3–115.5], $p<0.05$) (Fig. 3a). By using Mann-Whitney-*U test*, the group “Abd” showed significantly higher NGAL levels compared to “noAbd”, $p = 0.0192$. By using matched-pair-analysis with the criterion of ISS±5, the group “Abd” also showed significantly higher NGAL levels ($n = 38$; 73.1 ng/ml [IQR=61.6–126.0]) compared to “noAbd” ($n = 38$; 56.6 ng/ml [IQR=40.8–95.5]), $p = 0.0042$, Fig. 3b.

NGAL did not correlate significantly with the ISS [data not shown].

NGAL in the two days post-trauma

Fig. 3c demonstrates the two-day timeline of NGAL for the three patient groups. Significantly higher NGAL levels were found in the “HS Abd” group on the day of admission (d0) compared to the other two patient groups ($p<0.001$ *vs.* “noAbd” and $p<0.05$ *vs.* “Abd”). On d1 there was a slight rise in NGAL levels compared to d0. NGAL levels on d1 were significantly higher in the “HS Abd” group compared to the group “noAbd” ($p<0.01$) and in the group “Abd” compared to the group “noAbd” ($p<0.05$). Following the peak of NGAL levels on d1, NGAL levels decreased over time to d2, where no significant differences in NGAL levels were noticed among the three patient groups.

NGAL correlates with certain clinical parameters for hemorrhagic shock

The NGAL levels at admission to the ER significantly correlated with the following clinical hemorrhagic shock parameters: base deficit ($R=-0.19$, $p = 0.044$), lactate value ($R = 0.20$, $p = 0.0294$), SI ER ($R = 0.24$, $p = 0.0106$), SBP ER ($R=-0.19$, $p = 0.0496$), pH value ($R=-0.18$, $p = 0.0481$), the amount of PRBC units transfused within the first 24 h ($R = 0.35$, $p = 0.0001$) and leukocyte counts ($R = 0.42$, $p<0.0001$).

There were no significant correlations between NGAL levels and Hb value, the amount of FFP units transfused within the first 24 h, INR, PTT, platelet counts, fibrinogen and temperature.

ROC analysis for optimal cut-off level of ngal in abdominal injury and in hemorrhagic shock

Receiver operating characteristic curve analysis shows an optimal cut-off level of NGAL of 48.33 ng/ml for detecting abdominal injury (90% sensitivity [CI: 80% - 96%] and 39% specificity [CI: 24% -

