in vivo *33*: 1573-1580 (2019)
doi:10.21873/invivo.11639

Predictive Value of Osteoprotegerin and Neutrophil Gelatinase-associated Lipocalin on Multiple Organ Failure in Multiple Trauma

HENNING PETERS[1], CHRISTIAN MACKE[1], PHILIPP MOMMSEN[1], CHRISTIAN ZECKEY[1,2], JAN-DIERK CLAUSEN[1], CHRISTIAN KRETTEK[1], CLAUDIA NEUNABER[1] and MARCEL WINKELMANN[1]

[1]*Trauma Department, Hannover Medical School, Hannover, Germany;*
[2]*Department of General, Trauma and Reconstructive Surgery, Ludwig-Maximilians-Universität München, Munich, Germany*

Abstract. *Background/Aim: Multiple organ dysfunction syndrome (MODS) is the leading cause of late posttraumatic mortality. This study analyzed the prognostic values of osteoprotegerin (OPG) and neutrophil gelatinase-associated lipocalin (NGAL/lipocalin 2) compared to interleukin-6 (IL-6) in multiply injured patients. Patients and Methods: A retrospective observational cohort study on multiply injured patients with an injury severity score (ISS) of ≥16 was performed. OPG, NGAL and IL-6 blood concentrations were measured. Statistical analysis comprised receiver-operating-characteristic (ROC) analysis with the corresponding area under the curve (AUC). Results: Thirty-nine patients with a mean ISS of 34±11 were included. Fourteen patients (36%) developed MODS and 8 patients (21%) died. Plasma levels of NGAL, OPG, and IL-6 were significantly elevated in the MODS+ group. Each biomarker positively correlated with MODS score and diagnosis of MODS. Conclusion: NGAL and OPG might be indicative of MODS and could have the potential to be biomarkers in the early detection of patients at risk of posttraumatic MODS.*

Multiple organ dysfunction syndrome (MODS) is the leading cause of late posttraumatic mortality and accounts for up to 50% of deaths beyond the first 48 h (1, 2). Although the incidence has remained stable or decreased slightly, it still ranges from 25 to 40% (1, 3-7). Moreover, the mortality risk among patients with MODS is still high (approx. 33%), despite the fact that it has decreased in recent years (42.6% in 2002 *vs*. 33.3% in 2011 in the German trauma registry) (7, 8). Since the lack of striking outcome improvements indicates that reversing MODS is difficult, prevention might be the best option. Thus, early detection of patients with an increased risk of developing MODS is crucial to help avoid lethal consequences.

Severe traumata induce massive secretion of pro-inflammatory cytokines ("cytokine storm") and a subsequent increase in anti-inflammatory cytokines. Thus, imbalance between the early systemic inflammatory response and the later compensatory anti-inflammatory response may be accountable for organ dysfunction and increased susceptibility to infections (9-11). Based on these findings, biomarkers should have the potential to estimate the individual risk profile and detect imminent multiple organ failure, therefore scoring systems like the MODS score according to Marshall or sequential organ failure assessment score (SOFA score) have been adapted for identification of disease manifestations (12, 13). Interleukin (IL)-6 is, due to its central role in the regulation of posttraumatic inflammation, the most commonly, but certainly not routinely used parameter for the laboratory prediction of MODS (11, 14-18). However, IL-6 is a rather global biomarker and its release is susceptible to a broad range of external influences like surgeries.

Paracrine osteoprotegerin (OPG) is a member of the TNF-receptor superfamily. OPG expression is broadly distributed over a variety of tissues. Its best-understood effect is to mediate a decrease in bone resorption via the RANK-RANKL pathway (19-22). However, OPG also acts as a soluble decoy receptor for TNF-related apoptosis-inducing ligand (TRAIL), which is capable of inducing tumor cell apoptosis (23, 24). But there are also hints that OPG plays a

Correspondence to: Marcel Winkelmann, MD, Trauma Department, Hannover Medical School, Carl-Neuberg-Strasse 1, D-30625 Hannover, Germany. Tel: +49 17615322684, Fax: +49 5115328805, e-mail: winkelmann.marcel@mh-hannover.de

Key Words: Multiple organ failure, multiple organ dysfunction syndrome, multiple trauma, multiple injury, lipocalin.

role in immune regulation. It inhibits increased T-cell and dendritic cell survival and thereby may downregulate the immune response (25-28). Additionally, there are hints that OPG could also play a role in the regulation of B-cell development (29-31). The potential role of OPG in multiple trauma and posttraumatic MODS is largely unknown.

Neutrophil gelatinase-associated lipocalin (NGAL) is also known as lipocalin-2 (LCN2) or oncogene 24p3. It is derived from the distal tubule and has been shown to be a marker of impaired renal glomerular function and structural tubular injury. Various studies have demonstrated the potential utility of NGAL as a biomarker of acute kidney injury (AKI) (32-37). Plasma NGAL has been shown to be as good as urinary NGAL in predicting AKI (33). However, there remain important issues. A reliable cut-off value does not yet exist. There are no reliable data on NGAL in multiple trauma except for a study that demonstrated an association with death in patients with combat casualties (37). Nevertheless, AKI is frequently part of MODS, and severely injured patients are at particular risk of developing AKI as well as MODS.

Taking the current literature into consideration, biomarkers have the potential to improve the prediction of MODS although there is no commonly accepted standard parameter yet. However, IL-6 is so far the most widely used but yet a not very specific biomarker. The purpose of this study was to analyze the prognostic values of OPG and NGAL for the development of posttraumatic MODS compared to IL-6 in multiply injured patients. We hypothesized that OPG as well as NGAL are at least comparable in predicting MODS in multiply injured patients.

