Immunoproteasome in the Plasma of Pediatric Patients With Moderate and Major Burns, and Its Correlation With Proteasome and UCHL1 Measured by SPR Imaging Biosensors

Ewa Matuszczak, PhD, MD,* Artur Weremijewicz, MD,* Marta Komarowska, MD,* Anna Sankiewicz, MD,† Diana Markowska, MD,† Wojciech Debek, PhD MD,* Ewa Gorodkiewicz,† Robert Milewski, PhD MD,‡ and Adam Hermanowicz, PhD, MD*

The aim of this study was to determine the immunoproteasome concentration in blood plasma of pediatric patients with moderate and major burns and its correlation with circulating proteasome and ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) with surface plasmon resonance imaging biosensor. The study population comprised of 30 patients with moderate (n = 21) and severe burns (n = 9), aged 9 months to 14 years. The control group represented 18 healthy, age-matched patients, admitted for herniotomy. Exclusion criteria were as follows: admission to the hospital later than 6 hours after burn, cardiovascular or immunological diseases, and severe preexisting infections. Mean concentrations of immunoproteasome, 20S proteasome, and UCHL1 in the blood plasma of children with burns—4 to 6 hours, 12 hours, 3 days, 5 days, and 7 days after thermal injury—were above the levels measured in controls. The immunoproteasome, 20S proteasome, and UCHL1 concentrations in the blood plasma of their patients were highest 12 hours after burn, slowly decreased over time, and on the 5th day still were higher than in controls ($P < .05$). There was a strong correlation between immunoproteasome and 20S proteasome concentrations 6 hours and 5 days after burn, and moderate correlation 12 hours after burn ($P < .05$). The immunoproteasome concentration is elevated after burn injury and slowly reaches the normal range during the wound healing process. There is strong correlation between immunoproteasome and 20S proteasome concentrations in the serum of children with moderate and major burns. They did not find such correlation between immunoproteasome and UCHL1 concentrations. Immunoproteasome concentration do not correlate with age or sex.

The proteasome can be found in all eucariotic cells and is the central nonlysosomal protein degrading system, according to Ostrowska et al "responsible for degradation of damaged proteins, short-lived proteins, and for the processing of intracellular antigens" [1,2]. The ubiquitin-proteasome system is also responsible for regulation of cell cycle, "proliferation, gene transcription and differentiation, apoptosis, signal transduction, and antigen processing" (Sixt et al) [3].

The 20S proteasome is a barrel shaped complex consisting of four rings, and each unit has seven subunits. The central proteolytic chamber is formed by two outer rings (consisting of α subunits) and two inner rings (consisting of β subunits) [4].The catalytically active center of the 20s proteasome is formed by β1, β2, and β5 subunits [4].

According to Angeles et al, "Immunoproteasome is a subtype of proteasome that contains three major catalytic subunits: β1i (also known as low molecular mass peptide 2 [LMP2}; proteasome subunit beta 9 [PSMB9], β2i (also known as multicatalytic endopeptidase complex-like 1 [MECL-1; PSMB10}; and β5i (also known as LMP7; PSMB8)" [5].

*From the *Department of Pediatric Surgery, Medical University of Bialystok, Poland;*
†Department of Electrochemistry, University of Bialystok, Poland;
‡Department of Statistics, Medical University of Bialystok, Poland

Address correspondence to Ewa Matuszczak, PhD, MD, Waszyngtona 17, 15-274 Bialystok, Poland. Email: ewamat@tlen.pl

doi:10.1093/jbcr/iry011

Interferon-γ influences proteasomes, and as a result immunoproteasomes are formed. The immunoproteasome is responsible for processing of antigens, and expansion and/or survival of T cells, in T helper cell differentiation in patients with inflammation of the brain, in inflammatory cytokine production, and in autoimmune disease [5].

Immunoproteasome can be found in different cells, eg, in nervous system: neurons, astrocytes, microglia, and endothelial cells [6,7]. Based on animal studies, Chen et al found that "The increase of immunoproteasome LMP2 and LMP7 is probably involved in the inflammatory pathophysiological mechanism of ischemia stroke," and "selective inhibition of the immunoproteasome subunit LMP7 results in neuroprotection in middle cerebral artery occlusion (MCAO)" [6].

