Research in Veterinary Science 96 (2014) 460–463

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Interleukin-6 dynamics as a basis for an early-warning monitor for sepsis and inflammation in individual pigs

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ARTICLE INFO

Article history:
Received 14 November 2013
Accepted 25 March 2014

Keywords:
Disease outcome
Sepsis and inflammation
Early warning monitoring
IL-6 dynamics

ABSTRACT

Static interleukin-6 (IL-6) levels of pigs contain considerable individual differences, which obstruct the practical use of IL-6 for disease monitoring purposes. It was hypothesised that interleukin-6 (IL-6) dynamics could be used to quantify these individual differences and carries critical information of the individual pig infection status. Time series of IL-6 responses in 25 pigs were analysed before and after infection by *Actinobacillus pleuropneumoniae*. The results indicated that amplitude increases of IL-6 fluctuations of individual pigs rather than static IL-6 values should be used as indicator of the infection state. This study shows the added value for IL-6 time series analyses of individual pigs. These results are a first step towards the development of objective individualised methods for monitoring and early detection of sepsis and inflammation processes in pigs by integrating animal response dynamics.

IL-6 is often suggested as a key player in the immune response to infection (Borghetti et al., 2009; Kopf et al., 1994). It is well established that the expressions of IL-6 are up-regulated in inflammatory responses to microbial infections. However, previous studies show considerable individual variations in static blood concentrations of IL-6 (Hulten et al., 2003) and other acute phase proteins (Heegaard et al., 2011) in response to infection, which complicate the use of these biomarkers for real-time health monitoring purposes.

Many complex biological processes are involved in sepsis and inflammation (Cinel and Opal, 2009), making it a challenging task to quantify infection and inflammation processes in real-time. In a recent study (Scheffer et al., 2009), an approach was presented to detect sudden changes in complex dynamical systems based on time series data obtained from these systems. These authors suggested the existence of generic early-warning signals in time series which may indicate approaching thresholds for critical changes in complex dynamical systems (Scheffer et al., 2009). More specifically, the pattern of fluctuations in detrended time series was proposed as possible indicator of sudden dynamical transitions. Therefore, we hypothesised that changes of IL-6 fluctuation patterns contain critical information related with the infection state of individual pigs. Accordingly, we aimed at applying data-based modelling methods to quantify the dynamic properties (slow trends and fast fluctuations) of interleukin-6 time series in individual pigs before and after infection by *Actinobacillus pleuropneumoniae* as a first step in developing an early warning monitor for sepsis and inflammation processes. Based on earlier work, it might be expected that the dynamics of biomarkers are related with disease outcome and well-being (e.g. Jansen et al., 2009; Van Loon et al., 2010).

Experiments were approved by the ethical commission of Ghent University (EC2009/029–30/03/2009). Thirty early-weaned outbred pigs were obtained from Rattlerow Seghers Holding N.V. (Lokeren, Belgium). The pigs were catheterised three days before infection. At challenge, 25 animals were endobronchially inoculated with 1×10^7 CFU *A. pleuropneumoniae* (biotype 1-serotype 9 strain, no. 13261; Van Overbeke et al., 2001) under anaesthesia (Table 1, dataset 1 and 2). Five pigs received sterile medium and were used as control group (Table 1, dataset 3). Based on the blood sampling protocol, the 25 infected pigs were divided into two groups, whereas more samples were collected before infection for the pigs of dataset 2. For the control pig group, the blood samples were collected according to the sampling protocol of dataset 1. For every blood sample the IL-6 value was measured by commercially available ELISA kits (Porcine IL-6 Duoset, R&D Systems), resulting in an IL-6 time series for every pig. In addition, all pigs were clinically scored by a veterinarian.

For each pig, changes of IL-6 time series characteristics were quantified by means of static blood IL-6 values and IL-6 fluctuation patterns. All calculations were performed in Matlab using the Statistics Toolbox for the statistical comparisons and the Captain Toolbox for

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http://dx.doi.org/10.1016/j.rvsc.2014.03.014
0034-5288/

Table 1
Overview of the pig experiments with corresponding blood sampling protocols.

Dataset	Number of pigs	Blood sampling before infection (h before infection)	Blood sampling starting from moment of infection (h after infection)	Infection
1	20	4 samples (−72, −48, −24, −2)	≥8 samples (0, 2, 4, . . . , until max. 76 hours after infection for surviving pigs)	Yes
2	5	12 samples (−32, −30, −28, −26, −24, −22, −20, −18, −8, −6, −4, −2)	≥8 samples (0, 2, 4, . . . , until max. 24 hours after infection for surviving pigs)	Yes
3	5	4 samples (−72, −48, −24, −2)	≥8 samples (0, 2, 4, . . . , until max. 76 hours)	No (sterile medium)

the time series analysis (Taylor et al., 2007). To calculate the fluctuation patterns, the IL-6 time series were first standardised and afterwards detrended using an Integrated Random Walk Model (IRW) with Noise Variance Ratio (NVR) of 0.1 (Taylor et al., 2007). For each individual pig, the IL-6 residuals (fast IL-6 dynamics) were determined by subtracting the slow trend from each raw IL-6 time series. According to Scheffer et al. (2009), an increase in the amplitude of fluctuations in the residuals is expected in a time series containing a dynamical transition. Therefore, the obtained residuals of the IL-6 times series were standardised and the area under the cumulative sum function was quantified as measure of changes in the fast IL-6 fluctuation patterns (fast component of IL-6 dynamics; for more details on change detection with the cumulative sum function, see Basseville and Nikiforov, 1993). In a time series with increasing fluctuations, this value is expected to be lower compared to a time series with random fluctuations. Afterwards, these values were rescaled (by the factorial n!, where n is the number of samples in the analysed time window and the factorial of the positive integer n, denoted n!, is the product of all positive integers less than or equal to n) to enable correct comparisons. For dataset 1, data of a short period after infection (2 h before until 14 h after infection) were used for the development of the IRW-models. For dataset 2, the data generated at the day of catheterisation (14 hours) were compared with the data of the last 14 hours (dead or end experiment for surviving pigs). The area under the ROC curve (AUC) was calculated for each variable in order to determine the discriminating power for distinguishing between infected and non-infected pigs (Delong et al., 1988). When a high AUC was obtained for one of the variables, the optimal threshold was calculated with corresponding true positive rate (TPR) and true negative rate (TNR).