Patients and Methods

A retrospective observational cohort study was performed at the Hannover Medical School, a level I trauma center in a metropolitan area. Multiply injured patients with an injury severity score (ISS) of ≥16, who had an accident between 01/2009 and 12/2012 were included. Participants had to have a minimum age of 16 years and be admitted primarily within 6 h after trauma. Participants took part in a larger central plasma databank project ("Serothek"). Written consent was obtained from the nearest relatives in cases where the patient was unconscious or unable to consent. Once the patient was able to consent, written consent was acquired as soon as possible. Data gathering included a blood sample, genotyping, and documentation of clinical data. The study was approved by the institutional review board (application number 4980).

A standardized arterial blood sample for plasma cytokine measurements was taken at day 1, 2, 3, 5, 7, 10, and 14. A 10 ml sample was immediately centrifuged at 500 g for 10 min. The plasma was extracted and preserved at –80˚C. Cytokine analysis was performed with cytometric bead arrays. The analysis of NGAL was performed with a human Lipocalin-2/NGAL Quantikine ELISA kit (R&D Systems Europe Ltd., Abingdon, United Kingdom) with a sensitivity of 0.04 ng/ml and a standard range of 0.2-10 ng/ml. Measurement of OPG was performed with a human OPG FlowCytomix™ simplex kit (eBioscience, Frankfurt, Germany) with a sensitivity of 7.9 pg/ml and a standard range of 27-20.000 pg/ml and IL-6 with a human inflammation 20plex FlowCytomix™ multiplex kit with a sensitivity of 1.2 pg/ml and a standard range of 27-20.000 pg/ml. All analyses were performed in a timely manner in a specialized traumatology research lab at the Hannover Medical School. Patients with incomplete plasma samples prior to decease or discharge were excluded.

Demographic and clinical data acquisition was performed parallel to blood collection. The data included age, sex, mortality, initial Glasgow coma scale (GCS), length of intensive care stay as well as hospital stay, and transfusion requirements [packed red blood cells (PRBC), fresh frozen plasma (FFP) and platelet concentrate (PC)]. Injury pattern was classified according to the Abbreviated Injury Scale (AIS) using the AIS 2005 update 2008 (38). Total injury severity was calculated according to the ISS (39). The final analysis was performed between 11/2017 and 03/2018 as a part of the superior central plasma databank project.

The primary endpoint of this study was the development of MODS diagnosed on the basis of MODS score by Marshall *et al*., a generally accepted indicator: MODS was deemed present if the sum of single organ dysfunctions was >8 on at least one day, representing a severe multiple organ failure with a considerably increased mortality (12). Secondary endpoints were mortality as well as other posttraumatic complications, namely systemic inflammatory response syndrome (SIRS), sepsis, and acute respiratory distress syndrome (ARDS). Diagnoses of SIRS and sepsis were made using the 2010 revised criteria of S-2k guidelines of the German Sepsis Society and the German Interdisciplinary Association of Intensive Care and Emergency Medicine (40). ARDS was diagnosed according to the Berlin definition (41). Manifestations of ARDS were present in cases of occurrence within 1 week, with bilateral radiographic infiltration of the lung, and reduced oxygenation (< 300 mmHg) according to the Horovitz score (PaO_2/FiO_2).

Statistical analysis was performed with IBM SPSS (Version 22, IBM, Armonk, NY, USA). For the comparison of plasma concentrations and other continuous variables (length of intensive care stay, *etc*.) of the different study groups (MODS+ *vs*. MODS–) one-way or repeated measures analysis of variance (ANOVA) was performed. The Greenhouse-Geisser adjustment was used to correct for violations of sphericity. After testing the Gaussian distribution with the Shapiro-Wilk-test, the statistical analysis included parametric tests (Student's *t*-test). For non-Gaussian distributed data, we used non-parametric tests (Mann-Whitney test for independent data and Wilcoxon test for dependent data). Fisher's exact test (exact chi-squared-test) was used for the analysis of contingency tables. Evaluation of the diagnostic values of IL-6, NGAL and OPG as MODS predictors was performed with receiver-operating-characteristic (ROC) analysis and the area under the curve (AUC). Odds ratios and 95% confidence intervals (95%CI) were calculated as well. Cut-off points were defined on the basis of the Youden index (J) and the optimal cut-off point (c*) is the one that optimizes the differentiating ability of the biomarker when equal weight is given to sensitivity and specificity (42). The significance level was set at $p<0.05$. The updated STARD 2015 reporting guideline for diagnostic accuracy studies was used (43).

Results

Demographic and outcome data. Thirty-nine patients with a mean age of 41.4±20.2 years were included. The mean follow-up time was 35±24 days (median follow-up time 28

Table I. *Demographic and clinical data of study population as well as of MODS+ and MODS– subgroups.*