The impact of immunoproteasome on autoimmunity and inflammation raised an interest in understanding its cellular function. In our research, we assessed the immunoproteasome concentration in serum of pediatric patients with moderate and major burns, to verify if it is a part of the metabolic response to thermal injury, and its correlation with circulating proteasome and ubiquitin carboxyl-terminal hydrolase L1 (UCHL1), using a surface plasmon resonance imaging (SPRI) biosensor.

To determine the immunoproteasome concentration, usually the enzyme-linked immunoabsorbent (ELISA) test and semiquantitative western blotting are used [8–15]. The drawback methods is the use of indirect labels, which may be the cause of losing of the protein functional properties. The SPRI biosensor can become a good alternative. According to Sankiewicz et al, "This is a label-free, surface-sensitive spectroscopic technique used to examine the interaction between biomolecules" [16]. SPRI is based on detection of "changes in the refractive index within a short distance from the surface of a thin metal film caused by molecules bound to the metal surface" (also Sankiewicz et al) [16]. The researched substance is captured from the solution by the antibody or inhibitor [14–16].

So far several SPRI biosensors were used for clinical research to determine, eg, lysosomal proteases, UCHL1, and immunoproteasome and circulating proteasome [17–25].

MATERIALS AND METHODS

Thirty children with thermal injuries who were hospitalized between 2014 and 2015 were studied. We included study patients with moderate burns n = 21 (5–10% TBSA burn, 2–5% full-thickness burn) and patients with severe burns n = 9 (>10% TBSA burn, >5% full-thickness burn), which parents gave written informed consent for both clinical and biochemical follow-up. Patients were aged 9 months to 14 years (median = 2.07 ± 1.91 years). There were 11 girls and 19 boys. The severity of the burn was assessed according to American Burns Association. All children in this study were treated conservatively, according to the same protocol, including antibioticotherapy. Patients' characteristics are shown in Table 1. The control group represented 18 healthy patients, with the same age characteristics admitted for herniotomy between 2014 and 2015. Exclusion criteria were as follows: admission to the hospital later than 6 hours after thermal injury, cardiovascular or immunological diseases, and severe preexisting infections. The study protocol had the approval of the Ethics Committee of University of Bialystok.

METHODS

Venous blood samples (1–2 ml) were drawn 2 to 6 hours (on the admission), and 12 hours after the thermal injury, and on the subsequent days 3, 5, and 7. Blood samples were collected in EDTA tubes, plasma prepared according to the standard protocols and stored at –80°C.

CHIP PREPARATION

Gold Chip Was Manufactured as Described in a Previous Paper

Antibody immobilization for UCHL-1 determination was done according to the method described by Gorodkiewicz et al in their previous paper: "chips were then immersed in 20 mM of cysteamine ethanolic solutions for 2 hours and, after rinsing with ethanol and water, dried again under a stream of nitrogen. The specific rabbit monoclonal antibody solution in a PBS buffer (10 µg/ml) was activated with NHS (50 mM) and EDC (200 mM). Activation of the antibody was done by adding the mixture of NHS and EDC (1:1) in a carbonate buffer solution (pH 8.5) into the antibody solution and with vigorous stirring for 5 minutes at the room temperature. Three microliters of this solution were placed on the active places with the amine-modified surface and incubated at for 1 hour" [16–18].

Table 1. Characteristics of the study patients

	Patients with thermal injuries n = 30	
Characteristic	**Moderate burns n = 21**	**Major burns n = 9**
Gender	Female n = 9 Male n = 12	Female n = 1 Male n = 8
Age	1.5–7 y.o. (median: 2.07 y.o.)	1–9 y.o. (median:2.07 y.o.)
Burns depth (degree)	epidermis and upper layers of the dermis (Second Degree Burns)	epidermis (Second Degree Burns) or most of the dermis (Third Degree Burns)
	Partial thickness BSA: 5–10%, n = 21	Full thickness burn BSA: 10–15%, n = 8 Full thickness burn BSA: 6%, n = 1
Burn surface area (BSA)	Trunk n = 11 Upper limb and trunk n = 6	Trunk n = 4 Upper limb n = 3
Burned area	Lower limb and trunk n = 4	Lower limb n = 2
Antibioticotherapy	Yes	Yes
Preexisting medical disorders	None	None

