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RESEARCH ARTICLE

Kinetics of c-reactive protein (CRP) and serum amyloid A protein (SAA) in patients with community-acquired pneumonia (CAP), as presented with biologic half-life times

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Abstract

Context: In management of community-acquired pneumonia (CAP), excellent biomarkers for inflammation would be helpful in our practice.

Objectives: Kinetics of c-reactive protein (CRP) and serum amyloid A (SAA) was characterized, using their biologic half-life times.

Materials and methods: Time course of CRP and SAA levels in the successfully treated 36 CAP patients were investigated and their half-life times were determined and compared.

Results & Discussions: SAA and CRP declined in an exponential mean and the biologic half-life times of SAA levels was 34.9 ± 28.7 h, significantly shorter than that of CRP, 46.4 ± 21.7 h ($p = 0.0014$). *Conclusion*: The kinetic evidence, presented as biologic half-life times of CRP and SAA, helps us make a clinical assessment of CAP patients.

Keywords: Community-acquired *pneumonia (CAP);* serum amyloid A (SAA), C-reactive protein (CRP), biologic half-life time, kinetics

Introduction

When antimicrobial treatment against community-acquired pneumonia (CAP) is started, the pathogenic microorganisms responsible are not always identified, and thus the choice of antimicrobials is made in an empiric means. Thus, practice guidelines, including one by the Japanese Respiratory Society (JRS) (The committee for the Japanese Respiratory Society, 2006), as well as another by the Infectious Disease Society of America (IDSA) and the American Thoracic Society (ATS) (Mandell, 2007), stated that this antimicrobial choice should be evaluated within 2 or 3 days, based on how effective the treatment is and on which microorganism has been isolated by then. Excellent biomarkers would make this evaluation easier and more confirming, even for the health care professionals who do not major in pulmonary infections. Thus, in order to investigate whether or not each biomarker is available in the management of CAP, its kinetics in CAP patients should be characterized.

Since pneumonia is an inflammatory disorder, the biomarkers of inflammation are promising by providing a quantitative assessment of pneumonia and the antimicrobial treatment.

Serum amyloid A protein (SAA) has been reported to be a sensitive biomarker of inflammation, with which even the smallest inflammatory reaction among individuals with atherosclerosis can be detected (Johnson, 2004). SAA is a good indicator for acute exacerbations of chronic obstructive pulmonary disease (COPD)

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Table 1. Comparison between A-DROP and CURB-65.

Items	A-DROP	CURB-65
Consciousness	Confusion	Confusion
Blood BUN levels	>21 mg/dL and/or dehydration	≥7 mmol/L
Respiratory condition	SpO2 ≤ 90	RR ≥30/min
BP	<90 mmHg	Systolic BP ≤90 and/or diastolic BP ≤60
Age	≥70 (male), ≥75 (female)	≥65

BP, blood pressure; BUN, blood urea nitrogen; RR, respiratory rate.

(Bozinovski, 2007). SAA is also reported to be increased among patients with infectious diseases and is associated with C-reactive protein (CRP) values both in bacterial and viral infections (Lannergard, 2003).

Although many published studies, using rodents artificially administrated SAA protein, found that blood SAA levels declined in an exponential way and thus had a biologic half-life time (Hoffman & Benditt 1983; Kindy 1998; Kluve-Beckerman 1997; Tape C & Kisilevsky 1990; Wada, 1998). However, the kinetics of SAA are still controversial: the biologic half-life time of SAA was estimated to be shorter than 1 h in rodents (Hoffman & Benditt 1983; Kindy 1998; Kluve-Beckerman 1997; Tape C & Kisilevsky 1990; Wada, 1998), while it appeared between 12 and 24 h in human volunteers who were administered with endotoxin (Hudgins, 2003). Thus, in order to establish the evidence of the kinetics of SAA and CRP on the clinically practical bases, this study determined the biologic half-life times of SAA, in comparison with CRP, by exclusively investigating CAP patients who were successfully treated with an appropriate antimicrobial.

Methods

Diagnosis and enrollment

A total of 223 patients with CAP were hospitalized in our department from the 1 June 2005 to the 31 March 2007. They were diagnosed as having CAP and treated according to the JRS guidelines (The committee for the Japanese Respiratory Society, 2006). The severity of the patients' illness was evaluated using the A-DROP scoring system by the JRS guideline, or modified Japanese version of CURB-65 (Neill, 1996), assessing five items, including consciousness, blood urea nitrogen (BUN) levels, respiratory condition, blood pressure (BP), and age (The committee for the Japanese Respiratory Society, 2006), as in the CURB-65 score system (Table 1).