	Total	MODS+	MODS–	*p*-Value
Age [years], mean±SD	41.4±20.2	42.9±21.6	40.6±20.1	0.7[2]
Male sex, n (%)	32 (82)	11 (79)	21 (84)	0.7[1]
Injure Severity Score (ISS), median (IQR), mean±SD	33 (18)	37,5 (15)	27 (11)	*0.005[1]
	34.0±11.0	40.5±10.9	30.6±9.2	
Abbreviated Injury Scale				
AIS_{Head}, mean±SD	2.9±1.8	3.4±1.5	2.6±1.9	0.3[1]
AIS_{Face}, mean±SD	0.8±1.0	0.6±0.8	0.9±1.1	0.5[1]
AIS_{Thorax}, mean±SD	3.0±1.8	3.4±1.7	2.8±1.9	0.2[1]
$AIS_{Abdomen}$, mean±SD	1.1±1.3	1.6±1.2	0.8±1.2	0.1[1]
$AIS_{Extremities}$, mean±SD	2.6±1.4	3.0±1.4	2.3±1.4	0.2[1]
$AIS_{External}$, mean±SD	0.2±0.6	0.3±0.8	0.2±0.5	0.8[1]
Initial GCS, median (IQR), mean±SD	9 (12)	5 (6)	14 (12)	*0.02[1]
	8.9±5.2	6.4±3.9	10.1±5.4	
Duration of intensive care [days], mean±SD	24.0±13.0	24.8±18.1	24.1±9.0	0.5[1]
Duration of in-patient care [days], mean±SD	35±24	30.1±29.9	38.2±20.3	*0.04[1]
Duration of mechanical ventilation [hours], mean±SD	497±288	531±364	478±242	0.9[1]
Transfusion requirement				
PRBC [units], mean±SD	19.3±12.7	21±14	18±12	0.6[1]
FFP [units], mean±SD	10.8±10.2	12±9	10±11	0.3[1]
PC [units], mean±SD	1.8±3.5	3±5	1±2	0.1[1]
Mortality, n (%)	8 (21)	6 (43)	2 (8)	*0.02[2]
SIRS, n (%)	20 (51)	5 (36)	15 (60)	0.2[2]
Sepsis, n (%)	11 (28)	4 (29)	7 (28)	1.0[2]
ARDS, n (%)	16 (41)	7 (50)	9 (36)	0.5[2]

p means significance level of differences between MODS+ and MODS– patients; *significance by means of [1]Mann-Whitney-*U*-test and [2]Fisher's exact test.

days). Seven (18%) patients were female and 32 (82%) were male. The mean ISS was 34±11. The thorax was the most severely injured body region with a mean AIS of 3.0±1.8, followed by the head with 2.9±1.8. The mean duration of intensive care was 24±13 days with a mean duration of mechanical ventilation of 497±288 hours. Fourteen patients (36%) developed MODS and eight patients (21%) died within in-patient care, although the mean expected mortality added up to 36±19%. Moreover, 15 patients (39%) developed SIRS, 10 patients (26%) sepsis, and 15 patients (39%) ARDS. For a concise overview of the demographic and outcome data within the MODS+ (n=14) and MODS– (n=25) groups, please refer to Table I. Patients that developed MODS were injured more severely (median ISS 37.5 *vs*. 27, $p=0.005$), presented with a lower Glasgow Coma Scale score (median GCS 5 *vs*. 14, $p=0.02$) and had increased mortality (43 *vs*. 8%, $p=0.02$). Except for the duration of overall in-patient care (30.1±29.9 *vs*. 38.2±20.3 days, $p=0.04$), there were no more statistically significant differences between the two groups. In the binomial logistic regression with a model comprising dichotomized ISS (≤33 *vs*. >33) and dichotomized GCS (≥9 *vs*. <9) with a correct predicted occurrence of MODS in 74% of cases and a Nagelkerke's pseudo-R^2 of 0.26, neither ISS [odds ratio 4.0 (95%CI=0.9-17.9), $p=0.07$] nor GCS [odds ratio 3.2 (95%CI=0.7-14.3), $p=0.1$] could be verified as independent risk factors for the development of MODS.

Cytokine concentration. Plasma levels of NGAL, OPG and IL-6 were significantly elevated in the MODS+ group [NGAL 210.3±93.9 ng/ml *vs*. 145.4±92.3 ng/ml, $p<0.001$; OPG 250.4±251.3 ng/ml *vs*. 128.5±90.3 ng/ml, $p<0.001$; IL-6 310.9±454.6 pg/ml *vs*. 204.6±414.8 pg/ml, $p=0.001$]. For detailed information about plasma levels at different times please refer to Figure 1 and Tables II-IV. A repeated measures ANOVA with a Greenhouse-Geisser correction determined that mean performance levels showed a statistically significant difference between NGAL measurements, $F(3.45, 106.91)=3.65$, $p=0.01$, $\eta^2=0.11$, OPG measurements, $F(1.80, 55.77)=11.93$, $p<0.001$, $\eta^2=0.28$ and IL-6 measurements, $F(1.43, 44.44)=11.09$, $p=0.001$, $\eta^2=0.26$. To ensure that this was not attributable to the significantly increased ISS, the patient population was dichotomized into two groups according to the median ISS (33). There were no significant differences in plasma chemokine levels. Cytokine levels correlated with MODS significantly (Spearman-Rho: NGAL: 0.35, $p<0.001$; OPG: 0.35, $p<0.001$; IL-6: 0.21, $p=0.001$), but only OPG