Inhibitor—PSI Immobilization for Proteasome Determination

According to Gorodkiewicz et al and Sankiewicz et al, "PSI inhibitor (Z-Ile-Glu(OBut)-Ala-Leu-H)) at a concentration of 80 nM was activated with NHS (50 mM) and EDC (200 mM) in a carbonate buffer (pH = 8.5) environment and then placed on the thiol (cysteamine)-modified surface and incubated at 37°C for 1 hour" [17,18]. For receptor immobilization in the process of immunoproteasome determination, we used commercial inhibitor ONX 0914, as described by Sankiewicz et al [18]. According to Sankiewicz et al and Gorodkiewicz et al, "after receptor immobilization the biosensor were rinsed with water. Next, blood plasma samples (10× diluted) were placed directly on the prepared biosensor. The volume of the sample applied on each measuring field was 3 µl. Time of the interaction with receptor was max 10 minutes. As described by Gorodkiewicz et al, "The biosensor was washed with water and HBS-ES buffer solution pH = 7.4 (0.01 M 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid, 0.15 M sodium chloride, 0.005% Tween 20, 3 mM EDTA), BIOMED, Lublin, Poland) to remove unbound molecules from the surface" [16,18].

SPRI measurements were performed on self-made apparatus made, which detailed account can be found in previous paper [18]. SPRI measurements were performed as described by Sankiewicz et al, "the measurements were performed at a fixed angle of incident light and the reflectivity was simultaneously measured across an entire chip surface. The contrast values obtained for all pixels across a particular sample single spot were integrated. The signal was measured twice on the basis of registered images, after immobilization of the receptor (antibody or inhibitor) and then after interaction with analyte. The SPRI signal, which is proportional to coupled biomolecules, was obtained from subtraction between the signal before and after interaction with a biomolecule" [18].

Also a background correction was applied as described by Sankiewicz et al, "some of the sites on the biosensor covered with PBS buffer were used as a control. Nonspecific binding was monitored by measuring the SPRI signal at a site on the chip without the receptor (ligand). Minimization of nonspecific binding was achieved by preparing samples in PBS buffer (NaCl and KCl concentration ~200 mM) at pH 7.4" [18].

Blood plasma samples from healthy children admitted for planned surgeries, and from our patients with burns, were 10-times diluted with PBS buffer. The drops of blood plasma were transferred onto the chip surface for 10 minutes [18]. As described by Gorodkiewicz in a previous paper, "A whole section of 12 spots was covered. The surface was washed with distilled water 10-times and after drying, the SPRI measurement was performed. An average value taken from 12 pair of measurements was considered as a single result" [17]. Concentration was evaluated from the calibration curve of immunoproteasome and 20S proteasome and UCHL1 [18]. As control surfaces, the same sample solution was run over

surface derivatized with nonreacting biomolecules to delineate the level of nonspecific binding.

STATISTICS

The Mann–Whitney *U* test and the Kruskal–Wallis *H* test with Dunn's post hoc correction to control for multiple testing were used to compare differences between groups. Statistical analyses were calculated with the STATISTICA PL release 10.0 program. A two-tailed $P < .05$ was considered significant. Correlations were examined by linear regression (r) using the Spearman test.

RESULTS

Mean concentrations of immunoproteasome, 20S proteasome, and UCHL1 in the blood plasma of children with burns—4 to 6 hours after, 12 hours after, 3 days, 5 days, and 7 days after thermal injury—were higher than in controls ($P < .05$) (Figures 1, 2, and 3). The immunoproteasome, 20S proteasome, and UCHL1 concentrations in the blood plasma of patients with burns were highest 12 hours after thermal injury and were 5-folds higher for immunoproteasome, 6-folds higher for 20S proteasome, and 79-folds higher for UCHL1 than levels measured in controls, and the difference was statistically significant. The immunoproteasome, 20S proteasome, and UCHL1 concentration measured 3 days, 5 days, and 7 days after the thermal injury, slowly decreased over time, and still were higher than concentrations measured in controls ($P < .05$). There was a strong correlation between immunoproteasome and 20S proteasome concentrations 6 hours and 5 days after thermal injury, and moderate correlation 12 hours after the burn ($P < .05$). We did not find statistically significant difference in plasma levels of immunoproteasome in children with moderate and severe burns. An upward trend in immunoproteasome levels correlating with thermal injury severity could be observed, but the group of children with severe burns was too small to draw conclusions (data not shown). We did not find the correlation between immunoproteasome and UCHL1 concentrations in the blood plasma of our patients after burns. In our group of patients with thermal injuries, there were no severe complications (eg, death, sepsis). All patients were treated with the same protocol and discharged home in good general state on the final phase of wound healing process.

