Procalcitonin adds to diagnosis, but does not reduce initial antibiotics in febrile neutropenic children

David Lodahl & Henrik Schrøder

ABSTRACT

INTRODUCTION: The immune system is suppressed during chemotherapy. This makes diagnosis of severe life-threatening infections more difficult and it also intensifies the clinical course of such infections. Hence, empirical use of broad-spectrum antibiotics is mandatory. We investigated if procalcitonin (PCT) measurement may improve diagnostic accuracy.

MATERIAL AND METHODS: In a prospective observational study, we included all admissions due to febrile episodes in a cohort of children below 16 years of age. PCT and C-reactive protein (CRP) were analyzed using LUMI test and VITROS CRP slides, respectively.

RESULTS: We recorded 230 febrile episodes in 85 children. Severe systemic infection was found in 61 (27%) of these episodes. PCT performed better than CRP (p value ≤ 0.01). The discriminative power of PCT was significant already from admission. For CRP, discriminative power was significant after 48 hours. The cut-offs for PCT and CRP were 0.4 ng/ml and 336 nmol/ml to achieve sensitivities of 93%. The specificities for PCT and CRP were 45% and 22%, respectively. Severely infected patients were not found, either by PCT or by CRP in four (7%) cases. PCT levels rose in response to infection in the neutropenic population.

CONCLUSION: PCT measurement considerably improves biochemical information; however, the sensitivity is too low to safely alter the recommended administration of empirical antibiotics at admission.

ABBREVIATIONS

ANC = absolute neutrophile count
CNS = coagulase-negative staphylococci
CRP = C-reactive protein
FE = febrile episode
FUO = fever of unknown origin
No-SI = no systemic infection
PCT = procalcitonin
SI = systemic infection
WBC = white blood cell count

ORIGINAL ARTICLE

Department of Paediatrics, Aarhus University Hospital, Skejby

Dan Med Bul 2011;58(3):A4233

The immune system is suppressed during chemotherapy. Patients often develop severe neutropenia, and life-threatening infections can develop rapidly. Infections may be caused by high- or low-virulence bacteria. Diagnosis is difficult because of the absence of or changes in general signs of infection including formation of pus and leukocytosis [1]. Blood culture takes time and a negative blood culture does not exclude bacteraemia. This has led to the empirical use of broad-spectrum antibiotic treatment in paediatric patients with neotropenia, even where signs of infection are vague [2].

Throughout the past 15 years, our knowledge of the role of procalcitonin (PCT) in infectious diseases has increased [3]. The accuracy with which PCT may be measured has risen considerably [4, 5]. PCT in non-infected patients is low, < 0.05 ng/ml [6]. The origin and pathways by which PCT production is triggered remain unresolved [6, 7]. Bacterial endotoxin and severe infections are the best known stimuli for both C-reactive protein (CRP) and PCT production [8-10] (**Figure 1**). It is crucial for the diagnosis of a sepsis condition in the neutropenic population that the test used does not depend on an intact cellular immune response [3, 7, 8, 11, 12].

This study investigated if PCT can improve the diagnostic accuracy of severe bacterial infection in a population of children with cancer undergoing chemotherapy. We compared the sensitivity and specificity of PCT and CRP. CRP is the most widely used inflammatory marker [1, 11, 13, 14].

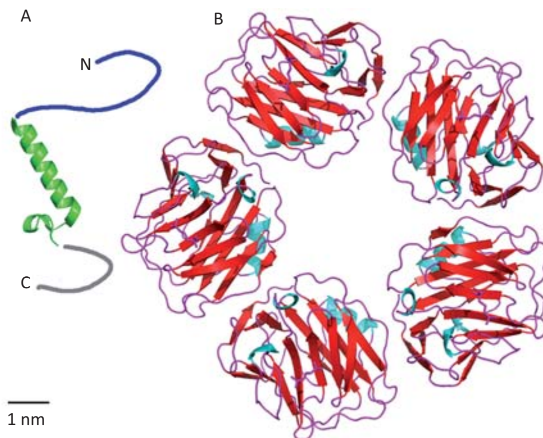
MATERIAL AND METHODS

This prospective observational study was performed from 25 September 2000 to 28 June 2001. It included all admissions due to febrile episodes (FE) in a cohort of children below the age of 16 years treated with chemotherapy for oncological or haematological diseases at the Department of Paediatrics, Division of Haematology/oncology, Aarhus University Hospital, Skejby. The inclusion criteria were an axillary temperature ≥ 38.5 °C once or ≥ 38.0 °C twice within 4-6 hours.

