Journal of Surgery
Graeff P, et al. J Surg 7: 1616
www.doi.org/10.29011/2575-9760.001616
www.gavinpublishers.com
Research Report

Unexpected Low Resistin Level Could be a Factor for Outcome Prediction in Multiply Injured Patients

Graeff P*, Winkelmann M, Clausen J, Wilhelmi M, Krettek C, Neunaber C

Hannover Medical School, Trauma Department, Carl-Neuberg-Straße 1, 30625 Hannover, Germany

***Corresponding author:** Pascal Graeff, Hannover Medical School, Trauma Department, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

Citation: Graeff P et al. (2022) Unexpected Low Resistin Level Could be a Factor for Outcome Prediction in Multiply Injured Patients. J Surg 7: 1616. DOI: 10.29011/2575-9760.001616

Received Date: 27 October, 2022; **Accepted Date:** 04 November, 2022; **Published Date:** 09 November, 2022

Abstract

Objective: Resistin plays a crucial role in the development of diabetes and several other internal diseases. Lately it was discovered as a predictor of septic and SIRS events in neonatal and paediatric ICU treatments as well. Regarding patients suffering a multiple trauma it was not considered yet even though it is expressed by macrophages and therefore might be one of the first proteins to be identified after a multiple trauma.

Method: We used serum samples of patients with multiple trauma we collected and stored before. The patients were selected into three groups: ISS<25 for less severe traumatized patients, ISS>25 for severe traumatized patients and death during treatment.

Results and Conclusion: With these samples we performed an ELISA to look for Resistin and IL-6. Resistin was highest in the ISS<25 group and slightly lower in the ISS>25 group with an increase in both. In the third group Resistin almost did not show any change in concentration. These findings show an unexpected result after performing an ANOVA test with statistically significant differences in the levels we measured but could as well lead to the goal of finding a new early marker for the severity of trauma and maybe an outcome parameter.

Keywords: Biomarker; Multiple Trauma; Outcome; Resistin

Introduction

A multiple trauma, also called a polytrauma in Europe, is per definition a combination of two or more injuries that are greater or equal to 3 on the Abbreviated Injury Score (AIS) and one or more additional diagnoses (pathologic condition), that is hypotension (systolic blood pressure ≤ 90 mm Hg,), unconsciousness (GCS score ≤ 8), acidosis (base deficit ≤ −6.0), coagulopathy (PTT ≥ 40 seconds or INR ≥ 1.4), and age (≥70 years). [1] In most definitions an Injury Severity Score (ISS) of 16 and more is considered as multiple trauma. [2,3] Until today it is the leading cause of death in the population below the age of 50. [4] Current knowledge states that there are multiple hits after a multiple trauma that are life-threatening. The first hit is at the accident site with hypoxia, haemorrhagic shock and direct organ injuries. [5] The second hit occurs due to reperfusion, operative intervention and infections in the early posttraumatic phase with the liberation of cytokines [5], both pro- and anti-inflammatory, which can lead to a Systemic Inflammatory Response Syndrome (SIRS), resulting in multiple organ failure. [6] If the anti-inflammatory cytokines predominate [6] or macrophage activation is over induced as a response to an infection which is common in multiply injured patients [7,8].

Therefore, it is of particular interest to find a sufficient and reliable laboratory parameter which helps to assess the likelihood of complications individually in every patient, to treat patients more efficiently at an early stage. Besides the established parameters and scoring systems such as ISS and the SOFA score treating

physicians are looking for specific blood parameters which help to evaluate the severity of a multiply injured patient. This might help to get more certainty in the initial phase of what to expect as an outcome and whether the SIRS could hit harder. Being able to respond to it as soon as possible or to find a way in preventing a SIRS would be a huge benefit in multiple trauma treatment. [8] This is the reason why the search for markers that allow an assessment of complications or severe courses of multiple trauma in the later course has been going on since the uprising of infection markers began. With every new discovery of a marker the hope began to rise anew. Whether it was the C-Reactive Protein (CRP) [9], Interleukin (IL) -6 [10] or Procalcitonin (PCT) [11], the results were promising but never matched the expectations.