The blood inflammatory markers leukocyte count (WBC), SAA, and CRP were measured at "the first occasion" on admission, "the second occasion" between day 3 and day 5, when our initial treatment was re-evaluated, and "the third occasion" between day 7 and day 14 before discharge. Of these patients, 36 had data on WBC, SAA, and CRP levels available on all three occasions, and their initial antimicrobial treatment was successful. They were retrospectively enrolled in order to describe the kinetics of SAA.

Since this was a retrospective observational study, the institutional ethics committee approved the study and waived informed consent.

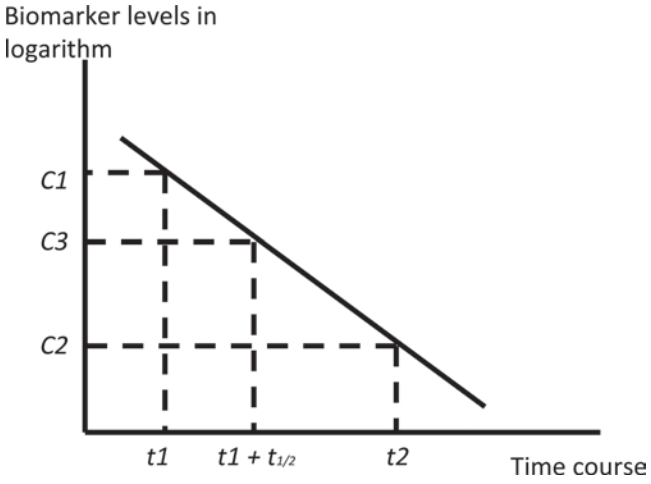

Figure 1. Biologic half life times, $t_{1/2}$, in the enrolled individual were determined, using the following formula: $t_{1/2}$=(t2-t1)/log2(C1/C2), where C1 and C2 are blood biomarker levels which are measured at time points t1 and t2, respectively. A time point (t1 + $t_{1/2}$) was estimated when blood biomarker levels become just a half of C1 (C3), on the basis of that the three time points and concentration, i.e., (t1, C1), (t2, C2) and (t1 + $t_{1/2}$, C3) were on the process of exponential elimination. These estimated half life times ($t_{1/2}$) were used for the regression analysis.

Measurement

Blood was taken from the enrolled patients, and blood levels of CRP and WBC were determined in the usual clinical laboratory in our hospital. SAA was measured using Latex immuno-coagulation (Eiken Kagaku, Tokyo, Japan). The half-life times of CRP and SAA in each patient were determined either in the initial phase between the first occasion and the second, or in the later phase between the second occasion and the third.

Estimation of biologic half-life time

Based on previous reports suggesting that CRP and SAA in humans and rodents declined in an exponential way, the biologic half-life times ($t_{1/2}$) of CRP and SAA in the blood were determined in each enrolled individual, with the data of blood concentrations at two time points (Figure 1), using the following formula:

$$t_{1/2} = \frac{\text{t2-t1}}{\log 2\left(\frac{\text{C1}}{\text{C2}}\right)}$$

where C1 and C2 are blood biomarker levels which are measured at time points t1 and t2, respectively. A time point (t1 + $t_{1/2}$) was estimated when blood biomarker

levels become just a half of C1 (C3), on the basis of that the three time points and concentration, i.e., (t1, C1), (t2, C2) and (t1 + $t_{1/2}$, C3) were on the process of exponential elimination (Figure 1). These estimated half life times ($t_{1/2}$) were used for the regression analysis.

Statistical analysis

Data are presented as means ± SD. The statistical analysis was performed by regression analysis, paired *t*-test, and the Mann-Whitney test as appropriate, using Prism 5 (San Diego, CA, USA). Statistical significance was defined as a p value < 0.05.

Results

Characteristics of enrolled CAP patients

Severity assessment based on the A-DROP scoring system showed that 13 patients in the total of 36 were classified as stage 0, 6 as stage 1, 6 as stage 2, 7 as stage 3, and 4 as stage 4 (Table 2). Pathogens were identified in 48% of the cases, including *Streptococcus pneumoniae* in 14 cases (39%), *Mycoplasma pneumoniae* in 2 cases (6%), and *Haemophilus influenzae* in 1 case (3%). Although some patients had hepatic dysfunction and/or renal dysfunction, as shown in the deteriorated BUN, creatinine, and/or hepatic transaminase levels (Table 2), this did not affect the course of treatment.