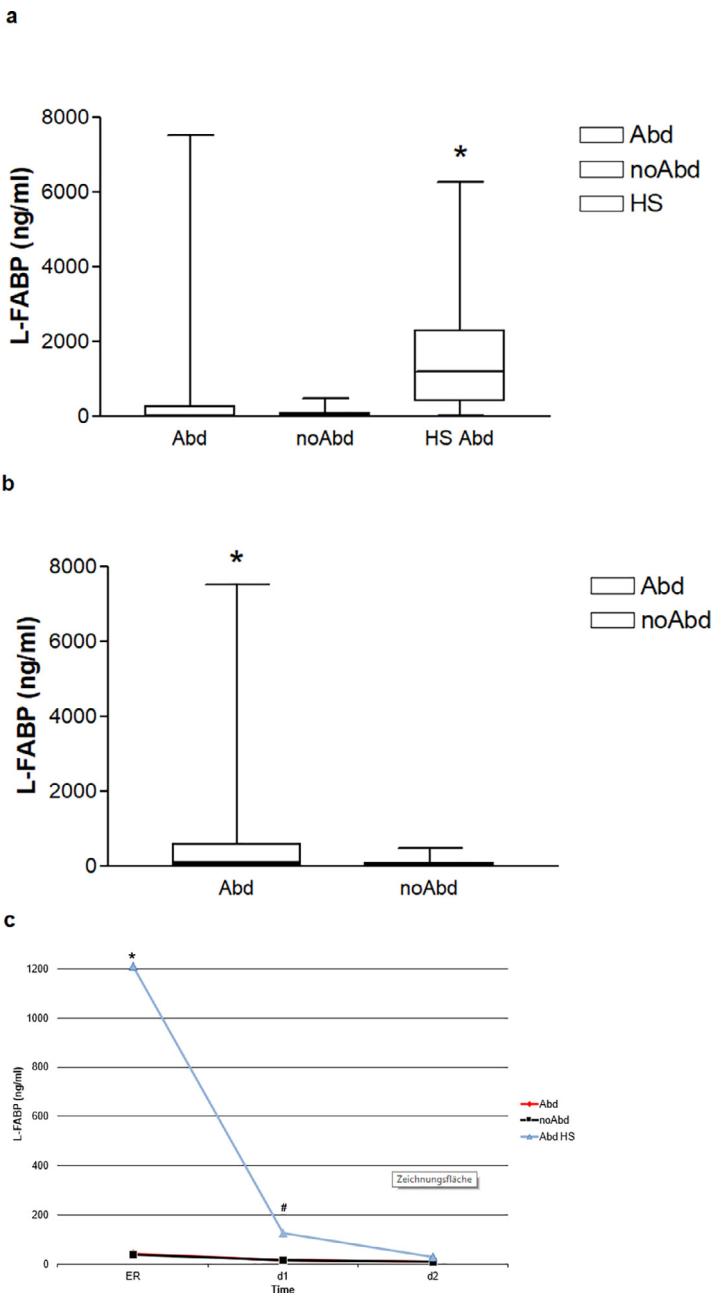

Fig. 1. a:L-FABP levels on admission to the emergency room. Median (interquartile range [IQR], Min and Max) liver fatty acid-binding protein (L-FABP) values in the three study groups based on the presence or absence of abdominal injury and hemorrhagic shock on admission to the emergency room. Abd: abdominal injury; ER: emergency room; HS Abd: abdominal injury and hemorrhagic shock; noAbd: without abdominal injury Abd ($n = 67$); noAbd ($n = 41$); HS Abd ($n = 10$) * $p<0.001$ HS *vs.* noAbd and *vs.* Abd **Figure 1b:L-FABP levels on admission to the emergency room by using matched-pair-analysis.** Median (interquartile range [IQR], Min and Max) liver fatty acid-binding protein (L-FABP) values in both study groups based on the presence or absence of abdominal injury by using matched-pair-analysis with the criteria of an ISS±5 on admission to the emergency room. Abd: abdominal injury; noAbd: without abdominal injury Abd ($n = 38$); noAbd ($n = 38$) * $p = 0.0016$ Abd *vs.* noAbd **Figure 1c: Time course of L-FABP levels.** Time course of median liver fatty acid-binding protein (L-FABP) levels (ng/ml) of all three patient groups based on the presence or absence of hemorrhagic shock and abdominal injury. Abd: abdominal injury; d1: day 1; d2: day 2; ER: emergency room; HS Abd: hemorrhagic shock with abdominal injury; noAbd: without abdominal injury * $p<0.001$ HS Abd. *vs.* Abd and *vs.* noAbd # $p<0.01$ HS Abd. *vs.* Abd and *vs.* noAbd.

56%]) and 80.80 ng/ml for detecting hemorrhagic shock (100% sensitivity [CI: 69% - 100%] and 55% specificity [CI: 42% - 68%]). The AUC is = 0.64 (CI: 0.53 - 0.75) for abdominal injury and = 0.76 (CI: 0.64 – 0.89) for hemorrhagic shock. The PPV for NGAL for the detection of abdominal injury and for the detection of hemorrhagic shock were 71% (CI: 60% - 80%) and 28% (CI: 14% - 45%), respectively. The NPV for NGAL for the detection of abdominal injury and for the detection of hemorrhagic shock were 70% (CI: 47% - 87%) and 100% (CI: 89% - 100%), respectively.

Patient characteristics of the abdominal subgroups

Overall, 68 patients suffered an abdominal injury. 52 of them had isolated injured visceral organs and they were divided into four abdominal subgroups according to the isolated injured visceral organ: “Spleen”, “Liver”, “Kidney/urogenital”, and “Intestinal” were enrolled in this study.

Table 3 depicts the patientsdemographic and injury characteristics of the four abdominal subgroups. No significant differences