Another promising candidate was adiponectin, as it is already known that adiponectin levels are increased during the course of sepsis, and here especially in the preseptic phase. [12] On top of that an organ-protective character in septic situations was found for adiponectin. [13] Another hormone secreted by adipocytes is Resistin, which was then tested as an indicator for septic events in children [14] and especially in neonates. [15,16] Resistin was first introduced in 2001 by the group of Steppan et al. from the University of Pennsylvania. [17] Originally detected in rodents, it was later found in the human blood samples as well. The first scientific achievement was the link between Resistin and diabetes mellitus type II, thus giving it the name it has today consisting of the two words “resistance to insulin”. The secretion of this hormone is different in various animal groups. In rodent it is mainly produced by adipose tissue making it an adipokines, whereas in humans it is secreted mostly by cells of the immune system such as macrophages [18].

In this present study the correlation between Resistin levels in blood samples of multiply injured patients in the early onset after multiple trauma were correlated with the development of septic complications as well as SIRS and Multi Organ Failure (MOF) in the later posttraumatic course. Since every multiple trauma has an inflammatory component the question which came to mind is, whether Resistin would increase in the early state of multiple trauma and how it would change in the following days? The hypothesis is that the Resistin levels in the early state after multiple trauma are higher in more severe injured patients compared to less injured ones.

Method

Sample Collection

All Samples were acquired from multiply injured patients with an ISS of 16 or more who were admitted to our intensive care unit. Written informed consent was obtained from all patients or in case of a patients inability to consent it was given by a legal representative.[19] Samples were collected in serum tubes on the day of admission as well as on day 1,2,4,7 and 10 after the admission. Samples were immediately cooled down in a 4°C refrigerator until further processing. All probes were then centrifuged to separate blood cells from serum. Afterwards the supernatant was transferred into an Eppendorf tube. These were stored at -80°C until further investigation. The study protocol and process of sample donation comply with the Declaration of Helsinki and were approved by the institutional review board (Hannover Medical School - Votum No. 4980).

Group Distribution

The samples were separated into three categories by their ISS and the outcome (Table 1). The first category (a) comprised four patients with an ISS<25, since it is a good cut for a severe trauma which was first published for children [20], and samples collected before 2014. We choose these relatively early samples due to the fact that we started to use a cytosorb dialysis since 2015 in case of severly injured patients. It is unclear whether cytosorb dialysis changes resistin levels or alters the underlying mechanisms. The second group (b) included four patients with an ISS>25 and the third group (c) consisted of four patients who did not survive (non-survival group) the treatment on our Intensive Care Unit (ICU). As it can be seen in table 1 the distribution of the sex is strongly in favour of male since these were nine and only three female patient samples tested. The mean age at the accident was 28 years without a mismatch between groups.

Groups	Age at accident	Outcome	sex	ISS	Leading diagnoses
ISS<25 **(a)**	21J	survived	male	17	blunt thoracic trauma, blunt abdominal trauma, minor fractures
	24J	survived	male	17	brain concussion, blunt thoracic trauma, blunt abdominal trauma
	19J	survived	male	22	blunt thoracic trauma, maxillofacial trauma, minor extremity trauma
	37J	survived	female	25	brain contusion with subarachniodal hemorrhage, blunt thoracic trauma, pelvic-ring fracture
ISS>25 **(b)**	24J	survived	male	50	III° traumatic brain injury with intracerebral bleeding and axonal injury, maxillofacial fracture,
	24J	survived	male	34	III° traumatic brain injury, blunt abdominal trauma, lumbar burst fracture
	26J	survived	male	50	III° traumatic brain injury, pelvic fracture, blunt abdominal fracture
	41J	survived	male	75	reanimation with ROSC, dens fracture, blunt abdominal trauma, lung contusion
Non-survival **(c)**	22J	died during therapy	male	43	III° traumatic brain injury, blunt thoracic trauma, pelvic fracture
	54J	died during therapy	female	27	dislocated fracture C4/6, infratentorial infarction after vertebral dissection
	30J	died during therapy	male	75	reanimation with ROSC, c-fracture cervical spine 4-6
	20J	died during therapy	female	66	brain contusion with subarachniodal hemorrhage, blunt thoracic trauma, pelvic fracture, blunt