DISCUSSION

The proteasome system is crucial for the protein breakdown of skeletal muscle in patients with thermal injuries [26]. Tumor necrosis factor alpha

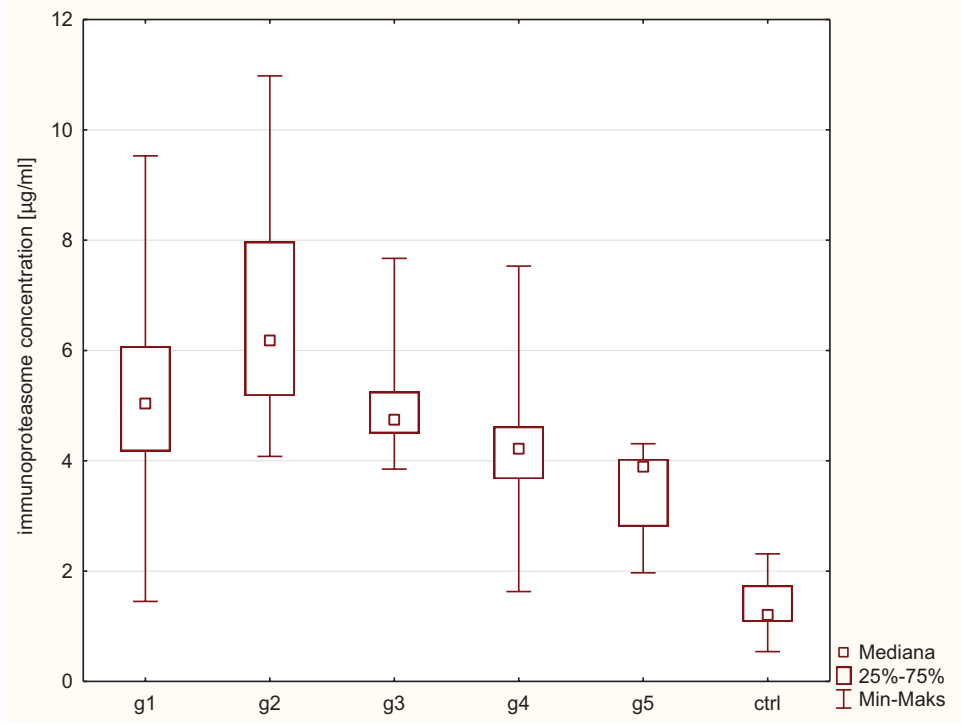

Figure 1. Concentrations of immunoproteasome in the blood plasma of children with burns, g1: 4 to 6 hours after, g2: 12 hours after, g3: 3 days, g4: 5 days, and g5: 7 days after thermal injury, ctrl = control group.