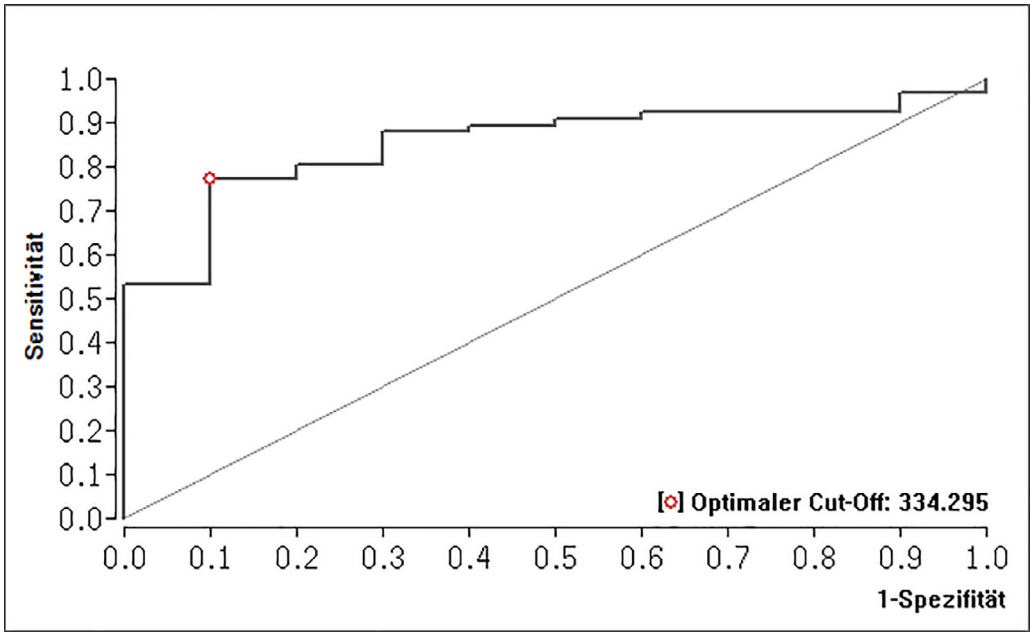

Fig. 2. Receiver operating curve of L-FABP for detection of hemorrhagic shock Receiver operating characteristic curve showing the optimal cut-off for L-FABP levels (334.3 ng/ml) in predicting the presence or the absence of hemorrghagic shock with 90% sensitivity and 78% specificity. L-FABP: Liver fatty acid-binding protein.

Table 3
Summary of the patient's demographic, injury characteristics and in-hospital outcome in the abdominal subgroups.

Patient's characteristics	Spleen (n = 15)	Liver (n = 14)	Kidney (n = 13)	Intestinal (n = 10)	p-value (all groups)
Age	29 (24–42)	37 (24–51)	51 (42–63)	38 (26–57)	0.03
Sex (male, n,%)	12 (80%)	10 (71%)	12 (92%)	9 (90%)	0.47
ISS	22 (17–29)	18 (11–29)	27 (14–37)	10 (9–13)	0.03
AIS Head Face Chest	0 (0–2) 0 (0–0) 3 (1–3)	0 (0–0) 0 (0–1) 3 (0–3)	0 (0–3) 0 (0–0) 2 (1–3)	0 (00) 0 (0-–0) 0	0.05 0.08 0.06 0.15
Abdominal Extremity	3 (3–4) 2 (0–2)	3(2–3) 1 (0–3)	3 (2–3) 0 (0–4)	(0-–0) 3 (3–3) 0 (0–1)	0.36
Injury pattern (n, blunt: penetrating)	15:0	11:3	13:0	3:7	0.00004
ICU stay (days)	7 (6–9)	5 (1–7)	6 (2–14)	3 (1–10)	0.27
Hospital stay (days)	12 (10–20)	10 (6–15)	11 (7–29)	8 (6–13)	0.32

Values are reported as median (interquartile range, IQR) and as percentages.
AIS: Abbreviated Injury Scale Score; ICU: Intensive care unit; intestinal: isolated injuries of the intestine; ISS: Injury Severity Score; kidney: isolated injuries of the kidney; liver: isolated injuries of the liver; spleen: isolated injuries of the spleen.

were found between the four abdominal subgroups with isolated visceral organ injuries regarding gender, AIS scores, hospital and ICU length of stay and mortality. There were significant differences between these four groups concerning age, ISS and injury pattern (p<0.05).

Table 4 outlines the physiological characteristics of the abdominal subgroups.

There were no significant differences between the four abdominal subgroups.

L-FABP as an organ-specific marker in trauma of the liver and of the kidney

The median L-FABP-levels were significantly higher in the group "liver" (135.0 ng/ml [IQR=23.7–393.8]) and in the group "kidney" (69.3 ng/ml [IQR=27.3–244.6]) compared to the "intestinal" group (14.2 ng/ml [IQR=9.3–23.1]), p<0.05 (Fig. 4).

There was no significant difference in the median of the L-FABP levels when comparing the groups "liver" and "kidney" with the group "spleen" (33.3 ng/ml [IQR=17.3–56.1]).

L-FABP correlates with clinical parameters of injuries to the liver

The L-FABP levels at admission to the ER of all patients (n = 119) significantly correlated with the clinical parameters of liver injuries on the day of admission: AST value (R = 0.74, p<0.0001) and ALT value (R = 0.71, p<0.0001).

Interestingly, the L-FABP levels of the patients in the subgroup "liver" (n = 14) at admission to the ER correlated significantly much higher with AST and ALT value (R = 0.86, p = 0.0002 and R = 0.89, p<0.0001, respectively).

L-FABP correlates with creatinine as a clinical parameter of an injury to the kidney

The L-FABP levels of all patients (n = 119) at admission to the ER significantly correlated with the clinical parameter creatinine on the day of admission (R = 0.29, p = 0.0019).

A higher significant correlation could be found with the L-FABP levels at admission to the ER of all patients (n = 119) with creatinine on the following day (d1) with R = 0.31 (p = 0.001) and

Table 4
Summary of the physiologic characteristics in the abdominal subgroups.