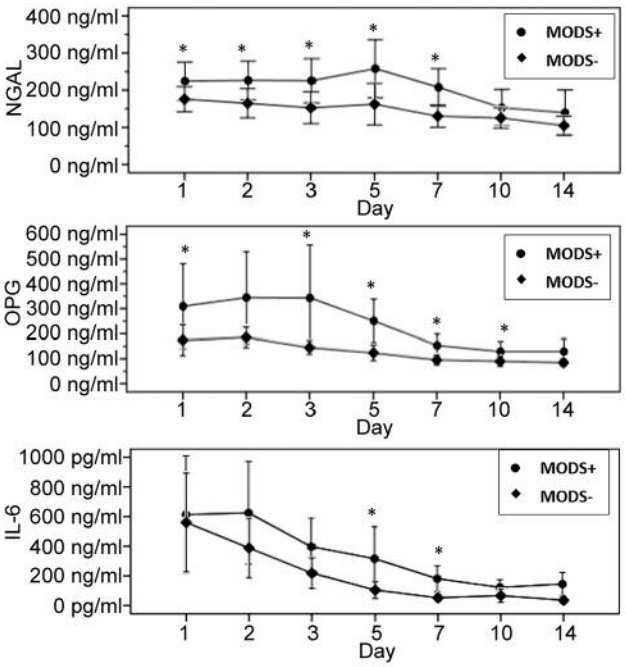

Figure 1. *Plasma levels of NGAL, OPG and IL-6 in patients with (MODS+) and without (MODS–) multiple organ dysfunction syndrome. NGAL: Neutrophil gelatinase-associated lipocalin; OPG: osteoprotegerin; IL: interleukin; MODS: multiple-organ dysfunction syndrome; mean and 95 percent confidence interval; significant differences between measurements according to repeated measures ANOVA with Greenhouse-Geisser correction [NGAL F(3.45, 106.91)=3.65, p=0.01, η^2=0.11, OPG F(1.80, 55.77)=11.93, p<0.001, η^2=0.28 and IL-6 F(1.43, 44.44)=1.09, p=0.001, η^2=0.26]; *p<0.05 at the respective time (Mann-Whitney-U-test).*

Table II. *Plasma levels of NGAL in MODS+ and MODS– groups at different times.*

Day	NGAL [ng/ml], mean±SD		*p*-Value
	MODS+	MODS–	
1	224.7±80.2	175.8±81.3	0.04
2	226.7±85.3	164.9±95.2	0.01
3	226.0±98.2	152.8±103.0	0.02
5	258.4±123.2	162.4±134.6	0.07
7	208.1±70.2	130.3±71.9	0.04
10	153.4±64.0	125.4±66.2	0.20
14	140.0±79.2	104.9±59.9	0.13

NGAL: Neutrophil gelatinase-associated lipocalin.

Table III. *Plasma levels of OPG in MODS+ and MODS– groups at different times.*

Day	OPG [ng/ml], mean±SD		*p*-Value
	MODS+	MODS–	
1	309.0±295.8	174.0±150.2	0.01
2	343.4±320.9	185.4±100.9	0.17
3	342.1±371.4	144.6±65.4	0.006
5	250.0±144.6	123.6±75.6	0.01
7	152.5±67.9	95.3±46.2	0.02
10	127.9±56.7	90.1±49.1	0.03
14	128.1±65.4	84.8±33.4	0.17

OPG: Osteoprotegerin.

Table IV. *Plasma levels of IL-6 in MODS+ and MODS– groups at different times.*

Day	IL-6 [pg/ml], mean±SD		*p*-Value
	MODS+	MODS–	
1	553.7±686.9	560.0±807.8	0.7
2	565.3±600.6	388.1±487.9	0.2
3	336.7±333.7	250.8±218.0	0.2
5	255.8±357.2	104.2±136.3	0.03
7	120.8±130.9	52.2±58.7	0.04
10	62.1±71.9	66.7±109.2	0.3
14	84.4±100.7	35.9±56.5	0.054

IL: Interleukin.

Table V. *Area under the curve and 95 percent confidence interval for predicting MODS at different times*

Day	AUC (95%CI)		
	NGAL	OPG	IL-6
1	0.71 (0.52-0.9)	0.72 (0.55-0.89)	0.5 (0.27-0.72)
2	0.75 (0.57-0.92)	0.63 (0.43-0.84)	0.63 (0.44-0.82)
3	0.73 (0.56-0.9)	0.75 (0.55-0.95)	0.6 (0.4-0.8)
5	0.77 (0.62-0.93)	0.74 (0.55-0.93)	0.7 (0.53-0.87)
7	0.81 (0.66-0.96)	0.75 (0.55-0.94)	0.7 (0.52-0.89)
10	0.65 (0.42-0.88)	0.71 (0.54-0.89)	0.6 (0.4-0.81)
14	0.68 (0.46-0.89)	0.66 (0.45-0.87)	0.72 (0.53-0.91)

NGAL: Neutrophil gelatinase-associated lipocalin; OPG: osteoprotegerin; IL: interleukin.

correlated with ISS (Spearman-Rho: 0.18, p=0.004). To define the maximum potential effectiveness of the biomarkers, we observed the AUC and calculated the Youden index (J) with the optimal cut-off point (c*) for each significantly increased biomarker at different times (days 1, 2, 3, 5, 7, 10 and 14). Table V shows the AUC for NGAL, OPG and IL-6. Detailed information about the optimal cut-off points according to Youden's index are shown in Table VI.

Table VI. *Cut-off values according to Youden's index (J) for predicting MODS at different times*

Day	NGAL				OPG				IL-6			
	PL [ng/ml]	Se	Sp	J	PL [ng/ml]	Se	Sp	J	PL [pg/ml]	Se	Sp	J
1	209.7	0.58	0.88	0.46	187.1	0.58	0.8	0.38	332.0	0.58	0.56	0.14
2	219.3	0.56	0.88	0.43	410.1	0.33	0.96	0.29	350.1	0.67	0.71	0.38
3	148.5	0.67	0.67	0.33	217.0	0.56	0.88	0.43	306.5	0.33	0.83	0.17
5	95.4	1.0	0.5	0.5	168.5	0.56	0.88	0.43	43.5	0.89	0.5	0.39
7	154.7	0.78	0.79	0.57	176.0	0.44	0.96	0.4	37.6	0.89	0.63	0.51
10	123.9	0.78	0.71	0.49	72.5	1.0	0.54	0.54	27.1	0.78	0.63	0.4
14	106.9	0.67	0.75	0.42	181.8	0.33	1.0	0.33	16.6	0.78	0.58	0.36

PL: Plasma levels; Se: sensitivity; Sp: specificity; J: Youden's J; NGAL: neutrophil gelatinase-associated lipocalin; OPG: osteoprotegerin; IL: interleukin.