At admission, clinical evaluation included observation for: catarrhal inflammation, coughing, other respiratory tract symptoms, headache, otalgia, diarrhoea, stomach ache, skin symptoms, arthralgia, myalgia, general malaise and vital signs. PCT and routine blood samples were drawn from central venous catheters (CVC) on admission and daily thereafter. Routine samples were: haemoglobin, thrombocytes, electrolytes, CRP, total

FIGURE 1

Molecular structures of procalcitonin and C-reactive protein. **A**) The molecular structure of procalcitonin is as yet unknown. It is hence represented as solution confirmation of calcitonin between the N- and C-terminal parts of procalcitonin, indicated by curved lines. **B**) C-reactive protein pentamer. Figures were prepared by the PyMOL Molecular Graphics System based on PDB ID's 2JXZ (Calcitonin) and 1B09 (CRP).



white blood cell count (WBC), absolute monocyte count (AMC), absolute neutrophile count (ANC). Aerobic and anaerobic blood cultures were drawn before administering antibiotics. On clinical indication, cultures were repeated and supplemented by samples from: blood, nose, upper pharynx, skin lacerations and rectum which were tested for viral and bacterial pathogens. Chest X-rays were performed on clinical indication.

Cases of catarrhal inflammation, gastroenteritis or confirmed viral antigens were classified as viral infections. If the child's general condition was affected or if WBC < 1.0 or ANC < 0.5 × 10^9/l, first-line antibiotics were administered: gentamycin 6 mg/kg in one daily dose and ampicillin and dicloxacillin 150 and 50 mg/kg/day, respectively, in three daily doses. Blood samples and clinical evaluations were repeated at least daily. The antibiotic strategy was continuously adapted to the test results and the clinical situation.

The PCT test result was blinded to all health professionals. CRP was part of standard care. Final classification of the cause of fever was made by the attending physician when it was possible to evaluate all test results including bacterial cultures and the total clinical course in a fever episode. The evaluation by the attending physician was the gold standard by which we classified the FEs as systemic infections (SI) or non-systemic infections (No-SI). SI cases were defined as culture-positive bacteraemia. No-SI cases were defined as all other causes of fever.

SI and No-SI fever episodes were further subdivided into a total of nine subgroups. A small control group of the cohort underwent PCT analysis at a time when they were not admitted with fever (**Table 1**).

CRP analysis was performed by the hospital's standard laboratory, using VITROS CRP slides.

TABLE 1

Median, minimum and maximum values of procalcitonin and C-reactive protein in non-systemically infected, systemically infected and nine subgroups (c through k) of the 230 febrile episodes.

			Procalcitonin			C-reactive protein		
Main group	Subgroup	n	median, ng/ml	min.-max. , ng/ml	p value[a]	median, nmol/l	min.-max., nmol/l	p value[a]
Control[b]		13	0.4	0.3-0.5		48	26-233	
No-SI	FUO[c]	87	0.4	0.1-11.8	–	732	64-4297	–
	Viral[d]	34	0.4	0.1-7.7	–	431	47-2954	–
	Local bacterial[e]	18	0.4	0.1-9.2	–	760	315-3928	–
	Non-microbial[f]	13	1.2	0.2-35.1	–	781	145-3024	–
	Lower resp. tract[g]	17	1.5	0.2-23.2	–	957	380-5007	–
	Total No-SI[c-g]	169	0.5	0.1-35.1	–	717	47-5007	–
SI	Grampos. CNS only[h]	16	0.9	0.4-31.0	0.005	519	199-3324	0.346
	Grampos. excl. CNS[i]	18	1.0	0.3-6.9	0.008	1,119	144-3126	0.146
	Total grampos[h+i]	34	1.0	0.3-31.0	0.000	662	144-3324	0.692
	Gramneg.[j]	26	12.1	0.2-202.7	0.000	1,722	373-4992	0.001
	Fungal[k]	1	1.3	–	0.529	1,676	–	0.541
	Total SI[h-k]	61	1.3	0.2-202.7	0.000	892	144-4992	0.021

CNS = coagulase-negative staphylococci; FUO = fever of unknown origin; No-SI = no systemic infection; SI = systemic infection.
a) p values express the probability that the total SI[h-k] or an SI subgroup[(h, i, h+i, j or k)] has the same distribution as the total No-SI[c-g] group;
b) Non-infected children with no febrile episodes; c) Exclusion diagnosis, i.e. cause of fever could not be identified; d) Cases of catarrhal inflammation and gastroenteritis. Viral antigen was isolated in only four cases; e) Positive culture or inflammation outside the bloodstream or lungs; f) Eight cases of cytorabin fever, three cases of toxic mucositis and two cases of neoplastic disease; g) Cases with clinical symptoms of pneumonia, two cases had pneumocystis pneumonia; h) Cases of grampositive coagulase-negative staphylococci in the bloodstream; i) Cases of grampositive bloodstream infection, exclusive coagulase-negative staphylococci; h+i) Total grampositive bloodstream infection; j) Gramnegative bloodstream infections (rich on endotoxin); k) Fungal bloodstream infection.