SAA is associated with CRP, but not WBC

Plasma CRP levels were 16.2 ± 8.1 mg/dL on the first occasion, significantly reduced to 8.0 ± 5.3 mg/dL on the second occasion ($p < 0.05$), and 2.8 ± 2.8 mg/dL on the third occasion ($p < 0.05$) (Figure 2A). In parallel, plasma SAA levels were 1348 ± 811 μg/mL on the first occasion, significantly reduced to 618 ± 606 μg/mL on the second ($p < 0.05$), and 153 ± 265 μg/mL on the third ($p < 0.05$) (Figure 2B). On the other hand, WBC was 12.1 ± 5.1 × 10^3/μL on the first occasion, significantly reduced to 8.3 ± 3.9 × 10^3/μL on the second, and significantly reduced to 6.9 ± 3.2 × 10^3/μL on the third (Figure 2C).

Table 2. Characteristics of enrolled CAP patients.

N	36
(M/F)	(24/12)
Age	65 ± 18
A-DROP[a] score	
Score 0	13
Score 1	6
Score 2	6
Score 3	7
Score 4	4
Score 5	0
AST (IU/L)	37 ± 34
ALT (IU/L)	26 ± 24
BUN (mg/dL)	19.5 ± 11.5
Cr (mg/dL)	1.3 ± 1.5
Total cholesterol (mg/dL)	135 ± 40
LDL-C (mg/dL)	37 ± 18
HDL-C (mg/dL)	82 ± 32
TG (mg/dL)	76 ± 28

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density-lipoprotein cholesterol; TG, triglycerides

[a]by Japanese Respiratory Society (JRS).

SAA levels were positively associated with CRP levels in the first occasion (|R| = 0.682, $p < 0.05$) (Figure 2D). This association was also observed at the second occasions (|R| = 0.682, $p < 0.05$) and third (|R| = 0.682, $p < 0.05$). In contrast, no associations between SAA levels and WBC were observed throughout the course (Figure 2E).

The severity of CAP estimated by A-DROP score was not associated to either CRP or SAA levels in the first occasion: CRP levels were 14.2 ± 6.9 in those with A-DROP score 0, 22.4 ± 12.0 in A-DROP 1, 17.2 ± 8.8 in A-DROP 2, 15.4 ± 7.2 in A-DROP 3 and 21.0 ± 5.1 in A-DROP 4, whereas SAA levels were 1195 ± 747 in those with A-DROP score 0, 1540 ± 523 in A-DROP 1, 1191 ± 567 in A-DROP 2, 1478 ± 1019 in A-DROP 3 and 1763 ± 1150 in A-DROP 4.

Changes of CRP and SAA in the second occasion were plotted in a semi-logarithmic scale with the adjustment of those levels in the first occasions as 1.0 (Figure 3A and B), however, the regression analyses failed to draw the significantly declining slope (95% confidential intervals (CI) were −0.1631 to 0.001782 for CRP and −0.1945 to 0.04831 for SAA). In contrast, further regression analyses on the CRP and SAA in the third occasion, with the adjustment of those in the second occasion as 1.0, drew the significantly declining slope: their 95% CI were −0.1686 to −0.02040 for CRP and −0.3469 to −0.03800 for SAA (Figure 3C and D). These analyses on the biologic half-life times, calculated from two time points of each individual, indicated that SAA and CRP declined in an exponential way.

Discussions

Our study depicted the kinetics of CRP and SAA in CAP patients: their blood levels significantly declined in the initial phase between first and second occasions (Figure 2A and B), although the declines were not in an exponential way (Figure 3A and B). This was because these biomarkers for inflammation peaked several hours after the initial measurements. In contrast, our result showed that CRP and SAA values in the later phase between the second occasion and the third declined in an exponential way (Figure 3C and D) and in the normal distribution. We thus calculated half-life times, using the data in the later phase: half-lives of CRP and SAA in CAP patients are 46.4 ± 21.7 h and 34.9 ± 28.7 h, respectively, and the latter are significantly shorter.