Physiologic charcteristics	Spleen (n = 15)	Liver (n = 14)	Kidney (n = 13)	Intestinal (n = 10)	p-value (all groups)
L-FABP (ng/ml, ER)	33.3 (17.3–56.1)	135.0 (23.7–393.8)	69.3 (27.3–244.6)	14.2 (9.3–23.1)	0.02
NGAL (ng/ml, ER)	88.6 (59.3–118.0)	61.6 (50.1–75.3)	93.1 (55.0–128.0)	67.6 (43.0–71.4)	0.14
SBP (mm Hg, pre-hospital)	130 (122–133)	115 (89–128)	141 (110–167)	133 (126–142)	0.25
SBP (mm Hg, ER)	130 (117–169)	145 (116–164)	160 (120–170)	150 (150–170)	0.71
Heart rate (beats/min, pre-hospital)	100 (91–119)	110 (88–119)	82 (74–107)	90 (87–110)	0.25
Heart rate (beats/min, ER)	110 (95–115)	98 (90–108)	106 (90–112)	100 (88–113)	0.53
PRBC transfusion within 24 h (Units)	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–0)	0.68
PRBC transfusion total (Units)	0 (0–2)	0 (0–2)	1 (0–4)	0 (0–0)	0.40
FFP transfusion within 24 h (Units)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0.44
FFP transfusion total (Units)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0.44
Hemoglobin (g/dl, ER)	13.8 (13.1–14.8)	12.8 (11.8–14.4)	14.2 (13.4–14–4)	13.1 (9,7–14.9)	0.60
INR (ER)	1.1 (1.0–1.1)	1.1 (1.0–1.1)	1.1 (1.0–1.1)	1.3 (1.0–1.4)	0.36
Fibrinogen (mg/dl, ER)	236 8195–290)	237 (198–257)	294 (231–299)	192 (154–221)	0.09
PLT count (x 10^3/µl, ER)	224 (207–252)	231 (217–268)	223 (171–262)	210 (119–260)	0.65
Lactate (mg/dl, ER)	22 (15–40)	23 (17–36)	24 (17–34)	33 (24–46)	0.19
Leukocytes (U/nl, ER)	17.1 (9.3–23.5)	11.2 (10.3–16.0)	13.1 (10.0–16.1)	9.1 (6.7–13.5)	0.20
Interleukin-6 (pg/ml, ER)	87.2 (34.5–155.5)	42.9 (10.3–167.1)	85.7 (14.9–137.8)	38.8 (13.7–85.6)	0.62
AST (U/l, ER)	54 (40–90)	42 (26–229)	70 (39–111)	28 (24–39)	0.049
ALT (U/l, ER)	47 (31–73)	43 (20–124)	47 826–83)	24 (16–33)	0.049
Creatinine (mg/dl, ER)	1.0 (0.9–1.2)	1.0 (0.9–1.2)	1.2 (1.0–1.3)	1.0 (0.9–1.1)	0.07

Values are reported as median (interquartile range, IQR).
AST: alanine transferase; ALT: aspartate transferase; ER: emergency room; FFP: fresh frozen plasma; INR: International normalized ratio; intestinal: isolated injuries of the intestine; kidney: isolated injuries of the kidney; L-FABP: liver fatty acid binding protein; liver: isolated injuries of the liver; NGAL: neutrophil gelatinase-associated lipocalin; PLT: Platelets; PRBC: packed red blood cells; PTT: partial thromboplastin time; SBP: systolic blood pressure; spleen: isolated injuries of the spleen.

between the L-FABP levels at day 1 (d1) with creatinine on day 2 (d2) with $R = 0.4$ ($p<0.0001$).

Furthermore, L-FABP levels on day 1 (d1) correlated significantly higher with creatinine of d2 in the group "Abd" ($R = 0.47$, $p = 0.0003$) and "HS Abd" ($R = 0.94$, $p = 0.0167$).

In the group "noAbd" there was no significant correlation between L-FABP levels and creatinine value at any time.

Interestingly, in patients of the abdominal subgroup "kidney" a significant and strong correlation existed between L-FABP levels of d1 and creatinine of d2 ($R = 0.72$, $p = 0.0118$).

NGAL fails to be an organ-specific marker in trauma of the kidney

The median NGAL levels were not significantly different in the four abdominal subgroups on any day in the timeline (spleen: 88.6 ng/ml [IQR = 59.3 – 118.0]; liver: 61.6 ng/ml [IQR = 50.1 – 75.3]; kidney: 93.1 ng/ml [55.0 – 128.0]; intestinal: 67.6 ng/ml [43.0 – 71.4]).

NGAL levels correlate with creatinine as a clinical parameter of an injury to the kidney

The NGAL levels at admission to the ER of all patients ($n = 119$) significantly correlated with the clinical parameter creatinine on the day of admission ($R = 0.41$, $p<0.0001$).