Table 1: Group distribution. An overview of the baseline characteristics is given for every group. The last column describes the main diagnoses. In the group (b) with an ISS>25 nearly everyone had a brain injury whereas only 2/4 of the third group (c) had a brain injury. Other relevant diagnoses were blunt thoracic and/or abdominal traumata. We included 9 males and 3 females. This phenomenon is due to the fact, that far more multiple injured patients treated on our ICU were males.

Elisa

Undiluted serum samples were evaluated with R&D® quanitkine test kits for human Resistin (Human Resistin Quantikine ELISA Kit) as well as IL-6 (Human IL-6 Quantikine ELISA Kit) according to the manufacturer's instructions. The results were delivered by the ELISA Reader as absorption measurements.

Statistics

Statistical evaluation was performed using SigmaPlot™ 13. Evaluation of Gaussian distribution was performed by a Shapiro-Wilk test and showed parametric data. Therefore, evaluation was performed with a Normality pre-test (Shapiro-Wilk) and ANOVA supported by a Student-Newman or a Dunn's post-hoc test for the comparing examination of the mean groups. Furthermore an additional examination with the RM-ANOVA was performed for the comparison of the serial blood levels in each patient. In addition, the mean calculated results were evaluated with a paired t-test. A value of $p<0.05$ was considered as significant difference.

Patient and Public Involvement' (PPI) Statement

--How were the research question(s) and outcome measures developed and informed by their priorities, experience, and preferences?

There was no information about the outcome measures and the priorities etc. since there was no active involvement of the patients in the execution of this study.

-How were patients involved in the design of this study?

Since the study was performed in some cases 10 years after the samples were taken and some patients have died during the therapy there was no involvement for the patients in the study.

-How were they involved in the recruitment to and conduct of the study?

There was no patient involvement in the recruitment and conduct of the study since there was no active patient involvement.

-Were they asked to assess the burden of the intervention and time required to participate in the research?

No assessment of the burden of the intervention and time required was performed since the patients were not actively participating in the study and the samples were taken as part of their daily routine blood controls.

-How were (or will) they be involved in your plans to disseminate the study results to participants and relevant wider patient communities?

The results of the study will be disseminated with the participants and wider communities after the manuscript is accepted and published.

-How were (or will) they be involved in your plans to disseminate the study results to participants and relevant wider patient communities?

The results of the study will be disseminated with the participants and wider communities after the manuscript is accepted and published.

Result

Individual and Grouped Mean Comparison of IL-6

First we analysed each group individually for IL-6. Figure 1 a-c depicts the IL-6 values on each time point for every patient of all groups. Additionally, the mean values for each time point were calculated. The comparison of the different patients in each group shows a huge inconsistency regarding the IL-6 values. Comparing the mean values of each group (Figure 1) a slight but not significant difference was noticed. Mean values of group (a) were slightly higher compared to group (b) whereas group (c) showed the highest IL-6 levels on the day of admission. Despite the slightly higher Il-6 values, group (a) and (b) showed a comparable course of mean IL-6 values in the study period, with an inclination in the beginning, a drop on day 4 and afterwards an inclination again. In group (c) the mean IL-6 level reached a maximum on the day of admission and decreased continuously until day 4. By analysing the results in this group, it has to be taken into account, that the measurement at day 7 on the right only includes one person due to the fact that all other patients died before the seventh blood sample could be taken leaving one sample on day 7.