Discussion

Multiply injured patients are at particular risk of posttraumatic MODS. In the present study, 36% of the patients developed MODS. NGAL as well as OPG appeared to indicate MODS earlier compared to IL-6.

The incidence of MODS in the current study population is in accordance with the literature that reports an incidence from 25 to 40% (1, 3-7). Froehlich *et al*. reported an incidence of 32.7% in a comparable study population from the German trauma registry. Although the mean injury severity was lower compared to our own patients (ISS 28±12 *vs*. 34±11), patients that developed MODS had been injured significantly more severely in that study as well (7). Mortality fluctuates considerably among different studies. However, each study supports the assumption that patients with manifest MODS are more likely to die within in-patient care (7, 14-17, 44). Thus, due to higher mortality during the course of treatment in the MODS+ group, mean duration of in-patient care is decreased.

The data of this study indicate slight advantages of NGAL and OPG compared to IL-6 as predictors of MODS with an increased AUC. However, the sensitivity and specificity leave a lot to be desired. Neither NGAL nor OPG alone are yet applicable to identify patients at risk of MODS to a high degree.

IL-6 is certainly a key cytokine in posttraumatic inflammation. Different studies have reported a correlation between elevated IL-6 levels and the incidence of MODS (14-17, 44). Usually, an early increase in IL-6 is found and associated with an increased risk of subsequent MODS (16, 17, 44). To some extent, this is in accordance with our own results. IL-6 levels are most elevated in the early phase of posttraumatic inflammation. This is likely to be due to its role as a pro-inflammatory cytokine that stimulates the immune response after trauma. In contrast to the results of Frink *et al*., who reported a marked difference in IL-6 from the very beginning, we found considerably elevated IL-6 levels even in patients without MODS (44). On the contrary, if MODS became present, IL-6 remained elevated for a longer time compared to patients without MODS, who showed a rapid decrease in IL-6 after the initial peak (44). However, Maier *et al*. reported a secondary increase in IL-6 in patients with late onset MODS. In this study, IL-6 had an AUC of 0.70 for the prediction of late onset MODS (14). This is comparable to the AUC of NGAL and OPG but not IL-6 in the early post-traumatic phase in our own study population. AUC of IL-6 is slightly lower especially during the first three days after trauma. One consideration might be that the markedly higher injury severity of our patients induced increased cytokine production and equalized the differences between patients with and without MODS to some extent, but this remains speculative (17, 45). Even more speculative appears the critical assessment of OPG's role in multiple injuries. According to the available literature, OPG is not yet established as a predictor of MODS. If we take into consideration that OPG may downregulate the immune response, the increase in OPG levels up to the third day in the MODS+ group perhaps might reflect a counter reaction against pro-inflammatory immune response. Since MODS is associated with intensified immune system activity, could elevated OPG levels be regarded as an indication of a pro-inflammatory immune response (25-28)? On the other hand, increased OPG could be attributed to a greater injury severity, since ISS was significantly increased in the MODS+ group. There is also the possibility that the increase in OPG in patients with MODS is due to additional, still insufficiently understood, functions of OPG. However, this is just a conjecture that cannot be supported by the present data and is highly speculative. The available data is somewhat better for NGAL. Neutrophil gelatinase-associated lipocalin has been shown to be a predictor of acute kidney injury in burn patients as well as combat casualties (37, 46-48). Moreover, Stewart *et al*. have associated NGAL with

increased mortality in severely injured patients (37). Acute kidney injury is often an inherent part of MODS. In the present study population, each patient with AKI manifestations (n=7) developed MODS. Looking at it the other way around, not every patient with MODS manifestations had an AKI, though. Whether increased NGAL is mainly attributable to renal dysfunction or is a predictor of MODS independent of AKI cannot be verified by the present data due to the limited sample size. Moreover, the coinciding outcomes of AKI and reduced renal function as part of MODS impairs the utility of NGAL to predict MODS since it already includes the former.

There are several limitations of this study that have to be considered. Elevated levels of NGAL as well as OPG are to a certain extent attributable to a generally increased cytokine expression. However, both NGAL and OPG demonstrated the potential to be useful in laboratory diagnosis of MODS compared to IL-6, which has been examined most frequent in literature. Apart from that, there is no standardized method for NGAL and OPG measurement yet. The performance of available assays is relatively variable (49). Moreover, the impact of NGAL and OPG might be overestimated due to the limited number of patients, since outliers could exert a greater influence. Comparable data are lacking almost completely. In addition, we failed to demonstrate reliable cut-off values, since values are fluctuating and are time-dependent and sensitivity and specificity are dissatisfying. Comparable, commonly accepted values are lacking too. Therefore, the generalizability of the study results is limited.

Conclusion

The incidence of MODS in severely injured patients is still high and associated with an increased risk of death. In conclusion, both NGAL and OPG might be indicative of MODS and could have the potential to be biomarkers in the early detection of patients that are at particular risk of posttraumatic MODS. Further research is necessary to analyze the prognostic value of NGAL and OPG in the diagnosis of MODS and determine reliable cut-off values with acceptable sensitivity and specificity.

Conflicts of Interest

The Authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Henning Peters: data acquisition, data analysis and interpretation, manuscript draft, manuscript revision; Christian Macke: data analysis and interpretation, manuscript draft; Philipp Mommsen: study conception and design, data analysis and interpretation; Christian Zeckey: study conception and design, data analysis and interpretation; Jan-Dierk Clausen: manuscript draft, manuscript revision; Christian Krettek: study conception and design, data analysis and interpretation; Claudia Neunaber: study conception and design, data acquisition, data analysis and interpretation; Marcel Winkelmann: study conception and design, data acquisition, data analysis and interpretation, manuscript draft, manuscript revision.