Furthermore, the NGAL levels of all patients ($n = 119$) on day 1 (d1) correlated significantly with the creatinine of d1 ($R = 0.49$, $p<0.0001$) and NGAL levels of all patients of d2 correlated significantly with creatinine of d2 ($R = 0.37$, $p = 0.0003$).

The highest significant correlations could be found between the NGAL levels at d1 and the creatinine of d1 in the group "Abd" ($R = 0.47$, $p = 0.0003$), "noAbd" ($R = 0.42$, $p = 0.0094$) and "HS Abd" ($R = 0.86$, $p = 0.0238$).

Interestingly, in patients of the abdominal subgroup "kidney" a significant and strong correlation existed between the NGAL levels of d0 and the creatinine of d0 ($R = 0.76$, $p = 0.0149$) and between the NGAL levels of d1 and the creatinine of d1 ($R = 0.79$, $p = 0.0088$).

Discussion

Almost 50% of early mortality in severely traumatized patients is caused by massive bleeding [1]. The main causes for late mortality are SIRS, sepsis and MODS [10,11]. These in turn are caused by delayed diagnosis [1–3], tissue hypoperfusion [39,40] and intestinal damage [4,41] resulting from hemorrhagic shock.

The present study investigated the association between L-FABP and abdominal injury, as well as the association between L-FABP and hemorrhagic shock, using L-FABP as a marker for intestinal damage and tissue hypoperfusion.

Furthermore, because of the presence of L-FABP in the liver and in the kidney and the presence of NGAL in the kidney, we also investigated whether L-FABP and NGAL are markers for the specific detection of an injury to these tissues.

L-FABP is a marker for abdominal injury

FABP levels reveal a significant relationship with the severity of trauma, as assessed by the ISS [42,43]. In our own study, we similarly reported a significant correlation between L-FABP levels and ISS [28].

In the present study, the ISS was significantly different between the groups because of the inclusion criteria, in that, independently from the ISS, every patient with an abdominal trauma was included. To exclude this relevant impact on the measured L-FABP levels, matched-pair-analysis concerning the ISS was performed.

L-FABP is a primary marker for abdominal injury, as it has been shown that L-FABP is significantly elevated in patients with abdominal injury compared with patients without abdominal injury. This result confirms our previously published findings [27,28].

L-FABP is a promising biomarker for the detection of hemorrhagic shock

The novel finding of the data presented is, that patients with hemorrhagic shock exhibited significantly higher L-FABP levels at admission to the ER than patients without. We were able to calculate an optimal cut-off level for L-FABP to detect hemorrhagic