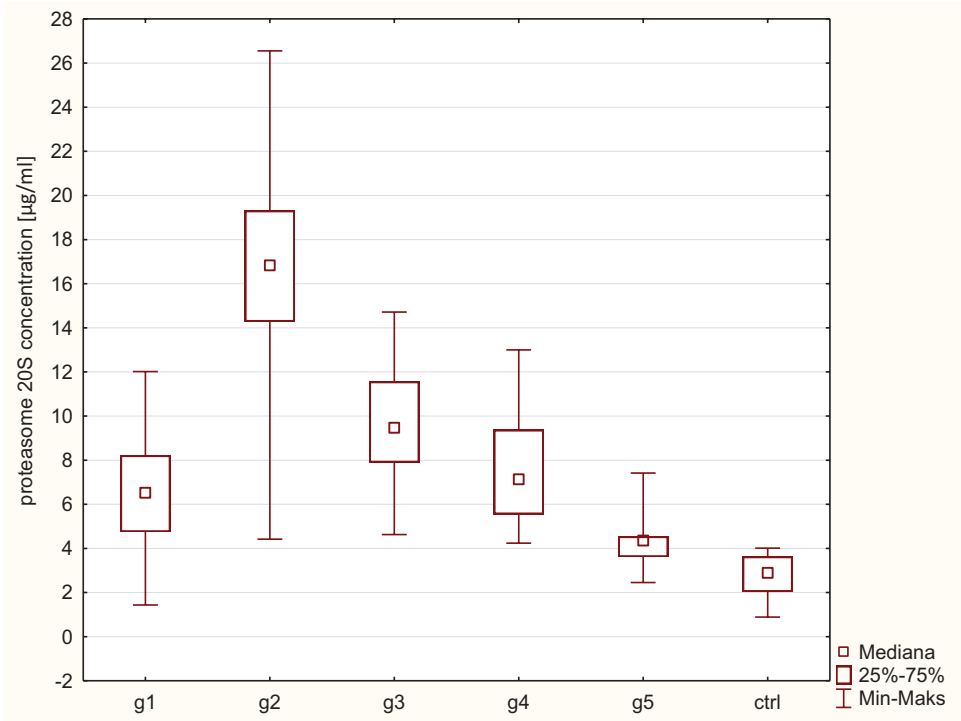

Figure 2. Concentrations of 20S proteasome in the blood plasma of children with burns, g1: 4 to 6 hours after, g2: 12 hours after, g3: 3 days, g4: 5 days, and g5: 7 days after thermal injury, ctrl = control group.

and burn activate the proteasome system in skeletal muscle, which enhance the degradation of protein, and lead to negative nitrogen balance after thermal injury [26].

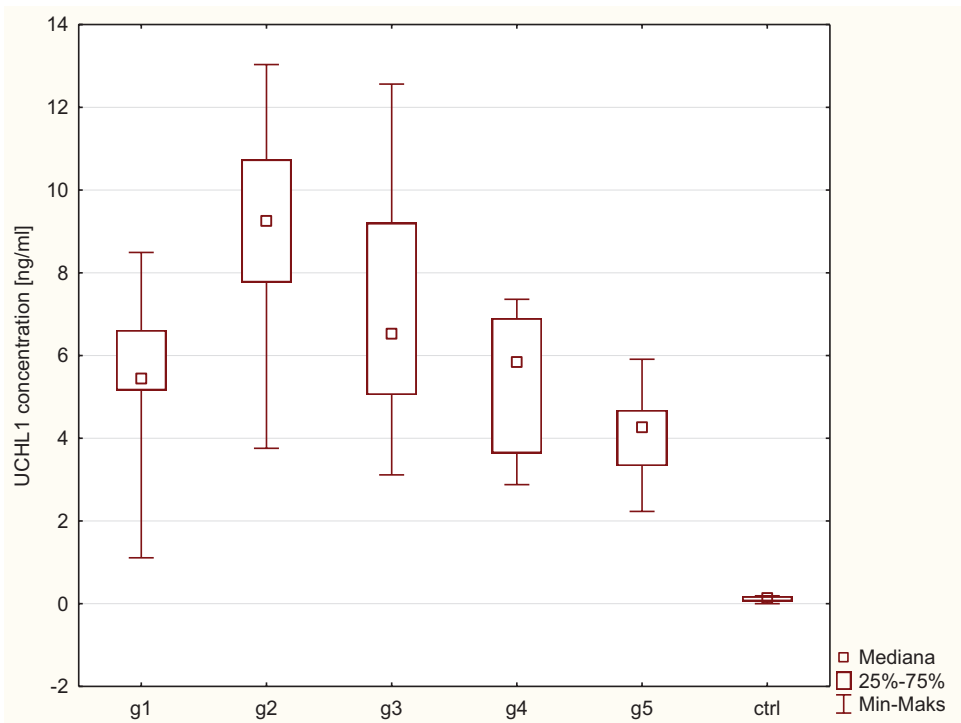

Figure 3. Concentrations of UCHL1 in the blood plasma of children with burns, g1: 4–6 hours after, g2: 12 hours after, g3: 3 days, g4: 5 days, and g5: 7 days after thermal injury, ctrl = control group.

Proteasome is not only a dominating component in protein degradation pathway, but also plays a key role in many regulatory processes—"signal transduction, cell differentiation, and stress response" as indicated by Ostrowska et al and Basler et al [1,4]. Wada et al were the first to detect circulating 20S proteasomes in human serum [23]. Also Lavabre-Bertrand et al found high levels of c-proteasomes in patients with "carcinomas of breast, stomach, kidney, colon, lung, testes, and liver" [27,28]. High levels of circulating proteasome were also found in patients suffering from liver diseases, in septic and trauma patients, eg, after burns [28–31].