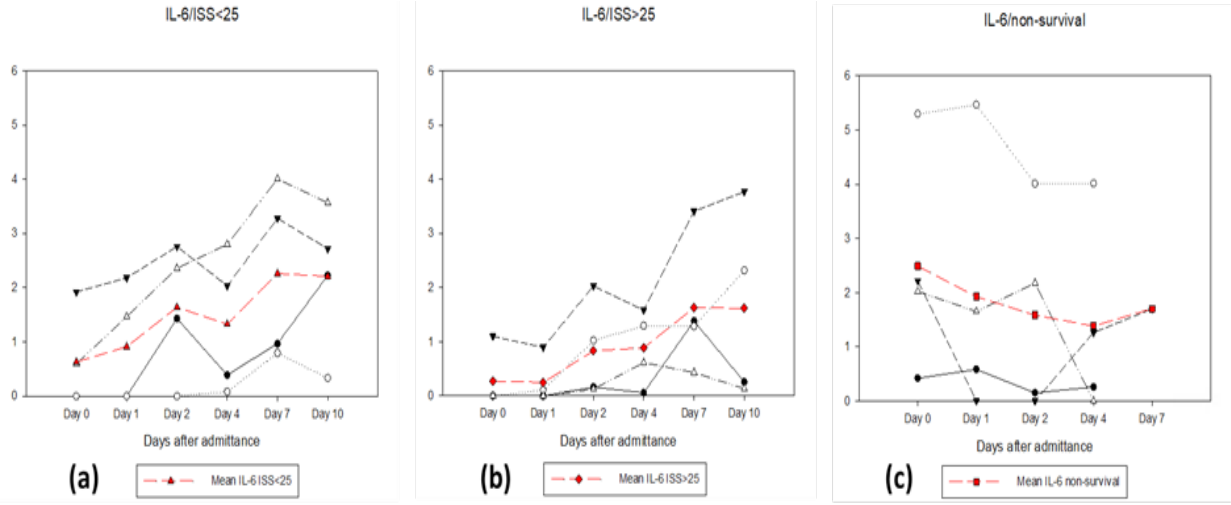

Figure 1: Individual course of IL-6 in the different groups. IL-6 concentrations over the first 10 days after admission. (a) ISS<25, (b) ISS>25 and (c) non-survival. The red marked lines show the calculated mean for every single group.

To get a better understanding of the Il-6 value course we compared the mean values (Figure 2) of each group. We saw an Il-6 value of 2.6 in the non-survival group (c) at the day of hospital admission whereas the survival groups (a) and (b) showed values below 1. Values of group (c) declined until day 4 whereas values of group (b) and (c) increased until day 4. The bread even point is at day three where the dead patients at 1.8. The more serious injured (b) have a concentration around 1. In the end the concentration of the ISS< 25 (a) are 1.5 and ISS>25 (b) are 2.2. No statistical difference were found when comparing the mean values of all groups at each time point.

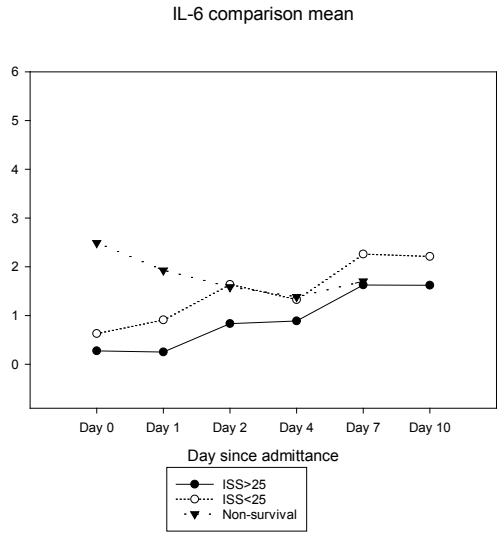

Figure 2: Mean IL-6 Comparison of groups (A)-(C). Comparison of the mean trends for the IL-6 groups as mentioned. In this Graph the red marked lines of Figure 1 is delineated.