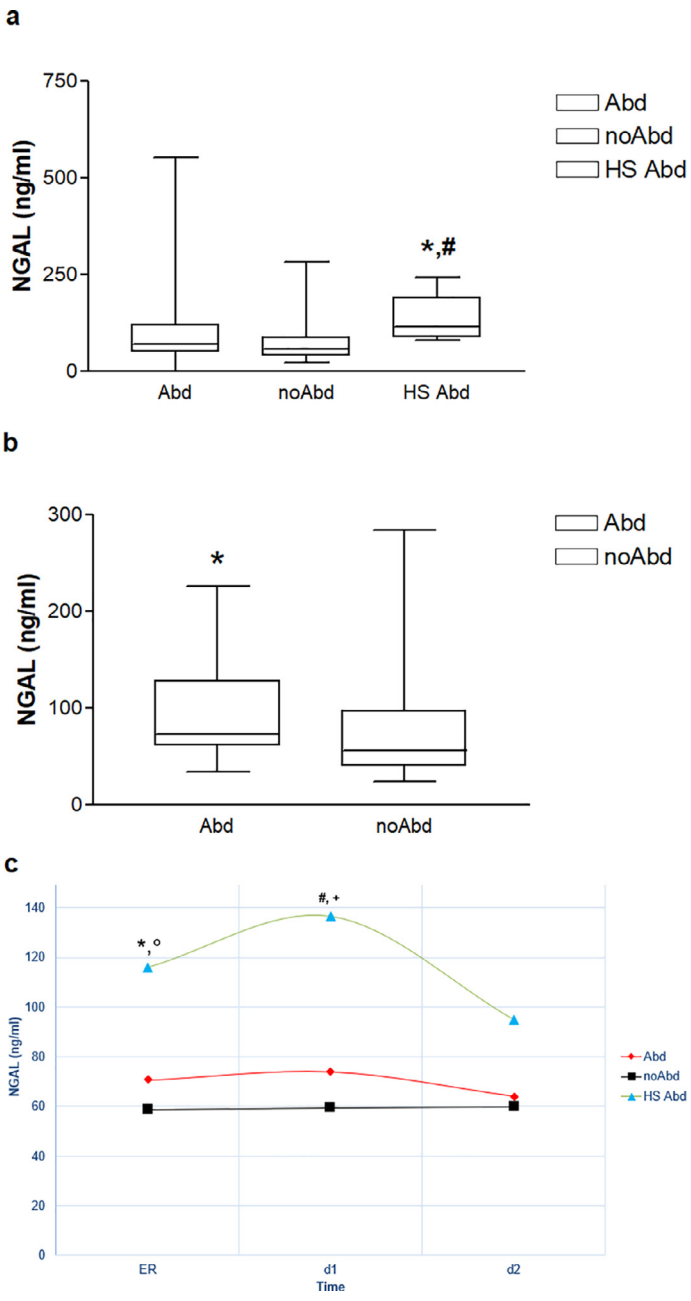

Fig. 3. a: NGAL levels on admission to the emergency room. Median (interquartile range [IQR], Min and Max) neutrophil gelatinase-associated lipocalin (NGAL) values in the three study groups based on the presence or absence of abdominal injury and hemorrhagic shock on admission to the emergency room. Abd: abdominal injury; ER: emergency room; HS Abd: abdominal injury and hemorrhagic shock; noAbd: without abdominal injury Abd ($n = 67$); noAbd ($n = 41$); HS Abd ($n = 10$) * $p<0.05$ HS *vs.* Abd # $p<0.001$ HS *vs.* noAbd **Figure 3b: NGAL levels on admission to the emergency room by using matched-pair-analysis.** Median (interquartile range [IQR], Min and Max) neutrophil gelatinase-associated lipocalin (NGAL) values in both study groups based on the presence or absence of abdominal injury by using matched-pair-analysis with the criteria of an ISS±5 on admission to the emergency room. Abd: abdominal injury; noAbd: without abdominal injury Abd ($n = 38$); noAbd ($n = 38$) * $p = 0.0042$ Abd *vs.* noAbd **Figure 3c: Time course of NGAL levels.** Time course of median neutrophil gelatinase-associated lipocalin (NGAL) levels (ng/ml) of all three patient groups based on the presence or absence of hemorrhagic shock and abdominal injury. Abd: abdominal injury; d1: day 1; d2: day 2; ER: emergency room; HS Abd: hemorrhagic shock with abdominal injury; noAbd: without abdominal injury * $p<0.05$ HS Abd. *vs.* Abd ° $p<0.001$ HS Abd *vs.* noAbd # $p<0.01$ HS Abd *vs.* noAbd + $p<0.05$ Abd *vs.* noAbd.

shock of 334.3 ng/ml with a sensitivity of 90% and a specificity of 78%. This demonstrates the potential usefulness of L-FABP as an early marker for hemorrhagic shock most likely indicating intestinal hypoperfusion.

The detection of intestinal hypoperfusion and the resulting intestinal damage is still an unsolved problem in the clinical setting, due to the lack of direct access and specific markers. [44,45] In a previous study, we were able to demonstrate that I-FABP is a promising biomarker for the detection of intestinal hypoperfusion with a sensitivity of 85% and a specificity of 81%. [46] Thus, L-FABP appears to be an even better biomarker for the detection of hemorrhagic shock because it has a higher sensitivity and a comparable specificity than I-FABP.

To the best of our knowledge, this study is the first to present a compelling evidence for a significant correlation between L-FABP levels and hemorrhagic shock. In a previous study, a correlation between L-FABP and intestinal hypoperfusion was found. [6] All other studies to date have only examined I-FABP concerning a correlation with low Hb values, low mean arterial pressure (MAP) and elevated SI. [42,43] In addition, low blood pressure and SI are unreliable parameters in determining the presence of hemorrhagic shock, particularly because of compensatory mechanism and lack of sufficient sensitivity or specificity to detect early hemorrhage. [47] The most commonly used biomarkers for the detection of hemorrhagic shock are lactate value and base deficit [48–51]. L-FABP correlates well with these two parameters in the present study. Furthermore, L-FABP correlates with other routinely used and clinically relevant parameters such as SI, Hb value, SBP, pH value and the amount of transfused PRBC and FFP units. In addition, a weak but significant correlation was observed for disturbances in INR, PTT and fibrinogen.

Overall, our results suggest, that L-FABP, like I-FABP, can be used as a novel biomarker for the detection of hemorrhagic shock most likely indicating intestinal hypoperfusion, which is known to trigger post-traumatic inflammation by bacteria translocation in the intestine. To avoid this and the resulting elevated late mortality, the early recognition and treatment of hemorrhagic shock is of the utmost importance. The two clinical parameters of SI and low blood pressure are unreliable due to initially occurring compensatory mechanisms. Therefore, the early release of L-FABP could be useful for the recognition of an impending hemorrhagic shock, in order to avoid its development.