The immunoproteasome is an alternative form of the constitutive proteasome, which regulates the production of inflammation mediators (such as IL-1 and tumor necrosis factor-α), and finally can cause the dysfunction of blood-brain barrier [32,33]. Compared with the constitutive proteasome, immunoproteasomes produce more peptides with a hydrophobic C-terminus, which are well suitable for the presentation on MHC class I molecules [34].

Immunoproteasome deficiency or inhibition affects T-cell survival, expansion, and differentiation, cytokine production, and progression of autoimmune conditions [34]. Moreover, mutations in LMP7 and LMP2 in humans cause complex autoimmune and inflammatory phenotypes [34,35].

Study by Callahan et al showed that the up-regulation of LMP2 and LMP7 (two major catalytic subunits of immunoproteasome) is a consequence of heat shock in murine and human cell lines [36]. According to Wang et al, "Generation of immunoproteasomes by heat may reflect redundant pathways of generation of immunoproteasome-dependent epitopes under this condition" [37]. The inhibition of LMP7 (subunit of the immunoproteasome) has been proven effective for the treatment of autoimmune conditions in different mouse models and attenuates LCMV-induced meningitis, so immunoproteasome can possibly be a promising drug target [4].

UCHL1 is also a part of ubiquitin-proteasome system, which functions as a ubiquitin ligase and a deubiquitinating enzyme [37]. So UCH-L1 can obstruct the degradation process of the target protein. It stabilizes cell proteins, eg, β catenin, and activates kinases, eg, CDK4 [37]. UCHL1 is expressed mainly in central nervous system [36–38]. According to some authors, UCHL1 level can be the signal of brain damage. Current research found UCHL1 also in different kinds of cells [37].

Sixt et al and Egerer et al observed that levels of proteasome may "be elevated up to 20-fold in patients with various autoimmune diseases, eg, autoimmune myositis, systemic lupus erythematosus, primary Sjogren syndrome, rheumatoid arthritis, vasculitis, systemic scleroderma, autoimmune hepatitis" [3,39]. Those authors found the correlation between the levels of proteasome and clinical state of petients. We made the same observation, connected with trend in the levels of immunoproteasome, 20S proteasome, and UCHL1 reflecting the clinical course in our patients with thermal injuries. According to our study, the peak in the concentrations of the immunoproteasome, 20S proteasome, and UCHL1 in blood plasma of patients 12 hours after burn, and its slow decrease, suggests that 20S proteasome and UCHL1 are released from tissues damaged by burn, and immunoproteasome is a part of the metabolic response to thermal injury.

We observed an upward trend in immunoproteasome levels correlating with thermal injury severity, but probably the group of children with severe burns was too small to show statistical significance. Our observation did not fully elucidate the clinical relevance of concentration of immunoproteasomes in blood plasma of children with burns. Also the impact of immunoproteasome on various immunological aspects raises an interest in understanding its cellular function in autoimmunity and inflammation. Nonetheless, we believe that evaluation of plasma levels of immunoproteasome, 20S proteasome, and UCHL1 may be helpful in the assessment of the efficacy of treatment, in the course of thermal injuries.

In our study, we did not find correlation of immunoproteasome, 20S proteasome, and UCHL1 levels with gender or age, which is consistent with previous observations [40–42].

CONCLUSIONS

The immunoproteasome concentration is elevated after burn injury and slowly reaches the normal range during the wound healing process. There is a strong correlation between immunoproteasome and 20S proteasome concentrations in the serum of pediatric patients with thermal injuries. We did not find such correlation between immunoproteasome and UCHL1 concentrations. Immunoproteasome concentration does not correlate with age or sex (supplementary material).

SUPPLEMENTARY MATERIAL

Supplementary data is available at *Journal of Burn Care & Research* online.