Individual and Grouped Mean Comparison of Resistin

We performed all Resistin measurements analogue to the IL-6 protocol. Most obvious is that the measurements (Figure 3) of the non-surviving patients have a constant course compared to those with an ISS<25 (a). The patients of the non-survival group showed a constant decrease of the Resistin levels whereas the patients in group (a) and (b) showed undulating Resistin levels during the study period. Comparing the mean Resistin levels (Figure 4) of all 3 groups we found increasing Resistin levels in group (a) and (b) and a nearly steady Resistin level until day 4 in group (c). There was a statistical significance among all treatment groups of p<0,001 in an ANOVA that compared the different courses of the parameters. The comparison of the mean values had a significance of p<0,001 and a significance of p<0,05 in the multiple pairwise comparison (Student-Newman) of all three mean courses against each other.

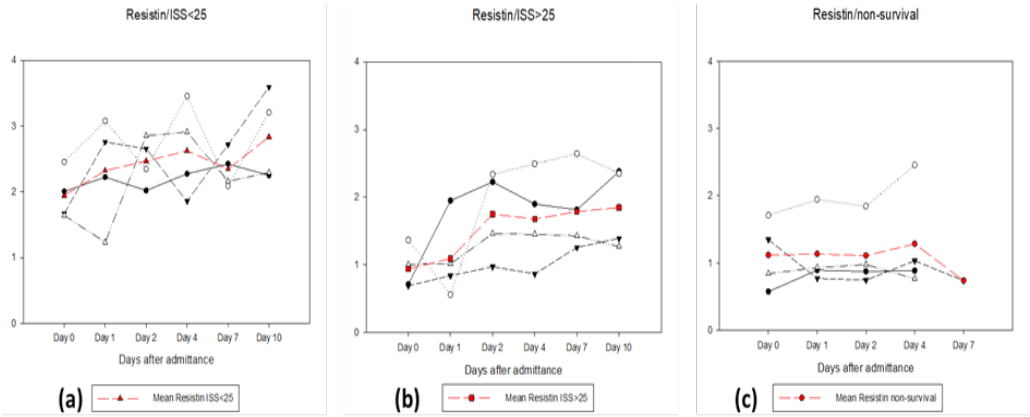

Figure 3: Individual course of Resistin in the different groups. The graph (a) describes the trend of the Resistin measurements in the patients with an ISS<25. The single measurements of every patient will not provide a clear trend since they are alternating. By looking at the mean line painted in red you can see an increase until day 4 and a dip in day 7 until the measurement increases again. The graph (b) shows a steady trend in every individual patient. The mean line has an increase and a small decrease at day 4. Afterwards it increases again. The graph (c) on the right shows that the trends are steady as well. In contrast to the measurements before there is neither a visible increase nor is there a clear decrease at day 4. Day 7 is a measurement which was only performed in one patient since the other patients died previously.

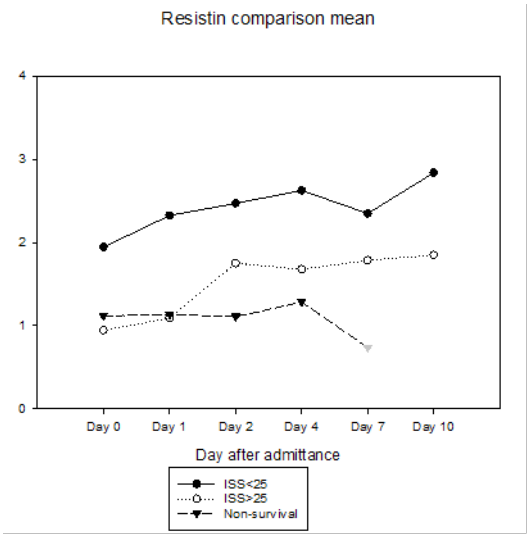

Figure 4: Mean Resistin Comparison of groups (a)-(c). This graph shows the comparison of the mean trends of Patients with an ISS<25, ISS>25 and non-surviving patients. The highest level of Resistin can be seen in the patients with an ISS<25 with a dip at day 7. The level of Resistin was nearly the same in the group of patients with an ISS>25 and those who died. But unlike the group of non-surviving patients, the ISS group had an increase at day 2 and a light dip at day 4.