Acknowledgements

The corresponding author received an internal grant from the Hannover Medical School for non-monetary resources. There was no influence on study design or manuscript preparation.

References

1 Ciesla DJ, Moore EE, Johnson JL, Burch JM, Cothren CC and Sauaia A: A 12-year prospective study of postinjury multiple organ failure: Has anything changed? Arch Surg *140(5)*: 432-438, 2005. PMID: 15897438. DOI: 10.1001/archsurg.140.5.432

2 Dewar D, Moore FA, Moore EE and Balogh Z: Postinjury multiple organ failure. Injury *40(9)*: 912-918, 2009. PMID: 19541301. DOI: 10.1016/j.injury.2009.05.024

3 Nast-Kolb D, Aufmkolk M, Rucholtz S, Obertacke U and Waydhas C: Multiple organ failure still a major cause of morbidity but not mortality in blunt multiple trauma. J Trauma *51(5)*: 835-841; discussion 841-832, 2001. PMID: 11706328.

4 Brattstrom O, Granath F, Rossi P and Oldner A: Early predictors of morbidity and mortality in trauma patients treated in the intensive care unit. Acta Anaesthesiol Scand *54(8)*: 1007-1017, 2010. PMID: 20626360. DOI: 10.1111/j.1399-6576.2010. 02266.x

5 Minei JP, Cuschieri J, Sperry J, Moore EE, West MA, Harbrecht BG, O'Keefe GE, Cohen MJ, Moldawer LL, Tompkins RG, Maier RV, Inflammation and the Host Response to Injury Collaborative Research P: The changing pattern and implications of multiple organ failure after blunt injury with hemorrhagic shock. Crit Care Med *40(4)*: 1129-1135, 2012. PMID: 22020243. DOI: 10.1097/CCM.0b013e3182376e9f

6 Kahl JE, Calvo RY, Sise MJ, Sise CB, Thorndike JF and Shackford SR: The changing nature of death on the trauma service. J Trauma Acute Care Surg *75(2)*: 195-201, 2013. PMID: 23823614. DOI: 10.1097/TA.0b013e3182997865

7 Frohlich M, Lefering R, Probst C, Paffrath T, Schneider MM, Maegele M, Sakka SG, Bouillon B, Wafaisade A, Committee on Emergency Medicine IC and Trauma Management of the German Trauma Society Sektion NIS: Epidemiology and risk factors of multiple-organ failure after multiple trauma: An analysis of 31,154 patients from the traumaregister dgu. J Trauma Acute Care Surg *76(4)*: 921-927; discussion 927-928, 2014. PMID: 24662853. DOI: 10.1097/TA.0000000000000199

8 Sauaia A, Moore EE, Johnson JL, Chin TL, Banerjee A, Sperry JL, Maier RV and Burlew CC: Temporal trends of postinjury multiple-organ failure: Still resource intensive, morbid, and lethal. J Trauma Acute Care Surg *76(3)*: 582-592, discussion 592-583, 2014. PMID: 24553523. DOI: 10.1097/TA.0000000 000000147

9 Roberts CS, Pape HC, Jones AL, Malkani AL, Rodriguez JL and Giannoudis PV: Damage control orthopaedics: Evolving concepts in the treatment of patients who have sustained orthopaedic trauma. Instr Course Lect *54*: 447-462, 2005. PMID: 15948472.