These observations were confirmed by a previous study, investigating the kinetics of CRP levels and SAA levels after a single intravenous endotoxin administration (Hudgins, 2003). The peak formation of CRP and SAA was at 24 h after endotoxin administration, and then both SAA and CRP started decreasing. SAA levels

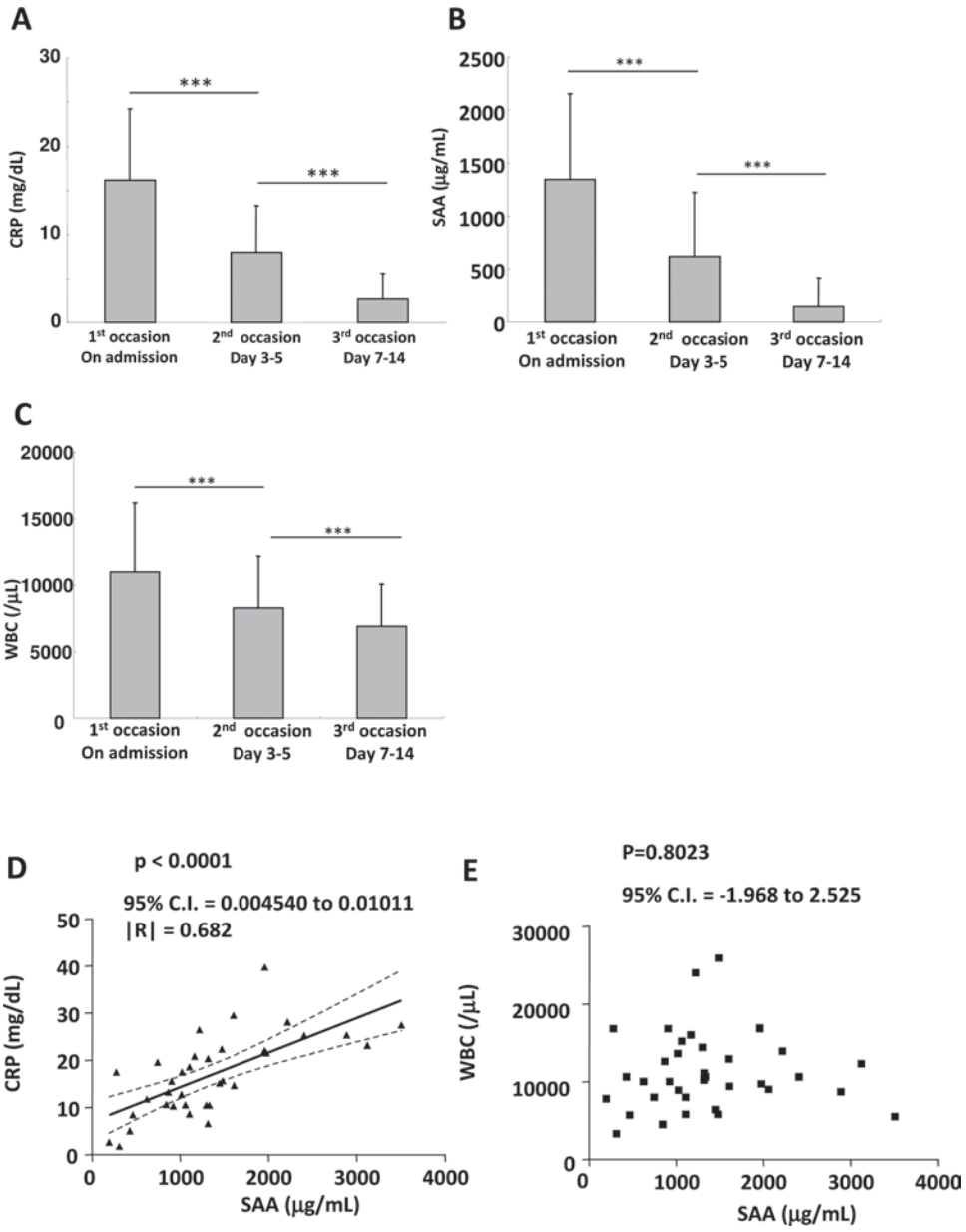

Figure 2. Resolution of community-acquired pneumonia (CAP)-induced inflammation as represented by reductions in c-reactive protein (CRP) (A), serum amyloid A (SAA) (B), and leukocyte count (WBC) (C) levels. The levels of these inflammation biomarkers decreased significantly after antimicrobial treatment. Data are presented as means ± SD. A significant positive association between SAA and CRP is seen ($|R| = 0.632$ to 0.682, $p < 0.01$) (D), whereas no association between SAA and WBC is evident (E). Data are presented as means ± SD.