Blood for the PCT analysis was drawn in Vacuette Serum Separator Clot Activator sample tubes. Samples were frozen at –20 °C until quantitative PCT determination was performed. Samples drawn outside normal working hours were stored at 4-5 °C until the following day.

PCT was analyzed using the immunoluminometric assay (ILMA) LUMI test from BRAHMS Diagnostica GmbH. The test was performed manually [5]. The detection limit was approx. 0.1 ng/ml and the functional assay sensitivity (FAS) approx. 0.3 ng/ml. All measurements were performed twice.

Statistical analyses

Each new admission was statistically analysed as a separate case whether or not the patient had previously been included in the study. For comparison of two groups, the Mann-Whitney U test was used. For comparison of more than two groups, the Kruskal Wallies test was used. To compare sensitivity and specificity, receiver-operating characteristic (ROC) curves and area under ROC curve (AUC) were calculated and compared.

To compare the sensitivity and specificity found in our study with relevant literature [15], three PCT cut-off levels were set at 1.0, 0.5 and 0.4 ng/ml. To compare the specificity of CRP and PCT, three cut-off values for CRP were chosen in order to give identical sensitivities for CRP and PCT. To evaluate the significance of any differences in specificity, McNemar's test was performed.

Statistical analyses were performed using SPSS, SigmaPlot and STATA. p-values < 0.05 were considered statistically significant.

No outliers were excluded from testing or reporting.

Ethics

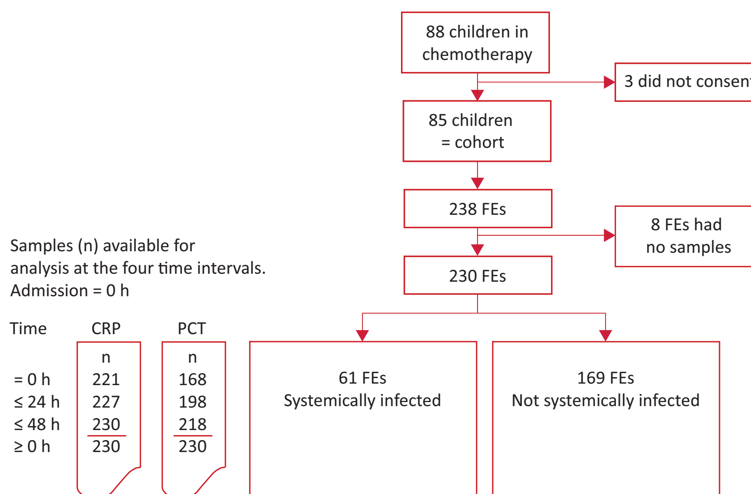
The study was conducted in accordance with the Helsinki Declaration. Prior to patient enrolment, the project was approved by the Local Ethics Committee and the Danish Data Agency Board. Written informed consent was obtained from parents of patients.

RESULTS

A total of 88 children with cancer were treated with chemotherapy at our centre during the study period. A total of 85 consented to participate and constituted the studied cohort. Age ranged from four months to 15 years (median 5.7 years): 45 had haematological cancer, 40 had solid tumours. Altogether, the children were admitted 238 times due to an FE, and the mean number of admissions per child was 2.8 (range 0-10). In eight of these FEs, no PCT or CRP samples were taken. In 230 FEs, we consecutively recorded all data as presented below. PCT and CRP were measured 787 and 998 times, respectively. In a few FEs, CRP was measured more frequently than PCT because of rapid clinical deterioration. Among the 230 FEs, 198 and 218 had PCT sampled within the first 24 and 48 hours, respectively. Similarly, 227 and 230 had CRP sampled within the first 24 and 48 hours, respectively (**Figure 2**). A total of 110 (48%) of the FEs were neutropenic with an ANC < 0.5 × 10^9/l at admission.