L-FABP is a specific biomarker for the detection of kidney or liver injury in traumatized patients

L-FABP is expressed not only in enterocytes of the small intestine but also in liver cells and in proximal tubule of the kidney [19,20]. It is a specific biomarker for injury of the liver and the kidney. In abdominal subgroups there were significantly higher L-FABP levels in patients with isolated organ injuries of the liver and of the kidney compared to patients with isolated injuries of the intestine. In comparison with patients with isolated injuries of the spleen it failed to be significant but there was a clear trend in median L-FABP levels, with higher levels in patients with liver or kidney injuries compared to spleen injuries. The ISS was significantly different between the four abdominal groups. Because of the small patient groups, a matched-pair-analysis was not possible. However, there was a strong and clear correlation between L-FABP on the day of admission and AST as well as ALT on the day of admission. Interestingly, the correlation was $r = 0.86$ (AST) and $r = 0.89$ (ALT) in patients with liver injuries.

Furthermore, there was a significant correlation between L-FABP levels on the day of admission and creatine levels on the following day. Interestingly, the correlation was also strong in patients with a

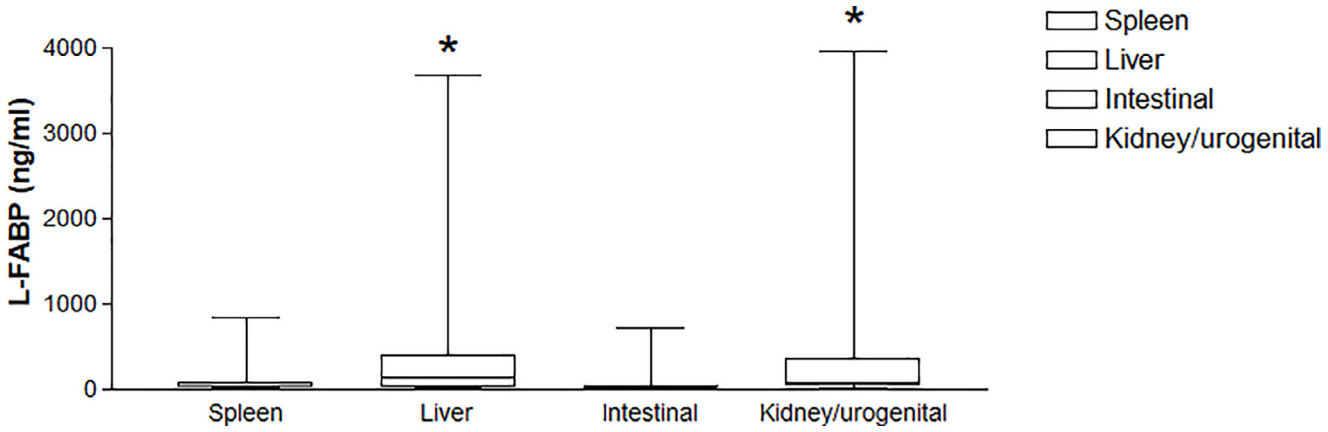

Fig. 4. L-FABP levels of the four abdominal subgroups with isolated organ injuries on admission to ER. Median (interquartile range [IQR], Min and Max) liver fatty acid-binding protein (L-FABP) values in the four abdominal subgroups with isolated organ injuries on admission to the emergency room (ER). intestinal: isolated injuries of the intestine; kidney: isolated injuries of the kidney; liver: isolated injuries of the liver; spleen: isolated injuries of the spleen. spleen (n = 15); liver (n = 14); intestinal (n = 10), kidney (n = 13) * p<0.05 Liver and Kidney *vs.* intestinal.

renal impairment. Thus, it appears that L-FABP levels increase earlier than creatinine levels in the detection of renal injury.

To our knowledge, this is the first study to investigate the use of L-FABP as a specific biomarker for the detection of kidney or liver injury in traumatized patients. Overall, our results suggest with a clear trend that L-FABP is a specific biomarker for the detection of kidney and liver injury.

NGAL is a novel biomarker for the detection of abdominal injury and hemorrhagic shock

NGAL was described in neutrophils and it is also normally expressed at very low levels in epithelial cells of several human tissues, including the proximal tubule of the kidney, lungs, stomach and colon. [30] Its expression is markedly induced in injured epithelia. [31,32] In the present study, NGAL levels are significantly higher in patients with hemorrhagic shock compared to patients with abdominal injury with a sensitivity of 100% and a specificity of 55%. Furthermore, NGAL levels significantly rose in patients with abdominal injury compared to no abdominal injury with a sensitivity of 90% and a specificity of 39%. Therefore, NGAL did not correlate with the ISS of the patients. One reason for this new finding could be the expression of NGAL in the epithelial cells of the colon. [30] Hemorrhagic shock results in a circulatory dysfunction causing decreased tissue oxygenation. [52] The tissue hypoxia could lead to an induction of NGAL production. In the present study, NGAL showed the highest levels on day 1 after trauma, supporting this hypothesis.