48 h after endotoxin administration were between 400 and 450 mg/L, which was reduced to 137 mg/L at 72 h. The SAA blood levels became one-third within 24 h. In contrast, CRP levels at 48 h were between 10 mg/dL and 15 mg/dL, and then decreased to 6.2 mg/dL at 72 h, indicating that the CRP levels were halved in 24 h. This report, as well as our result, demonstrated that SAA disappeared quicker in an exponential way than CRP.

Our data on half-life times suggested that a 30% reduction should be attained in CRP levels at 23.9 ± 11.2 h and in SAA levels at 18.0 ± 14.8 h. This suggests that 97.5% of successfully treated CAP patients showed a 30% reduction in CRP levels within 46.3 h after the resolution of the inflammatory reaction. Our data thus provided kinetic evidence for the clinical observation that CRP levels in successfully treated patients showed a 30% reduction within 48 or 72 h after antimicrobial administration (The committee for the Japanese Respiratory Society, 2006). SAA has the further advantage of a shorter biologic half-life.

SAA is synthesized mostly in the liver in response to interleukin 1 (IL-1), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), and LPS (Kluve-Beckerman, 1997; Wada, 1998). This pro-inflammatory cytokines-linked signal transduction includes some transcriptional factors, such as nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and C/EBP, which are also shared by CRP synthesizing signals (Jensen, 1998; Uhlar, 1999). The activation of the signals leads to the transcription of both molecules, SAA and CRP, at the same time.

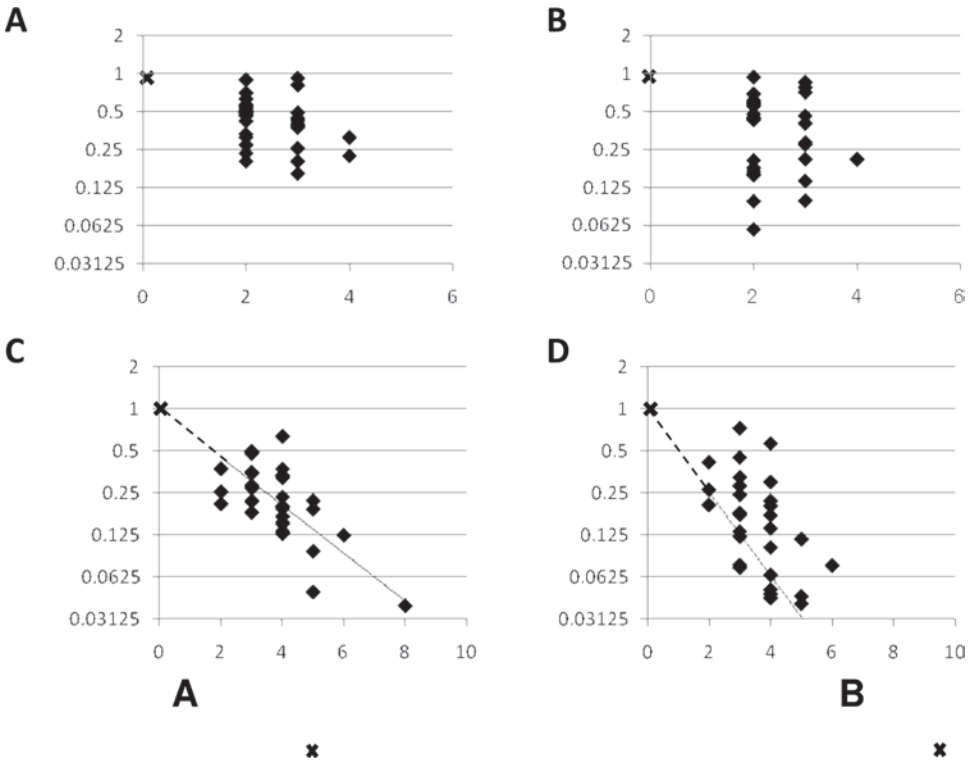

Figure 3. Blood CRP levels at the second occasion (A), as well as SAA (B), with an adjustment of those levels at the first occasion as 1 (cross), were plotted together with the time course. In the same way, blood CRP levels at the third occasion (C), as well as SAA (D), with an adjustment of those at the second occasion as 1 (cross). The regression analyses demonstrated that the changes in the second occasions did not significantly decline in CRP (A: 95% CI = −0.1631 to 0.001782) and SAA (B: 95% CI = −0.1945 to 0.04831). In contrast, decline in the third occasions were significant in CRP (C: 95% CI = −0.1686 to −0.02040) and SAA (D: 95% CI = −0.3469 to −0.03800). D'Agostino & Pearson ominibus K test indicated that changes CRP and SAA were in the normal distribution ($p = 0.0005$ for CRP, and $p < 0.0001$ for SAA).