FIGURE 2

Flowchart of 230 febrile episodes in 85 children. Classified into two groups by the attending physicians. CRP = C-reactive protein; FE = febrile episode; PCT = procalcitonin.



We had 13 controls. A total of 61 FEs were SI, 169 FEs were No-SI. Eight positive blood cultures were considered the result of contamination.

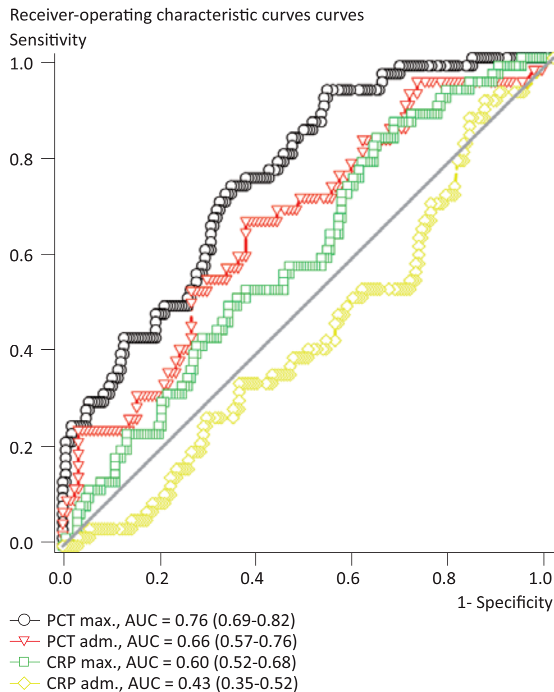
Both PCT and CRP could discriminate between SI and No-SI. Within the SI group, PCT and CRP levels varied. Both PCT and CRP levels were significantly higher in gramnegative than in grampositive bacteraemia with p values of 0.002 and 0.037, respectively. CRP reacted significantly less to coagulase-negative staphylococci (CNS) infections than to other grampositive infections (p value = 0.03). PCT reacted similarly to CNS infections and other grampositive infections (p value = 0.73).

PCT could significantly differentiate all three (CNS, other grampositive infections and gramnegative infections) from No-SI. CRP only differentiated gramnegative infections from No-SI (Table 1). Within the No-SI FEs, PCT and CRP levels varied significantly (Table 1).

The discriminative power rose over time, even after 48 hours, as the AUC increased over time (**Figure 3**). PCT allowed us to discriminate between SI and No-SI already at admission, and the discriminative power rose over time. CRP only allowed us to discriminate between SI and No-SI if samples were taken more than 48 hours after admission. Within all time intervals, PCT had a sig-

FIGURE 3

Receiver-operating characteristic curves for procalcitonin (PCT) and C-reactive protein (CRP). Adm. = level at admission, (95% confidence intervals); AUC = area under curve; Max. = level at maximum, (95% confidence intervals).



nificantly better discriminative power (higher AUC) than CRP (p values ≤ 0.01).

PCT had a significantly higher specificity than CRP at equal levels of sensitivity (p values ≤ 0.01). PCT was more sensitive at admission than CRP. The sensitivity was 93% at the lowest shown cut-offs of PCT (0.4 ng/ml) and CRP (336 nmol/l). The specificity was 45% and 22%, respectively (**Table 2**).

In the neutropenic subgroup of our patients, we found PCT to be a reliable marker of SI patients. In fact, the highest and 14th highest PCT ever measured (203 and 33.2 ng/ml, respectively) were found in two gramnegative infected patients with an ANC of 0.0. The overall correlation between PCT and ANC levels was –0.2 (p value < 0.05 Spearman's rho), which implies that PCT was highest at times when ANC was lowest. We recomputed and compared all ROC curves and specificities of the 48% of the patients who were neutropenic on admission and found similar results (data not shown). However, in the 52% who were not neutropenic, CRP reached discriminative power sooner, i.e. not at admission, but within 24 hours (data not shown).

DISCUSSION

In line with other studies, we confirm that PCT and CRP can be used as markers of infection in neutropenic populations. The fraction of neutropenic children was lower in our study than in similar studies [8, 11, 12]. Carnino et al found PCT (and CRP) to react more, Hatzistilianou et al less, in deeply neutropenic patients [3, 7]. Neither of the two studies, however, considered the distribution of grampositive and gramnegative bacteria.