Direct Comparison of Resistin and IL-6 for Every Single Group

The direct comparison of Resistin and IL-6 levels (Figure 5) show similar trends in group (a) and (b). The ISS<25 (a) group had a significance of p=0,006. The ISS>25 (b) group had a significance of p=0,007 and the last group one of p=0,027. The mean courses of Resistin and Il-6 levels in group (a) were ascending in a comparable manner. The dip was noticed on day 4 for IL-6 and on the seventh day for Resistin. In case of group (b) the courses showed similar qualities until day 4 but afterwards the Resistin course flattened in comparison to Il-6. The comparison in the non-survival group showed a decrease in IL-6 levels after an initially high, whereas Resistin has an equal initial value compared to group (b) but has a steady trend. Furthermore, it is noticeable that the Resistin values are higher in the ISS<25 (a) and ISS>25 (b) whereas in the non-survival group (c) were lower.

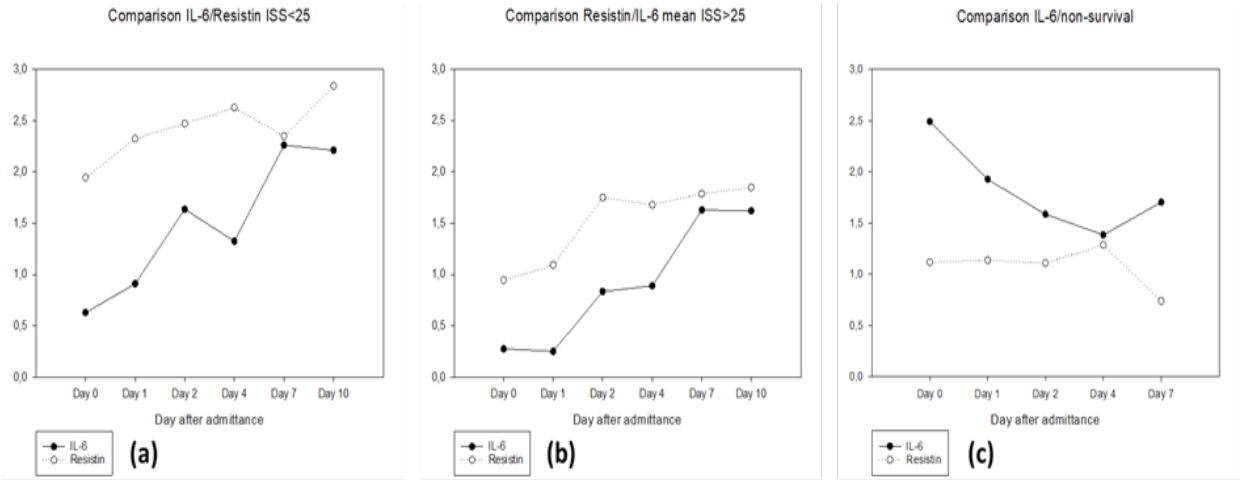

Figure 5: Individual comparison of the mean Resistin/IL-6 in the different groups. The graphs above are showing the direct comparisons of IL-6 and Resistin between the three already known groups. Resistin is shown by the dotted line appearing grey and IL-6 is displayed as the solid black line.