REFERENCES

1. Ostrowska H, Kruszewski K, Kasacka I. Immunoproteasome subunit LMP7 is up-regulated in the ischemic kidney in an experimental model of renovascular hypertension. Int J Biochem Cell Biol 2006;38:1778–85.
2. Kloetzel PM, Ossendorp F. Proteasome and peptidase function in MHC-class-I-mediated antigen presentation. Curr Opin Immunol 2004;16:76–81.
3. Sixt SU, Dahlmann B. Extracellular, circulating proteasomes and ubiquitin - incidence and relevance. Biochim Biophys Acta 2008;1782:817–23.
4. Basler M, Mundt S, Bitzer A, Schmidt C, Groettrup M. The immunoproteasome: a novel drug target for autoimmune diseases. Clin Exp Rheumatol 2015;33(4 Suppl 92):4–9.
5. Angeles A, Fung G, Luo H. Immune and non-immune functions of the immunoproteasome. Front Biosci (Landmark Ed) 2012;17:1904–16.
6. Chen X, Zhang X, Wang Y, et al. Inhibition of immunoproteasome reduces infarction volume and attenuates inflammatory reaction in a rat model of ischemic stroke. Cell Death Dis 2015;6:1626.
7. Kremer M, Henn A, Kolb C, et al. Reduced immunoproteasome formation and accumulation of immunoproteasomal precursors in the brains of lymphocytic choriomeningitis virus-infected mice. J Immunol 2010;185:5549–60.
8. Niewerth D, Franke NE, Jansen G, et al. Higher ratio immune versus constitutive proteasome level as novel indicator of sensitivity of pediatric acute leukemia cells to proteasome inhibitors. Haematologica 2013;98:1896–904.
9. Roccaro AM, Sacco A, Aujay M, et al. Selective inhibition of chymotrypsin-like activity of the immunoproteasome and constitutive proteasome in Waldenstrom macroglobulinemia. Blood 2010;115:4051–60.
10. Orre M, Kamphuis W, Dooves S, et al. Reactive glia show increased immunoproteasome activity in Alzheimer's disease. Brain 2013;136:1415–31.
11. Vasuri F, Capizzi E, Bellavista E, et al. Studies on immunoproteasome in human liver. Part I: absence in fetuses, presence in normal subjects, and increased levels in chronic active hepatitis and cirrhosis. Biochem Biophys Res Commun 2010;397:301–6.
12. Oh KI, Seo JN. Expression pattern of immunoproteasome subunits in human thymus. Immune Netw 2009;9:285–8.
13. Eleftheriadis T, Pissas G, Antoniadi G, Liakopoulos V, Stefanidis I. Proteasome or immunoproteasome inhibitors cause apoptosis in human renal tubular epithelial cells under normoxic and hypoxic conditions. Int Urol Nephrol 2016;48:907–15.
14. Steiner G. Surface plasmon resonance imaging. Anal Bioanal Chem 2004;379:328–31.
15. Helmerhorst E, Chandler DJ, Nussio M, Mamotte CD. Real-time and Label-free bio-sensing of molecular interactions by surface plasmon resonance: a laboratory medicine perspective. Clin Biochem Rev 2012;33:161–73.
16. Sankiewicz A, Laudanski P, Romanowicz L, et al. Development of surface plasmon resonance imaging biosensors for detection of ubiquitin carboxyl-terminal hydrolase L1. Anal Biochem 2015;469:4–11.
17. Gorodkiewicz E, Ostrowska H, Sankiewicz A. SPR imaging biosensor for the 20S proteasome: sensor development and application to measurement of proteasomes in human blood plasma. Mikrochim Acta 2011;175:177–84.
18. Sankiewicz A, Markowska A, Lukaszewski Z, Puzan B, Gorodkiewicz E. Methods for 20S immunoproteasome and 20S constitutive proteasome determination based on SPRI biosensors. Cel Mol Bioeng 2017;10:174.
19. Laudanski P, Gorodkiewicz E, Ramotowska B, Charkiewicz R, Kuzmicki M, Szamatowicz J. Determination of cathepsins B, D and G concentration in eutopic proliferative endometrium of women with endometriosis by the surface plasmon resonance imaging (SPRI) technique. Eur J Obstet Gynecol Reprod Biol 2013;169:80–3.
20. Gorodkiewicz E, Guszcz T, Roszkowska-Jakimiec W, Kozłowski R. Cathepsin D serum and urine concentration in superficial and invasive transitional bladder cancer as determined by surface plasmon resonance imaging. Oncol Lett 2014;8:1323–7.
21. Sankiewicz A, Guszcz T, Mena-Hortelano R, Zukowski K, Gorodkiewicz E. Podoplanin serum and urine concentration in transitional bladder cancer. Cancer Biomark 2016;16:343–50.