Furthermore, NGAL showed a weak but significant correlation to the clinical parameters of hemorrhagic shock such as base deficit, lactate value, SI, SBP, pH value, the amount of transfused PRBC units and leukocyte counts. However, there was no correlation to the Hb value or markers of disorders.

Overall, our results confirm NGAL's potential as an additional novel biomarker for abdominal injury and hemorrhagic shock.

NGAL failed to be an organ-specific biomarker for the detection of kidney injuries in severely traumatized patients

The potential of NGAL as a marker for kidney damage has been examined in several studies. [29,53-56] As far as we know, NGAL is not examined in traumatized patients for the detection of kidney injury except as a parameter for acute kidney failure in burns. [55] Only one study examined NGAL as a parameter for blunt kidney injury, but in rats, rather than humans. [57]

In the analysis of the abdominal subgroups, NGAL levels were not significantly different across the four groups. In correlation to the creatinine values, the NGAL levels showed a significant correlation to the creatinine levels of the same day. Interestingly, in patients with an isolated injury of the kidney, the correlation was very strong and clear on day the of admission and the first following day.

Thus, the results suggest that NGAL failed to be an organ-specific biomarker for kidney injury but that it is a marker for acute kidney failure, confirming the above-mentioned studies.

Limitations

The present analysis has several limitations, most importantly the limited number of patients enrolled in the abdominal subgroups and in the group of hemorrhagic shock. As the sensitivity and specificity are determined from the optimal cut-off level in the ROC analysis, and are being evaluated on the same data, they will be subject to an overall favourable bias, that could be appreciable with the relatively small sample sizes. Future studies should involve larger cohorts of patients and controls to confirm our findings and to analyze the sensitivity and specificity of L-FABP and NGAL levels to detect hemorrhagic shock, abdominal injuries and injuries to specific abdominal organs in particular. In detail, it may be of importance to determine whether L-FABP and NGAL are independently early-detectable and sensitive markers of hemorrhagic shock. Such markers would allow early improvement or perhaps even monitoring of shock therapy. Likewise, since we observed an early decrease of the initially elevated I-FABP levels to normal values, the usefulness of FABP assays in the clinical setting needs to be evaluated. Otherwise, the rise in NGAL levels to the first day after trauma needs to be evaluated for clinical usefulness in the early recognition of hemorrhagic shock. On the other hand, future studies should examine not only serum but also urinary NGAL levels for the detection of hemorrhagic shock.

Furthermore, this study included only measurements of L-FABP and NGAL levels until the second post-traumatic day and the meanings of high elevated L-FABP or NGAL levels on the following course on intensive care units could not be interpreted. So, future studies should examine a longer post-traumatic timeline.

For early detection of hemorrhagic shock, there is currently no bedside-test available for the rapid measurement of L-FABP or NGAL available. Up to now, L-FABP and NGAL testing is performed using an ELISA test, taking two to four hours for measurement and therefore limiting its clinical relevance in the acute setting. Oth-

erwise, measurement using ELISA is easy and the materials costs only 12 (NGAL) and 13 (L-FABP) Euros per patient. Introducing the measurement on clinical routine would stimulate the development of a point-of-care approach, as it was already developed for H–FABP, with a bedside test having results available within 15 min for acute coronary syndrome or myocardial infarction [58,59].

Conclusion

The novel findings of this study are that L-FABP is not only a marker for abdominal injury but also for hemorrhagic shock. Furthermore, NGAL also seems to be a novel biomarker for abdominal trauma and the detection of hemorrhagic shock, in which L-FABP showed a stronger correlation to the clinical parameters of hemorrhagic shock and a higher sensitivity and specificity for detection than NGAL.

At the same time, this study further revealed the novel finding, that L-FABP also shows potential as an early and specific biomarker for the detection of isolated kidney and liver injuries. NGAL failed to be organ-specific in the detection of kidney injury in traumatized patients.

Ethics approval and consent to participate

Institutional Ethics Committee approval (408/16, in accordance with the Declaration of Helsinki and reported following the Strengthening the Reporting of OBservational studies in Epidemiology, STROBE guidelines).

Written informed consent was obtained from all enrolled subjects or their nominated legally authorized representatives on behalf of the participants in accordance with the ethical standards.

Consent for publication

Not applicable.

Availbility for material and data

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

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Author's contributions

M. Voth, R. Verboket, D. Henrich and I. Marzi designed the study, developed the methodology, performed the analysis and wrote the manuscript.

Competing interests

The authors declare that they have no competing interests.

Acknowledgement

Not applicable.

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