We believe the inherent risk of infection with some low-virulence grampositive agents is greater in immunosuppressed individuals than in others. If the PCT response and the endotoxin level are related as described by Dandona et al [9], differences in the distribution of low-virulence grampositive bacteria and high-virulence endotoxin-producing gramnegative bacteria may result in different overall PCT responses in different populations. In our study, PCT and CRP values differed significantly between grampositive and gramnegative bacteraemias. This has also been shown by others [1, 8, 12, 13] and it has been contradicted by von Lilienfeld et al who stated that this finding could have been caused by contamination [11]. We have shown that PCT differentiated better and more quickly than CRP between febrile episodes with bacteraemia and all other febrile episodes. This finding corresponds to similar reports [1, 3, 6-8, 13, 14]. However, the reaction time was too long and the sensitivity too low for both CRP and PCT to safely withhold broad-spectrum antibiotics upon admission. This is in agreement with previous studies [4, 10].

In previous studies on the same PCT test as ours, PCT cut-offs varied from 0.4 to 1.15 ng/ml [1, 8, 10, 15]. At a PCT cut-off of 0.4 ng/ml, combined with a CRP cut-off of 336 nmol/l, we missed only one low-virulence SI case.

Our reported test characteristics of PCT might have been biased by three design weaknesses:

1) The cut-off might have been set to high. In other studies using newer, fully automated and more sensitive tests for PCT detection, it was suggested that cut-offs should be as low as 0.1 ng/ml to safely categorize patients as No-SI [2, 5]. However, this cut-off is under the detection limit of the test we used.

2) Peak PCT levels have been measured falsely low. PCT has a shorter half-life than CRP. PCT levels may therefore often have peaked and then dropped to a somewhat lower level between two blood samples.

3) Our gold standard might have had a low sensitivity and specificity. If, for example, the attending physician falsely categorized a No-SI FEs as an SI, and PCT correctly categorized the FE as No-SI, we would falsely have shown PCT with a low sensitivity when, in fact, it was more specific. Our reported ability of CRP and PCT to discriminate between SI and No-SI febrile episodes is an ability to find the same results as those found by the attending physician. This is not necessarily tantamount

TABLE 2

Variation in C-reactive protein (CRP) and procalcitonin (PCT) sensitivity and specificity at different cut-off values. Cut-offs for PCT were chosen to match relevant literature. Cut-offs for CRP were chosen to provide identical sensitivities at peak levels.

		Admission, %		Peak level, %	
	Cut- off	sensitivity	specificity	sensitivity	specificity
PCT	0.4 ng/ml	54	67	93	45
CRP	336 nmol/l	39	58		22
PCT	0.5 ng/ml	44	74	79	55
CRP	537 nmol/l	21	76		39
PCT	1.0 ng/ml	26	85	61	70
CRP	679 nmol/l	8	83		46

to an ability to discriminate between true SI and No-SI febrile episodes [2, 4, 10, 16]. One way of bypassing the problem of an incorrect clinical evaluation is to compare outcomes of antibiotic treatment provided in accordance with the PCT response [16]. Further research is needed to establish if early termination or a narrowing of the broad- spectrum antibiotic treatment is safe when PCT levels are consistently low. This, however, could have devastating consequences, especially if PCT is unable to detect certain infections. Our data are insufficient to safely elaborate on the levels of PCT over time. Others have shown that increasing or sustained, high levels of PCT are predictors of an adverse outcome [3, 10, 17, 18].

In accordance with others, we have described how PCT and CRP levels rose in response to different causes of FEs in a cohort. Individual patients influenced the final results differently [8, 11]. The more FEs, the higher the influence. We do not know what role PCT plays in the immune system. We do not know if PCT is a mediator or a marker [19]. We also do not know if individual PCT responses and individual susceptibilities to infections are related [20]. To safely apply our findings to other patients, it should be clarified whether all patients have the same PCT response to the same infectious conditions.

CONCLUSION

PCT reacts more to gramnegative endotoxin-producing bacteria than to grampositive bacteria. PCT reacts more quickly and is more sensitive and specific to systemic bacterial infections than CRP. PCT reacts well in neutropenic children. However, neither CRP nor PCT reacts quickly enough, nor are they sufficiently sensitive to safely exclude invasive bacteraemia upon admission. Thus, initiation of treatment with broad-spectrum antibiotics in this population still relies on a clinical decision.

CORRESPONDENCE: *David Lodahl,* Department of Anaesthesia, Aarhus University Hospital, Skejby, 8200 Aarhus N, Denmark.
E-mail: david.lodahl@skejby.rm.dk

ACCEPTED: 16 November 201

CONFLICTS OF INTEREST: LUMI-test for PCT detection was freely supplied by BRAHMS Diagnostica GmbH. The Danish Medical Research Counsel provided a scholarship.