Figure 1 (left) shows the boxplot of the static blood IL-6 values at 14 h after infection for the infected pig group and the control group, which received sterile medium. Since it is expected that IL-6 levels increase in response to the infection, a one-tail two-sample *t*-test was used for comparison. The IL-6 concentrations of the infected pig group (dataset 1) were not significantly different from the control group (one-tail two-sample *t*-test: $P > 0.5$; $\text{mean}_{\text{Inf}} = 5.5$ ng/ml; $\text{mean}_{\text{Con}} = 8.8$ ng/ml). This result suggests that it was not possible to use single IL-6 blood values for infection monitoring at pig group level, which was also confirmed by the low AUC value ($\text{AUC} = 0.54$, $\text{SE}_{\text{AUC}} = 0.19$). Afterwards, individual changes of IL-6 were calculated (difference between the IL-6 value at 14 h after infection and the value at 2 h before infection of the same pig). No significant difference was found between the infected group and the control group (one-tail Wilcoxon rank sum test: $P = 0.14$; Fig. 1, right). At individual level, a small improvement of the discriminating power was found ($\text{AUC} = 0.66$, $\text{SE}_{\text{AUC}} = 0.17$), but both results show that IL-6 increases measured by one or two IL-6 blood values were insufficient for accurate infection monitoring. Therefore, the IL-6 responses were also dynamically analysed based on the measured time series data. For every IL-6 time series, the slow trend was removed using the IRW models (Fig. 2, top left; Taylor et al., 2007; Scheffer et al., 2009). The obtained residuals of the infected pigs (dataset 1) and the control pigs (dataset 3) are illustrated in Fig. 2 (top, right). For the infected pigs, the residuals increased starting from 4 to 6 h after infection, whereas the residuals of the control pigs behaved like random noise. These results are in line with the study of Scheffer et al. (2009), since they suggested that an increase in the amplitude of fluctuations in the residuals can be an early sign of a dynamical system undergoing a sudden transition. A significant difference was found by comparing this fast fluctuation component of both pig groups (one-tail two-sample *t*-test: $P = 0.001$; Fig. 2, left bottom). In addition, ROC curve analysis showed a high discriminative power for this fast IL-6 component ($\text{AUC} = 0.88$,

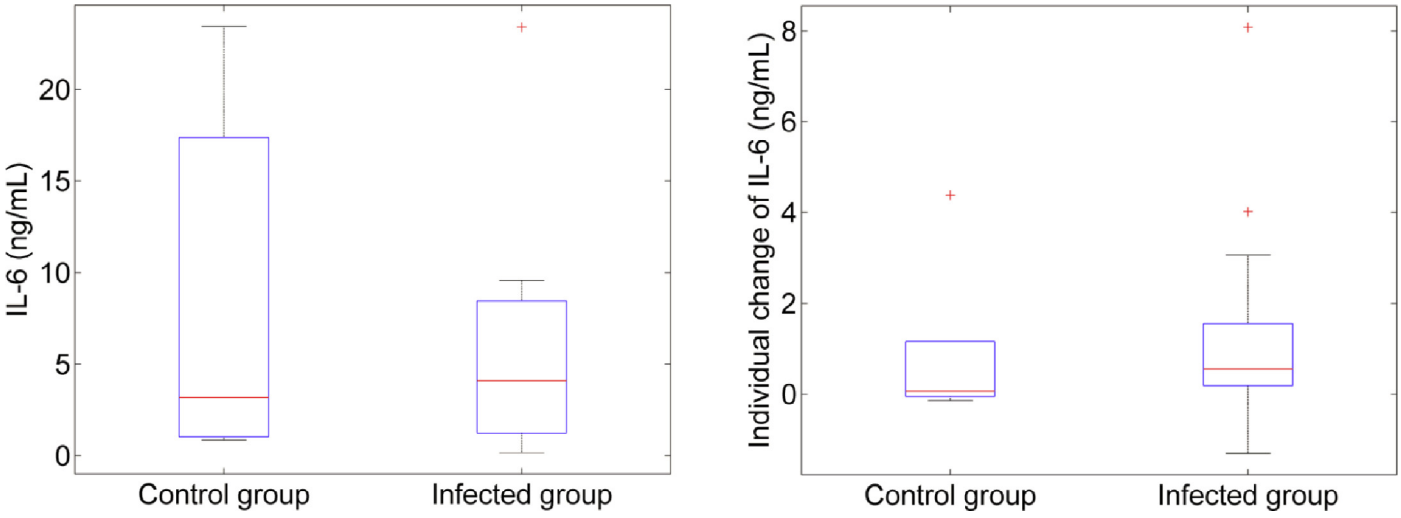

Fig. 1. Comparison of IL-6 concentrations. Boxplots of IL-6 concentrations with the median, interquartile range (box), 1.5 times the interquartile range (whiskers) and outliers (crosses). Left: comparison of infected (dataset 1, n = 20) and control (dataset 3, n = 5) pigs at the pig group level. Right: comparison of individual IL-6 changes from 2 h before to 14 h after infection.