22. Matuszczak E, Tylicka M, Dębek W, Tokarzewicz A, Gorodkiewicz E, Hermanowicz A. Concentration of UHCL1 in the serum of children with acute appendicitis, before and after surgery, and its correlation with CRP and prealbumin. J Invest Surg 2017;31(2):1–6
23. Matuszczak E, Tylicka M, Dębek W, Sankiewicz A, Gorodkiewicz E, Hermanowicz A. Overexpression of ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) in serum of children after thermal injury. Adv Med Sci 2017;62:83–6.
24. Matuszczak E, Tylicka M, Hermanowicz A, Debek W, Sankiewicz A, Gorodkiewicz E. Application of SPR imaging biosensor for the measurement of 20S proteasomes in blood plasma of children with thermal injury. Ann Clin Lab Sci 2016;46:407–11.
25. Tokarzewicz A, Romanowicz L, Sveklo I, Matuszczak E, Hermanowicz A, Gorodkiewicz E. SPRI biosensors for quantitative determination of matrix metalloproteinase-2. Anal Methods 2017;9:2407–14.
26. Tan Y, Peng X, Wang F, You Z, Dong Y, Wang S. Effects of tumor necrosis factor-alpha on the 26S proteasome and 19S regulator in skeletal muscle of severely scalded mice. J Burn Care Res 2006;27:226–33.
27. Wada M, Kosaka M, Saito S, Sano T, Tanaka K, Ichihara A. Serum concentration and localization in tumor cells of proteasomes in patients with hematologic malignancy and their pathophysiologic significance. J Lab Clin Med 1993;121:215–23.
28. Lavabre-Bertrand T, Henry L, Carillo S, et al. Plasma proteasome level is a potential marker in patients with solid tumors and hemopoietic malignancies. Cancer 2001;92:2493–500.
29. Wong BR, Parlati F, Qu K, et al. Drug discovery in the ubiquitin regulatory pathway. Drug Discov Today 2003;8:746–54.
30. Golab J, Bauer TM, Daniel V, Naujokat C. Role of the ubiquitin-proteasome pathway in the diagnosis of human diseases. Clin Chim Acta 2004;340:27–40.
31. Harris JR. Release of a macromolecular protein component from human erythrocyte ghosts. Biochim Biophys Acta 1968;150:534–7.
32. Muchamuel T, Basler M, Aujay MA, et al. A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. Nat Med 2009;15:781–7.
33. Basler M, Dajee M, Moll C, Groettrup M, Kirk CJ. Prevention of experimental colitis by a selective inhibitor of the immunoproteasome. J Immunol 2010;185:634–41.
34. Bitzer A, Basler M, Krappmann D, Groettrup M. Immunoproteasome subunit deficiency has no influence on the canonical pathway of NF-κB activation. Mol Immunol 2017;83:147–53.
35. Brehm A, Krüger E. Dysfunction in protein clearance by the proteasome: impact on autoinflammatory diseases. Semin Immunopathol 2015;37:323–33.
36. Callahan MK, Wohlfert EA, Ménoret A, Srivastava PK. Heat shock up-regulates lmp2 and lmp7 and enhances presentation of immunoproteasome-dependent epitopes. J Immunol 2006;177:8393–9.
37. Wang R, Zhang M, Zhou W, Ly PT, Cai F, Song W. NF-κB signaling inhibits ubiquitin carboxyl-terminal hydrolase L1 gene expression. J Neurochem 2011;116:1160–70.
38. Diaz-Arrastia R, Wang KK, Papa L, et al.; TRACK-TBI Investigators. Acute biomarkers of traumatic brain

injury: relationship between plasma levels of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein. J Neurotrauma 2014;31:19–25.
39. Egerer K, Kuckelkorn U, Rudolph PE, et al. Circulating proteasomes are markers of cell damage and immunologic activity in autoimmune diseases. J Rheumatol 2002;29:2045–52.
40. Matuszczak E, Tylicka M, Dębek W, Hermanowicz A, Ostrowska H. The comparison of C-proteasome activity in the plasma of children after burn injury, mild head injury and blunt abdominal trauma. Adv Med Sci 2015;60:253–8.
41. Tylicka M, Matuszczak E, Dębek W, Hermanowicz A, Ostrowska H. Circulating proteasome activity following mild head injury in children. Childs Nerv Syst 2014;30:1191–6.
42. Matuszczak E, Tylicka M, Dębek W, Hermanowicz A, Ostrowska H. Correlation between circulating proteasome activity, total protein and c-reactive protein levels following burn in children. Burns 2014;40:842–7.