ACKNOWLEDGEMENTS: We wish to extend our gratitude to *J.P. Morth* for assistance with Figure 1.

LITERATURE

1. Kitanovski L, Jazbec J, Hojker S et al. Diagnostic accuracy of procalcitonin and interleukin-6 values for predicting bacteremia and clinical sepsis in febrile neutropenic children with cancer. Eur J Clin Microbiol Infect Dis 2006;25:413-5.
2. Schuetz P, Christ-Crain M, Muller B. Procalcitonin and other biomarkers to improve assessment and antibiotic stewardship in infections – hope for hype? Swiss Med Wkly 2009;139:318-26.
3. Hatzistilianou M, Rekliti A, Athanassiadou F et al. Procalcitonin as an early marker of bacterial infection in neutropenic febrile children with acute lymphoblastic leukemia. Inflamm Res 2010;59:339-47.
4. Chawes BL, Rechnitzer C, Schmiegelow K et al. Procalcitonin til tidlig diagnostik af bakteriæmi hos børn med cancer. Ugeskr Læger 2007;169: 138-42.
5. Steinbach G, Rau B, Debard AL et al. Multicenter evaluation of a new immunoassay for procalcitonin measurement on the Kryptor System. Clin Chem Lab Med 2004;42:440-9.
6. Semeraro M, Thomee C, Rolland E et al. A predictor of unfavourable outcome in neutropenic paediatric patients presenting with fever of unknown origin. Pediatr Blood Cancer 2010;54:284-90.
7. Carnino L, Betteto S, Loiacono M et al. Procalcitonin as a predictive marker of infections in chemoinduced neutropenia. J Cancer Res Clin Oncol 2010;136:611-5.
8. Fleischhack G, Cipic D, Juettner J et al. Procalcitonin – a sensitive inflammation marker of febrile episodes in neutropenic children with cancer. Intensive Care Med 2000;26(Suppl 2):S202-S211.
9. Dandona P, Nix D, Wilson MF et al. Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab 1994;79:1605-8.
10. Jensen JU, Heslet L, Jensen TH et al. Procalcitonin increase in early identification of critically ill patients at high risk of mortality. Crit Care Med 2006;34:2596-602.
11. von Lilienfeld-Toal M, Dietrich MP, Glasmacher A et al. Markers of bacteremia in febrile neutropenic patients with hematological malignancies: procalcitonin and IL-6 are more reliable than C-reactive protein. Eur J Clin Microbiol Infect Dis 2004;23:539-44.
12. Prat C, Sancho JM, Dominguez J et al. Evaluation of procalcitonin, neopterin, C-reactive protein, IL-6 and IL-8 as a diagnostic marker of infection in patients with febrile neutropenia. Leuk Lymphoma 2008;49: 1752-61.
13. Fleischhack G, Kambeck I, Cipic D et al. Procalcitonin in paediatric cancer patients: its diagnostic relevance is superior to that of C-reactive protein, interleukin 6, interleukin 8, soluble interleukin 2 receptor and soluble tumour necrosis factor receptor II. Br J Haematol 2000;111:1093-102.
14. Hatzistilianou M, Rekleity A, Athanassiadou F et al. Serial procalcitonin responses in infection of children with secondary immunodeficiency. Clin Invest Med 2007;30:E75-E85.
15. Sakr Y, Sponholz C, Tuche F et al. The role of procalcitonin in febrile neutropenic patients: review of the literature. Infection 2008;36:396-407.
16. Christ-Crain M, Muller B. Procalcitonin in bacterial infections – hype, hope, more or less? Swiss Med Wkly 2005;135:451-60.
17. Secmeer G, Devrim I, Kara A et al. Role of procalcitonin and CRP in differentiating a stable from a deteriorating clinical course in pediatric febrile neutropenia. J Pediatr Hematol Oncol 2007;29:107-11.
18. Charles PE, Tinel C, Barbar S et al. Procalcitonin kinetics within the first days of sepsis: relationship with the appropriateness of antibiotic therapy and the outcome. Crit Care 2009;13:R38.
19. Nylen ES, Whang KT, Snider RH et al. Mortality is increased by procalcitonin and decreased by an antiserum reactive to procalcitonin in experimental sepsis. Crit Care Med 1998;26:1001-6.
20. Rittirsch D, Flierl MA, Ward PA. Harmful molecular mechanisms in sepsis. Nat Rev Immunol 2008;8:776-87.