10 Sears BW, Stover MD and Callaci J: Pathoanatomy and clinical correlates of the immunoinflammatory response following orthopaedic trauma. J Am Acad Orthop Surg *17(4)*: 255-265, 2009. PMID: 19307674.
11 Volpin G, Cohen M, Assaf M, Meir T, Katz R and Pollack S: Cytokine levels (il-4, il-6, il-8 and tgfbeta) as potential biomarkers of systemic inflammatory response in trauma patients. Int Orthop *38(6)*: 1303-1309, 2014. PMID: 24402554. DOI: 10.1007/s00264-013-2261-2
12 Marshall JC, Cook DJ, Christou NV, Bernard GR, Sprung CL and Sibbald WJ: Multiple organ dysfunction score: A reliable descriptor of a complex clinical outcome. Crit Care Med *23(10)*: 1638-1652, 1995. PMID: 7587228.
13 Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, Reinhart CK, Suter PM and Thijs LG: The sofa (sepsis-related organ failure assessment) score to describe organ dysfunction/failure. On behalf of the working group on sepsis-related problems of the european society of intensive care medicine. Intensive Care Med *22(7)*: 707-710, 1996. PMID: 8844239.
14 Maier B, Lefering R, Lehnert M, Laurer HL, Steudel WI, Neugebauer EA and Marzi I: Early versus late onset of multiple organ failure is associated with differing patterns of plasma cytokine biomarker expression and outcome after severe trauma. Shock *28(6)*: 668-674, 2007. PMID: 18092384.
15 Giannoudis PV, Harwood PJ, Loughenbury P, Van Griensven M, Krettek C and Pape HC: Correlation between il-6 levels and the systemic inflammatory response score: Can an il-6 cutoff predict a sirs state? J Trauma *65(3)*: 646-652, 2008. PMID: 18784579. DOI: 10.1097/TA.0b013e3181820d48
16 Lausevic Z, Lausevic M, Trbojevic-Stankovic J, Krstic S and Stojimirovic B: Predicting multiple organ failure in patients with severe trauma. Can J Surg *51(2)*: 97-102, 2008. PMID: 18377749
17 Jastrow KM, 3rd, Gonzalez EA, McGuire MF, Suliburk JW, Kozar RA, Iyengar S, Motschall DA, McKinley BA, Moore FA and Mercer DW: Early cytokine production risk stratifies trauma patients for multiple organ failure. J Am Coll Surg *209(3)*: 320-331, 2009. PMID: 19717036. DOI: 10.1016/j.jamcollsurg.2009.05.002
18 Haasper C, Kalmbach M, Dikos GD, Meller R, Muller C, Krettek C, Hildebrand F and Frink M: Prognostic value of procalcitonin (pct) and/or interleukin-6 (il-6) plasma levels after multiple trauma for the development of multi organ dysfunction syndrome (mods) or sepsis. Technol Health Care *18(2)*: 89-100, 2010. PMID: 20495248. DOI: 10.3233/THC-2010-0571
19 Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J and Boyle WJ: Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell *93(2)*: 165-176, 1998. PMID: 9568710
20 Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Sato Y, Goto M, Yamaguchi K, Kuriyama M, Kanno T, Murakami A, Tsuda E, Morinaga T and Higashio K: Identity of osteoclastogenesis inhibitory factor (ocif) and osteoprotegerin (opg): A mechanism by which opg/ocif inhibits osteoclastogenesis *in vitro*. Endocrinology *139(3)*: 1329-1337, 1998. PMID: 9492069. DOI: 10.1210/endo.139.3.5837
21 Matsuzaki K, Udagawa N, Takahashi N, Yamaguchi K, Yasuda H, Shima N, Morinaga T, Toyama Y, Yabe Y, Higashio K and Suda T: Osteoclast differentiation factor (odf) induces osteoclast-like cell formation in human peripheral blood mononuclear cell cultures. Biochem Biophys Res Commun *246(1)*: 199-204, 1998. PMID: 9600092. DOI: 10.1006/bbrc.1998.8586
22 Fuller K, Wong B, Fox S, Choi Y and Chambers TJ: Trance is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. J Exp Med *188(5)*: 997-1001, 1998. PMID: 9730902. DOI: 10.1084/jem.188.5.997
23 Emery JG, McDonnell P, Burke MB, Deen KC, Lyn S, Silverman C, Dul E, Appelbaum ER, Eichman C, DiPrinzio R, Dodds RA, James IE, Rosenberg M, Lee JC and Young PR: Osteoprotegerin is a receptor for the cytotoxic ligand trail. J Biol Chem *273(23)*: 14363-14367, 1998. PMID: 9603945. DOI: 10.1074/jbc.273.23.14363
24 Reid P and Holen I: Pathophysiological roles of osteoprotegerin (opg). Eur J Cell Biol *88(1)*: 1-17, 2009. PMID: 18707795. DOI: 10.1016/j.ejcb.2008.06.004
25 Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D and Galibert L: A homologue of the tnf receptor and its ligand enhance t-cell growth and dendritic-cell function. Nature *390(6656)*: 175-179, 1997. PMID: 9367155. DOI: 10.1038/ 36593
26 Wong BR, Josien R, Lee SY, Sauter B, Li HL, Steinman RM and Choi Y: Trance (tumor necrosis factor [tnf]-related activation-induced cytokine), a new tnf family member predominantly expressed in t cells, is a dendritic cell-specific survival factor. J Exp Med *186(12)*: 2075-2080, 1997. PMID: 9396779. DOI: 10.1084/jem.186.12.2075
27 Yun TJ, Chaudhary PM, Shu GL, Frazer JK, Ewings MK, Schwartz SM, Pascual V, Hood LE and Clark EA: Opg/fdcr-1, a tnf receptor family member, is expressed in lymphoid cells and is up-regulated by ligating cd40. J Immunol *161(11)*: 6113-6121, 1998. PMID: 9834095.
28 Schoppet M, Henser S, Ruppert V, Stubig T, Al-Fakhri N, Maisch B and Hofbauer LC: Osteoprotegerin expression in dendritic cells increases with maturation and is nf-kappab-dependent. J Cell Biochem *100(6)*: 1430-1439, 2007. PMID: 17171649. DOI: 10.1002/jcb.21129
29 Yun TJ, Tallquist MD, Aicher A, Rafferty KL, Marshall AJ, Moon JJ, Ewings ME, Mohaupt M, Herring SW and Clark EA: Osteoprotegerin, a crucial regulator of bone metabolism, also regulates b cell development and function. J Immunol *166(3)*: 1482-1491, 2001. PMID: 11160187. DOI: 10.4049/jimmunol.166.3.1482
30 Ferrari-Lacraz S and Ferrari S: Do rankl inhibitors (denosumab) affect inflammation and immunity? Osteoporos Int *22(2)*: 435-446, 2011. PMID: 20571772. DOI: 10.1007/s00198-010-1326-y
31 To M, Ito K, Ausin PM, Kharitonov SA and Barnes PJ: Osteoprotegerin in sputum is a potential biomarker in copd. Chest *140(1)*: 76-83, 2011. PMID: 21127170; DOI: 10.1378/chest.10-1608
32 Ronco C: N-gal: Diagnosing aki as soon as possible. Crit Care *11(6)*: 173, 2007. PMID: 18001501. DOI: 10.1186/cc6162
33 Parikh CR, Coca SG, Thiessen-Philbrook H, Shlipak MG, Koyner JL, Wang Z, Edelstein CL, Devarajan P, Patel UD, Zappitelli M, Krawczeski CD, Passik CS, Swaminathan M, Garg AX and Consortium T-A: Postoperative biomarkers predict acute kidney injury and poor outcomes after adult cardiac surgery. J Am Soc Nephrol *22(9)*: 1748-1757, 2011. PMID: 21836147. DOI: 10.1681/ASN.2010111163