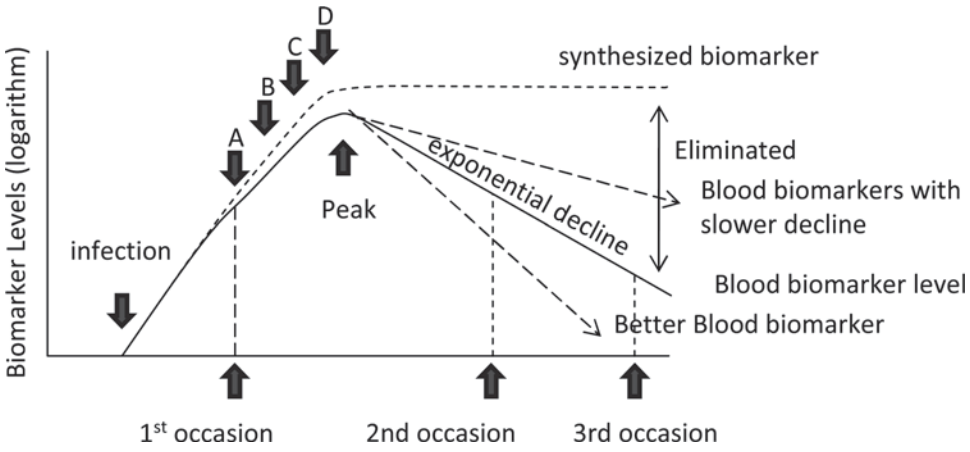

Figure 4. A scheme depicted the basic idea of this study on the kinetics of inflammatory biomarkers. IL-6 and TNF-α, induced by bacterial infection, stimulates macrophages and/or hepatocytes to produce CRP and SAA. After the first occasion, antimicrobial treatment was started (arrow A), which eradicated bacteria (arrow B), stopping the biomarker synthesis (arrow C). The synthesis and elimination of biomarkers are soon balanced (arrow D), around when blood biomarker levels form a peak, and thereafter, the biomarkers declined. In case of CRP and SAA, their declines follow an exponential way (Figure 3C and D) and thus the half-life times should be calculated with second and third time points. They might be one of the major properties of the biomarkers in the management of CAP patients. (Figure 4)

This probably explains the association between SAA levels and CRP levels (Figure 1D).

The CRP levels indicate prognosis of patients with hospital-acquired pneumonia (HAP) (The committee for the Japanese Respiratory Society, 2007), as well as in those with CAP (Coelho, 2007; Kohno, 2011). In parallel, this leads to the hypothesis that SAA levels and prognosis and/or severity of CAP patients are associated, although our study failed to confirm this because only successfully treated CAP patients were enrolled. According to our result, A-DROP scores were not associated either to CRP levels or to SAA levels in the blood, suggesting that they are independent index of the prognosis of CAP patients. This was confirmed by a recently published report, that

made a re-evaluation of A-DROP scoring system in Japan (Kohno 2011).

A scheme (Figure 4) depicted the basic idea of this study on the kinetics of inflammatory biomarkers. IL-6 and TNF-α, induced by bacterial infection, stimulates macrophages and/or hepatocytes to produce CRP and SAA. After the first occasion, antimicrobial treatment was started (arrow A), which eradicated bacteria (arrow B), stopping the biomarker synthesis (arrow C). The synthesis and elimination of biomarkers are soon balanced (arrow D), around when blood biomarker levels form a peak, and thereafter, the biomarkers declined. In case of CRP and SAA, their declines follow an exponential way (Figure 3C and D) and thus the half-life times should be calculated with second and third time points. They might be one of major properties of the biomarkers in the management of CAP patients (Figure 4).

Conclusions

Collectively, our study demonstrated that biologic half-life times of CRP and SAA in CAP patients are 46.4 ± 21.7 h and 34.9 ± 28.7 h, respectively. They were positively associated, suggesting that they share pro-inflammatory intracellular signals acitivated by bacterial infection. In addition, SAA had a shorter biologic half-life than CRP, with a probable implication that SAA might be a better indicator in CAP patients. Our study also provided the kinetic evidence, which enabled us to understand the time course of the inflammatory biomarkers in CAP patients.

Declaration of interest

The authors declare no conflict of interest.

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