Discussion and Conclusion

Looking into previous studies in which Resistin was examined it was described as a pro-inflammatory protein [21], which is why we expected high levels in severely injured patients. With our findings it is impossible to hold that presumption, since we found out that there is a significantly lower level of Resistin in non-surviving group. Another astonishing result was the significantly lower Resistin level for the serious injured patients (ISS>25) compared to the less serious injured (ISS<25). It has been shown before, that high Resistin levels are associated with multiple organ dysfunction and SIRS even without a septic component. By that, it can help to predict the course of patients admitted to the ICU. [22] Our results cannot fully support this theory for multiply injured patients. It is known that IL-6 values and the injury severity correlate [23], which can help to assess and predict the organ functions. [24] High IL-6 values as one aspect of the so called "cytokine storm" contribute to the development of a multi organ failure. [8] Therefore "cytokine dialysis" e.g. with a cytosorb filter can be an option to attenuate the course of SIRS and by that it might help to prevent irreparable organ damage. [25] It is obvious that if a "cytokine dialysis" is used, IL-6 values cannot be utilized to assess the course of the SIRS.

Therefore we were looking for other parameters and identified Resistin as a parameter which might play a role in severe trauma. The initial idea was that Resistin should be more increased in severely injured patients since it is secreted by macrophages. Considering the results of our study there is no clear evidence that can fulfil these expectations. In contrary to our initial hypothesis, the patients with an ISS<25 showed higher Resistin values at the time of admission compared to the group with an ISS>25. Additionally, the Resistin values in the non-survival group, which we expected to be the highest or at least as high as in the ISS>25 group, were even lower than those of the two comparative groups. Notable is that none of the examined patients went through a septic event. This is an interesting fact, since previous clinical interest focused on the detection of septic events. [26-28] In contrast to our initial hypothesis, our data suggests that patients with lower Resistin levels have a worse outcome.

Our study has several limitations. First of all we have small group sizes and in case of the non-survival group only 1 data set for day 7 was available. Another problem is that there is no clear normal Resistin value in Blood samples which makes it hard to interpret our data as high or low levels. Therefore a bigger study is needed and already planned.

Funding Details

This Study was financially supported by the Trauma Stiftung e.V. (Carl-Neuberg Straße 1, 30625 Hannover) with 5000€

References

1. Pape HC, Lefering R, Butcher N, Peitzman A, Leenen L, et al. (2014) The definition of polytrauma revisited: An international consensus process and proposal of the new 'Berlin definition'. The journal of trauma and acute care surgery 77: 780-786.
2. de Vries R, Reininga IHF, Pieske O, Lefering R, El Moumni M, et al. (2018) Injury mechanisms, patterns and outcomes of older polytrauma patients-An analysis of the Dutch Trauma Registry. PloS one 13: e0190587.
3. Marzi I, Lustenberger T, Störmann P, Mörs K, Wagner N, et al. (2019) [Increasing overhead ressources of the trauma room]. Der Unfallchirurg 122: 53-58.
4. Sakran JV, Greer SE, Werlin E, McCunn M (2012) Care of the injured worldwide: trauma still the neglected disease of modern society. Scandinavian journal of trauma, resuscitation and emergency medicine 20: 64.
5. Keel M, Trentz O (2005) Pathophysiology of polytrauma. Injury 36: 691-709.
6. Ashare A, Powers LS, Butler NS, Doerschug KC, Monick MM, et al. (2005) Anti-inflammatory response is associated with mortality and severity of infection in sepsis. American journal of physiology Lung cellular and molecular physiology 288: L633-640.
7. Neunaber C, Zeckey C, Andruszkow H, Frink M, Mommsen P, et al. (2011) Immunomodulation in polytrauma and polymicrobial sepsis - where do we stand? Recent patents on inflammation & allergy drug discovery 5: 17-25.
8. Hildebrand F, Pape HC, Krettek C (2005) [The importance of cytokines in the posttraumatic inflammatory reaction]. Unfallchirurg 108: 793-794.
9. Nast-Kolb D, Waydhas C, Jochum M, Duswald KH, Machleidt W, et al. (1992) [Biochemical factors as objective parameters for assessing the prognosis in polytrauma]. Der Unfallchirurg 95: 59-66.
10. Foëx BA, Lamb WR, Roberts TE, Brear SG, Macartney I, et al. (1993) Early cytokine response to multiple injury. Injury 24: 373-376.
11. Mimoz O, Benoist JF, Edouard AR, Assicot M, Bohuon C, et al. (1998) Procalcitonin and C-reactive protein during the early posttraumatic systemic inflammatory response syndrome. Intensive care medicine 24: 185-188.
12. Hillenbrand A, Xu P, Zhou S, Blatz A, Weiss M, et al. (2016) Circulating adipokine levels and prognostic value in septic patients. Journal of inflammation (London, England) 13: 30.
13. van Meurs M, Castro P, Shapiro NI, Lu S, Yano M, et al. (2012) Adiponectin diminishes organ-specific microvascular endothelial cell activation associated with sepsis. Shock (Augusta, Ga) 37: 392-398.
14. Saboktakin L, Bilan N, Ghalehgolab Behbahan A, Poorebrahim S (2019) Relationship Between Resistin Levels and Sepsis Among Children Under 12 Years of Age: A Case Control Study. Frontiers in pediatrics 7: 355.
15. Aliefendioglu D, Gursoy T, Caglayan O, Aktas A, Ovali F (2014) Can resistin be a new indicator of neonatal sepsis? Pediatrics and neonatology 55: 53-57.
16. Khattab AA, El-Mekkawy MS, Helwa MA, Omar ES (2018) Utility of serum resistin in the diagnosis of neonatal sepsis and prediction of disease severity in term and late preterm infants. Journal of perinatal medicine 46: 919-925.

17. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, et al. (2001) The hormone resistin links obesity to diabetes. Nature 409: 307-312.

18. Schwartz DR, Lazar MA (2011) Human resistin: found in translation from mouse to man. Trends in endocrinology and metabolism: TEM 22: 259-265.

19. Baker SP, O'Neill B, Haddon W, Jr., Long WB (1974) The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. The Journal of trauma 14: 187-196.

20. Brown JB, Gestring ML, Leeper CM, Sperry JL, Peitzman AB, et al. (2017) The value of the injury severity score in pediatric trauma: Time for a new definition of severe injury? The journal of trauma and acute care surgery 82: 995-1001.

21. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A (2005) Resistin, an adipokine with potent proinflammatory properties. Journal of immunology (Baltimore, Md : 1950) 174: 5789-5795.

22. Koch A, Gressner OA, Sanson E, Tacke F, Trautwein C (2009) Serum resistin levels in critically ill patients are associated with inflammation, organ dysfunction and metabolism and may predict survival of non-septic patients. Crit Care 13: R95.

23. Stensballe J, Christiansen M, Tønnesen E, Espersen K, Lippert FK, et al. (2009) The early IL-6 and IL-10 response in trauma is correlated with injury severity and mortality. Acta anaesthesiologica Scandinavica 53: 515-521.

24. Frink M, van Griensven M, Kobbe P, Brin T, Zeckey C, et al. (2009) IL-6 predicts organ dysfunction and mortality in patients with multiple injuries. Scandinavian journal of trauma, resuscitation and emergency medicine 17: 49.

25. Ishihara S, Ward JA, Tasaki O, Brinkley WW, Seraile LG, et al. (1999) Effects of long-term hemofiltration on circulating mediators and superoxide production during continuous endotoxin administration. The Journal of trauma 46: 894-899.

26. Karampela I, Christodoulatos GS, Dalamaga M (2019) The Role of Adipose Tissue and Adipokines in Sepsis: Inflammatory and Metabolic Considerations, and the Obesity Paradox. Current obesity reports 8: 434-457.

27. Gokmen Z, Ozkiraz S, Kulaksizoglu S, Kilicdag H, Ozel D, et al. (2013) Resistin--a novel feature in the diagnosis of sepsis in premature neonates. American journal of perinatology 30: 513-517.

28. Macdonald SPJ, Bosio E, Neil C, Arendts G, Burrows S, et al. (2017) Resistin and NGAL are associated with inflammatory response, endothelial activation and clinical outcomes in sepsis. Inflammation research : official journal of the European Histamine Research Society 66: 611-619.