34 Pickering JW and Endre ZH: The clinical utility of plasma neutrophil gelatinase-associated lipocalin in acute kidney injury. Blood Purif *35(4)*: 295-302, 2013. PMID: 23712081. DOI: 10.1159/000351542

35 Haase-Fielitz A, Haase M and Devarajan P: Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury: A critical evaluation of current status. Ann Clin Biochem *51(Pt 3)*: 335-351, 2014. PMID: 24518531. DOI: 10.1177/0004563214521795

36 Ronco C, Legrand M, Goldstein SL, Hur M, Tran N, Howell EC, Cantaluppi V, Cruz DN, Damman K, Bagshaw SM, Di Somma S and Lewington A: Neutrophil gelatinase-associated lipocalin: Ready for routine clinical use? An international perspective. Blood Purif *37(4)*: 271-285, 2014. PMID: 25012891. DOI: 10.1159/000360689

37 Stewart IJ, Glass KR, Howard JT, Morrow BD, Sosnov JA, Siew ED, Wickersham N, Latack W, Kwan HK, Heegard KD, Diaz C, Henderson AT, Saenz KK, Ikizler TA and Chung KK: The potential utility of urinary biomarkers for risk prediction in combat casualties: A prospective observational cohort study. Crit Care *19*: 252, 2015. PMID: 26077788. DOI: 10.1186/s13054-015-0965-y

38 Gennarelli TA, Wodzin E and Medicine AftAoA: Abbreviated injury scale 2005: Update 2008. Association for the Advancement of Automotive Medicine: Barrington, 2008. ISBN-13: 978-0000002020

39 Baker SP, O'Neill B, Haddon W, Jr. and Long WB: The injury severity score: A method for describing patients with multiple injuries and evaluating emergency care. J Trauma *14(3)*: 187-196, 1974. PMID: 4814394.

40 Reinhart K, Brunkhorst FM, Bone HG, Bardutzky J, Dempfle CE, Forst H, Gastmeier P, Gerlach H, Grundling M, John S, Kern W, Kreymann G, Kruger W, Kujath P, Marggraf G, Martin J, Mayer K, Meier-Hellmann A, Oppert M, Putensen C, Quintel M, Ragaller M, Rossaint R, Seifert H, Spies C, Stuber F, Weiler N, Weimann A, Werdan K, Welte T, German Sepsis S, German Interdisciplinary Association of Intensive C and Emergency M: Prevention, diagnosis, therapy and follow-up care of sepsis: 1st revision of s-2k guidelines of the german sepsis society (deutsche sepsis-gesellschaft e.V. (dsg)) and the german interdisciplinary association of intensive care and emergency medicine (deutsche interdisziplinare vereinigung fur intensiv- und notfallmedizin (divi)). Ger Med Sci 8, 2010. DOI: 10.3205/000103

41 Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L and Slutsky AS: Acute respiratory distress syndrome: The berlin definition. JAMA *307(23)*: 2526-2533, 2012. PMID: 22797452. DOI: 10.1001/jama.2012.5669

42 Youden WJ: Index for rating diagnostic tests. Cancer *3(1)*: 32-35, 1950. PMID: 15405679. DOI: 10.1002/1097-0142(1950)3:1<32::aid-cncr2820030106>3.0.co;2-3

43 Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, Lijmer JG, Moher D, Rennie D, de Vet HC, Kressel HY, Rifai N, Golub RM, Altman DG, Hooft L, Korevaar DA, Cohen JF and Group S: Stard 2015: An updated list of essential items for reporting diagnostic accuracy studies. BMJ *351*: h5527, 2015. PMID: 26511519. DOI: 10.1136/bmj.h5527

44 Frink M, Pape HC, van Griensven M, Krettek C, Chaudry IH and Hildebrand F: Influence of sex and age on mods and cytokines after multiple injuries. Shock *27(2)*: 151-156, 2007. PMID: 17224789. DOI: 10.1097/01.shk.0000239767.64786.de

45 Lenz A, Franklin GA and Cheadle WG: Systemic inflammation after trauma. Injury *38(12)*: 1336-1345, 2007. PMID: 18048040. DOI: 10.1016/j.injury.2007.10.003

46 Hong DY, Lee JH, Park SO, Baek KJ and Lee KR: Plasma neutrophil gelatinase-associated lipocalin as early biomarker for acute kidney injury in burn patients. J Burn Care Res *34(6)*: e326-332, 2013. PMID: 23511281. DOI: 10.1097/BCR.0b013e31827d1f36

47 Yavuz S, Anarat A, Acarturk S, Dalay AC, Kesiktas E, Yavuz M and Acarturk TO: Neutrophil gelatinase associated lipocalin as an indicator of acute kidney injury and inflammation in burned children. Burns *40(4)*: 648-654, 2014. PMID: 24060429. DOI: 10.1016/j.burns.2013.09.004

48 Sen S, Godwin ZR, Palmieri T, Greenhalgh D, Steele AN and Tran NK: Whole blood neutrophil gelatinase-associated lipocalin predicts acute kidney injury in burn patients. J Surg Res *196(2)*: 382-387, 2015. PMID: 25890435. DOI: 10.1016/j.jss.2015.03.033

49 Hjortrup PB, Haase N, Wetterslev M and Perner A: Clinical review: Predictive value of neutrophil gelatinase-associated lipocalin for acute kidney injury in intensive care patients. Crit Care *17(2)*: 211, 2013. PMID: 23680259. DOI: 10.1186/cc11855

Received June 5, 2019
Revised July 1, 2019
Accepted July 2, 2019